Response Variability and Timing Precision of Neuronal Spike Trains In Vivo

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Reich, Daniel S., Jonathan D. Victor, Bruce W. Knight, Tsuyoshi Ozaki, and Ehud Kaplan. Response variability and timing precision of neuronal spike trains in vivo. *J. Neurophysiol.* 77: 2836–2841, 1977. We report that neuronal spike trains can exhibit high, stimulus-dependent temporal precision even while the trial-to-trial response variability, measured in several traditional ways, remains substantially independent of the stimulus. We show that retinal ganglion cells and neurons in the lateral geniculate nucleus (LGN) of cats in vivo display both these aspects of firing behavior, which have previously been reported to be contradictory. We develop a simple model that treats neurons as ''leaky'' integrate-and-fire devices and show that it, too, can exhibit both behaviors. We consider the implications of our findings for the problem of neural coding.

INTRODUCTION

The variability of neurons' response patterns has been studied extensively and is widely considered to be larger (see Shadlen and Newsome 1994 for a review). For retinal ganglion cells in intact mammals, one particular measure of trial-to-trial variability, the standard deviation of the cell's responses to multiple cycles of a sinusoidal grating, is consistently independent of contrast, region (center or surround) or fraction of the receptive field stimulated, and overall illumination (Croner et al. 1993; Reich et al. 1994). On the other hand, retinal ganglion cells in rabbits and salamanders respond with high temporal precision to a flickering full-field light stimulus (Berry et al. 1996), as do cells in rat neocortical slices that are directly injected with time-varying current (although these cells respond less precisely to steady stimulation) (Mainen and Sejnowski 1995). On the basis of their results, Mainen and Sejnowski suggest that the ''intrinsic noise'' of neurons is low. Moreover, even in the so-called ''higher areas'' of the visual cortex of awake monkeys, neurons can respond to visual stimuli by firing spikes at the same times in every stimulus presentation (Bair and Koch 1996). Thus there continues to be substantial debate regarding the size of variability at the various levels of the mammalian visual system (Gur et al. 1996; Softky and Koch 1993).

Although these widely divergent reports of the size and nature of neuronal variability may appear inherently paradoxical, they are not. We show here that neurons in vivo routinely exhibit, in the same spike trains, simultaneously, both high, stimulus-dependent temporal precision and stimulus-independent variability among repeated responses to a given stimulus, even while the response itself changes dramatically. This behavior was found in every cell examined in our study. A simple theoretical model for neuronal firing—the leaky or ''forgetful'' integrator (Knight 1972)—also produces this sort of spiking behavior and provides a straight-forward resolution of the apparent paradox.

METHODS

Experiments

We recorded extracellularly the activity of lateral geniculate nucleus (LGN) neurons and their retinal inputs in anesthetized, paralyzed cats (Kaplan et al. 1987). Experiments were performed on two male and one female adult cats. The behavior described in this paper was seen in all four retinal ganglion and LGN cells from which recordings suitable for this type of analysis were made. Anesthesia was induced by intramuscular injections of xylazine (Rompun, 1 mg/kg) and ketamine (Ketaset, 10 mg/kg) and was maintained throughout surgery and the recording process with intravenous injections of thiopental (Pentothal, 2.5%, 2–6 $mg \cdot kg^{-1} \cdot hr^{-1}$). Paralysis was induced and maintained with vecuronium (Norcuron, 0.25 $mg \cdot kg^{-1} \cdot hr^{-1}$) or pancuronium (Pavulon, 0.3–0.5 $mg \cdot kg^{-1} \cdot hr^{-1}$). Gas-permeable hard contact lenses were used to prevent corneal drying, and artificial pupils (3 mm diam) were placed in front of the eyes. Blood pressure, heart rate, and expired CO_2 were continuously maintained within the physiological range. The optical quality of the animals' eyes was checked regularly by ophthalmoscopy. Our experimental protocol was approved by the Rockefeller University Animal Care and Use Committee and was in accordance with National Institutes of Health guidelines for the use of higher mammals in neuroscience experiments.

A tungsten–in-glass electrode (5–10-μ tip) recorded spikes from a single LGN neuron and from its retinal ganglion cell input in the form of extracellular synaptic (S) potentials (Bishop et al. 1958). The electrode signal was amplified and monitored conventionally. Visual stimuli were created on a white CRT (Conrac, 135 frames/s, 100 cd/m^2 mean luminance) by specialized equipment developed in our laboratory. The eyes were refracted to focus at the viewing distance of 114 cm. LGN spikes and S potentials were timed to the nearest 0.1 ms.

Model

The model we used is based on the evolution of a dimensionless state variable whose value, compared to a stochastic firing threshold, determines when the cell fires (Knight 1972). The state variable evolves by

$$V(t) = \int_0^t f(t) e^{-(t-t')/\tau} dt',$$

where t is the time since the previous spike, τ is the time constant of the leak, and

$$f(t) = A_0 + A_1 \cos(\omega t + \varphi)$$

is the sinusoidally modulated input. This model is a limiting case of the Hodgkin-Huxley equations for neuronal firing (Knight 1972), and the state variable $V(t)$ plays the role of the intracellular voltage. $V(t)$ is recalculated every 0.1 ms. The cell fires when $V(t)$ reaches a threshold whose value is chosen randomly from a uniform distribution (in our simulation, between 0.7 and 0.9); a new threshold is calculated after each spike is fired. (Versions of the simulation in which the threshold changes every 0.1 ms or in which the probability of firing increases exponentially as the state variable nears the threshold yield similar results.) The single parameter that was varied in this study was A_1, the amplitude of the sinusoidal stimulus. The constant parameters were $A_0 = 50.0$ imp/s (IPS); $\omega/2\pi = 4$ Hz; $\varphi = \pi$; $\tau = 20$ ms. The first few stimulus cycles were discarded to ensure steady-state behavior of the model.

Analysis

We calculated *1*) high-resolution (1-ms bins) peristimulus time histograms, representing the binned nerve impulses fired in response to multiple presentations of the same stimulus, to show the firing rate at any time during the response cycle and *2*) mean rates and stimulus-locked Fourier fundamental components of these response records to evaluate the amplitude and variability of the neuron's response. We also calculated three separate measures of response variability: *1*) the vector standard deviation of the single-cycle Fourier fundamental component (Croner et al. 1993), which is the square root of the sum of the variances of the real and imaginary parts of the Fourier components, taken separately; *2*) the standard deviation of the single-cycle Fourier fundamental amplitudes (a phase-independent measure that is always smaller than the amplitude and phase measure); and *3*) the standard deviation of the spike count in each trial (also known as the mean rate variability).

RESULTS

Figure 1 shows the peristimulus time histogram (PSTH) of an LGN neuron's response to 1,024 cycles of a drifting 4-Hz sinusoidal grating, presented at three contrasts (0, 32, and 100%). There are no preferred times for spikes to occur in the unmodulated (0%) case. At 32% contrast, the first spike in each response tends to be fired at a reliable and reproducible time (the large peak), but later spikes are more widely distributed (Fig. 1*B*). At 100% contrast, however, there are several large but narrow peaks in the PSTH that occur after the initial spike (Fig. 1*C*). As a measure of temporal precision akin to that used by other investigators (Mainen and Sejnowski 1995), we fit each peak in the PSTH to a Gaussian distribution and measured the width of each distribution in the 100% contrast situation. By this measure, the temporal precision for the first few spikes of this cell's response was on the order of ±5 ms. Figure 1*D* is a raster plot of all the spike times for the responses whose PSTH is shown in Fig. 1*C*. The slow drift seen in the rasters indicates that our measure of precision is conservative, and the small, gradual change in response pattern that occurs through trial number 250 could be due to a change in eye position. For the sake of completeness, we have shown the responses to all 1,024 cycles of the stimulus.

Similar behavior is found in retinal ganglion cells. Figure 2*A* shows the PSTH of the response of an X-type, ON-center retinal ganglion cell to 1,024 cycles of a 4-Hz drifting grating at 32 and 100% contrast. Also shown is a plot of response versus contrast (on a semilogarithmic scale), which demonstrates that, whereas the response amplitude increases significantly with contrast, the three measures of response variability remain relatively constant. Preferred firing times are again evident in the 100% case, with a precision of approximately ±5 ms for the first few spikes; we show one of the Gaussian distributions used to measure this timing precision. Thus response variability (measured in any of the three ways shown in the figure) is independent of whether spikes are precisely timed (in the high-contrast regime) or not (in the low-contrast regime).

Figure 2*B* shows the response of an X-type, OFF-center cell to the same stimulus. The temporal precision is on the order of ±3 ms. Note that the response variability for this cell is not constant over low contrasts; that is because this OFF cell fires very few spikes at low contrast. However, once the mean rate exceeds 3 IPS at 2% contrast, the response variability reaches its maximum value and remains flat thereafter, even while the firing precision (and mean firing rate) increases dramatically. Thus despite the differences from the ON-center cell at low contrasts, the data from this cell support our conclusion that response variability is stimulus-independent whereas firing precision is stimulus-dependent, at least over a reasonable range of contrasts.

Fourier analysis reveals that the precise spike timing is not an artifact of the frame rate (135 Hz) of the stimulating CRT. For the data presented in Fig. 1*C*, for example, the nearest peaks in the power spectrum of the spike train occur at 121 and 140 Hz; for the data in Fig. 2*A* there is a small peak at 90 Hz; and for the data in Fig. 2*B*, the nearest peaks are at 120 and 145 Hz. Moreover, the interpeak intervals vary throughout the response cycle.

We show now that a simple model (namely, a leaky integrate-and-fire neuron with a stochastic threshold, which fires in response to sufficient input but which forgets its previous input at an exponential rate) robustly exhibits, in the same response discharge, high, stimulus-dependent temporal firing precision together with stimulus-independent response variability. The key parameter of the model is the rate at which the neuron forgets its previous input. Responses to strongly modulated stimuli typically include a period of intense firing alternating with a quiet period when no spikes are fired (i.e., when the model cell is relatively inhibited). If the time constant of the leak (a stimulus-independent parameter) is shorter than the quiet period (a stimulus-dependent response property), the neuron ''forgets'' the residual input that is integrated during the active portion of the response. Therefore the neuron's initial state is restored after the inactive period of every stimulus cycle at high modulation depth, and response fluctuations do not accumulate from cycle to cycle.

Figure 3*A* shows PSTHs of the model's response to 1,024 cycles of a 4-Hz sinusoidal grating at 0, 16, and 100% contrast. Although the range of variation of the stochastic threshold is the same in all three cases, the firing precision is high at high contrast and low at lower contrasts. Figure 3*B* plots the model's response versus contrast, showing that all measures of response variability are independent of contrast,

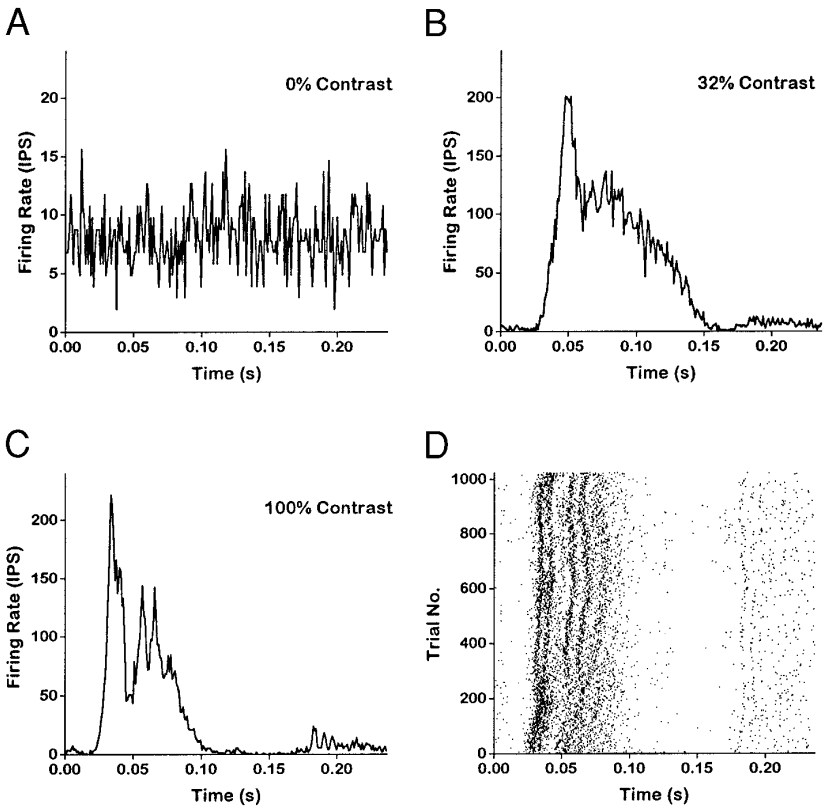

FIG. 1. Neurons in vivo can fire action potentials at precise, reproducible times. Peristimulus time histograms (PSTHs) from an X-type, ON-center cell in the cat lateral geniculate nucleus for 1,024 cycles of a 4 Hz, 0.15 cycles/deg, drifting sinusoidal grating. Vertical axis represents the firing rate in imp/s (IPS) in each 1-ms bin. *A*: 0% contrast. *B*: 32% contrast. *C*: 100% contrast. Note: vertical scale in *A* is 10 times greater than in *B* and *C*. *D*: raster plot of the data from *C*, showing that the cell's response is relatively stationary across response cycles.

whereas the response amplitude, measured as the Fourier fundamental or the mean rate, generally increases with contrast.

Note that at 100% contrast the firing precision is lower for spikes that occur later in the response cycle, as the width of the histogram's sharp peaks increases with time. This change occurs because errors in absolute spike time (due to the stochastic threshold) accumulate as successive spikes are fired during each response cycle. Figure 3*C* shows the probability distribution of the internal state variable of the model (which resets to zero after each spike is fired) at three different times during the stimulus cycle (50, 75, and 190 ms), all at 100% contrast. At 50 ms, before any spikes have been fired, the distribution is very narrow because there has been no opportunity for variability to accumulate. Because of the model's stochastic threshold, the first spike in each trial occurs at a slightly different time (the 1st peak in the 100% contrast histogram in Fig. 3*A* is narrow but has a nonzero width). Each spike causes the state variable to reset to zero, so the amount of input that has accumulated by 75 ms varies depending on when the first spike was actually fired. Hence the wider distribution at 75 ms. The distribution of the state variable at an intermediate time (e.g., 65 ms in the middle of the 1st peak of the histogram) is bimodal, because on some trials the cell has already fired and the state variable is near zero, whereas on others the cell has not yet fired and the state variable is near threshold. At 190 ms, after all the spikes have occurred, the distribution of the state variable is wider than at 50 or 75 ms, because a great deal of variability has accumulated after many spikes have fired. The difference in the width of the distribution between 75 and 190 ms is sufficient to account for the precise firing of the first few spikes in each response and the imprecise firing of the last few.

Note that either a stochastic threshold or additive (stimulus-independent) background noise in the cell's membrane potential is sufficient to ensure that the model does not respond identically to every presentation of the stimulus. Both of these types of input noise yield qualitatively similar PSTHs. In fact, our rather abstract model with a fluctuating firing threshold, and the more physiologically realistic model in which the noise is introduced by a stimulus-independent fluctuating input current, are very closely related. Imagine a simulation in which we followed the state variable from one spike to the next in the absence of the fluctuating input current and then followed it in a large sample of cases with the noisy current present. At the moment that each noisy run crosses threshold, subtract from the state variable the value it had at that moment in the noise-free run. Our collection of differences has a probability distribution that can be regarded as an equivalent distribution of threshold values for the stochastic-threshold case and will yield an identical distribution of interspike interval values for that spike pair. If the noise does not dominate the result, the two kinds of systems have the same response characteristics. However, the actual distribution of the state variable at any time is different (i.e., the state variable has intrinsic variability that is reflected in its distribution even before the 1st spike is fired).

DISCUSSION

Our results indicate that analyzing the size and nature of neuronal variability is not straightforward and that several

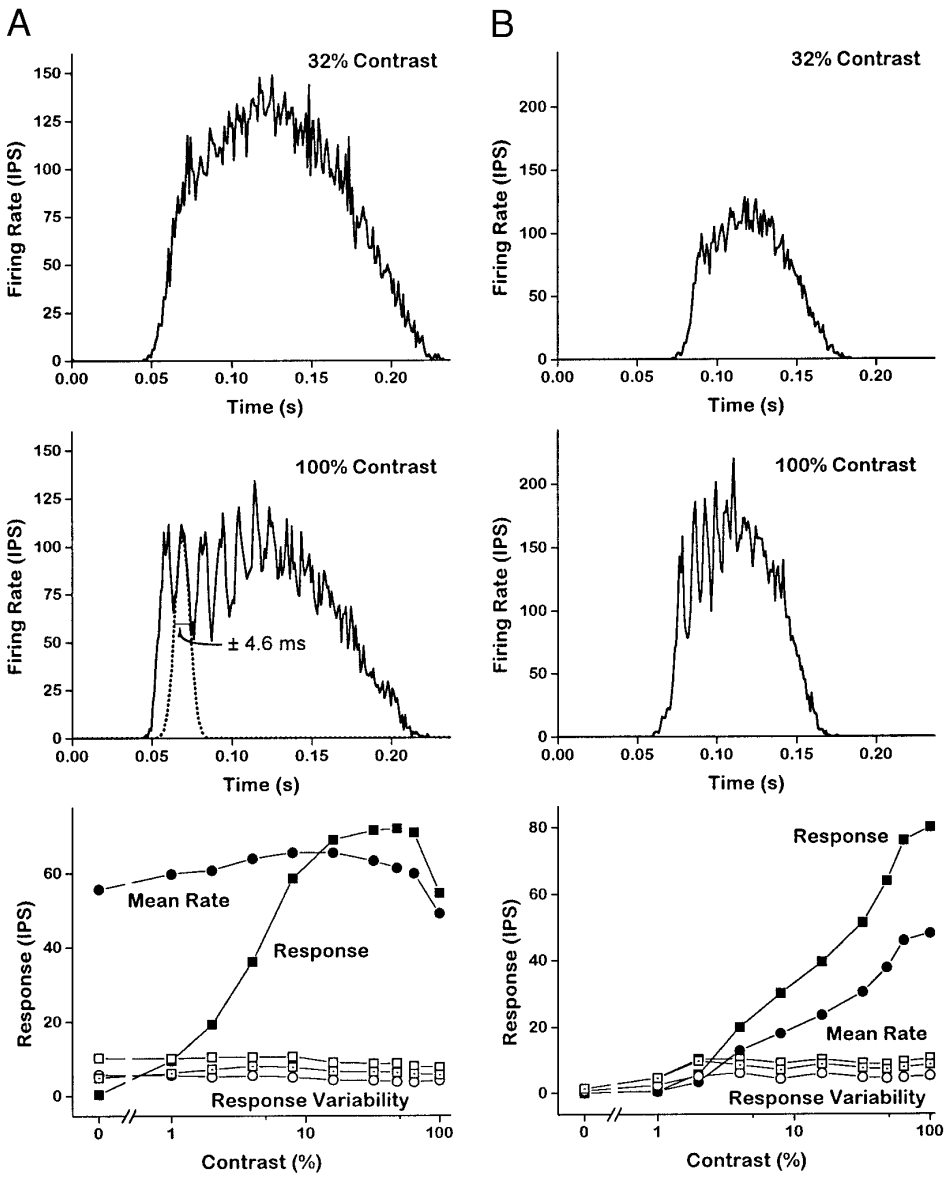

FIG. 2. Spike timing can be precise even while response variability is substantial. *A*: X-type, ON-center retinal ganglion cell recorded as sypnaptic (S) potentials in the cat lateral geniculate nucleus (LGN). Stimulus was a drifting grating (4 Hz, 1.2 cycles/deg, 1,024 cycles). PSTHs for the responses to stimuli at 32 and 100% contrast. Again, the vertical axis shows the firing rate in each 1-ms bin. *Bottom plot*: cell's response vs. contrast, on a semilogarithmic scale. Five data points plotted for each contrast: mean firing rate (●), amplitude of the mean Fourier fundamental (■), variability of the mean rate (○), variability of the Fourier fundamentals (□), and phase-independent variability of the Fourier fundamentals (⊡). *B*: as in *A*, but here the cell is an X-type, OFF-center retinal ganglion cell stimulated with a drifting grating at 32 and 100% contrast (4 Hz, 1.05 cycles/deg, 1,024 cycles). Note that the cell exhibited a 1–2 min period of nonstationary firing (obvious in the raster diagrams, not shown here) during 2 of the stimulus conditions (4 and 16% contrast); this corresponded to roughly 250–500 cycles of the 4-Hz stimulus. Including these trials in the response and response variability calculations yielded unexpectedly high values at those 2 contrasts; however, deleting those trials at the 2 contrasts (and analyzing the 750–1,000 remaining ones) resulted in values that clearly followed the trend for the 8 other contrasts we tested. We plot the data analyzed without the ''variant'' firing for the sake of clarity.

measures of variability must be considered. For example, we consider the response variability, measured as the standard deviation of the fundamental Fourier components for each response cycle (considering or disregarding the phase of the response) and as the standard deviation of the spike count in each cycle, as well as the variability of timing of individual spikes. It is not obvious that there is any simple relationship between these various measures of variability.

These two general types of variability—one substantially independent of the stimulus and the other strongly dependent on the stimulus—can both be generated by a single process: background noise that affects the neuron's membrane potential or, equivalently, fluctuations in the firing threshold. This noise could come, for example, from the barrage of excitatory and inhibitory inputs that converge on many cells in the brain (Shadlen and Newsome 1994). In our model, all the various potential sources of neuronal noise are included in a single parameter, the fluctuation in the firing threshold of the model cell.

The firing precision we report here is lower than that recently reported for neurons in a rat neocortical slice (Mainen and Sejnowski 1995). This result is not surprising; our experiments were performed in vivo, where synaptic activity is higher, and in a different region of the brain. In addition, our stimuli were delivered through the natural neural pathway, in which variability could accumulate at each of several stages. Moreover, the firing precision we found is on the same order of magnitude as that reported in the retinae of salamanders and rabbits (Berry et al. 1996) and even in the medial temporal visual area (area MT) of awake monkeys (Bair and Koch 1996).

Several investigators have proposed that the timing of individual spikes, and not merely the mean firing rate or the Fourier fundamental, carries information that is accessible to the nervous system (Abeles et al. 1994; Bialek et al. 1991; Bullock 1968; Hopfield 1995; Richmond et al. 1987; Softky 1995; Victor and Purpura 1996). Our data do not provide information about which response measure the neuron uses

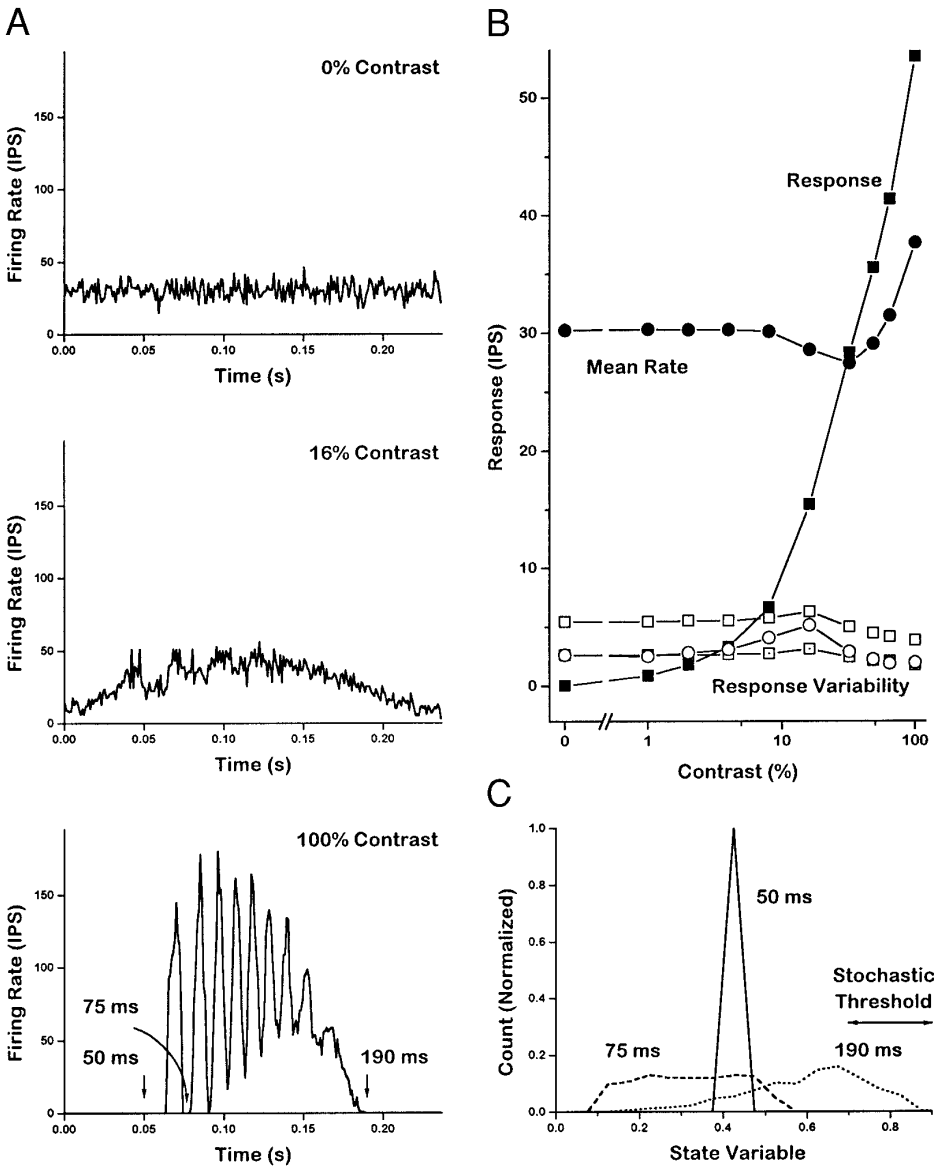

FIG. 3. A leaky (forgetful) integrate-and-fire model, with noise added to its firing threshold, simultaneously displays high timing precision and substantial response variability. *A*: PSTHs of the model's response to 1,024 presentations of a 4-Hz sinusoidal stimulus at 0, 16, and 100% contrast. Arrows at 50, 75, and 190 ms in the 100% panel indicate times for which the state variable's distribution is shown in *C*. *B*: model's response vs. contrast. *C*: probability distribution of the model's state variable, akin to the membrane potential of a real neuron, at 3 times during the cycle, in response to stimulation at 100% contrast. See Fig. 2 for definitions of symbols.

or which source of variability plays a greater role in information processing in the brain. However, our demonstration that visual neurons stimulated at high contrast can fire with high temporal precision, whereas the same neurons stimulated at lower contrasts do not, raises the question of whether this phenomenon occurs only under extreme conditions or whether it is present, albeit less obviously, in responses to weaker stimuli. A finding that spike timing is important for responses to weakly modulated stimuli (not reported here) would suggest that the nervous system can extract timing information from a blur of different spike times, that it could distinguish spikes that provide information about the stimulus from those that are due to noise and that therefore fall at the ''wrong'' time. Such an algorithm could involve, for example, comparing the timing of spikes in several neurons that carry similar information. Alternatively, the mode of information transmission may switch from detection (in a regime in which spike times are imprecise and timing is not used to convey information) to discrimination (in a regime in which spike times are precise).

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