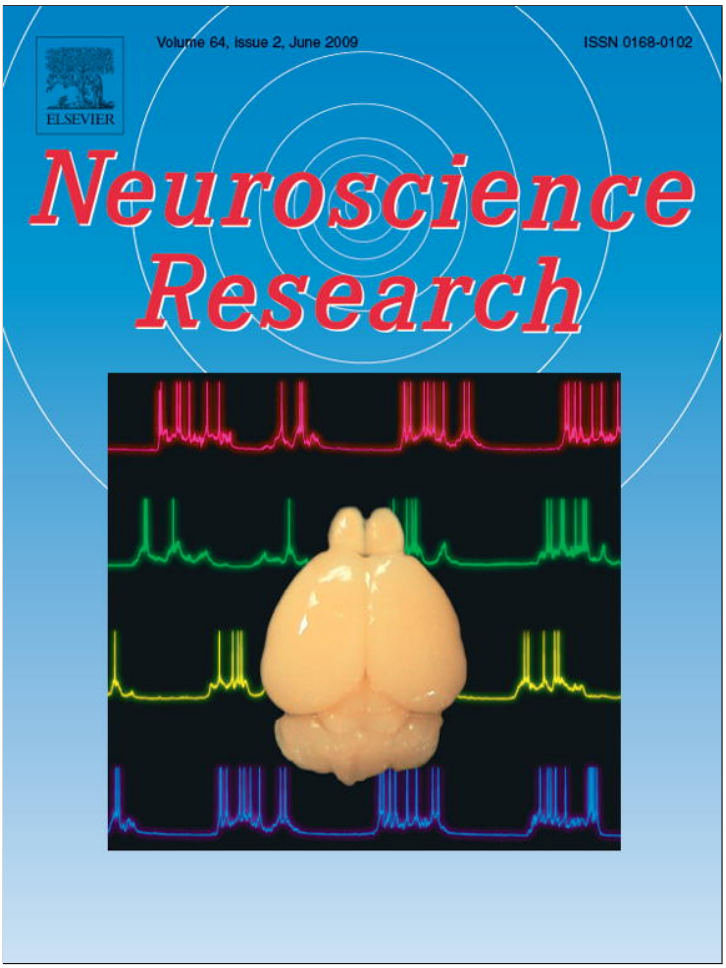

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Rapid communication

Laterality of neocortical slow-wave oscillations in anesthetized mice☆

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ABSTRACT

In the slow-wave (SW) state, the vast majority of cortical neurons exhibit mostly synchronized oscillatory activity. In this study, we examined the right–left hemispheric difference in slow-wave timings in urethane-anesthetized mice. We found that interhemispheric cross-correlograms of local field potentials (LFPs) peaked asymmetrically. Double *in vivo* whole-cell patch-clamp recordings also revealed the interhemispheric temporal disparity of slow wave-relevant synaptic barrages. The data suggest the hemispheric laterality in the slow wave origin.

Slow-wave (SW) sleep, which corresponds to the stages III and IV of non-rapid eye movement sleep associated with so-called "deep" sleep, is characterized by electroencephalogram (EEG) with slow frequencies of usually less than 1 Hz. This sleep state is believed to play roles in memory consolidation/elimination and synaptic homeostasis (Tononi and Cirelli, 2006). During SW sleep, virtually all neocortical neurons display synchronous slow oscillations that alternate between depolarizing phases with massed action potentials and hyperpolarizing phases with firing cessation (Steriade et al., 1993).

Analyses of EEG (Massimini et al., 2004; Murphy et al., 2009) and intracellular membrane potential (Volgushev et al., 2006) demonstrate that SWs originate locally and propagate over the cerebral cortex through cortical network interactions (Sanchez-Vives and McCormick, 2000; Timofeev et al., 2000). Recent evidence indicates that SWs are preferentially initiated at the anterior cortical region and spread down to posterior regions (Massimini et al., 2004; Murphy et al., 2009; Volgushev et al., 2006; Amzica and Steriade, 1995). Little is as yet known, however, about the directional SW flow across brain hemispheres. In the present study, we report the interhemispheric temporal asymmetry of SW activity.

Male mice (ICR, 20–24 postnatal days) were anesthetized with urethane (1.0–1.5 g/kg, i.p.). On either or both side(s) of the skull (±1.3 mm lateral to the bregma), craniotomy (1 mm × 1 mm square) was made at 2.90 mm (frontal, frontal association cortex), −0.75 mm (parietal, primary somatosensory cortex), and −2.45 mm (occipital, secondary visual cortex) anterior to the bregma. After removal of the dura, the exposed cortex was covered with 1.5% agar at a thickness of 0.5 mm.

Local field potentials (LFPs) were recorded with two electrodes placed ipsilaterally or contralaterally into the frontal, parietal, and occipital cortex. For the electrodes, borosilicate glass capillaries with inner filaments were pulled (~1 MΩ) with a P-97 puller (Sutter Instruments, Novato, CA, USA) and filled with artificial cerebrospinal fluid (in mM): NaCl 127, KCl 1.6, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, and glucose 10. The tips of two neighboring electrodes were separated by 0.4 mm (ipsilateral) or 2.6 mm (contralateral) and lowered to 150–200 μm in depth from the cortex surface, which corresponded to the upper border of layer II, with a DMX-11 electric manipulator (Narishige, Tokyo, Japan). Signals were low-pass filtered at 0.2 kHz to clearly detect the bimodality of SW activity. Voltage-clamp recordings were obtained from layer II/III neurons at depths of 150–300 μm in the left and right frontal cortex with borosilicate glass electrodes (5–7 MΩ) filled with solution (in mM): K-gluconate 135, KCl 4, $CaCl_2$ 0.1, HEPES 10, EGTA 1, Mg-ATP 4, and Na_2GTP 0.4 at pH 7.2. The neurons were voltage-clamped at −90 mV. All recordings were amplified by MultiClamp 700B and analyzed by pCLAMP 9.2 (Molecular Devices, Union City, CA, USA). Signals were digitized at

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20 kHz. For averaged data, values are represented as the mean ± standard deviation. Cross-correlograms was used to reveal the time relations between the SW activity of two recording loci. The correlation was calculated from two recording traces with a time period of at least 2 min, which roughly corresponds to >30 cycles of slow oscillations.

Because anesthetized animals show neocortical SWs that are almost identical to those seen in natural SW sleep (Steriade et al., 1993), we addressed SWs in urethane-anesthetized mice. The SW activity was monitored by LFP recordings from the left frontal cortex and its homotopic locus in the contralateral side (left–right, $n = 37$ mice). As control experiments, LFPs were recorded ipsilaterally from two adjacent loci in the frontal cortex (left–left, $n = 13$ mice, right–right, $n = 11$ mice).

SW oscillations consisted of biphasic periods of (i) high frequency-oscillation states, which correspond to SW arrival at the recording site, and (ii) silent states (Fig. 1A and B). The phases of these active-and-silent cycles seemed to be synchronized between two recording sites. However, the cross-correlogram between contralaterally recorded LFPs showed a peak at a time difference of either about +20 or −20 ms, whereas ipsilateral LFPs exhibited a sharp peak at about 0 ms (Fig. 1C and D). The mean absolute value of the time lag that gave the maximal cross-correlation value was 18.8 ± 4.6 ms (contralateral) and 0.2 ± 1.2 ms (ipsilateral) (Fig. 1E). The lag in the contralateral (but not ipsilateral) recordings was significantly higher than 0 ($p < 0.001$, Z-test), indicating that at the millisecond level, SWs occurred with a left–right delay. In this analysis, the positive time lag indicates earlier SW generation in the left hemisphere, and *vice versa*. In 24 (65%) of 37 mice, left SWs preceded right ones, whereas 12 mice (32%) showed the opposite direction, and one mouse showed no time lag (Fig. 1D). Thus, the left-preceding-right type was significantly dominant ($p = 0.03$ for 24 *versus* 12; F test for a proportion).

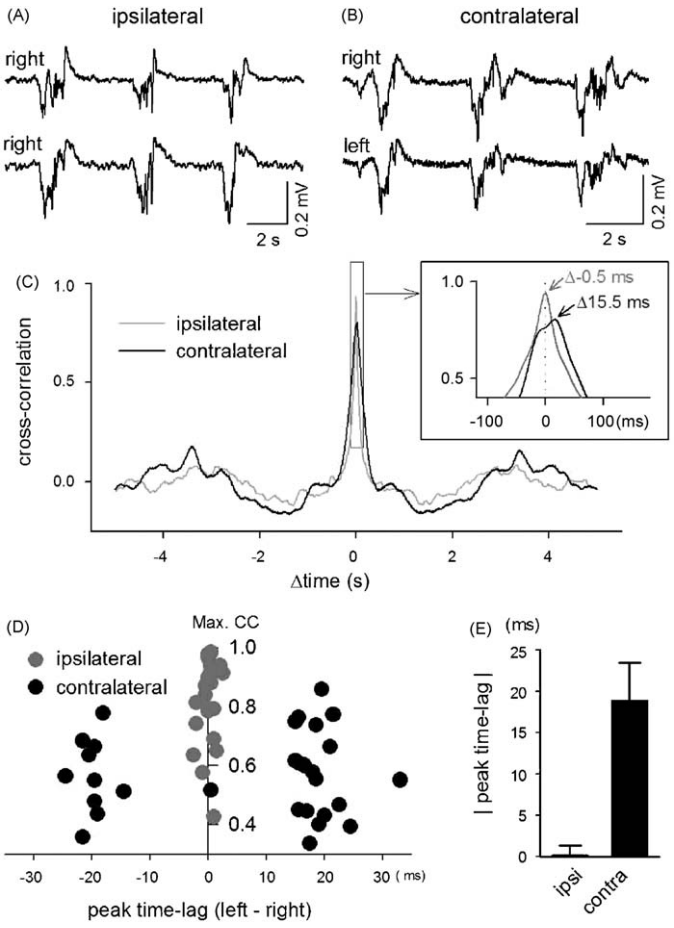

Fig. 1. Interhemispheric time lags between SWs. (A and B) Representative LFP traces simultaneously recorded from the ipsilateral (A) and contralateral (B) sides of the frontal cortex show synchronous oscillations alternating between active and silent periods at the SW rhythm. (C) Cross-correlograms between the two LFP traces. The time axis of the boxed region is magnified in the inset. The cross-correlogram of the contralateral (gray) but not ipsilateral (black) LFP recordings had an asymmetric peak, indicating lateralized SW generation. (D) Distributions of the maximal cross-correlation values (Max. CC) and the time lags that yield the maximal values for ipsilateral (gray) and contralateral (black) LFP recordings. (E) The mean (±SD) absolute values of the peak time lags, calculated from the panel D.

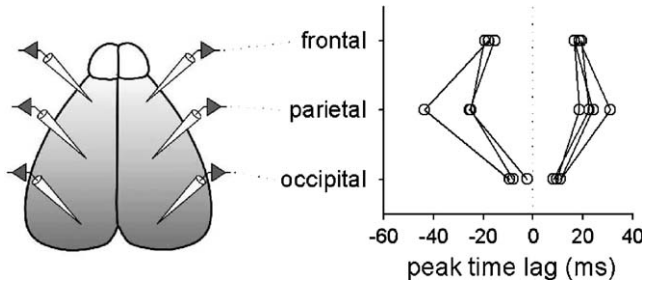

Fig. 2. Consistent direction of SW flow in identical animals. Two LFP electrodes were sequentially inserted into both hemispheres of the frontal, parietal, and occipital cortex. Data from the same mice are linked by the lines. Note the '±' signs of the lags are consistent within each animal ($n = 7$ mice).

We next compared the time lags in various cortical areas within the same animals. LFPs were bilaterally recorded by two electrodes that were sequentially inserted into the frontal, parietal, and then occipital cortex (Fig. 2). The time-lag data were collected from seven mice (Fig. 2). The lag was the largest in the parietal cortex (mean absolute value, 27.1 ± 8.1 ms) and the smallest in the occipital cortex (8.5 ± 2.9 ms). This regional difference was statistically significant ($p = 0.0006$, $t_{7.51} = 5.71$, Welch test). Interestingly, within animals, the signs of the lags were invariant among the cortical areas. In other words, the hemispheric dominance was consistent across the antero-posterior axis of the neocortex in the same mouse.

To further confirm the SW laterality, we carried out *in vivo* dual whole-cell voltage-clamp recordings from frontal cortical neurons. Cortical neurons received intermittent barrages of excitatory synaptic inputs during SWs (Fig. 3A), and the synaptic barrages were almost synchronized between both hemispheres, but again,

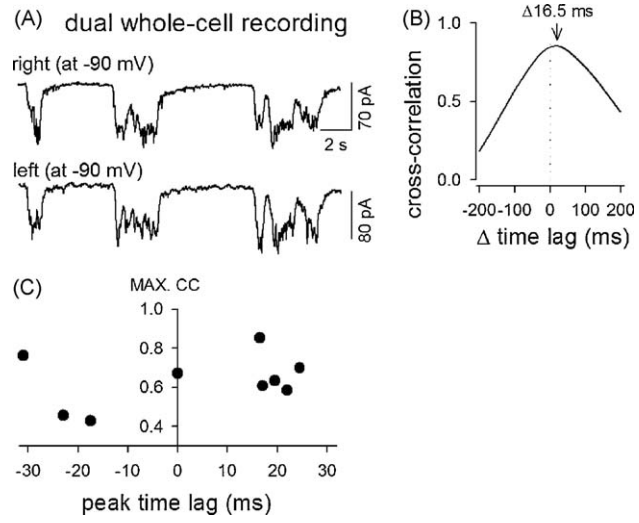

Fig. 3. Interhemispheric time lags of SW oscillations in whole-cell recorded cortical neurons *in vivo*. (A) Typical synaptic input patterns in two voltage-clamped neurons at both sides of the frontal cortex. (B) Cross-correlogram of the traces shown in (A) indicates a non-zero peak time lag. (C) Summary of the peak time lag data ($n = 9$ neuron pairs).

the cross-correlogram had a peak time lag of 16.5 ms, a value close to that in LFPs (Fig. 3B). Similar time lags were observed in eight of nine mice, whereas there was no delay in one case (Fig. 3C).

In both extracellular and intracellular recordings, we found the time lag of SW activity between the right and left neocortex in urethane-anesthetized mice. This indicates that the SW origin is not equally distributed in both hemispheres but rather is rooted in a single side of the brain. A recent human study shows the right-and-left difference in EEG rhythms by computing the directed transformation function (Bertini et al., 2007). Furthermore, a recent analysis with high-density EEG source modeling demonstrates that SWs are more likely to originate in the insula and cingulate cortex, and that these "hot spots" seem to more active in the left hemisphere than in the right (Murphy et al., 2009). But our finding, to our knowledge, is the first evidence for the directed interhemispheric SW flow.

In the same animal, the sign of the time lag was maintained across different cortical areas, suggesting that SWs initiate from a single hot spot in the same hemisphere and thereafter propagate ipsilaterally and contralaterally throughout the brain. We found a very few cases of the nearly zero-lagged SWs (LFP, $n = 1$ mouse; whole-cell, $n = 1$ mice). This may indicate that there are a few mice that show symmetric SW generation, but more probably, it is simply due to the inappropriate recording periods. In these data, indeed, left-preceding and right-preceding SWs seemed to frequently alternate, and as a result, the cross-correlogram is peaked at nearly zero (data not shown).

Given that the homotopic callosal fibers are highly dense (Lomber et al., 1994; Wahl et al., 2007) and that a human EEG study estimates the SW propagation velocity to be as fast as 2.7 ± 0.2 m/s (Massimini et al., 2004), the interhemispheric delay of about 20 ms, as observed here, appears too large. However, multiple intracellular recordings in anesthetized cats demonstrate that cells ipsilaterally located at an interval of 1.5 mm have asymmetric peaks with time lags of 12.0 ± 11.2 ms and also that neurons between the areas 4 and 18 showed time lags of 124.0 ± 86.8 ms (Amzica and Steriade, 1995). Thus, it is likely that the propagation velocity varies across areas and species, being smaller in non-human mammals.

The asymmetry can be accounted for by use-dependent local regulation (Tononi and Cirelli, 2006). In rodents, for example, unilateral sensory stimulation (Vyazovskiy et al., 2000) and preferential paw use (Vyazovskiy and Tobler, 2008) are both known to lead to a contralateral EEG power increase. It is hence possible that the SW time lags resulted from preferential use of the unilateral hemisphere. There is a large body of evidence on functional and morphological asymmetry of rodents' brain. Rodents usually exhibit a tendency toward preferential use of either the right or left paw (Tang and Verstynen, 2002). As for brain morphology, the right hemisphere is consistently larger, as measured by dry/wet tissue weights and external cerebral dimensions (Kolb et al., 1982). The left–right asymmetry was also identified in the spine head size and the subunit composition of glutamate receptors on dendrites of hippocampal pyramidal cells (Kawakami et al., 2003; Shinohara et al., 2008). Neonatal exposures to a novel environment is reported to produce a volumetric increase of the right hippocampus (Verstynen et al., 2001), accompanied by a leftward shift of preferential paw use (Tang and Verstynen, 2002). In this respect, the left-biased SW generation seems consistent with literatures demonstrating a bias toward right-handedness of naturally grown rodents (Waters and Denenberg, 1994).

In conclusion, we found the bihemispherical asymmetry in SW activity, as measured by LFP and postsynaptic currents in the mouse neocortex. The right–left dominance varies from mouse to mouse, but the dominance is consistent across cortical areas within animals. Preferential use of the cerebral hemisphere might exist in naïve mice, contributing to behavioral habits, such as handedness.

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