Orientation Selectivity of Cortical Neurons During Intracellular Blockade of Inhibition

Sacha Nelson; Louis Toth; Bhavin Sheth; Mriganka Sur


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8. To perform a global study of the detailed rupture process of large earthquakes, teleseismic records of body waves generally have proved the most useful, as well as the most abundant, data set. Recent work with near-field, broad-band body waves to study the source, such as those recorded by TERRAscope in southern California, would not be possible on a global scale because of the lack of dense coverage by broad-band seismometers.


10. We used records from the Southern California Seismic Network, the Northern California Seismic Network, or the Washington Regional Seismic Network, depending on the availability of data. The similarity of the waveform produced by stacking records at each of the three arrays for a particular event justifies using the arrays interchangeably according to which array recorded and archived a given event.

11. During the period from April 1980 to June 1992, 1049 earthquakes occurred between 35° and 90° from California, with seismic moments greater than 1.6 x 10^16 dyne-cm and depths greater than 100 km. Events with nodal P-wave radiation coefficients <0.1 were excluded, as were a few events for which the end of rupture could not be reliably inferred or for which the rupture appeared to stop and then restart. The remaining 122 events were used in (9) and in the analysis presented here.

12. The envelope of a time series is given by the square root of the sum of the squares of the time series and its Hilbert transform [E. R. Kanasewich, Time Sequence Analysis in Geophysics (Univ. of Alberta Press, Alberta, Canada, 1981)].

13. The frequency content of our stacks is controlled by the response of the instruments used in the short-period regional arrays, which is peaked between 0.2 and 2 Hz to the source spectrum, which is peaked in velocity around the corner frequency (which varies from 0.2 to 2 Hz for these earthquakes). Hence, we obtain envelopes of band-limited seismic radiation. The envelope is similar in shape to the source time function if the shape of the source spectrum does not change during the rupture. Only for band-limited radiation can we get a noise-free and detailed picture of the time-dependent rupture.

14. In this scheme, each envelope is stretched in time so that rupture begins at normalized time 0 and ends at normalized time 1. Use of this scheme to study the relationship between average and the durations of the envelopes, removing the effects of earthquake size and duration and preserving only the shape. Thus, the resulting average envelope shape is not affected by changes in fault parameters that affect the duration in a simple way, such as rupture velocity or stress drop over a constant spectral source zone. Furthermore, this scheme has the advantage that the first-order effects of directivity (those due to unilateral rupture), which could affect the duration of the stack (and, hence, the duration of the envelope), do not affect the envelope shape.

Hence, the use of this scheme ensures that the resulting average envelope shape could not be affected much by systematic differences in direc
tivity, if they are present.

15. The shape of the average envelope for the deepest group of earthquakes is consistent with the common practice in source modeling of parameta
tizing the time function as the convolution of two boxcars (one representing the duration of rupture over the entire fault and the other the duration of slip at a particular point on the fault).

16. Vidale and Houston found durations of 122 deep and intermediate earthquakes from stacks and found a decrease in the duration (when corrected for earthquake moment) of about a factor of 2 from 100 to 600 km depth. If the parameters that describe rupture (which include stress drop, fault geometry, the ratio of rupture velocity to shear ve
erocity, and the intermittency of slip) have no system-
tic trend with depth, durations should decrease with depth at the same rate that the shear velocity increases with depth, according to a simple scaling model. But shear velocity increases only about 20% between 100 and 600 km in depth. Thus, the obser-
vation that durations decrease by a factor of 2 requires that one or more of the rupture parameters change significantly with depth. However, a simple increase in stress drop with depth, which would decrease the duration, was not sufficient to change the envelope shape from asymmetric to symmetric (Fig. 3A). Furthermore, work by Houston and Williams (9) showed that increasing stress drops do not change significantly between 100 and 600 km in depth. Nor would an increase with depth in rupture velocity as a proportion of shear velocity, which could explain the decrease in duration with depth, produce the difference in envelope shapes.

17. Seismic moments are taken from the Harvard Centroid Moment Tensor Program (9) measured durations of 122 earthquakes.


22. The lengths relevant to our seismic observations are on the scale of kilometers rather than meters. Thus, variations in properties over kilometers is probably more pertinent than, for example, abso-

23. Green and Burnley (3), for example, found, that in experiments on germanate olivine under devia-
tor stress, spinel-filled anti-cracks (analogous to Mode I cracks in brittle failure) [C. Scholz, The Mechanics of Earthquakes and Faulting (Cam-
bridge Univ. Press, Cambridge, 1990), chap. 1] appear to link up into throughgoing Mode II and II structures, on which shear slip occurs. This pro-

24. The ability to respond selectively to con-
tours of a particular orientation is a com-
mon feature of visual cortical neurons and is believed to underlie the first stages of the perception of form. Attempts to understand the cellular mechanism of orientation selec-
tivity have led to conflicting interpreta-
tions. Intracellular recording studies (1-4) have generally supported the hypothesis that orientation selectivity arises from the pattern of convergence of excitatory affer-
ents from the lateral geniculate nucleus (LGN) (5). Other studies, especially those that employ local application of bicucul-
lin, a blocker of receptors for GABA_A, have demonstrated the importance of inhi-

bition in maintaining selectivity (6, 7). An

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Sachin Nelson,* Louis Toth, Bhavin Sheth, Mriganka Sur

Neurons in the primary visual cortex of the cat are selectively activated by stimuli with particular orientations. This selectivity can be disrupted by the application of antagonists of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) to a local region of the cortex. In order to determine whether inhibitory inputs are necessary for a single cortical neuron to show orientation selectivity, GABA receptors were blocked intracellularly during whole cell recording. Although the membrane potential, spontaneous activity, subfield antagonism, and directional selectivity of neurons were altered after they were perfused internally with the blocking solution, 18 out of 18 neurons remained selective for stimulus orientation. These results indicate that excitatory inputs are sufficient to generate orientation selectivity.

Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

*To whom correspondence should be addressed.
unresolved issue is whether inhibitory synapses directly prevent responses to nonoptimal stimuli or whether their effect is mediated indirectly by neighboring interconnected cortical neurons. This distinction is likely to be of general importance in understanding the function of inhibitory circuits throughout the cerebral cortex.

In order to distinguish between direct and indirect effects of cortical inhibition, we developed a method of blocking inhibitory conductances in single cortical neurons by perfusing them intracellularly with pipette solutions that contained cesium fluoride (CsF), did not contain adenosine triphosphate (ATP) or guanosine triphosphate (GTP) (8), and to which we added the chloride-channel blockers picrotoxin (PTX; 4 cells) (9), or 4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid (DIDS; 14 cells) (10). We assessed the efficacy of the inhibitory blockade in several ways. First, we tested in vitro the effects of our solutions on voltage-clamped responses to the CaVA agonist muscimol and to electrical stimulation of synaptic inputs (11). Rat visual cortical neurons perfused internally with control solution had large inhibitory responses to applied muscimol (Fig. 1A) or to electrical stimulation (Fig. 1, C and D), whereas cells perfused with CsF-DIDS solution had almost no response to the inhibitory agonist (Fig. 1B) and had purely excitatory responses to electrical stimulation (Fig. 1, E and F) (12).

Next, we confirmed that CsF-DIDS also blocked inhibition in vivo by attempting to evoke inhibitory responses in cat neurons, both electrically and visually (13). In control cells, electrical stimuli delivered to the LGN resulted in small excitatory postsynaptic potentials (EPSPs) that were largely obscured by overlapping inhibitory postsynaptic potentials (IPSPs) (Fig. 2A) [see also (1, 3)]. In neurons perfused with CsF-DIDS (Fig. 2B), however, electrical stimuli evoked large EPSPs but no IPSPs. Control recordings from simple cells stimulated with stationary light bars showed inhibition when the stimulus was turned "on" in an "off" subfield or when it was turned "off" in an "on" subfield, as previously reported (3, 14). During recordings from neurons perfused with CsF-DIDS or CsF-PTX solutions, however, blockade of inhibition revealed a small underlying excitation at the onset of a light bar in the "off" subfield and at its offset in the "on" subfield (Fig. 2C). These results are similar to those observed after iontophoretic application of bicuculline (6) and are consistent with the hypothesis that inhibition contributes to the antagonism between "on" and "off" subfields in cortical simple cells (15).

Fig. 1. Tests of inhibitory blockade in rat visual cortical neurons that were voltage-clamped in vitro. Traces are averages of three (A) or five (B), (C), and (D) responses. In (A) and (B), muscimol (100 μM) was applied by puffing pipette (arrow). (A) Responses during perfusion with control solution. (B) Largest responses that could be evoked in another cell after 10 min of perfusion with CsF-DIDS solution. Duration of muscimol puff was 60 ms in (B), as compared with only 10 ms in (A). (C) and (E) Synaptic currents evoked by electrical stimulation (arrow) of afferents during 800-μs voltage steps from a holding potential of −52 mV. Step voltages ranged from −82 mV (C) or −97 mV (E) to 23 mV by 15-mV increments. (C) and (E) Currents evoked in a cell perfused with control solution were primarily outward at potentials above −60 mV. In this cell and in two others, the quaternary lidocaine derivative QX-314 (10 mM) was added to the control solution to improve the voltage clamp. Similar currents were evoked in cells perfused with control solution not containing QX-314. Peak current (measured at time indicated by filled circle) is plotted against membrane potential in (C). (D) Currents evoked in another cell from the same slice as the cell in (C) and perfused with CsF-DIDS are inward at potentials below 0 mV. For clarity, the response at −97 mV is not shown. Peak current (filled circle) and current at 25 ms after peak (open circle) are plotted as filled and open circles in (F) and (H). Peak muscimol and synaptic currents evoked at 0 mV. Numbers of cells tested are indicated below each bar.

Fig. 2. Effects of inhibitory blockade on responses of cat primary visual cortical neurons to electrical and visual stimulation in vivo. Each trace is an average of five responses. (A and B) Responses to electrical stimulation (1 mA, 0.1 ms) of the LGN. (A) Control cell response consists of small initial EPSP followed by a large multiphasic IPSP (arrow). (B) Response consists of a large EPSP (arrow), without a subsequent IPSP, in a cell recorded with CsF-DIDS. Electrically evoked IPSPs were observed in all control cells (n = 3) and were absent in all cells perfused with CsF-DIDS that were tested (n = 6). Four of the cells perfused with CsF-DIDS responded at latencies of 1.6 to 2.0 ms, which indicates monosynaptic input. (C) Responses to visual stimulation with optimally oriented, stationary bars of light presented at two different receptive field locations in a simple cell recorded with CsF-DIDS. Stimulus duration is indicated by the heavy line below trace 2. Membrane potential (V_m) = −36 mV.

We tested the effects of intracellular blockade of inhibition on the selectivity of neurons for the orientation of a fixed or moving bar in 18 cells, including 7 simple cells, 6 complex cells, and 5 additional cells that had not been adequately tested with stationary stimuli to be classified as simple or complex. Both simple cells (Fig. 3A) and complex cells (Fig. 3B) maintained a high degree of orientation selectivity. In six of the cells tested during inhibitory blockade, we presented stimuli at orientations that varied in increments of 30°. Tuning curves constructed from these responses were similar to those obtained extracellularly from other cells (Fig. 4, A and B). In six addi-
tional cells, we measured selectivity both extracellularly (while patched on to the cell but before the achievement of whole-cell configuration) and intracellularly (after blockade of inhibition). The degree of orientation selectivity remained similar (Fig. 4, C through E).

The effects of intracellular perfusion on direction selectivity were more heterogeneous. In three of the six cells studied before and after blockade there was an apparent loss of direction selectivity (Fig. 4, F through H). However, some of the remaining 12 cells, which were studied only after blockade, remained quite directionally selective, and overall there was no substantial difference in the direction of distribution of selectivities in the blocked and control cells (Fig. 4, F and G). We observed, as recently reported (15), that spiking responses were often more directionally selective than were subthreshold responses. Hence, loss of directional tuning in some of our cells could have resulted directly from inhibitory blockade (16) or, secondarily, from membrane depolarization.

Although we observed changes in spontaneous firing as well as in electrically and visually evoked synaptic responses that were indicative of the effective blockade of inhibition, we did not observe any substantial change in orientation selectivity. Our results are consistent with the hypothesis that the orientation selectivity of cortical neurons is due primarily to the pattern of convergent excitatory input they receive from thalamic afferents (1, 5) and from other cortical neurons (17). Our results are not consistent with the hypothesis that inhibitory synapses selectively counteract the effect of excitation at non-optimal orientations. The apparent discrepancy between our data and those obtained when GABA receptors are blocked by application of antagonists to a local region of the visual cortex (6, 7) may reflect the importance of inhibition in regulating recurrent cortical excitation (17). We hypothesize that inhibition evoked at near optimal orientations may help to raise the cell's threshold for firing, thus sharpening the spike response to more broadly tuned excitatory input (18).

In our experiments, the firing threshold was presumably maintained near a normal level, despite inhibitory blockade, by the intracellular injection of hyperpolarizing current. Because cortical neurons receive the majority of their excitatory input from neighboring cortical neurons (19), the effect of a reduction in the firing threshold in an ensemble of neurons should be more dramatic than the same effect when confined to a single neuron (20).

REFERENCES AND NOTES

11. Techniques for whole cell tight-seal recording in slices were based on published reports (M. G. Braiton, J. J. Lurco, A. Kriegstein, J. Neurosci. Methods 30, 203 (1988)). Slices 400 μm thick, prepared from the primary visual cortex of rats 3 to 6 weeks old, were maintained at the surface of oxygenated artificial cerebrospinal fluid at 35°C. Control solution contained 120 mM potassium gluconate, 10 mM KCl, 10 mM Hepes, 5 mM MgATP, 3 mM MgSO4, and 1 mM GTP. Ca2+ -free solution contained 120 mM NaCl, 10 mM KCl, 10 mM Hepes, 5 mM EGTA, and 0.1 mM DIDS. Solutions were set to 270 to 280 mosm
Peptide nucleic acids (PNA) incorporating nucleic acid bases into an achiral polyamide backbone bind to DNA in a sequence-dependent manner. The structure of a PNA–ribonucleic acid (RNA) complex was determined with nuclear magnetic resonance methods. A hexameric PNA formed a 1:1 complex with a complementary RNA that is an antiparallel, right-handed double helix with Watson–Crick base pairing similar to the “A” form structure of RNA duplexes. The achiral PNA backbone assumed a distinct conformation upon binding that differed from previously proposed models and provides a basis for further structure-based design of antisense agents.

A novel therapeutic strategy is to titrate the concentration of a target enzyme or receptor by altering its expression either at the transcriptional level (antigene) or translational level (antisense) by means of an agent directed against the nucleic acid sequence encoding the target (1). Although this approach has been shown to work in mammalian and plant cells (2) with the use of natural and modified nucleic acids (3), significant obstacles to using such gene-targeted agents to treat human disease remain unsolved (4). PNA incorporating the nucleic acid bases adenine, cytosine, thymine, and guanine into a polyamide backbone have been described (5) and are of interest as gene-targeting agents. They are made with standard peptide chemistries fully compatible with automated solid-phase synthesis, bind more tightly to their DNA targets than does the cognate DNA strand, and are sensitive to mismatch (6).

We conducted a nuclear magnetic resonance (NMR) investigation to describe the structure of a PNA-RNA complex containing all four common bases. The sequence GAACCT-K# (bis-succinyl) and r(GAGUUUC) followed by NMR indicated only a 1:1 complex formed at all ratios, with six imino resonances appearing from reduced solvent exchange rates (Fig. 2A). NMR spectra of the uncomplexed PNA indicated that many conformers were present in slow chemical exchange (Fig. 2C) because of cis-trans equilibria about the secondary amide bond, $\chi_2$, of each PNA residue, whereas in the PNA-RNA complex only one resonance was detected for each proton (Fig. 2B). These data suggest that the PNA backbone adopts a single $\chi_2$ conformation when bound to the complementary RNA strand.

A complete set of two-dimensional (2D) homonuclear NMR data (9) provided proton resonance assignments of the PNA-RNA complex by standard methodologies (10). The single thymine residue (T1) was easily identified by both double-quantum (2Q) and total correlation spectroscopy (TOCSY) spectra (9). Observation of strong nuclear Overhauser effect (NOE) cross peaks to two adjacent pyrimidine H5,H6 proton pairs identified these as the flanking cytosine bases C4 and C5. NOE cross peaks between cytosine H5 and 4-NH$_2$ resonances were followed to the guanine imino proton, whereas NOEs among the six imino resonances and aro-