Visually Evoked Oscillations of Membrane Potential in Cells of Cat Visual Cortex

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In response to visual stimulation, cells of the cat visual cortex fire rhythmically at frequencies between 30 and 60 hertz. This rhythmic firing can be synchronized among cells in widespread areas of the visual cortex. The visual stimulus conditions under which this process occurs suggest that the synchronization may contribute to the integration of information across broadly displaced parts of the visual field. An intricate mechanism must control the regularity of firing and its synchronization. In vivo whole-cell patch recordings from cells in area 17 have now shown that robust oscillations of membrane potential underlie the regularity of firing seen extracellularly. In the cells studied, the characteristics of the oscillations of membrane potential suggest that such oscillations are produced by rhythmic activity in synaptic inputs. These rhythmic synaptic inputs form the most likely mechanism for the synchronization of activity in neighboring cortical cells.

In response to visual stimulation, cells in area 17 of the cat visual cortex fire rhythmically at frequencies between 30 and 60 Hz (*1–4*). Rhythmic activity in a single cell is often synchronized to that in nearby cells and to oscillations of the local field potential (*4–6*). When an appropriate visual stimulus is used, oscillations synchronize not only between nearby cells (*1, 4*) but across different cortical columns (*1*), different cortical areas (*4, 6*), and across the two hemispheres (*3*). The characteristics of the visual stimulus that evoke this response synchronization have led to the proposal that the synchronization could be used for the perceptual linking of disparate, but related, parts of the visual image (*6–10*).

The cellular mechanisms that produce oscillations and their local synchronization in vivo are unknown. Several specific mechanisms have been suggested (*11*). First, intracellular recordings from the rat cortical slice preparation have revealed subpopulations of both inhibitory interneurons (*12*) and pyramidal cells (*13–15*), in which the membrane potential oscillates at frequencies ranging from 10 to 50 Hz by virtue of the intrinsic membrane properties of the cell. Such cells could entrain a large fraction of the local population into a coordinated oscillatory response (*12, 15, 16*). Second, cortical oscillatory responses may reflect coordinated rhythmic activity in afferent sources such as the retina and lateral geniculate nucleus (LGN) (*17*), structures that are known to exhibit varying degrees of oscillatory activity (*18–22*). Third, oscillatory responses could be generated by the cortical network, with mutually excitatory cells receiving recurrent feedback inhibition (*23, 24*).

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We recorded intracellularly from cells in area 17 of the cat visual cortex in vivo (*25*) using the whole-cell patch technique (*26*) and found that the rhythmic firing of cells that has been observed extracellularly is triggered by large, visually evoked oscillations in membrane potential. The visual response properties of the intracellularly recorded oscillations closely match the properties of the rhythmic firing observed extracellularly. Our results suggest that the oscillatory responses in the cells from which we have recorded are generated largely by rhythmic synaptic input, with little contribution from the cells' intrinsic membrane properties.

Twenty-eight cortical cells were studied, including 19 complex cells and 9 simple cells located in layers 2 through 5. Ten cells, nine simple and one complex, received monosynaptic input from the LGN as judged by the latency of the response to electrical stimulation of the LGN. The remaining 18 cells, all of which were complex, received polysynaptic input from the LGN (*27*). The resting potentials of the cells averaged −60 mV. The observed input resistances (100 to 200 megohms) were lower than those observed in cortical slices (*28*), possibly because of the constant barrage of synaptic activity present in vivo.

When a bright bar of high contrast was swept back and forth through the receptive field of a complex cell at the preferred orientation, motion in each direction evoked a single wave of depolarization characteristic of complex cells (Fig. 1, A and B). No action potentials were seen rising from the depolarizations because the cell was hyperpolarized with −130 pA of current injected through the recording electrode. Superimposed on the slow depolarization were oscillations of more than 10 mV in amplitude (Fig. 1A). These oscillations can be distinguished more clearly in Fig. 1C, where an expanded trace shows that the oscillations were regular, with a period of about 25 ms. Fourier analysis of this 250-ms epoch of the visual response confirmed that the dominant frequency of the oscillations lies near 40 Hz (Fig. 1D), well within the range of frequencies (30 to 60 Hz) observed extracellularly (*2, 4*).

The oscillations observed with hyperpolarization (Fig. 1) produced regularly occurring action potentials when the hyperpolarizing current used to obtain the records was turned off (Fig. 2B). The action potentials were synchronized with the subthreshold oscillation in membrane potential (Fig. 2, C and D). A spike-triggered average of the membrane potential (Fig. 2C) showed persistent oscillations during the periods both before and after the occurrence of an action potential centered at 0 ms, which indicates that each spike was synchronized with the oscillations in membrane potential. A spike autocorrelogram (Fig. 2D) calculated from

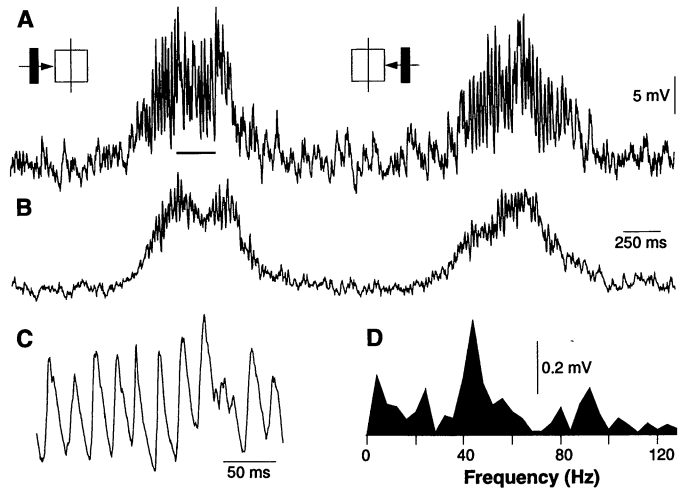


Fig. 1. (**A**) Intracellular records of the membrane potential of a complex cell recorded in response to a bright bar swept in two different directions across the receptive field at the preferred orientation. Resting membrane potential (V_{rest}) = −65 mV. Input resistance (R_{in}) = 200 megohms. Current (−130 pA) was injected through the recording electrode during the recordings. (**B**) An average of nine individual traces similar to those in (A). (**C**) A 250-ms epoch of activity taken from the record in (A) (horizontal bar) shown at an expanded time scale. (**D**) Frequency spectrum of the trace shown in (C).

the same data shows that the probability of firing before and after each spike waxes and wanes in synchrony with oscillations of the membrane potential; peaks in the probability of firing occur simultaneously with peaks in the membrane potential.

If the oscillations in membrane potential are responsible for the rhythmic firing of single cells observed extracellularly, it would be expected that oscillations in membrane potential, rhythmic firing of single cells, and oscillations in the extracellular field potential would each respond similarly to visual stimulation. Three similarities are apparent from observation of these different oscillatory signals. (i) The phase of oscillations of intracellular membrane potential, like those of extracellularly recorded field potentials (*1*, *2*, 6), is not locked to the visual stimulus. Averaging several intracellular responses to identical stimuli reduced the amplitude of the oscillations in membrane potential but not the amplitude of the visually evoked slow depolarization (Fig. 1, A and B). (ii) The amplitude of oscillations in membrane potential, like the amplitude of oscillations in extracellular field potentials (*2*), is dependent on stimulus orientation (*29*). (iii) Extracellularly recorded rhythmic firing of action potentials is observed more frequently in complex cells than in simple cells (*2*). The visually evoked intracellular oscillations were on average two times greater in complex cells than in simple cells. Out of 14 complex cells analyzed, 10 showed strong visually evoked oscillatory behavior, whereas only 2 of the 9 simple cells showed robust visually evoked increases in oscillation amplitude.

In vitro studies suggest that some cells of the visual cortex may oscillate because of their intrinsic membrane properties (*12*–*15*). In our in vivo intracellular records, however, the oscillations in membrane potential appeared to arise externally from synaptic inputs that oscillated at frequencies near 40 Hz. The best evidence for oscillatory synaptic input comes from a comparison of visually evoked responses recorded in the presence and absence of injected current (Fig. 2, A and B, and Fig. 3B). The application of hyperpolarizing current increased oscillation amplitude but did not affect oscillation frequency.

The current-induced increase in amplitude of the oscillations is best explained if the oscillations of membrane potential are produced by corresponding oscillations in excitatory synaptic input. The current-induced hyperpolarization would increase the driving force on excitatory synaptic currents, thereby increasing the size of the excitatory postsynaptic potentials (EPSPs) that arise from the oscillating synaptic input. It is difficult to know whether the current-induced increase in the size of the oscillations is caused by changes in the size of visually evoked EPSPs or by changes in the size of visually evoked inhibitory postsynaptic potentials (IPSPs) or by some alteration in the activity of intrinsic voltage-sensitive channels. The current did significantly increase the amplitude of the EPSPs evoked by electrical stimulation of the LGN (Fig. 3A). In each set of traces, there were EPSPs and IPSPs identifiable by their shapes and latencies; the current increased the size of the EPSPs and decreased the size of the IPSPs. Because many of the same synapses mediate both the visually and electrically evoked responses, it follows that the current-induced increase in the size of the oscillations is caused by an increase in the size of EPSPs.

Although hyperpolarizing current in-

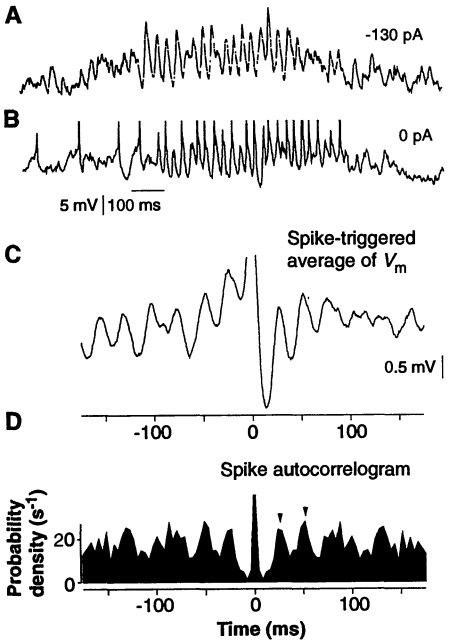


Fig. 2. (**A** and **B**) Response of the complex cell of Fig. 1 to one direction of bar motion with (A) and without (B) −130 pA of current injected into the cell. For clarity, action potentials have been truncated in (B). (**C**) Spike-triggered average of membrane potential (V_m) taken from eight traces similar to those in (B). Before calculating the average, we removed action potentials by a median filter technique. (**D**) Spike autocorrelogram calculated for the same responses used to obtain the spike-triggered average in (C). Arrowheads indicate peaks of probability of firing after an action potential.

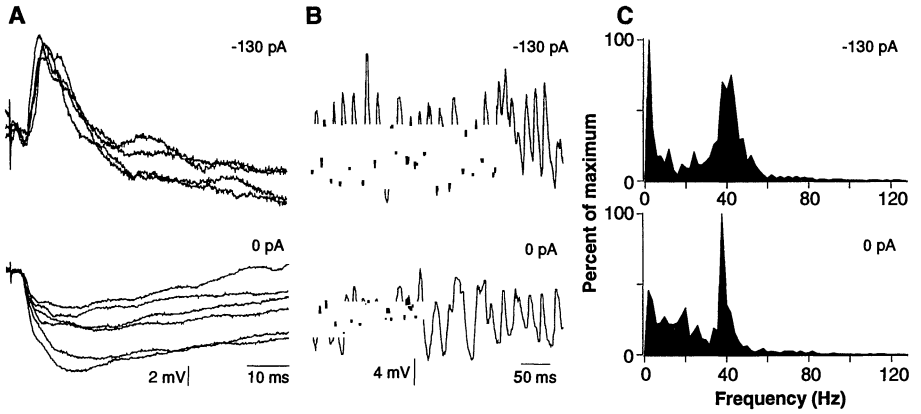
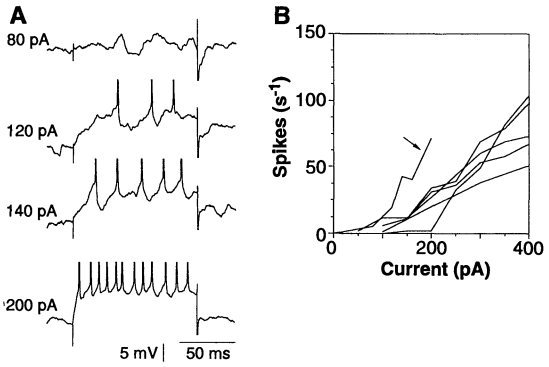


Fig. 3. (**A**) Response of the cell in Figs. 1 and 2 to supramaximal electrical stimulation of the LGN recorded with −130-pA current injected into the cell (top) and without that current (bottom). (**B**) Oscillations in membrane potential evoked by an optimally oriented bar swept through the receptive field, with injected current (top) and without (bottom). Spikes rising from the peaks of the oscillations in the bottom trace were removed with a median filter. (**C**) Average frequency spectrum of 14 traces recorded with injected current (top) and without (bottom). Peak frequency is near 40 Hz in both cases.

Fig. 4. (**A**) Response of the cell in Figs. 1 through 3 to depolarizing current pulses of different amplitudes recorded in the absence of visual stimulation. Action potentials have been truncated for clarity. (**B**) Average spike frequency calculated from traces like those in (A), plotted as a function of current pulse amplitude for this cell (arrow) and for five other cells.



creased the amplitude of the oscillations, it did not affect their frequency (Fig. 3C). Each power spectrum in Fig. 3C was obtained from 14 median-filtered records like those in Fig. 3B. Because the spectra were normalized to their peak amplitudes, they do not illustrate the twofold increase in the power of the oscillations (24- to 96-Hz range) caused by the injected current. The spectra do show, however, that the peak frequencies of the oscillations that occurred in the presence and absence of current are very similar: 40 Hz with current and 38 Hz without. This result suggests that active membrane processes, which are dependent on membrane potential, do not contribute significantly to the oscillations in this cell. If voltage-dependent ion channels did contribute to the oscillatory behavior, it is likely that the frequency would be altered by the 10- to 20-mV shift in resting potential produced by the injected current.

If voltage-sensitive ion channels were to contribute to the visually evoked oscillations in cortical cells, it would be expected, on the basis of previous in vitro experiments (*12–15*), that oscillations could also be triggered by depolarizing current pulses. Visual stimuli and current pulses would then trigger oscillations through the same mechanism, by depolarizing the membrane. In the cortical cells studied in vivo, however, depolarizing current pulses did not produce oscillations (Fig. 4A). These cells showed neither subthreshold oscillations of membrane potential nor any selective tendency to fire at 40 Hz. Depolarizing current pulses evoked trains of action potentials, but the frequency of the trains varied monotonically with the amplitude of the current pulse (Fig. 4B). This result implies that in these cells the depolarization induced by the visual stimulus did not trigger the oscillations through the activation of intrinsic, voltage-sensitive mechanisms.

Intracellular records show that the rhythmic firing of action potentials observed extracellularly is driven by large oscillations in membrane potential. In the cells from which we recorded, the membrane potential oscillations were in turn driven by oscillations in synaptic activity. There are three possible sources for these oscillations in synaptic activity: intrinsic membrane properties in the presynaptic cells (*12, 15*), oscillations of geniculate input (*22*), or intracortical feedback pathways (*23*). The presence of oscillating synaptic input in the recorded cells indicates that rhythmic synaptic activity can propagate through the cortical circuit and can presumably trigger the synchronization of activity in widely spaced groups of cortical cells.

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25. Experiments were performed on 2- to 3-kg young adult female cats. Anesthesia was induced with an intramuscular injection of ketamine hydrochloride [30 mg per kilogram of body weight (mg/kg)] and acepromazine (0.3 mg/kg). Anesthesia was maintained for the rest of the experiment with an intravenous infusion of sodium thiopental (30 mg/kg initial dose; 1 to 2 mg/kg per hour maintenance dose). Paralysis was induced and maintained with an infusion of pancuronium bromide (0.2 mg/kg per hour), and the animals were artificially respirated at a rate to maintain end-tidal CO_2 at 3.5 to 4.0% [D. Ferster, *Visual Neurosci.* **4**, 115 (1990)].
26. Intracellular current-clamp records were obtained with an in vivo variation of the whole-cell patch method [M. G. Blanton, J. J. Lo Turco, A. R. Kriegstein, *J. Neurosci. Methods* **30**, 203 (1989); O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers. Arch.* **391**, 85 (1981)]. Electrodes were introduced into the cortex through a small craniotomy. The dura overlying the cortex was removed, but the pia and arachnoid membranes were left intact. Electrode resistance ranged from 5 to 14 megohms when the electrodes were filled with a solution buffered to pH 7.4 containing 135 mM potassium gluconate, 5 mM Hepes, 2 mM $MgCl_2$, 1.1 mM EGTA, 0.1 mM $CaCl_2$, 3 mM adenosine triphosphate, 2 mM guanosine triphosphate, and 0.02 mM guanosine 3′,5′ monophosphate (adjusted to 280 mosmol). The depth of the neurons from the cortical surface ranged from 100 to 900 μm [D. Ferster and B. Jagadeesh, *J. Neurosci.* **12**, 1262 (1992)].
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