Slow and fast rhythms generated in the cerebral cortex of the anesthetized mouse

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Ruiz-Mejias M, Ciria-Suarez L, Mattia M, Sanchez-Vives MV. Slow and fast rhythms generated in the cerebral cortex of the anesthetized mouse. J Neurophysiol 106: 2910–2921, 2011. First published August 31, 2011; doi:10.1152/jn.00440.2011.—A characterization of the oscillatory activity in the cerebral cortex of the mouse was realized under ketamine anesthesia. Bilateral recordings were obtained from deep layers of primary visual, somatosensory, motor, and medial prefrontal cortex. A slow oscillatory activity consisting of up and down states was detected, the average frequency being 0.97 Hz in all areas. Different parameters of the oscillation were estimated across cortical areas, including duration of up and down states and their variability, speed of state transitions, and population firing rate. Similar values were obtained for all areas except for prefrontal cortex, which showed significant faster down-to-up state transitions, higher firing rate during up states, and more regular cycles. The wave propagation patterns in the anteroposterior axis in motor cortex and the mediolateral axis in visual cortex were studied with multielectrode recordings, yielding speed values between 8 and 93 mm/s. The firing of single units was analyzed with respect to the population activity. The most common pattern was that of neurons firing in >90% of the up states with 1–6 spikes. Finally, fast rhythms (beta, low gamma, and high gamma) were analyzed, all of them showing significantly larger power during up states than in down states. Prefrontal cortex exhibited significantly larger power in both beta and gamma bands (up to 1 order of magnitude larger in the case of high gamma) than the rest of the cortical areas. This study allows us to carry out interareal comparisons and provides a baseline to compare against cortical emerging activity from genetically altered animals.

SLOW OSCILLATIONS (<1 Hz) occurring during slow-wave sleep and ketamine anesthesia were originally characterized in the neocortex of the cat (Steriade et al. 1993). During this rhythmic activity, the cortical network switches between depolarized, active periods or up states and silent periods or down states. During up states, the recurrence within the network maintains persistent activity with a functional structure that often reproduces that occurring during the cortical processing in the awake state (Destexhe et al. 2007; Luccak et al. 2007; MacLean et al. 2005; Sakata and Harris 2009; Steriade and Timofeev 2003; Tsodyks et al. 1999). One of these features is the synchronization of activity in beta and gamma bands during up states (Compte et al. 2008; Hasenstaub et al. 2005; Steriade et al. 1996). In vivo rhythmic cortical slow oscillations and their propagation have been observed and characterized in different species including humans (Csercsa et al. 2010; Massimini et al. 2004; Nir et al. 2011), cats (Chauvette et al. 2010; MacLean et al. 2005; Volgushev et al. 2006), ferrets (Haider et al. 2006), and rats (Sakata and Harris 2009). Up and down states have been studied as well in the brain of mice in vitro (Ikegaya et al. 2004; Rigas and Castro-Alamancos 2007) and in vivo, both in cortex (Fellin et al. 2009; Petersen et al. 2003) and thalamus (Zhu et al. 2006). However, there has not been a systematic characterization across different cortical areas, despite this being interesting for sleep and memory studies (Diedelmann and Born 2010; Vyazovskiy et al. 2006) as well as for establishing a baseline for cortical function in genetically modified mice.

Here we present a detailed characterization of rhythmic spontaneous activity across different cortical areas in the anesthetized mouse, including patterns of propagation and high-frequency content. We find that prefrontal cortex presents differences with respect to all other studied areas (primary visual, somatosensory, and motor). An analysis of the firing of single units during up states is also included.

EXPERIMENTAL PROCEDURES

In vivo extracellular recordings. Mice were cared for and treated in accordance with Spanish regulatory laws (BOE 256; 25-10-1990), which comply with the European Union guidelines on protection of vertebrates used for experimentation (Strasbourg 3/18/1986). All experiments were approved by the Ethics Committee from the Hospital Clinic (Barcelona, Spain). Adult C57BL/6SJL mice 3–6 mo old were used for extracellular recordings (n = 40). Anesthesia was induced with intraperitoneal injection of ketamine (75 mg/kg) and medetomidine (1 mg/kg). Atropine (0.3 mg/kg) was administered to prevent respiratory secretions. Tracheotomy was performed to increase stability during recordings. After this procedure and administration of a maintaining dose of ketamine (37.5 mg/kg ip), the animal was placed in a stereotaxic frame and air enriched with oxygen was delivered through a thin silicon tube placed at 0.5–1 cm from the tracheal cannula. A continuous infusion of ketamine at 40 mg·kg−1·h−1 was delivered subcutaneously to maintain a constant level of anesthesia. Methylprednisolone (30 mg/kg) was also administered to prevent inflammation. Body temperature was maintained at 36–37.5°C. Bilateral craniotomies were made at 4 sites: AP 36–37.5°C. Bilateral craniotomies were made at 4 sites: AP 2.3 mm from bregma, L 0.4 mm or AP 2.5 mm, L 0.5 mm (medial prefrontal cortex); AP 0.5 mm, L 1.5 mm (primary motor cortex); AP −1.5 mm, L 2.5 mm (primary somatosensory cortex); and AP −2.5 mm, L 2.5 mm (primary visual cortex) (following Franklin and Paxinos 2008). Extracellular slow wave recordings were obtained with tungsten electrodes with impedances of 1–2 MΩ. Electrodes were placed in infragranular layers (0.4 lateral and 1.0–1.2 mm deep in prefrontal cortex, 0.9–1.1 mm in somatosensory cortex, 0.7–0.9 mm in visual cortex, 1.0–1.2 mm in motor cortex). Extracellular recordings were usually obtained bilaterally, amplified with either a NeuroLog (Digitimer) or a multichannel system (Multi Channel Systems). The signal was digitized at 20 KHz with a CED acquisition board and Spike 2 software (Cambridge Electronic Design).

In vivo slow wave propagation. Arrays of M = 16 aligned electrodes separated by 125 μm (Neuronexus) were used to record activity propagation. We computed the speed of up state propagation across the cortex, relying on the time lags between consecutive detected up state onsets from multiunit activity (MUA) of different electrodes. We selected...
time series containing at least \( N = 300 \) up states. The average of the onset times of the same traveling up state in the multielectrode array was taken as reference for each detected up state to compute the array of relative time lags. In other words, if \( T_{c,n} \) is the onset time of the \( n \)th up state in the \( c \)th electrode, the array of relative time lags is \( \Delta T_{c} = T_{c,n} - \sum_{n=1}^{M} T_{c}/M \).

Absolute values \( \Delta T_{c} > 150 \) ms were not considered, because these were very likely due to a failed up state detection. We explored the possible existence of different patterns of activity propagation by sorting the time lag arrays \( \Delta T_{c} \) of the detected up states in the low-dimensional space resulting from principal component analysis (PCA) (Jackson 1991). We found that the first three principal components always represented \( >50\% \) of the total variance. In this three-dimensional subspace each detected up state is a point. We worked out the “principal axis” of the point cloud, minimizing in the least-squares sense the sum of the distances of the points from this axis. Finally, the points are projected on such a principal axis in order to associate a scalar quantity to each up state and to rank them. Sorting \( \Delta T_{c} \), with such ranking, we found gradual but significant changes in the activity propagation patterns. We then pooled the up states in five equally sized groups (at least 60 time lag arrays per group), such that the first pool was \( \Delta T^{(1)} = [\Delta T_{c}]^{1/5} \), the second pool was \( \Delta T^{(2)} = [\Delta T_{c}]^{2/5} \), and so on. For each group of arrays we carried out the average time lag \( <\Delta T^{(n)}_{c}> = \sum_{n=1}^{5} \Delta T^{(n)}_{c}/n \) and its standard error (SE) for each electrode \( c \). Finally, the speed was computed in those recordings showing a monotonic change with electrode position of the average time lag within each propagation pattern. It was carried out by dividing the distance \( D^{(c)} \) between the electrodes showing the maximum and minimum average time lags by their lag difference: \( D^{(n)}/[\max(<\Delta T^{(n)}_{c}>)-\min(<\Delta T^{(n)}_{c}>)] \).

In vivo single unit with extracellular recordings. Glass recording electrodes (8–15 MΩ) were pulled on a Sutter Instruments P-97 micropipette puller (Novato, CA) from medium-walled glass and filled up with saline. Electrodes were placed in the primary motor, medial prefrontal cortex, and somatosensory cortex. A tungsten electrode similar to those described above was placed nearby the glass electrode, at \( <200 \) µm, to obtain a recording from the local network in the vicinity. Signals were digitized and acquired at 50 kHz for single-unit recordings and at 10 kHz for extracellular recordings. Agar was used to prevent the cortex from desiccation and to stabilize electrophysiological recordings.

Data analysis. MUA was estimated as the power change in the Fourier components at high frequencies of the extracellular recordings (Reig et al. 2010; Sanchez-Vives et al. 2010). We assume that the normalized MUA spectrum provides a good estimate of the population firing rate, because normalized Fourier components at high frequencies have densities proportional to the spiking activity of the involved neurons (Mattia and Del Giudice 2002). MUA were logarhythmically scaled in order to balance the large fluctuations of the nearby spikes. Up and down states were singled out by setting a threshold in the log(MUA) time series. The threshold was set to \( 60\% \) of the interval between the peaks in the bimodal distributions of log(MUA) corresponding to the up and down states. The peak related to the down state was used as reference, setting there log(MUA) = 0. Single-out sets of up and down state durations from each recording were used to estimate the nine parameters reported in Fig. 3. Up and down state durations (Fig. 3, B and C) were the averages across such sets. Frequency in Fig. 3A was the inverse of the average duration of the whole up-down cycles. Down-to-up and up-to-down transitions in Fig. 3, D and F were estimated as the slope of the average profile of log(MUA) around a small time interval around the transitions \( ([-10, 25]) \) ms for upward and downward transitions, respectively. The maximum relative firing rate in Fig. 3F was the maximum average log(MUA) following the down-to-up transition. The coefficient of variation (CV) in Fig. 3, G–I, was the fraction between the standard deviation and the mean value of the durations of up and down states and of the whole up-down cycles singled out for each recording, respectively.

All of the MUA off-line estimates and analyses were implemented in MATLAB (The MathWorks, Natick, MA). Data are displayed as means ± SE in all error bars in plots.

Single-unit recordings were analyzed with Spike2 software (Cambridge Electronic Design). Firing patterns were obtained through peri-event time histograms (PETHs) triggered to the onset of up states and a bin size of 20 ms. The length of the analyzed periods for the PETHs was 300 s.

To analyze the fast components of oscillations, up and down states were detected and their respective power spectra were calculated. Power spectrum density analysis was carried out by Welch’s method with \( 50\% \) overlapped windows of 2,000 samples. To compare power, an average relative power was calculated by averaging within three frequency bands (beta 15–30 Hz, low gamma 30–60 Hz, and high gamma 60–90 Hz) resulting from the quotient between the power in the up states and in the down states.

Statistical analysis. Unless otherwise stated, all comparisons between means were performed with one-way ANOVA followed by Fischer least significant difference post hoc tests, and their degrees of freedom and \( F \) values are shown in Supplemental Tables S1 and S2.

RESULTS

The spontaneous rhythmic activity generated in different regions of the mouse cortex (primary visual, somatosensory, and motor cortex and medial prefrontal) was recorded under ketamine anesthesia. Under these conditions, both slow oscillations and fast rhythms are generated in the thalamocortical network (Steriade et al. 1993, 1996). The slow oscillation (Fig. 1) consisted of periods of neuronal firing or up states (Fig. 1A, bottom) interspersed with rather silent periods or down states. The recordings showed the low-frequency nature of the slow waves (\(<1 \) Hz). During up states different frequencies were generated, including high-frequency fluctuations (Fig. 1A, top), as described in detail below. Such up states propagate across the cortical tissue (Luczac et al. 2007; Massimini et al. 2004; Sanchez-Vives and McCormick 2000), and several parameters of these waves were analyzed to describe the features of these oscillations and to compare across different cortical areas.

**General description of slow oscillation in the anesthetized mouse.** To characterize the slow waves in the anesthetized mouse, extracellular recordings from primary visual, somatosensory, motor, and medial prefrontal cortex were obtained by means of tungsten electrodes placed in the deep cortical layers. The recordings were bilateral, and up states usually occurred concurrently in both hemispheres with a small time lag measurable at the central peak of the waveform cross-correlation (Fig. 1, B and C). The distribution of time lags between left and right hemispheres is represented in Supplemental Fig. S1 (\( n = 29 \) bilateral recordings; \( n = 16 \) mice). It illustrates that there was no significant bias toward one hemisphere to initiate the activity. The average absolute value of the time lag of the peak (mean ± SD) was 7.1 ± 10.9 ms in visual cortex (\( n = 7 \)), 7.9 ± 9.4 ms in somatosensory cortex (\( n = 7 \)), 5.0 ± 4.9 ms in motor cortex (\( n = 6 \)), and 6.4 ± 3.8 ms in prefrontal cortex (\( n = 9 \)).

To characterize the emergent rhythmic activity, nine parameters of the oscillatory activity were quantified as in Sanchez-Vives et al. (2010): frequency of oscillation, up state duration, down state duration, slope of down-to-up
state transition, slope of up-to-down state transition, maximum relative firing rate, CV of the up state duration, CV of the down state duration, and CV of the up state–down state period. An operational definition of each one of these parameters is included in EXPERIMENTAL PROCEDURES.

Figure 2 illustrates representative recordings of all four recorded cortical areas and the analytical methods used to quantify their activity. We were interested in the comparative evaluation of these parameters across cortical areas, and therefore box plots were produced for primary visual (n = 7), primary somatosensory (n = 7), primary motor (n = 7), and medial prefrontal (n = 10), displaying the mean and median values (Fig. 3). These numbers of observations refer to the number of recordings included. Given that only one recording was obtained per area in each experiment, the number of recordings per area is equivalent to the number of animals. No significant differences in any of the analyzed parameters were found across hemispheres for the same cortical area.

The average oscillatory frequency of oscillation across all areas was 0.97 Hz; 54.8% of the recordings displayed frequencies between 0.6 and 1 Hz. No significant differences across areas were observed between the average duration of either up (Fig. 3B; 0.28 s) or down (Fig. 3C; 0.8 s) states.

Comparing across areas, prefrontal cortex was the area that exhibited larger differences (P < 0.05) compared with the other areas across a variety of parameters. The slope of down-to-up transitions in prefrontal recordings was significantly faster than in the other three cortical regions. The population firing rate was also higher in prefrontal cortex than in the other cortical areas (Fig. 3F). Another property of the rhythm in the prefrontal cortex was the higher regularity in oscillations, reflected in a lower CV of the duration of up states compared with the other three areas (Fig. 3G).

Some other specific differences between areas were also detected, such as a faster transition from up to down state in motor and prefrontal cortex than in the two primary sensory areas. Further information about the statistics of this section is given in Supplemental Table S1.

Firing properties of cortical neurons during slow waves. Recordings of single units (Fig. 4) were obtained by singling out 37 single neurons from motor, 9 from somatosensory, and 43 from prefrontal cortex. Their firing patterns were analyzed with respect to the population activity in the same location recorded as the local field potential (LFP). Almost all spikes were found to occur during up states, with a few exceptions that are described below. Figure 4, A–D, illustrates two different firing patterns that were observed: tonic firing during the whole duration of the up state (mean firing rate 24.7 Hz; n = 19 neurons) versus a sparse firing (mean firing rate 5.2 Hz; n = 70).

We were interested to determine the number of spikes that single neurons fire per up state. No differences were observed between neurons from motor and somatosensory cortex; thus we grouped them together. Of those 46 neurons, 52.2% fired in >90% of the up states, while 37.0% fired in 50–90% of up states and only 10.9% fired in <50% of active states (Fig. 4F). On the other hand, most of the neurons had a sparse firing with <2 spikes per up state in 47.8% of the neurons; 26.1% fired 2–6 spikes, and the same percentage had tonic firing and fired trains of >6 spikes during up states (Fig. 4E).

Neurons from prefrontal cortex showed some differences with neurons from primary cortices, and we report these separately. The difference was that prefrontal cortex neurons fired in more up states and with more spikes per up state. This is represented in Fig. 4, G and H; 72% of prefrontal cortex neurons fired in all up states, while only 3% fired in less than half the up states. On the other hand, 50% of neurons had a discharge of 2–6 spikes per up state.

Taking all neurons together, we conclude that most of the neurons participate with a relatively sparse firing in most of the up states when participating in slow oscillations.

We were interested to know how the firing of individual neurons was distributed during up states. Therefore, the firing rate of all 89 units was analyzed within up states, and PETHs were built around the onset of up states. We observed a different distribution of single units’ firing during up states between primary cortices and prefrontal cortex neurons, so they appear separated in the next considerations. The PETHs
Fig. 2. Analysis of population activity in 4 cortical areas. A: prefrontal cortex: from top to bottom: relative firing rate, LFP, raster plots of 100 aligned up states, and waveform average of the relative firing rate. This average is the one used for the calculation of the down-to-up and up-to-down slopes and the maximum firing rate of the analyzed periods. The shade corresponds to the SD. The red boxes represent the automatically detected up and down states. B–D: same as A but in motor, somatosensory, and visual cortices, respectively.
revealed two main groups of firing patterns: 1) neurons with a maximum firing rate at the initiation of the up state (primary cortices $n=15$; prefrontal cortex $n=23$) (Fig. 5, A and D) and 2) neurons with the maximum firing rate toward the center of the up state (primary cortices $n=28$; prefrontal cortex $n=15$) (Fig. 5, B and E). Of the remaining three neurons from primary cortices, one had a tonic firing evenly distributed during the duration of the up state (e.g., Fig. 5C) and two showed an increased firing rate preceding the termination of the up state. Of the remaining prefrontal cortex single units, three fired at the end of up states and two had rather a tonic discharge (Fig. 5F).

To compare across neurons and to exclude the variations in up state duration, up states were divided into five even windows (Fig. 5, G–I), and the firing rate of the unit was estimated for each fifth of the up state. In this way, a grand average of the PETHs for 46 neurons from primary cortices and 43 prefrontal cortex neurons was calculated (see Fig. 5G, inset). The average distribution of somatosensory and motor single units’ firing showed a preferential increase of firing rate toward the center of the up state, given that the most common neuronal pattern was that in Fig. 5B and E. Prefrontal cortex single units fired toward the beginning of the up state (Fig. 5G, inset).

To further take into account differences in up state duration, all the up states recorded for each neuron ($n=100–200$) were split into three groups according to length: short, medium, and large. When the firing rate distribution of single neurons was represented for these three groups (Fig. 5, G–I) it showed that the predominance of motor and somatosensory single-unit firing toward the center of the up state was not dependent on the duration of the up states. The same was the case for prefrontal cortex single units, which independently of the duration of up states tended to fire toward the beginning of up states (Fig. 5, G–I). In this way the single-unit firing rate distribution was evaluated in a total of 6,764 up states in the case of the primary cortices and 7,695 in the case of the prefrontal cortex.

A small number of neurons (3 of 46) had spikes in the 50-ms window preceding up states. These were the only spikes...
observed during down states. When analyzing these spikes preceding up states, we should take into account a number of possible sources of error. One of these is the fact that single units and LFP were analyzed with two closely located different electrodes. The maximum separation between the electrodes was 100 μm. If we consider that some up states could be detected earlier in one electrode than in the other, and we consider the slowest detected propagation speed (10 mm/s), we conclude that it would take 10 ms to travel from one electrode to the other, so the firing occurring earlier than 10 ms has to be considered as preceding the up state. Another possible source of error could be the method of detection of the up state in the population. Any method of up state detection requires the determination of a threshold. In the network, though, the up state starts building up before the threshold is reached. We estimate that on average we could consider that up to 30 ms preceding the threshold there could be activity building up. Thus, if we consider both confounds together, there can be a window of 40 ms during which the firing of single units could be considered as occurring during up states. Those occurring earlier are probably spikes actually preceding up states. From this we conclude that the spikes detected in these two neurons that appear to precede up states are probably part of the building up of the activity.

Propagation of slow waves in the mouse cortex. Wave propagation was evaluated with an array of 16 electrodes separated from each other by 125 μm. The electrode was placed at 1 ± 0.2-mm depth and parallel to the midline in the motor cortex and orthogonal to the midline in the visual cortex (Fig. 6, A and B, top insets). We included in the analysis 20 recordings of wave propagation, 12 from motor and 8 from visual cortex, and the propagation of at least 300 consecutive up states was evaluated.

Up states were detected with the same threshold algorithm for the spectral estimated MUA used previously to determine the properties of slow oscillatory activity (see EXPERIMENTAL PROCEDURES for details; Sanchez-Vives et al. 2010). The relative time lags between up state onsets singled out from the 16 electrodes were used to identify different patterns of activity propagation. Therefore, we performed a PCA that allowed the projection of the time lag patterns of different up states into a subspace suitable for sorting them and capable of retaining the most variance. These ranked up states were pooled into five equally sized groups. The average time lags within each group
are shown in Fig. 6 for three typical recordings: the different symbols and related connecting lines clearly display significantly different modes of propagation within the same recording session, which occur randomly in time.

Six of twelve cases showed a clear pattern of propagation from front to back in motor cortex, in agreement with the predominant direction of propagation of oscillations during slow-wave sleep in humans (Massimini et al. 2004). In visual cortex, the majority of cases (6 of 8) showed a monotonic variation of the average time lag along the multielectrode array. While the direction of the propagation along the axis of the electrode array was constant, the estimated speed of different waves varied continuously within a range of 8–93 mm/s. This is illustrated for both motor and visual recordings in Fig. 6, A and B. All 12 cases were highly similar to those illustrated here. The motor and visual distributions of velocities (Fig. 6, A and B, bottom insets) across time lag patterns and recordings are not significantly different (2-tailed Kolmogorov-Smirnov test, $P = 0.42$), although the two electrode arrays were placed in orthogonal positions.

The remaining seven cases showed more heterogeneity in the direction of propagation (see, e.g., Fig. 6C from a motor cortex recording). These patterns were compatible with waves propagating from other areas, e.g., with lateral propagation or with colliding waves coming from different directions. In these cases we could not confidently estimate the component of the speed of propagation along the axis of the electrode array.

Fast components of mouse neocortical slow oscillations. The frequency content of the LFP in the recordings from the four studied cortical areas (visual, somatosensory, motor, and prefrontal cortex) was analyzed. Spectrogram analysis confirmed the presence of fast rhythms in the up states (Fig. 7A). To study frequency content during up and down states in a systematic manner, a power spectrum analysis was carried out on recordings from prefrontal cortex ($n = 10$ mice; Fig. 7, B and C) and somatosensory ($n = 7$; Fig. 7E), motor ($n = 6$; Fig. 7D).
with respect to somatosensory cortex, and P cortex (states. The beta power ratio was 13.2 times larger in prefrontal somatosensory, and visual cortex in up with respect to down cortex. Reasons like a lower power during down states in prefrontal result of a specific increase during up states and not of other areas (n = 27, 6 of 12 recordings). Vertical dashed line is the average propagation speed: 30.0 ± 3.9 mm/s. B: same as in A for recordings in the visual cortex. Note that in this cortical area the array was placed in the coronal plane (see top inset), thus orthogonal to that in A. Bottom inset: distribution of speeds estimated from the up state pools with similar time lag patterns in the motor cortex recordings, illustrating a monotonic decrease of time lags from medial to lateral positions (n = 28, 6 of 8 recordings). Average speed: 23.4 ± 2.1 mm/s. C: another motor cortex example showing complex patterns of activity propagation.

DISCUSSION

This study is a systematic characterization of the emergent cortical activity in the mouse cerebral cortex during ketamine anesthesia. Continuous infusion of subcutaneous ketamine allowed us to obtain stable anesthesia levels reflected in a regular slow oscillatory frequency along the duration of the experiment (6–7 h). Under these conditions, up and down states were spontaneously generated by the cortical network, and nine parameters quantifying these were compared across visual, somatosensory, motor, and prefrontal cortices. We also explored the firing of individual neurons with respect to the network activity, wave propagation, and generation of beta and gamma synchronization during up states. This information should be valuable for understanding cortical network emerging activity in the mouse model. It is also useful as a baseline of spontaneous activity generated in the mouse, allowing us to compare against experimental or genetic manipulations. Given that slow-wave sleep has a role in memory consolidation (Diekelmann and Born 2010; Marshall et al. 2006), it is also relevant to have a quantitative study as a point of reference for behavioral studies.

Fig. 6. Speed of propagation of up state onsets in 2 cortical areas (motor and visual) of the mouse. A: average time lags of up state onsets recorded with arrays of 16 electrodes in primary motor cortex. Top inset: location and position of the recording array (discontinuous line). Time lags were grouped in 5 different pools with similar patterns of activity propagation (each with different symbols and gray levels) obtained with a principal component analysis (see text for details). Bottom inset: distribution of speeds estimated from the up state pools with similar time lag patterns in the motor cortex recordings, illustrating a monotonic propagation from front to back (n = 27, 6 of 12 recordings). Vertical dashed line is the average propagation speed: 30.0 ± 3.9 mm/s. B: same as in A for recordings in the visual cortex. Note that in this cortical area the array was placed in the coronal plane (see top inset), thus orthogonal to that in A. Bottom inset: distribution of speeds for the visual cortex recordings showing a monotonic decrease of time lags from medial to lateral positions (n = 28, 6 of 8 recordings). Average speed: 23.4 ± 2.1 mm/s. C: another motor cortex example showing complex patterns of activity propagation.

Fig. 7. Relative powers during up versus down states for the studied frequency bands, beta (15–30 Hz), low gamma (30–60 Hz), and high gamma (60–90 Hz). In the low gamma range, the relative power in somatosensory, motor, and prefrontal cortices. We also divided the gamma band into high gamma (60–90 Hz) and low gamma (30–60 Hz). In the low gamma range, the relative power in somatosensory, motor, and prefrontal cortices. We also divided the gamma band into high gamma (60–90 Hz) and low gamma (30–60 Hz). Further information on the statistics of this section is given in Supplemental Table S2.

The absolute values of beta and gamma power in up (Fig. 8, D–F) and down (Fig. 8, G–I) states are illustrated in Fig. 8, D–I, while the relative beta and gamma power in up versus down states is represented in Fig. 8, A–C. Figure 8 shows that the higher beta and gamma power in prefrontal cortex is the result of a specific increase during up states and not of other reasons like a lower power during down states in prefrontal cortex.

The beta power band was 3.5- to 7.2-fold higher in motor, somatosensory, and visual cortex in up with respect to down states. The beta power ratio was 13.2 times larger in prefrontal cortex (P < 0.005 with respect to motor cortex, P < 0.0001 with respect to somatosensory cortex, and P < 0.0001 with respect to visual cortex). Prefrontal cortex showed a markedly higher relative power in the gamma band (Fig. 7, B and C). We divided the gamma band into high gamma (60–90 Hz) and low gamma (30–60 Hz). In the low gamma range, the relative power in prefrontal cortex was 42.2, significantly larger than in other areas (P < 0.001 with respect to motor cortex, P < 0.0001 with respect to somatosensory cortex, and P < 0.0001 with respect to visual cortex), where values ranged from 3.6 to 5.8. Finally, in the high gamma band prefrontal cortex showed a relative power value of 124, which was significantly different from motor (P < 0.005), somatosensory (P < 0.005), and visual (P < 0.005) cortices, where the values ranged from 8.9 to 14. Further information on the statistics of this section is given in Supplemental Table S2.
Slow oscillations recorded in primary sensory cortices (visual and somatosensory) and primary motor cortex were highly similar, and most of the differences found in this study were observed in prefrontal cortex. Interestingly, the mean frequency of occurrence was the same in all areas, coherent with a traveling wave across the cortical network. However, the regularity of this frequency (1/CV of the cycle) was maximal in prefrontal cortex. During slow-wave sleep in humans it has been described that waves preferentially travel from frontal to occipital areas (Massimini et al. 2004). Even though any point in the cortical network can potentially start a new wave, the frontal cortex appears to be the most common initiator of a new wave (Massimini et al. 2004). Our multiple recordings following an anteroposterior alignment also found a preferential propagation from front to back in the mouse (Fig. 6A). The preferential origin of the rhythmic activity in the prefrontal cortex could be due to some of the distinctive features of this area that are discussed next.

In this study we found that up state duration, the down-to-up state transition slope, the maximum firing rate, and the CV of up state duration were significantly different in prefrontal cortex compared with the other three studied regions. These parameters were not statistically different between motor, somatosensory, and visual cortices, a fact perhaps due to all of these being primary areas and thus sharing some structural properties. Not only did we find higher population firing rate in prefrontal cortex, but also single-unit recordings revealed higher firing rates in prefrontal cortex during up states. Differences in the prefrontal cortical structure or in its connectivity with cortical and subcortical areas could translate functionally into some of the functional differences detected here. Interestingly, some of the differences observed in prefrontal cortex activity (higher population firing rate during up states, faster down-to-up state transition) are compatible with a higher interneuronal connectivity and recurrence in this area. The transition from down to up state reflects the recruitment of the local network for the cortical activation. When the recruitment is faster, there is a steeper transition from down to up state, as is the case when inhibition is progressively decreased (Sanchez-Vives et al. 2010) or temperature increased (Reig et al. 2010). That the prefrontal network activates faster than the primary cortices could be explained by different possible mechanisms: higher local connectivity and more efficient reverberation of activity, higher excitability, or less inhibition with respect to primary cortices. The finding of relatively more spinous neurons in prefrontal cortex in macaques and humans is suggestive of higher synaptic connectivity in this area (Elston 2000; Elston et al. 2001).

The down-to-up transition was then faster in prefrontal than in visual, somatosensory, and motor cortex. The transition from the up to down state, the silencing of the local network, was significantly faster in prefrontal and motor cortex than in visual and somatosensory cortex. This means that in prefrontal cortex, and partially in motor cortex, the initiation and termination of up states is more synchronized than in primary sensory cortices. We have observed a correlation between the down-to-up and up-to-down slopes previously, for example, in situations with increasingly blocked inhibition (Sanchez-Vives et al. 2010) or with increasing temperatures (Reig et al. 2010). The link between down-to-up and up-to-down slope is population firing rate during the up state. A fast depolarizing recruitment of the network usually leads to a high firing rate in
the population. A high firing rate efficiently recruits the potassium currents that could be terminating up states and that are activity dependent (Compte et al. 2003; Cunningham et al. 2006; Sanchez-Vives et al. 2010). Indeed, prefrontal and motor cortex have significantly higher firing rates than primary sensory cortices (Fig. 3F). In our previous observations, fast down-to-up and up-to-down slopes were concurrent not only with higher firing rates during up states but also with shorter up states. However, we do not observe in our statistics illustrated in Fig. 3B any significant differences in up state duration across areas.

Our data from prefrontal cortex are in agreement with the complementary hypothesis of an increased dynamical stability of up states, for example due to a more effective recurrent GABAergic and glutamatergic synaptic coupling (Amit and Brunel 1997; Brunel and Wang 2001). Stronger stability means that changes in time of the firing rates are compelled by “restoring forces” toward the low and high firing states, making them more robust to intrinsic fluctuations in the firing activity. A strong local synaptic reverberation capable of making the high-firing regime a preferred dynamical state of the network is a way to implement strong restoring forces. Such forces induce the neuronal activity to have highly nonlinear dynamics that could explain why both the drop from the up states and the chain reaction eliciting high-frequency reverberation from down to up states are faster in prefrontal than in primary sensory cortices. This is an attractive scenario that suggests the existence along the cortex of a hierarchy of excitability and characteristics of the local circuitry: starting from “caudal” peripheral areas rapidly adapting to fast sensorial stimuli and motor actions and ending with the “rostral” associative areas whose integrative role has to be more stim-

Fig. 8. Fast rhythms in the mouse cerebral cortex during slow oscillations in 4 cortical areas. A–C: comparison of relative power in 15–30 Hz (beta), 30–60 Hz (low gamma), and 60–90 Hz (high gamma) frequency bands across the 4 cortical areas (prefrontal, visual, motor, and somatosensory cortex). D–F: comparison of absolute power in up states in the beta, low gamma, and high gamma frequency bands across cortical areas. G–I: comparison of absolute power across areas in down states for the same frequency bands. Error bars are SE. *Significant difference *P < 0.01.
The spontaneous activity of individual neurons in the up states reflects the organization of the recurrent activity in the slow waves (Harris et al. 2010; Steriade et al. 1993). While the majority of our recorded neurons fired during >90% of the up states, most neurons participated in the active states sparsely, with <6 spikes per up state in the majority of cases (see RESULTS). The PETHs extracted from the firing of individual neurons during slow oscillations showed two main patterns depending on the distribution of spikes of single neurons during the local up state: those neurons that concentrated their firing at the beginning of the up state (more common in prefrontal cortex neurons) and those with a peak of their firing rate toward the middle of the up states (more frequent in motor and somatosensory neurons). Both patterns persisted regardless of up state duration (Fig. 5, G–I). These firing patterns are comparable to those found in previous studies in rodents (Erchova et al. 2002; Luczak et al. 2007) and explain how firing of neurons collaborates in the generation of the MUA that is present in the up states.

The slow oscillation is a wave that propagates along the cortical network (Amzica and Steriade 1995; Massimini et al. 2004; Sanchez-Vives and McCormick 2000; Volgushev et al. 2006). In this study we quantified the speed of propagation of the up states in two cortical areas of the anesthetized mouse: visual and motor cortex. In both of these areas values varied between 8 and 93 mm/s, with an average value of 23.4 and 30.0 mm/s in visual and motor cortex, respectively. Similar values (10–100 mm/s) were reported in the mouse with the use of voltage-sensitive dyes during the propagation of spontaneous depolarizations or up states during quiet wakefulness (Petersen et al. 2003). In multisite recordings from different cortical areas of the cat under ketamine-xylazine anesthesia, the speed of propagation of the slow waves also lies around 100 mm/s (Amzica and Steriade 1995). However, the speed of propagation of slow waves during slow-wave sleep by high-density EEG in humans is 1.2–7.0 m/s, the average speed being 2.7 m/s (Massimini et al. 2004). These higher speeds may reflect not only a slight overestimation due to obliquely propagating wave and volume conduction (Massimini et al. 2004) but also higher speeds of propagation in humans secondary to long-range connectivity. In contrast, the speed of propagation in ferret cortical slices is low, around 10 mm/s (Sanchez-Vives and McCormick 2000), given that it relies exclusively on local connectivity. In making the comparison between in vivo and in vitro recordings, the limits of our speed estimate should be considered: a linear array of electrodes was used, allowing an estimation of an upper limit of the velocity. Indeed, under the assumption of quasi-planar waves a correct estimate is available only when the direction of propagation is parallel to the orientation of the multielectrode array.

In the last part of our study, we describe the existence of high-frequency (beta and gamma) synchronization during up states in different cortical areas of the mouse. Even when beta and gamma frequencies have been functionally associated to cognitive functions like attention (for a review see Wang 2010), these frequencies also emerge spontaneously during up or activated states. This is the case not only during slow-wave sleep and anesthesia (Steriade et al. 1996) but also in cortical slices in vitro (Compte et al. 2008). We find that in the mouse, the power of beta, and particularly that of gamma, is strikingly higher in prefrontal than in motor and sensory primary cortical areas (Fig. 8). We can only speculate about the cellular or network basis for these differences, but they suggest that the prefrontal circuitry has specific properties to efficiently generate gamma rhythms. These properties may involve networks of fast-spiking inhibitory neurons or specific excitatory-inhibitory loops (Compte et al. 2008; Freund 2003; Hasenstaub et al. 2005; Paik et al. 2009; Tamas et al. 2000; Whittington et al. 1995). Gamma synchronization would then emerge out of the prefrontal circuit during up states, probably being upregulated in the awake, attentive animal. This trend for the prefrontal circuit to generate beta and gamma frequencies matches its role as the origin of top-down influences during perceptual, attentive, or memory tasks (Engel et al. 2001; Gregoriou et al. 2009; Lachaux et al. 2008; Palva et al. 2010; Womelsdorf et al. 2007).
RHYTHMS IN MOUSE CEREBRAL CORTEX


