Reliability of Spike Timing in Neocortical Neurons

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It is not known whether the variability of neural activity in the cerebral cortex carries information or reflects noisy underlying mechanisms. In an examination of the reliability of spike generation using recordings from neurons in rat neocortical slices, the precision of spike timing was found to depend on stimulus transients. Constant stimuli led to imprecise spike trains, whereas stimuli with fluctuations resembling synaptic activity produced spike trains with timing reproducible to less than 1 millisecond. These data suggest a low intrinsic noise level in spike generation, which could allow cortical neurons to accurately transform synaptic input into spike sequences, supporting a possible role for spike timing in the processing of cortical information by the neocortex.

Neurons transmit information by transforming continuously varying input signals into trains of discrete action potentials. The coding scheme used in this process is an unresolved issue that is critical to computational theories of brain function. Codes that utilize spike timing (*1*, *2*) can make more efficient use of the capacity of neural connections than those that simply rely on the average rate of firing (*3*). The simplest spike-timing code would be one output pulse for each input pulse, but synaptic currents in the cortex are too small and intracellular recordings in vivo look very noisy (*4*). Furthermore, cortical activity is characterized by highly irregular interspike intervals in both spontaneous (*5*) and stimulus-evoked conditions (*6*). These observations have led some to conclude that only statistical averages of many inputs carry useful information between cortical neurons (*7*). Another possibility, which we explore here, is that cortical neurons may respond reliably to relatively weak input fluctuations. Irregularity in spike timing may then reflect the presence of information. This is possible only if the intrinsic noise within neurons is small. Although some earlier studies have suggested that neurons may have low intrinsic noise (*8*, *9*), others have argued to the contrary (*10*).

The aim of the present report was to determine directly the temporal precision with which cortical neurons are capable of encoding a stimulus into a spike train. A rat cortical slice preparation was chosen so that the state of a single neuron and its input could be well controlled experimentally (*11*). Somatic whole-cell recordings were made in the current-clamp configuration (*12*), and spike trains were elicited with current injected through the recording electrode, near the presumed site of generation of action potentials (*13*). We assessed reliability by repeatedly presenting the same stimulus and evaluating the consistency of the evoked spike sequences.

First, repetitive firing was evoked with flat (dc) current pulses (0 to 250 pA, 0.9 s; Fig. 1A). The variability of spike counts from trial to trial was small [coefficient of variation (CV) = 0.10 ± 0.13; mean ± SD; n = 10 cells]. However, the small variances in interspike intervals (ISIs) summed to increase the desynchronization of corresponding action potentials over the course of the stimulus. The first spike of each train was tightly locked to the onset of the pulse (SD = 0.62 ± 0.25 ms; n = 8), whereas the timing of the last spike in the train was highly variable (SD = 31 ± 19 ms; n = 8). Thus, responses to flat pulse stimuli indicate reliability of spike count or average firing

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rate but lack of reliability in precise timing, as measured relative to stimulus onset.

Intracellular recordings from cortical neurons in vivo reveal large and rapid fluctuations of the membrane potential from many synaptic events (*4*). The integration of many independent excitatory and inhibitory synaptic currents would be expected to approach a Gaussian distribution at the soma. Accordingly, sequences of filtered Gaussian white noise generated by computer were added to the constant depolarizing pulse (*14*). As with flat pulse stimuli, spike count showed little variability (CV = 0.052 ± 0.029, n = 10). In contrast to the case with flat pulse responses, when any particular fluctuating current waveform was repeatedly injected the pattern of spikes elicited showed precise and stable timing throughout the length of the trial (Fig. 1B). Occasionally, from trial to trial, spikes could appear, disappear, or abruptly shift tens of milliseconds. In some cases, a single dropped or added spike disrupted the timing of several consecutive subsequent spikes. This behavior made it problematic to compute directly the variability in ISIs or the timing of a particular spike number; therefore, in further analysis we used the peristimulus time histogram (PSTH; Fig. 2A).

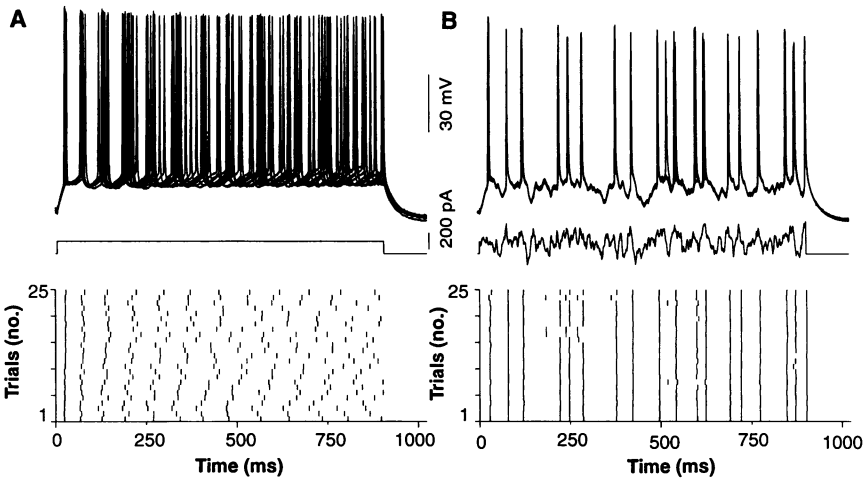

Fig. 1. Reliability of firing patterns of cortical neurons evoked by constant and fluctuating current. (**A**) In this example, a superthreshold dc current pulse (150 pA, 900 ms; middle) evoked trains of action potentials (approximately 14 Hz) in a regular-firing layer-5 neuron. Responses are shown superimposed (first 10 trials, top) and as a raster plot of spike times over spike times (25 consecutive trials, bottom). (**B**) The same cell as in (A) was again stimulated repeatedly, but this time with a fluctuating stimulus [Gaussian white noise, μ_s = 150 pA, σ_s = 100 pA, τ_s = 3 ms; see (*14*)].

Highly reproducible firing patterns were a robust phenomenon in the presence of stimulus fluctuations. Two measures of spike timing were calculated from the PSTH, which we termed the "reliability" and the "precision" (Fig. 2A). According to these measures, all the cells analyzed were capable of responding to fluctuating input currents with nearly 100% of spikes (high reliability) in clusters with an SD of less than 1 ms (high precision; Fig. 2B). The reliability of spike patterns was strongly correlated with the amplitude of stimulus fluctuations, σ_s (Fig. 2C). The firing rate also increased with the amplitude of fluctuations, particularly for cells showing strong adaptation to dc stimulation (*15*). The precision of spike timing depended on the time constant of stimulus fluctuations, τ_s (Fig. 2D). Precision and reliability dropped as stimuli were filtered at time constants increasing from 1 to 25 ms. The precision of most responses was in the range of 1 to 2 ms, a time scale much smaller than both the maximum firing rate of these cells and the time constant of fluctuations in the stimuli (*16*). The decrease in precision was paralleled by a reduction of reliability (*15*).

The reproducibility of spike patterns suggested that spikes were triggered preferentially by particular patterns of depolarizing and hyperpolarizing current in the stimulus. A reverse correlation of spike train and stimulus [spike-triggered average of the stimulus; see (*17*)] can reveal the stimulus waveform that tends to precede the generation of an action potential and can indicate the length of stimulus history that is relevant. Reverse correlations showed a strong tendency for spikes to be preceded by

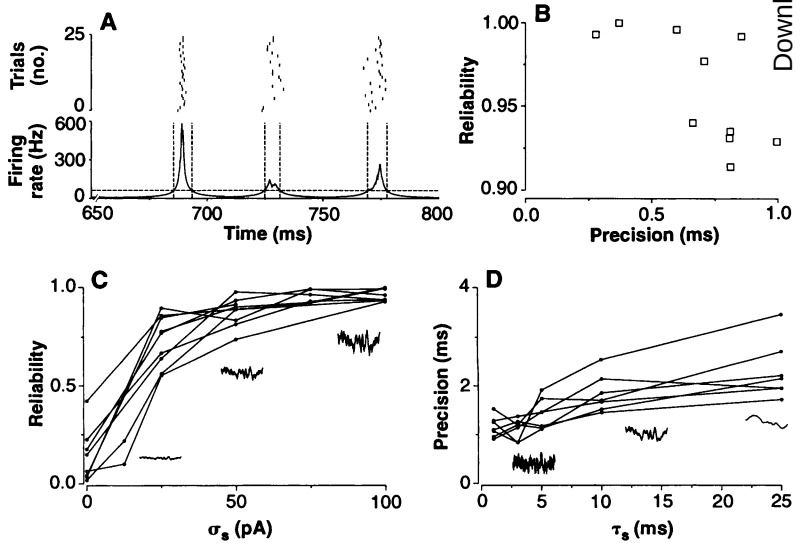

Fig. 2. Dependence of the reliability and precision of spike timing on stimulus current statistics. (**A**) The PSTH of spikes collected over 20 to 25 successive presentations of a particular stimulus waveform was used to quantify the consistency of spike patterns evoked by fluctuating stimuli of different types. Spikes during the first 100 ms after stimulus onset, during which most spike frequency adaptation occurred, were discarded. We smoothed the PSTH using an adaptive filter (centered on each time step and widened to capture 10 spikes) to yield an estimate of the instantaneous firing rate. A threshold (horizontal dotted line, set at three times the mean firing rate of the cell over a given block of responses) was used to select dramatic elevations in instantaneous firing rate, or "events." Because the minimum ISI was long compared to the duration of these events, at most one spike occurred during any event on any trial. We defined "reliability" as the fraction of total spikes that occurred during such periods of elevated firing rate. We defined temporal "precision" as the SD of spike times within any event, averaged over all events during a response. (**B**) Each square represents the most reliable block of responses recorded in one of ten cells (eight regular firing, two intrinsic bursting). For these responses, a stimulus mean, μ_s, between 100 and 300 pA and a fluctuation amplitude, σ_s, between 50 and 100 pA were used, yielding firing rates between 14 and 24 Hz. (**C**) Estimates of reliability for stimuli with various amplitudes of stimulus fluctuations. Each line on the graph connects measurements made for one of nine cells examined at four or five different values of σ_s (μ_s for these blocks was 100 to 300 pA, giving firing rates between 8 and 24 Hz). The input resistance of neurons examined was 222 ± 85 megohms; peak-to-peak voltage transients produced by these currents were less than 25 mV. (**D**) The temporal precision of responses obtained in seven cells for stimuli filtered at different time constants (τ_s = 1 to 25 ms, σ_s = 25 to 50 pA, μ_s = 100 to 200 pA). The average membrane time constant for these cells was 29.7 ± 5.9 ms.

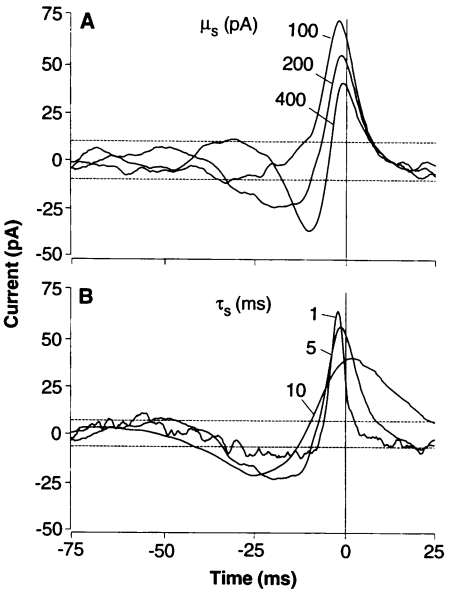

Fig. 3. Reverse correlations (spike-triggered stimulus averages) were computed over 25 consecutive trials for which stimuli were generated with equivalent parameters (μ_s, σ_s, τ_s) but different random seed [see (*14*)]. (**A**) Reverse correlations from blocks of trials with mean amplitudes shown (σ_s = 50 pA, τ_s = 3 ms). Firing frequency ranged from 10 to 25 Hz (corresponding to 225 to 554 spikes averaged). (**B**) Reverse correlations obtained with the different time constants of stimulus filtering shown (μ_s = 200 pA and σ_s = 50 pA; 282 to 322 spikes averaged). For (A) and (B) the trigger point (t = 0, vertical line) was at the inflection in the rising phase of the spike. The current values shown are relative to the mean current (μ_s). For a neuron generating spikes randomly, the average stimulus preceding a spike is not expected to differ from the average stimulus in general, which approaches a flat line with increasing samples. Departure from this expectation reveals a preference for particular stimulus waveforms. Confidence limits (dashed horizontal lines) were calculated as described in (*9*), and only the widest limits are shown. The data are from a single neuron for which reverse correlations were collected at a variety of stimulus parameters. Similar results were seen in all three other cells examined.

a depolarizing transient. At greater mean input currents (μ_s), the average depolarizing transient was reduced while a preceding hyperpolarizing transient was introduced (Fig. 3A). Varying the time constant of stimulus filtering revealed a preference for maximum stimulus slope 5 to 10 ms preceding the spike (Fig. 3B). Therefore, the time course of the reverse correlations was broadened by filtering of the stimulus, but the basic shape was preserved. Transients of this amplitude correspond to the arrival of about 10 excitatory postsynaptic currents of 5 to 10 pA within 10 ms.

These data demonstrate that repetitive firing in neocortical neurons is sufficiently reliable that currents resembling synaptic input may be repeatably encoded into spike patterns with millisecond precision. Therefore, it is likely that the intrinsic variability of the spike-generating currents and their susceptibility to nonsynaptic background noise make only minimal contributions to interspike interval variability under in vivo conditions.

Spike-triggered stimulus averages suggest that consistent temporal coding follows in part from a greater sensitivity of spike generation to transients than to steady-state depolarization. This analysis also indicates that properly timed hyperpolarizing events may increase the firing probability, possibly through a reduction of sodium channel inactivation. Stimuli without transients may be encoded reliably with respect to the mean rate but not with respect to the exact timing of spikes. The behavior observed is roughly compatible with a deterministic leaky-integrator or Hodgkin-Huxley model with a fixed level of additive background noise (*18*). However, other mechanisms such as spike frequency adaptation may contribute to the observed reliability.

We have deliberately isolated one step in the sequence of electrical and chemical events involved in the propagation of a neural signal. Although we find that reliable spike trains may be elicited by injected currents resembling integrated synaptic inputs, we have not addressed unreliability at other steps in the signaling process, particularly in synaptic transmission (*19*). Such variability would be expected to erode the fidelity of temporal coding but may be mitigated by particular activity patterns (*20*). Evidence for rapid modulation of firing rate (*21*) and repetition of particular spike interval patterns (*1*) suggests that it is possible for the neocortex to overcome these sources of noise.

Neurons in the peripheral auditory system can encode information on the basis of the timing of individual spikes (*22*). Although our finding that neocortical neurons also have the ability to generate precisely timed firing patterns does not prove that this timing has a physiological significance, it is consistent with theories of cortical information processing in which spike timing is important.

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11. As the purpose was to isolate the process of spike generation from synaptic transmission and dendritic integration, care was taken to eliminate sources of variability extrinsic to spike-generating currents themselves. To reduce spontaneous synaptic activity, D-2-amino-5-phosphonovaleric acid (D-APV; 20 μM) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM) were used to block glutamate receptors, and bicuculline methiodide (BMI; 5 μM) was used to block γ-aminobutyric acid ($GABA_A$) receptors. To mitigate the effects of possible long-term drift in recording conditions, reliability was measured over blocks of consecutive trials recorded during a period of less than 2 min, and blocks showing obvious instability (fluctuations in membrane potential or input resistance) were not included in the analysis. Data are reported for cells in which recordings were sufficiently stable and long-lasting to permit examination of repeated responses to stimuli of a range of parameters. Nevertheless, even under these conditions, relatively small sources of uncontrolled noise were still present. For instance, the background voltage noise measured over 200-ms periods at resting potential (−68.4 ± 5.1 mV, n = 10) was in the range of 0.05 to 0.5 mV (root mean square).
12. Coronal slices of occipital cortex (400 μm) were prepared from Sprague-Dawley rats 14 to 24 days old deeply anesthetized with ether and decapitated. After 1 to 6 hours incubation in an interface chamber, a slice was transferred to a submerged chamber (22° to 24°C) for recording and continuously perfused with oxygenated (95% O_2, 5% CO_2) Ringer solution containing (in millimolar) 126 NaCl, 1.25 NaH_2PO_4, 10 D-glucose, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, and 26 $NaHCO_3$. Tight-seal whole-cell recordings were obtained from pyramidal-shaped neurons of layer 5 under visual control [G. J. Stuart, H.-U. Dodt, B. Sakmann, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **423**, 511 (1993)]. Patch pipettes (3 to 8 megohms, thin-walled borosilicate glass, wax-coated to reduce capacitance) contained (in millimolar) 100 potassium gluconate, 25 KCl, 5 NaCl, 10 Hepes, 0.2 EGTA, 4 adenosine triphosphate, and 0.3 guanosine triphosphate; pH 7.2 with KOH. We recorded whole-cell potentials using a patch-clamp amplifier (Axopatch 200a) in the "fast" current-clamp mode, filtered at 1 kHz and digitized at 4 to 16 kHz. Trials were 1024 ms in duration and were col-

lected at intervals of 3 to 4 s. We performed bridge balance digitally off line using hyperpolarizing pulses of 50 to 100 pA preceding each trial. We detected spike times using thresholding of the first or second time derivative of the voltage. For all data collection and analysis we used Sun workstations with custom software written in C++ based on NEURON [M. Hines, in *Neural Systems: Analysis and Modeling*, F. H. Eeckman, Ed. (Kluwer, Boston, 1993), pp. 127–136].
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14. The arrival of many uncorrelated excitatory and inhibitory synaptic events will deliver a total current to a neuron that may be treated approximately as shot noise [S. Rice, in *Selected Papers on Noise and Stochastic Processes*, N. Wax, Ed. (Dover, New York, 1954), pp. 133–294]. The event rates and their amplitude waveforms determine the mean, variance, and frequency spectrum of the net current. Accordingly, the stimuli used were realizations of Gaussian white noise with chosen mean (μ_s) and SD (σ_s) of fluctuations. Convolution with the function $f(t) = t \exp(-t/\tau_s)$ gave low-pass filtering with a time constant τ_s, as could be expected from synaptic time courses and dendritic filtering. Unless otherwise noted, τ_s was 3 ms. The range of σ_s investigated, 0 to 100 pA, produced voltage transients up to about 25 mV peak to peak.
15. Z. F. Mainen and T. J. Sejnowski, data not shown.
16. There was no systematic relation between μ_s and reliability over the range of values investigated (50 to 300 pA producing a firing rate of 4 to 32 Hz), although in some cells reliability did increase or decrease with μ_s.
17. The reverse correlation reported is similar to the first-order response kernel of the neuron [P. Z. Marmarelis and V. Z. Marmarelis, *Analysis of Physiological Systems: The White Noise Approach* (Plenum, New York, 1978)].
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20. For example, bursts of action potentials may significantly increase the reliability (release probability) of a synapse through the mechanism of paired-pulse facilitation [R. S. Zucker, *Annu. Rev. Neurosci.* **12**, 13 (1989)].
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23. We are grateful to T. Zador, C. F. Stevens, C. Koch, and W. Bair for insightful comments and discussion. Z.F.M. is a Howard Hughes Medical Institute predoctoral fellow.

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