FAST OSCILLATIONS DURING THE UP STATES OF SLOW CORTICAL RHYTHMIC ACTIVITY *IN VITRO*

Harvey M, Puccini G, Descalzo VF , Reig R, Compte A and Sanchez-Vives MV.
Instituto de Neurociencias UMH-CSIC, San Juan de Alicante, 03550 Spain.

Rhythmic, synchronized activity in neuronal ensembles has been implicated in a number of sensory and cognitive processes including attention, learning, and perception. The mechanisms for the generation and organization of this activity is, however, unknown. Through the modulation of extracellular ionic concentrations slow (< 1 Hz) oscillations, resembling those seen during slow wave sleep, can be reliably induced in ferret visual cortex *in vitro* (Sanchez-Vives and McCormick 2000). These oscillations are composed of short (< 1 s) bouts of spiking activity (up states) occurring at 2-5 seconds intervals interspersed with periods of relative quiescence (down states). This *in vitro* preparation is particularly amenable to mechanistic studies for the generation and synchronization of rhythmic activity in cortical ensembles: 1. The stability of the preparation allows for the combination of intracellular, unit, and field recordings, 2. contributions of individual cortical layers can be easily assessed due to the relative accessibility and divisibility of the individual lamina. 3. The ability to induce, wash out, and recover the oscillation allows for the controlled study of generative mechanisms, and 4. the preparation is free from confounding thalamic and pre-thalamic input.

In normal slow wave sleep *in vivo* the high frequency activity observed during up states is often synchronized at gamma frequencies (Steriade et al. 1996). We wanted to know whether similar patterns of synchronization could be observed *in vitro*. With that purpose we recorded from ferret slices maintained in an interface chamber and bathed with a modified ACSF that resembles the *in situ* ionic concentrations. Slow rhythmic activity was then recorded as previously described (Sanchez-Vives and McCormick, 2000). Single or multiple (extracellular or intra / extracellular) recordings were obtained either from the same layer at different distances (1-8 mm) or from different layers in the same vertical column. No filtering was used to prevent any disturbance in the subsequent spectral analysis. When required, sinusoidal 50 Hz artifacts were removed offline by means of the spectral estimation of power at 50 Hz. Using time-resolved multi-taper spectral analysis (Mitra and Pesaran, Biophys. J. 76:691-1999) we examined local field potentials (LFP) and intracellular records during periods of slow, oscillatory activity. By proper normalization, we designed our analysis to detect the excess of each frequency's contribution to the total spectral power. This was subsequently checked on artificially generated signal-noise combinations. The analysis would therefore ignore spectral power increases if they were paralleled by equal power increases at all other frequencies. In addition, we calculated 95% confidence intervals on multi-taper spectral estimates based on the chi-squared distribution to assess the significance of the effects observed. Thus, we can determine at which points a particular frequency significantly dominates the signal with respect to the contribution of all other frequencies and how this correlates with the occurrence of up states and down states of the slow oscillation. These methods applied to recordings in various slowly oscillating slices revealed peaks in the normalized power spectra at lower gamma / higher beta frequencies (~ 15 to 40 Hz) which were temporally coherent with the up states of the oscillation in both the field and intracellular records. We take the presence of these peaks to be highly suggestive of the local synchronization of cortical ensembles at lower gamma frequencies during the activation of the cortical microcircuitry in the up states. Here we describe the

spatiotemporal organization of this activity, its laminar distribution, and the relationship between single units, field potentials and membrane potential of individual neurons.

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References

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