Laminar Analysis of Trial-To-Trial Variability in Rat Barrel Cortex

Robert Haslinger, Istvan Ulbert, Emery Brown, Christopher Moore, Anna Devor

Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown MA

Introduction

The response properties of cortex are not static. Identical stimuli may generate very different cortical responses, a phenomenon often thought to be due to interaction with ongoing activity (Arieli 1996; Kisley 1999). Here we investigate how the vibrissa evoked population spiking (multi unit activity or MUA) response of alpha chloralose anesthetized rat barrel cortex depends upon the *prestimulus magnitude and phase* of ongoing local field potential (LFP) oscillations. We correlate the magnitude and phase of the LFP oscillation immediately prior to vibrissa deflection with the size of the evoked MUA response in all cortical layers. The implications for cortical response properties as a function of Up and Down states are discussed (Shu 2003; Petersen 2003; Sachdev 2004) .

Methods

Surgical Procedures

Three Sprague Dawley rats weighing 200-350g were anesthetized with 1.5% halothane in oxygen for surgery. A tracheotomy was performed and cannulas were inserted in the femoral artery and vein for monitoring of blood pressure, blood gas and alpha chloralose administration. After surgery halothane was discontinued and anesthesia was maintained with 50mg/kg intravenous bolus of alpha chloralose followed by continuous infusion at 40 mg/(kg * hr). Following tracheotomy animals were mechanically ventilated with a mixture of air and oxygen. The animal was fixed in a stereotaxic frame, the skull over the barrel cortex was thinned and a well of dental acrylic was built around the edge of the thined skull. A craniotomy and durotomy were performed over the barrel cortex. The well was filled with a buffered saline containing 135 mM NaCl, 5 mM KCl, 5 mM Hepes, 1.8 mM CaCl2, 1 mM MgCl2. (Simons 1989; Armstrong-James 1992; Moore 1998).

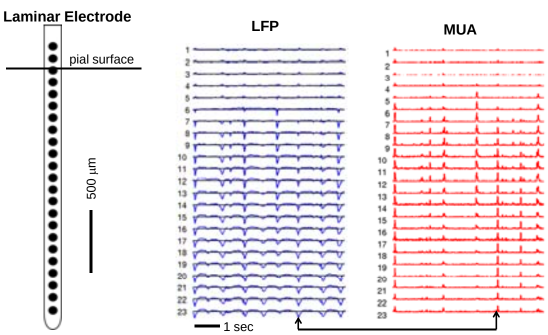
Cortical Laminar Electrodes

A linear micro-array with 23 contacts spaced 100 micrometers apart (laminar electrode) was slowly inserted into the barrel cortex perpendicular to the cortical lamina and the principle whisker identified as that which produced the largest evoked response. The recorded extracellular field potential was amplified and analogue filtered into a low pass (0.1-500 Hz) component recorded at 2KHz (the LFP) and a high pass (500-5000 Hz) component recorded at 20KHz (the MUA) which was rectified by taking its absolute value (Ulbert 2001; Devor 2003).

Vibrissa Stimulation Protocol

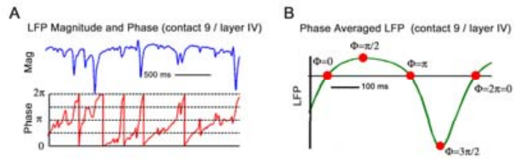
The principle whisker was deflected repeatedly by a computer controlled piezoelectric stimulator. The stimulator, positioned 3mm from the base of a whisker, deflected the whisker upwards and allowed a free return to the resting position (Devor 2003). The stimulus protocol employed 27 different stimulus amplitudes spaced linearly with a maximum vertical displacement of 1200 mm (969 degrees/s). 1080 stimulus presentations (40 of each amplitude) and 600 nulls (no stimulus) were used. The inter-stimulus interval was 1 second and the vibrissa deflections and nulls were randomized. This stimulus protocol was repeated 2-4 times in each rat.

Laminar Profile of Spontaneous Activity



Anesthetized rat barrel cortex exhibits strong spontaneous ~1Hz LFP oscillations in all lamina. These are generated by the synchronized fluctuation of large populations of neurons between depolarized (Up) and hyperpolarized (Down) states (Wilson 1996). Spontaneous spiking (MUA) is strongest in the Up state, which corresponds to negative deflections of the LFP (Steriade 1993).

Magnitude and Phase Together Describe LFP Oscillations

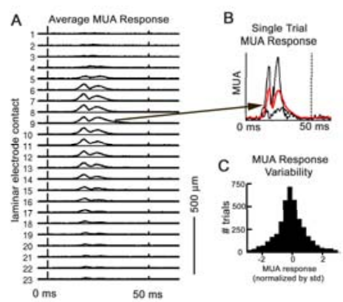


(A) Example of LFP oscillations recorded in layer IV (contact 9) and their phase. The LFP oscillations are distinctly non-sinusoidal, but their phase Φ, defined between 0 and 2π, can be rigorously calculated using a *Hilbert Transform* (Le Van Quyen 2001)

(B) 200 seconds of spontaneous LFP oscillations binned and averaged with respect to their phase. Note that each value of the LFP magnitude occurs at *two* distinct phases. In one case the LFP is increasing, in the other it is decreasing (e.g. $\Phi=0$ and $\Phi=\pi$.) Thus magnitude alone is not a complete description of the dynamics.

Each phase corresponds to a distinct feature of the waveform. $\Phi=0$ to the start of the positive LFP deflection (hyperpolarization/Down state), $\Phi=\pi/2$ to the maxima of the positive deflection (greatest hyperpolarization), $\Phi=\pi$ to the start of the negative LFP deflection (depolarization/Up state) and $\Phi=3\pi/2$ to the minimum of the negative LFP deflection (greatest depolarization/ Up state).

MUA Response to Vibrissa Deflection is Highly Variable



(A) Stimulus averaged (over 40 identical vibrissa deflections) MUA response of all cortical lamina

(B) The single trial responses (black lines) can be extremely different from the stimulus averaged response (red line). We quantify the responses into a single number by integrating the MUA between 0 and 50 ms (vertical dashed line)

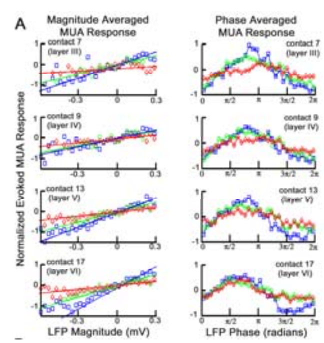
(C) Example of the distribution of MUA responses in cortical layer IV (contact 9). The integrated response has been centered about its mean (mean=0) and normalized by its standard deviation (std=1) for eventual comparison of results across animals (Linkenkaer-Hansen 2004).

MUA Response is a Function of Prestimulus LFP

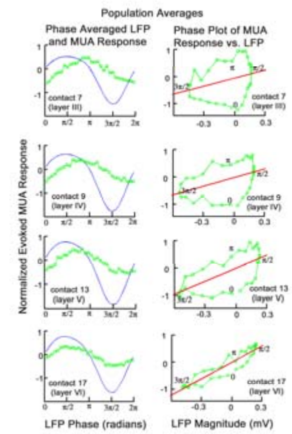
MUA response binned and averaged with respect to the *prestimulus* magnitude and phase of the LFP oscillations. Results from three different animals (blue squares, green circles, red diamonds) are shown. MUA response in units of its standard deviation (std=1) and centered about its mean (mean=0).

(A) In all layers, the magnitude averaged MUA response is largest for positive deflections of the LFP, corresponding to neuronal hyperpolarization. (Down state)

(B) Phase averaging reveals that the response is not a simple function of LFP magnitude. If it were, the response would peak at $\Phi=\pi/2$. Instead, the response is a smooth function of prestimulus phase which peaks between $\Phi=\pi/2$ and $\Phi=\pi$, depending upon cortical layer.



MUA Response Depends Upon The Recent History of the LFP



Population averages. **First column**: Phase averaged LFP (blue line) and MUA response (green squares) averaged across all three rats. **Second column**: phase plots of the LFP (x-axis) and MUA response (y-axis) shown in the first column. The direction of increasing phase (and time) is counter clockwise. Note that LFP magnitude is a good predictor of the MUA response only in layer VI . In other layers, magnitude averaging pools together the top and bottom of the loops, which are in fact distinct. The red lines are linear fits to show this pooling effect.

The response depends not only on the instantaneous magnitude of the prestimulus LFP but also on its recent history, or equivalently, what phase of the LFP oscillation the cortex is at when the stimulus arrives.

Conclusions

- The MUA response of rat barrel cortex to identical stimuli is highly variable.
- This variability is, at least in part, generated by spontaneous cortical oscillations.
- Phase can be used to rigorously describe the LFP oscillations, even though they are highly non-sinusoidal
- The MUA response is a strong function of both the magnitude and phase of the LFP immediately prior to vibrissa deflection
- Considering only the LFP magnitude implies the response is largest in the Down state.
- Phase plots show the response is not a monotonic function of LFP magnitude, in fact it depends strongly on the LFP's recent history. This suggests the response properties of barrel cortex are richer than a strict partitioning into Up and Down states would indicate.

References

Arieli A, Sterkin A, Grinvald A, and Aertsen A. *Science* 273: 1868 (1996)

Armstrong-James M, Fox K, and Das-Gupta A. *J Neurophysiol* 68: 1345 (1992)

Devor A, Dunn AK, Andermann ML, Ulbert I, Boas DA, and Dale AM. *Neuron* 39: 353 (2003)

Kisley MA and Gerstein GL. *J Neurosci* 19: 10541 (1999)

Le Van Quyen L, Foucher J, Lachaux J, Rodriguez E, Lutz A, Martinerie J, and Varela FJ. *J Neurosci Methods* 111: 83 (2001)

Linkenkaer-Hansen K, Nikulin VV, Palva S, Ilmoniemi RJ, and Palva JM. *J Neurosci* 24: 10186 (2004)

Moore CI and Nelson SB. *J Neurophysiol* 80: 2882 (1998)

Petersen C, Hahn T, Mehta M, Grinvald A, and Sakmann B. *PNAS* 100: 13638 (2003)

Sachdev R, Ebener FF, and Wilson CJ. *J Neurophysiol*, 92: 3511 (2004)

Shu Y, Hasenstaub A, and McCormick DA. *Nature* 423: 288 (2003)

Simons DJ and Carvell GE. *J Neurophysiol* 61: 311 (1989)

Steriade M, Nunez A, and Amzica F. *J Neurosci* 13: 3252 (1993)

Ulbert I, Halgren E, Heit G, and Karmos G. *J Neurosci Methods* 106: 69 (2001)

Wilson CJ and Kawaguchi Y. *J Neurosci* 16: 2397 (1996)