J Physiology (Paris) (1996) 90, 185–188

Voltage-clamp measurement of visually-evoked conductances with whole-cell patch recordings in primary visual cortex

L Borg-Graham, C Monier, Y Frégnac*

Équipe Cognisciences, Institut Alfred Fessard, CNRS, 1, avenue de la Terrasse, 91198 Gif-sur-Yvette, France

Summary — Whole cell patch recordings have been realized in the primary visual cortex of the anesthetized and paralyzed cat, in order to better characterize input resistance and time constant of visual cortical cells *in vivo*. Measurements of conductance changes evoked by visual stimulation were derived from voltage clamp recordings achieved in continuous mode at two or more different subtreshold holding potentials. They show that the magnitude of the conductance increase can reach up to 300% of the mean conductance at rest. The observation of similar changes for the preferred and antagonist responses, when flashing ON and OFF, a test stimulus in pure ON and OFF subfields supports the hypothesis of a role for shunting inhibition in the spatial organization of simple receptive fields.

visual cortex / patch clamp *in vivo* / receptive field / conductance / shunting inhibition

Introduction

In order to understand the biophysical mechanisms that underlie receptive field dynamics in the visual cortex, it is important to apply methods which allow a quantitative assessment of the interactions between synaptic inputs and the intrinsic state of the neuron. Here results are described from blind patch *in vivo* recordings in the cat's primary visual cortex in order to study supra- and sub-threshold receptive field (RF) and intrinsic firing properties. In particular, we have applied a voltage-clamp based technique for estimating the synaptic conductance changes of a cortical neuron in response to visual stimuli. This technique yields results different from those obtained with the more classical technique of measuring the voltage deflection in response to periodic current pulses, *eg* 0.1 nA 25 ms pulses, applied at 20 Hz (Berman *et al*, 1991).

Materials and methods

One hundred and thirty-five cells from the primary visual cortex of anesthetized and paralyzed kitten and adult cats were used in this study. Experimental methods concerning surgery, monitoring of the physiological state of the preparation, and visual stimulation have been described (Frégnac *et al*, 1992). Whole cell patch current and voltage clamp recordings (Axoclamp 2A amplifier, continuous mode) of up to 2 h recording time were made using 3–4 MΩ electrodes filled with K-gluconate or K-methylsulfate. Recordings were obtained by slowly advancing the electrode to depths up to 2000 μm while monitoring its resistance with current steps. Slight positive pressure was maintained during the descent to keep the electrode tip free until direct contact with cell membrane, indicated by an approximately 5% increase in resistance. At this point pressure was released, and subsequently formation of the giga-seal (2–8 GΩ) was observed either spontaneously or after slight suction applied by mouth. After the giga-seal stabilized (typically within a few minutes) additional suction was used to obtain whole cell access, with access resistances of 10–30 MΩ.

*Correspondence and reprints

Intrinsic properties of *in vivo* cortical cells

Recordings were made from the major physiological classes of cortical cells (simple and complex) and, in some cases, correlated with histology, including regular spiking (*eg* spiny stellate and pyramid), fast spiking (*eg* smooth stellate), and bursting (*eg* pyramidal) neurons (Mc Cormick *et al*, 1985; Martin, 1988; Mason and Larkman, 1990). As shown in table I, we have found that cell input resistances and time constants are closer to *in vivo* sharp recordings (9 to 75 MΩ in Bringuier *et al*, 1992) rather than whole cell recordings *in vitro* (165 MΩ for layer V pyramidal cells, 325 MΩ for layer II–III pyramidal cells; Perrais, 1995; Lambolez *et al*, 1996), suggesting that tonic synaptic activity makes a large contribution to the cell's resting condition. These characteristics are seen in cells in which the intrinsic firing properties are more reminiscent of slice recordings than those typically seen with conventional *in vivo* sharp recordings, in particular with respect to the 'noisiness' of the trace.

The mode of the distributions is included in these results since there was a tendency in some parameters to evolve as the experimental technique improved, in particular as the typical access resistance became lower.

Table I. Input characteristics.

	Mean (± *SD*)		*Mode*	*Number of cells*
Resting potential (mV)	–62	(10)	–62	112
Time constant (ms)	14	(0.7)	11	105
Input resistance (MΩ)	80	(5.3)	50	117

For example, cell input resistance and time constant were derived with the standard method of observing the voltage response to current steps. In this protocol it is crucial to separate the response of the electrode from that of the cell, which in turn is primarily a function of the difference in electrode *versus* cell time constants. Given the relatively large parasitic capacitance of the *in vivo* protocol, it is therefore important to minimize the electrode (access) resistance (and thus the electrode time constant) as much as possible, since this makes the electrode artifact more readily distinguishable. Also, simulations based on multicompartmental models of the passive properties of the dendritic tree (Borg-Graham, 1995) show that the access resistance correction factor in the equation below is a crucial parameter in the estimation of the relative phasic (synaptic) versus tonic input conductance.

Conductance changes during visual stimulation

Synaptic input in response to dark and light bars flashed in several positions of the RF was studied quantitatively as follows. The somatic component of synaptic conductance activation was estimated by recording under voltage clamp (continuous mode) at various holding potentials during mapping of the visual RF. This analysis estimates the synaptic input with the following basic formula:

$$G_{(syn)}(t) \approx \frac{I_1(t) - I_2(t)}{(V_1 - R_s I_1) - (V_2 - R_s I_2)} - G_{rest}$$

where the current (I) and voltage (V) traces are taken from two or more distinct protocols, and a correction factor is applied based on the estimated electrode series (access) resistance R_s, since the recordings were done in continuous mode without bridge compensation. When more than two clamp conditions were applied to a cell, the slope of the linear regression extracted from the I-V data at each time point was used to derive the first term on the right hand side of the formula. G_{rest} stands for the conductance measured at rest in the absence of visual stimulation. The advantage of this technique over the more standard method of evaluating responses (voltage deflections) to short current pulses in current clamp mode includes the following:

i) Under voltage clamp, the contribution of non-linearities local to the recording site are minimized.

ii) The capacitive component of the conductance seen with the current pulse method is avoided (Koch *et al*, 1990).

iii) The waveform-based calculation allows a direct extraction of the continuous synaptic input over the entire response.

The effective reversal potential of the composite synaptic event at time t was also estimated by extracting the V component of the intersection of the I-V characteristic associated with $G_{in}(t)$ (= $G_{syn}(t)$ + G_{rest} from above) with that of G_{rest}. This technique is essentially equivalent to the more classical technique of depolarizing or hyperpolarizing the cell as appropriate until the synaptic event reverses, signaling the 'reversal potential' of the event. However, the intersection of characteristic technique avoids the possible introduction of additional non-linearities when the cell is depolarized/hyperpolarized by the experimenter.

Our results suggest that light-evoked synaptic input can transiently change the conductance of the cell by a range of 50–300%, significantly larger than previously published measurements (only up to 25% in Berman *et al*, 1991). In addition, the magnitude of the conductance changes does not predict the asymmetry in the response firing (or in membrane potential changes observed in bridge mode) for the ON and OFF transitions of the same stimulus shown in unimodal subfields (either ON or OFF) of simple cells, as shown in figure 1. Similarly large conductance increases are observed for both the agonist and antagonist responses to a test stimulus flashed in a fixed position of the receptive field.

In some simple cells we find evidence that simple subfields receive complex subthreshold excitatory input, consistent with the 'Simplex' model proposed by Frégnac *et al* (1989) and later confirmed by *in vivo* recordings made with sharp electrodes (Frégnac and Debanne, 1993; Shulz *et al*, 1993). We also find that in some simple cell RF subfields there is no opponent antagonist response under current clamp, whereas a significant increase in the input conductance is found as derived from the voltage clamp recordings. These changes could correspond to a shunting effect of inhibitory input unseen during classical bridge mode recording.

Taken with the fairly low input impedances and fast time constants mentioned earlier, these findings suggest that the level of tonic and phasic modulation of synaptic

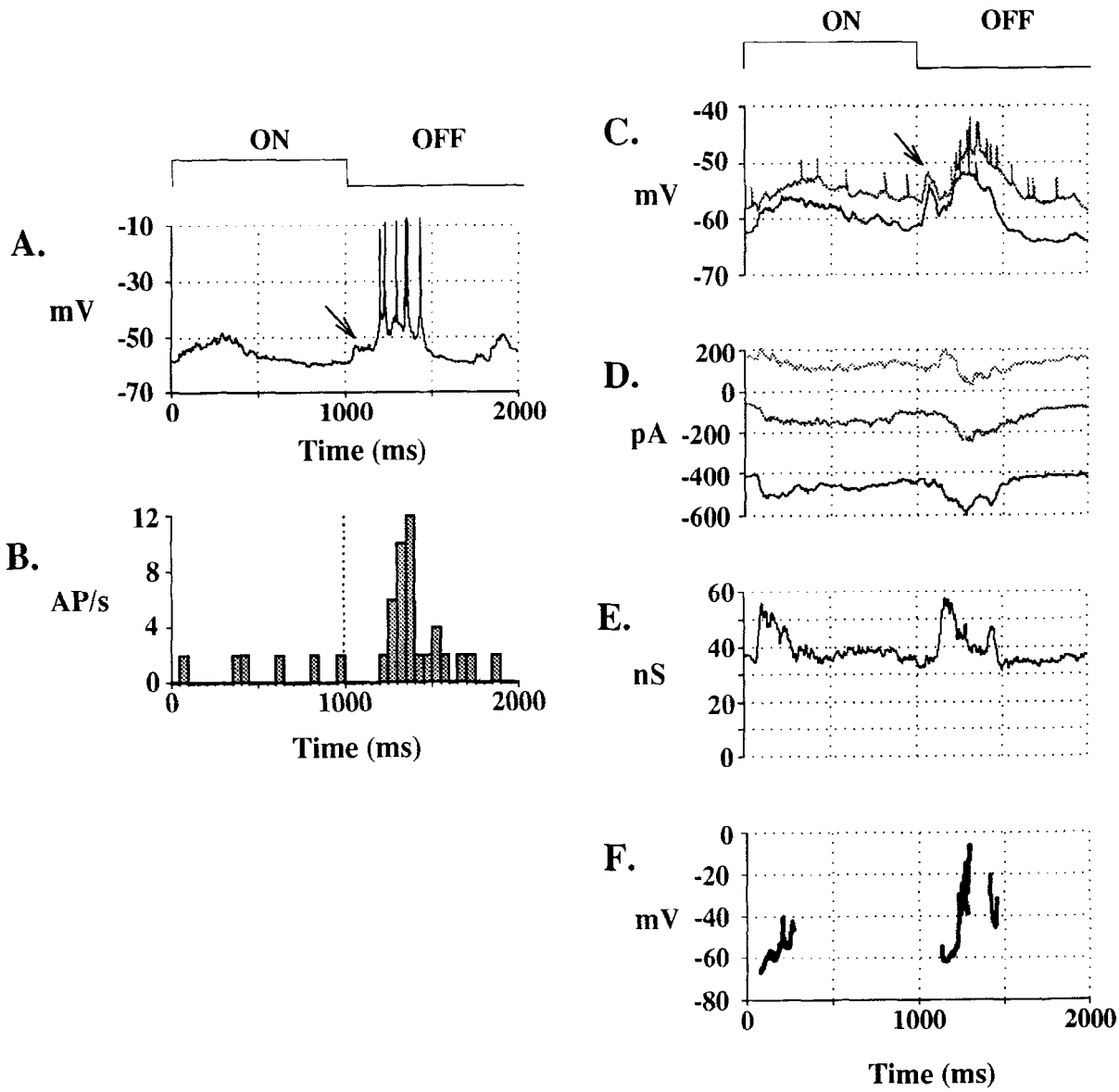

Fig 1. Recordings made from a simple cell in cat area 17, stimulated with a flashing light bar over an OFF subregion of the receptive field. **A.** Single current clamp trace, depolarized with 100 pA. **B.** Average PSTH (10 trials) from traces depolarized with 100 pA. **C.** Average current clamp traces (10 trials) at 0 (lower trace) and 100 pA (upper trace) holding current. **D.** Average voltage clamp traces (10 trials) at −85 (lower trace), −70 (middle trace) and −55 mV (upper trace) holding potentials. **E.** Total somatic input conductance, estimated from the linear regression of the voltage clamp traces in D, corrected with a 25 MΩ access resistance. **F.** Reversal potential of synaptic events estimated from **E**, for times t when $G(t) - G_{rest} > 5$ nS. Note that the reversal potential for the largest G transients is around rest during the initial phases of both the ON and OFF responses, suggesting a primarily inhibitory synaptic input which tends to transiently 'clamp' the cell in the vicinity of its resting potential, despite otherwise near- or supra-threshold non-spiking depolarizations (note initial brief spike-less EPSP of OFF response at the arrow in **A**, also seen in the averaged responses in **C**).

input to cortical cells dominates the 'resting' integrative properties of the neuron.

Acknowledgments

This work was supported by HFSP (grant RG 69–93), Fondation Fyssen, Fondation Philippe, and CNRS ATIPE Cogniscience.

References

Berman NJ, Douglas RJ, Martin KAC, Whitteridge D (1991) Mechanisms of inhibition in cat visual cortex. *J Physiol (Lond)* 440, 697–722

Borg-Graham L (1995) The Surf-Hippo Neuron simulation system (version 2.5). Public domain address: ftp://ftp.ai.mit.edu/pub/surf-hippo

Bringuier V, Frégnac Y, Debanne D, Shulz D, Baranyi A (1992) Synaptic origin of rhythmic visually evoked activity in kitten area 17 neurons. *NeuroReport* 3, 1065–1068

Frégnac Y, Debanne D (1993) Potentiation and depression in visual cortical neurons: a functional approach to synaptic plasticity. *In: Brain Mechanisms of Perception and Memory: from Neuron to Behavior* (Ono T, Squire L Raichle ME, Perrett DI, Fukuda M, eds) Oxford University Press, 533–561

Frégnac Y, Shulz D, Debanne D (1989) The role of neuronal coactivity in shaping visual cortical receptive field properties. *Biomed Res* 10, 29–35

Frégnac Y, Shulz D, Thorpe S, Bienenstock E (1992) Cellular analogs of visual cortical epigenesis: I. Plasticity of orientation selectivity. *J Neurosci* 12, 1280–1300

Koch C, Douglas R, Wehmeier U (1990) Visibility of synaptically induced conductance changes: theory and simulations of anatomically characterized cortical pyramidal cells. *J Neurosci* 10, 1728–1744

Lambolez B, Ropert N, Perrais D, Rossier J, Hestrin S (1996) Correlation between kinetics and RNA splicing of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors in neocortical neurons. *Proc Natl Acad Sci USA* 93, 1797–1802

Martin KAC (1988). From single cells to simple circuits in the cerebral cortex. *Q J Exp Physiol* 73, 637–702

Mason A, Larkman A (1990) Correlations between morphology and electrophysiology of pyramidal neurons in slices of rat visual cortex. II. Electrophysiology. *J Neurosci* 10, 1415–1428

Mc Cormick DA, Connors BW, Lighthall JW, Prince DA (1985) Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. *J Neurophysiol* 54, 782–806

Perrais D (1995) Kinetics and pharmacological characteristics of GABAA synaptic currents in rat visual cortex. Advanced Study Diploma (DEA de Neurosciences, in French), University Paris VI

Shulz D, Debanne D, Frégnac Y (1993) Cortical convergence of ON- and OFF-pathways, and functional adaptation of receptive field organization in cat area 17. *Prog Brain Res* 95, 191–206