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Reliability of spike encoding in neocortical neurons

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Abstract

The spontaneous and driven activity of cortical neurons have a high degree of irregularity (1), yet it is not known whether this variability carries information or simply reflects intrinsic noise. We have assessed reliability in the generation of spike trains, using intracellular recording and current injection in vitro, and found that when the identical fluctuating current stimulus was repeatedly injected, the evoked spike pattern was highly consistent, with the precision depending strongly on characteristics of the stimulus. These results indicate that action potential generation in neocortex is a highly reliable process which may enable neurons to faithfully encode incoming information into precisely-timed spike sequences.

It is central to our understanding of cortical function that neurons respond selectively to certain features of the environment with a change in their firing rate (2). It has also been widely observed that such responses are marked by a high degree of irregularity in the temporal patterning of action potentials (1), yet the significance of this variability is poorly understood. On one hand, variability of spike timing could arise from the noise inherent

in the biophysical processes involved in the generation (3) and transmission of neural signals (4). Alternatively, spike patterns may contain information about the environment above and beyond that conveyed by the mean rate (5). Spike variability would then be attributed to unobserved changes in stimuli or internal state rather than simply to intrinsic noise.

To test whether cortical information processing could plausibly rely on coding within the fine temporal structure of spike trains, we performed experiments on an *in vitro* cortical slice preparation chosen so that the state of a single neuron and its input could be well-controlled experimentally. Reliability was assessed with a simple paradigm: a given stimulus was repeatedly presented and the consistency of the sequence of spikes was evaluated. In order to isolate the process of spike-generation from those of synaptic transmission and dendritic integration, somatic whole-cell recordings were made in the current-clamp configuration (6), and spike trains were elicited using current injected through the recording electrode, near the presumed site of action potential generation (7).

First, simple current pulses (0-0.5 nA, 0.9 sec) were used to evoke trains of action potentials. The variability of spike count was small, and variability of interspike interval (ISI; compared across trials for each interval from 1 to n and then averaged across intervals) was low. However, the variance of ISIs rendered the time of occurrence of spikes increasingly desynchronized through the course of the pulse (Fig. 1A). Hence, the timing of the first spike of each train was tightly locked to the onset of the current pulse, while the timing of the last spike in the train was highly variable. Thus, responses to pulse stimuli indicate reliability of spike rate (as measured by total count or reciprocal ISI) but lack of reliability in timing (as measured relative to stimulus onset).

Naturally-occurring input is likely to differ considerably from the current pulse stimuli. *In vivo*, synaptic activity typically results in hyperpolarizing and depolarizing transients (8). Accordingly, signals with random dynamic modulation were generated by computer to approximate the summation of many synaptic currents as seen at the soma (9). When the identical input sequence (same random seed) was repeatedly injected, patterns of spikes with precise and stable timing were elicited (Fig. 1B). As with pulse stimuli, both spike count and ISIs showed little variability. In contrast to pulse stimuli, however, variability of spike timing was also extremely low. While variance of ISIs did not lead to desynchronization, a more discrete form of variability

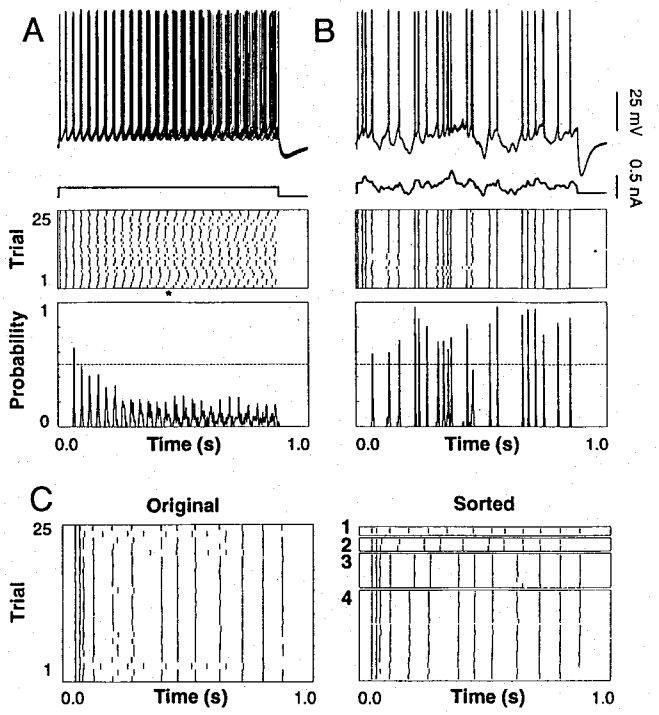

Figure 1: Firing patterns in cortical neurons are highly reliable only in the presence of stimulus transients. (A) A current pulse (0.2 nA, 900 msec) evoked a train of action potentials (30 Hz) in a regular-firing layer 5 pyramidal neuron. Ten consecutive responses are shown overlaid (top). Initial spikes were time-locked to stimulus onset but subsequent spike drifted out of phase. This can be seen in a rasterplot of spike times over 25 consecutive trials (middle) and a smoothed histogram calculated over these trials (bottom). Two sources of variability are apparent (i) variability of interspike intervals which can be seen to accumulates from spike to spike within a trial, and (ii) slow trial to trial drift (visible as coordinated lengthing and shortening of many consecutive intervals, *). (B) Same cell as (A), but rather than a pulse, a current waveform with random fluctuations (mean = 0.2 nA +/- 0.1 nA) was generated and the identical waveform presented repeatedly. In contrast to (A), all spikes were time-locked, and variability of spike timing was extremely low. (C) An example of the response of one cell to 25 consecutive repetitions of the identical stimulus with transients (left). It can be seen that cell spike times tend not to drift, but rather to shift abruptly, disappear, and appear at particular locations. This behavior is more readily seen when the spike sequences are reordered to group like trials (1-4, right). Secondary as well as primary sequences were reproducible.

was apparent: from trial to trial spikes could appear, disappear, or abruptly shift tens of msec (Fig. 1C). In some cases, a single missed or added spike disrupted the timing of many consecutive following spikes. Secondary and tertiary spike patterns were on occasion themselves repeated precisely.

To assess more systematically the dependence of spike time reliability on stimulus characteristics, a simple measure was used to compare responses at a range of different stimulus parameters. As indicated by Fig. 1, reliability increased with increasing amplitude of stimulus fluctuations (Fig. 2A). Firing rate also increased with amplitude of fluctuations, probably due to the ability of stimulus transients to rapidly activate and deinactivate the fast sodium conductance responsible for spiking. Reliability was strongly dependent on the mean amplitude (DC offset) of the stimulus current and correspondingly on mean firing rate (Fig. 2B). The form of the dependence varied with cell firing properties. For neurons with weak spike-frequency adaptation, reliability decreaseed monotonically with increasing input current and firing rate, whereas for neurons with strong adaptation reliability first rose, then fell, giving a narrow range which was optimal.

Finally, reliability depended on the frequency of stimulus fluctuations (Fig. 2C), as judged by varying the time constant of filtering applied to the stimulus. Reliability was highest at intermediate time constants (5 msec), presumably because faster transients were substantially filtered by the membrane time constant ($>$ 10 msec). Reliability decreased greatly for longer time constants (25 msec), suggesting that modulation on this slow a time scale does not lead to reliable spike encoding on the millisecond time scale.

The production of reliable spike patterns suggests that spikes are triggered by certain "preferred" features in the input, presumably related to transients in the current waveform. To characterize the preferred features, the approach of white noise analysis (12, 11) was employed. The reverse correlation of spike events and stimulus (a spike-triggered stimulus average) identifies which stimulus features tend to precede the generation of an action potential and gives an indication of the length of stimulus history that is relevant. Reverse correlations (Fig. 4) revealed a strong tendency for spikes to be preceded by a hyperpolarization (10-30 msec pre-spike) followed by a depolarization (0-10 msec pre-spike). Increased stimulus means (higher firing rates) reduced the average depolarizing transient while accentuating the hyperpolarizing transient (Fig. 3A). Varying the stimulus time-constant revealed a consistent

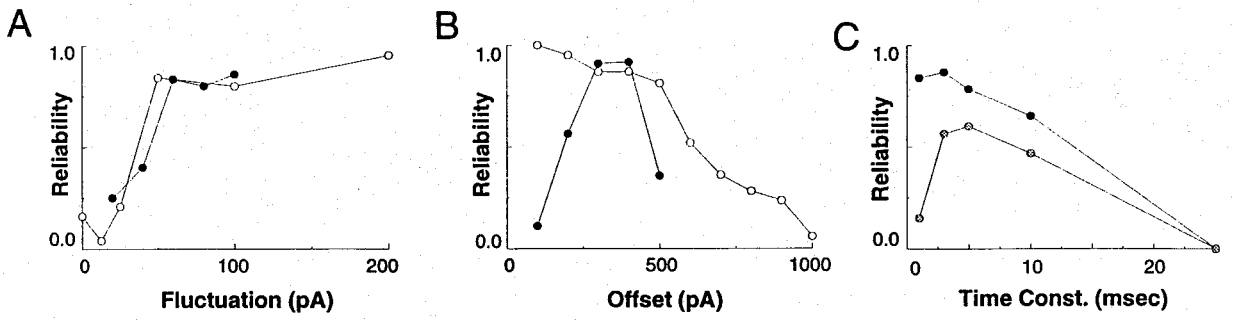

Figure 2: Reliability depends on stimulus characteristics. Reliability was defined operationally as the fraction of spikes in a response that were consistently evoked over repeated trials to within about one msec precision. A single random white-noise sequence was used to elicit 25 or 50 successive responses, and a post-stimulus spike time histogram was computed for these trials. The histogram was smoothed using a gaussian filter. This sets of time scale of interest (1 msec standard deviation) while avoiding binning artifacts. A threshold was then used to detect significant "events" within this spike probability estimate (Fig. 1A,B bottom). The number of events is divided by the maximum number of spikes on any trial within the set to obtain a number between 0 and 1. The same threshold level (0.5) and procedures were applied across all stimulus conditions to obtain an estimate of reliability biased for coincidences at the millisecond time scale. (A) Reliability was enhanced by increasing amplitude of stimulus transients. (B) Reliability decreased with increasing mean stimulus (DC current offset). A regular-firing cell (white circles) showed a monotonically decreasing relationship while a rapidly-adapting cell (black circles) demonstrated maximal reliability at intermediate stimulus intensities. (C) Reliability varied as a function of temporal characteristics of stimulus. The time constant of the low pass filter which was used to smooth the injected current was manipulated to yield stimuli with different frequency content. Maximal reliability was observed for stimuli with filtering near 5 msec, somewhat less than the membrane time constant of these cells.

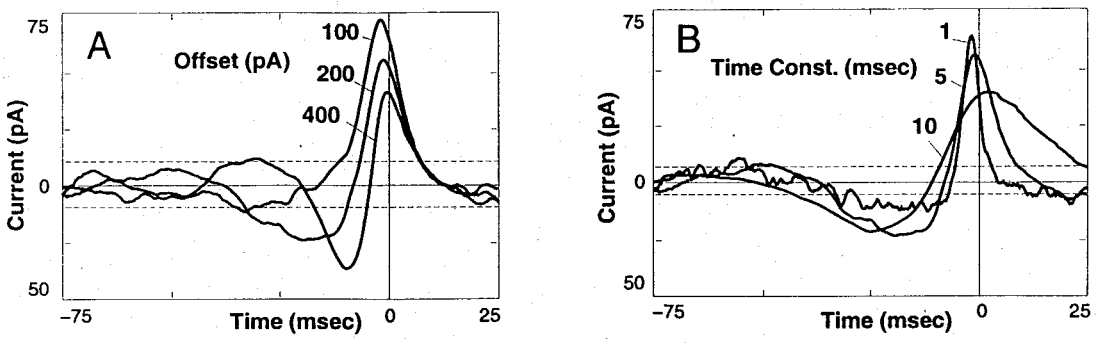

Figure 3: Spikes are correlated with stimulus transients. Reverse correlations of the spike train with the stimulus were computed with a set (25 trials) of stimulus waveforms generated with identical parameters (μ_s=200 pA,σ_s=50 pA,τ_s=3 msec, except where explicitly varied) but different random seeds (9), which is equivalent to a cumulative average of the stimulus surrounding each spike (200-1000 total) over the set of trials. For a neuron generating spikes randomly (irrespective of stimulus), the average stimulus surrounding a spike is not expected to differ from the average stimulus in general, which approaches a flat line as the number of samples averaged increases. Departure from this expectation reveals a preference for particular waveforms. Spikes detection employed a positive threshold function on the derivative of membrane potential. Spikes occurring too close to the beginning or end of a trial to sample the stimulus over the full averaging period were discarded. Confidence limits (dotted lines) were calculated as described in (11). (A) Reverse correlations determined for different mean amplitude (DC offset) of stimuli reveal spikes tend to follow particular transients in the stimulus pattern. For all stimulus offsets a rapid depolarization tends to precede each spike by approximately 5-10 msec. With larger depolarizing offsets (higher firing frequencies) the presence of a preceding hyperpolarization is recruited. Firing frequency ranged from 10 Hz (100 pA offset) to 30 Hz (400 pA offset). (B) Reverse correlations for different time constants of stimulus filter show a consistent tendency for maximum stimulus slope (dI/dt) to occur 5-10 msec before spike detection. Therefore, the time course of the reverse correlation is not likely to be an artifact of the choice stimulus time constant.

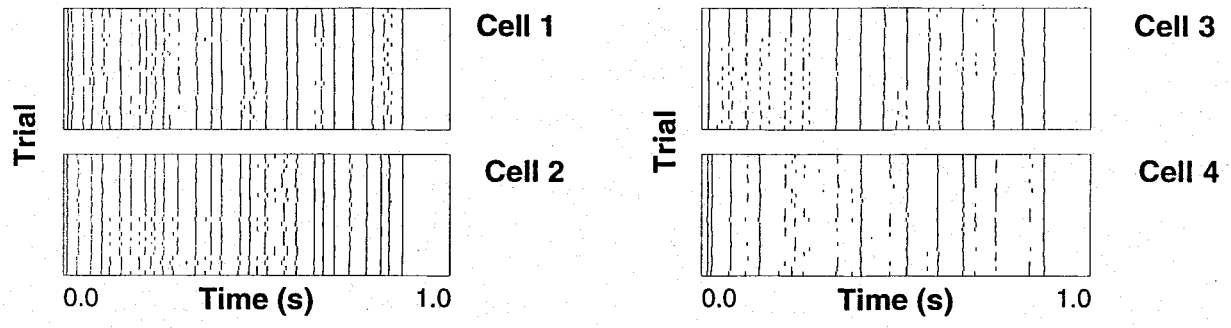

Figure 4: Intrinsic cell properties affect response pattern. Although individual cells show stereotyped spike patterns for a given stimulus, the identical stimulus does not generate the same pattern of spikes in different neurons. The response of four different pyramidal neurons to 25 presentations of the same current stimulus (μ_s=200 pA,σ_s=50 pA,τ_s=3 msec) are shown.

preference for maximum stimulus slope 5-10 ms preceding the spike (Fig. 3B).

These results indicate that a particular cell encodes a given input pattern into a consistent spike pattern based on generation of spikes in response to particular input transients. However, it was not the case that all cells generated the same patterns in response to the same stimulus waveform (Fig. 4). Thus, intrinsic cell properties and internal state may lead to differing reactions to identical incoming signals.

We have found that the mechanisms of action potential generation in neocortical neurons is sufficiently reliable and robust to extrinsic noise that stimuli may be repeatably encoded into firing patterns that are consistent to the millisecond level. Such specific and faithful temporal coding appears to follow from the preference of spike generation for stimulus transients, and thus depends on the presence of fluctuations in the input. Stimuli without transients are encoded reliably with respect to mean rate and interspike intervals, but not with respect to the exact timing of spikes. Reliability may decrease at extremely high firing rates or for very slow input modulation.

We have specifically isolated one step in the sequence of electrical and chemical events involved in the propagation of a neural signal. Although we find that the process of spike generation is highly reliable for injected currents with some resemblance to synaptic input, we have not taken into

account unreliability at other steps in the signaling process, particularly in synaptic transmission (4). Variability from these sources would be expected to erode the fidelity of temporal coding, but may be less significant than generally assumed.

Although the ability to generate precisely-timed firing patterns does not necessarily entail that this timing has a physiological significance, our data are consistent with the notion that coding by spike sequences or coincidence plays a general role in information processing the cortex (5, 13), and could help to explain recent evidence for reliability in the fine temporal structure in visual responses to identical stimuli in the behaving monkey (14).

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[6] Cortical slices were prepared from 14-24 day old Sprague-Dawley rats by standard methods. For recording, slices were transferred to a submerged chamber at room temperature (22-24 ° C) and continuously perfused with ringer solution (140 NaCl, 1.25 NaH_2PO_4, 10 D-glucose, 3.5 KCl, 1.3 MgCl, 2.5 $CaCl_2$, 26 $NaHCO_3$) oxygenated (95% O_2 /5% CO_2). Whole-cell patch clamp recordings were obtained from pyramidal neurons of layers 2/3 or 5 of occipital cortex under visual control [G.J. Stuart, H.U. Dodt, B. Sakmann, *Pflugers. Archiv. Eur. J. Physiol.* **423**, 511 (1993)]. Whole-cell currents were amplified with either a microelectrode bridge amplifier (Axoclamp 2A) or a patch clamp amplifier in "fast" current-clamp mode (Axopatch 200a). Patch pipettes (3-5 MΩ) contained (in mM): 140 K-gluconate, 60 KCl, 40 HEPES, 0.2 EGTA, 5 $MgCl_2$, 2 Mg-ATP, 0.3 Li-GTP, pH 7.2 with KOH. Trials were 1024 msec in duration and were collected at a intervals of 2 to 5 s. Whole-cell potentials were filtered at 1 kHz and digitized at 4 kHz. Bridge balance was performed digitally offline.

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[9] Stimuli are generated by obtaining a sequence of Gaussian-distributed random values using a computer random number generator. This sequence is convolved with a an alpha function $f(t) = t\exp(-t/\tau_s)$ to give low-pass filtering with time constant, τ_s, resembling that resulting from synaptic time courses and dendritic filtering. The final stimulus is offset with a D.C. bias and scaled to obtain a desired mean, μ_s, and standard deviation, σ_s.

[10] Although usual care is taken to minimize instrument noise and obtain stable recordings, synaptic receptor blockers (20 μM D-APV and 10 μM CNQX to block glutamate receptors, and 5 μM bicuculline to block $GABA_A$ receptors) are the only extraordinary measure taken to eliminate biological noise. To reduce the effects of longterm recording stabil-

ity, reliability is assessed over a block of consecutive trials taken over a period of a few minutes.

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