

Comparison of Discharge Variability In Vitro and In Vivo in Cat Visual Cortex Neurons

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SUMMARY AND CONCLUSIONS

1. In neocortical slices, the majority of neurons fire quite regularly in response to constant current injections. But neurons in the intact animal fire irregularly in response to constant current injection as well as to visual stimuli.

2. To quantify this observation, we developed a new measure of variability, which compares only adjacent interspike intervals and is therefore less sensitive to rate variations than existing measures such as the coefficient of variation of interspike intervals.

3. We find that the variability of firing is much higher in cells of primary visual cortex in the anesthetized cat than in slice. The response to current injected from an intracellular electrode in vivo is also variable, but slightly more regular and less bursty than in response to visual stimuli.

4. Using a new technique for analyzing the variability of integrate-and-fire neurons, we prove that this behavior is consistent with a simple integrate-and-fire model receiving a large amount of synaptic background activity, but not with a noisy spiking mechanism.

INTRODUCTION

The input-output relations of single cortical cells have not been well characterized because there is no method for measuring simultaneously the spiking output of a cell and the synaptic input that causes these spikes. In the absence of such measurements, the study of discharge variability is a useful tool for understanding how cortical cells process their synaptic input. The statistics of firing can illuminate some aspects of the interplay between synaptic input and cellular mechanisms. For example, the observed firing irregularity of motoneurons can be understood in terms of the temporal integration of synaptic events (Calvin and Stevens 1967, 1968; Fetz et al. 1991), but such analyses fail for retinal ganglion cells (Barlow and Levick 1969; Croner et al. 1993) and cortical cells (Softky and Koch 1993).

Firing variability is high in vivo in neocortex (Burns and Webb 1976; Dean 1981; Noda and Aday 1970) but the source of this variability is not clear (Shadlen and Newsome 1994; Softky 1995; Softky and Koch 1993; Usher et al. 1994). One explanation is that the spike generation mechanism introduces a stochastic component. However, this seems unlikely because neocortical cells in slices are known to fire in a highly repeatable manner in response to repeated injections of the same current waveform (Mainen and Sejnowski 1995). This report quantifies the low variability in

slice preparations and provides evidence that the spiking mechanism is not noisy in vivo either.

A number of investigators have examined the input-output relationships of single neurons in slice by the use of constant current injections (see Connors and Gutnick 1990). Because there are no fluctuations associated with the current passed through the electrode, a cell ought to fire more regularly when responding to constant current injection than to synaptic stimulation of various kinds. This is what Ratliff et al. (1968) found in the *Limulus* retina, for example. In contrast, some investigators (Ahmed et al. 1993; B. Ahmed and K. Martin, unpublished data) have found that visual cortical cells in vivo still fire very irregularly in response to constant current.

If the variability is in fact due to synaptic activity or some other source of constant background noise (as opposed to a noisy spike trigger mechanism, for example), injecting current into the cell should make it fire more regularly. Current input through the electrode should be able to overwhelm whatever current is causing the fluctuations. An analytical way of describing exactly how this happens for integrate-and-fire neurons is developed (see APPENDIX). At least when the cell is strongly stimulated, there should be a difference in variability between current and visual stimulation.

We developed a new technique for measuring variability that is less sensitive to changes in the mean firing rate and simpler to evaluate than previous methods. Using this method, we quantified the variability of the cells recorded by Ahmed et al. (1993) and found that for most cells there is indeed a small but statistically significant difference in variability between current and visual stimulation.

METHODS

We analyzed sharp electrode intracellular recordings in vivo and in vitro using similar methods. Electrodes in both cases were beveled to 100 M Ω . Slice recordings were derived from 89 cells in slices of adult cat visual cortex, areas 17, 18, and 19 (Berman 1991; N. J. Berman, R. J. Douglas, and K. A. C. Martin, unpublished data). The slices were maintained at 34–36°C. Current was passed through the electrode for 180 or 300 ms; data were sampled at 10 or 5 kHz, respectively. For each train, the first 50 ms after current injection began were ignored. Currents normally ranged from 0 to 1 nA, but in a few cases currents as large as 2.5 nA were used.

The in vivo sharp electrode recordings that we analyzed were derived from 33 cells in adult cat visual cortex areas 17 and 18

(Ahmed et al. 1993). The cats were anesthetized with sodium barbiturate and N_2O . See Douglas et al. (1991) for details of the experimental preparation. Visual stimulation consisted of a high-contrast bar moving in the preferred direction. The stimulus lasted 800 ms, and the response was sampled at 2.5 kHz. In some cases, as part of a different experimental protocol that will not be discussed in this paper, an optic chiasm shock interrupted the train in the middle. In these cases, each half of the response was treated independently from the other. Current injection trials lasted 350 ms (after stripping off ~ 10 ms to remove the electrical artifact) and were sampled at 5 kHz. The current was injected in the absence of visual stimuli. Within each block of current injection trials, three trials were conducted at each current level. The low number of trials prevented us from estimating the variability of the number of spikes. Currents normally ranged from 0 to 1 nA, but in a few cases currents of up to 2.5 nA were used.

Fourteen of the recorded cells were labeled intracellularly with horseradish peroxidase, so their laminar locations and morphologies were known accurately. One cell from lower layer III was probably a basket cell. All of the remaining filled cells were either pyramidal (12) or spiny stellate cells (1).

RESULTS

Measuring variability

A standard method of measuring the variability of a spike train is by the coefficient of variation of the interspike intervals (ISIs), C_V . If the spikes in a train occur at times t_i ($0 \leq i \leq N$), then the ISIs will be

$$\Delta t_i = t_i - t_{i-1} \quad (1)$$

for $1 \leq i \leq N$. The distribution of ISIs then has the standard deviation

$$\sigma_{\Delta t} = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (\Delta t_i - \langle \Delta t \rangle)^2} \quad (2)$$

The irregularity of the spike train can be measured by the relative width C_V of the ISI distribution

$$C_V = \frac{\sigma_{\Delta t}}{\langle \Delta t \rangle} \quad (3)$$

where $\langle . . . \rangle$ corresponds to an average over all ISIs in the train. This measure, and others such as the autocorrelation function, will not give an accurate estimate of the variability of a neuron if the mean rate changes over time (Fig. 1), because it compares ISIs that come from different mean rates. A changing mean rate is quite common: the firing rate may adapt after a stimulus, or oscillate (Poggio and Viernstein 1964; Werner and Mountcastle 1963), or switch between fast and slow firing rates (Zipser et al. 1993). In our data, firing rates in response to visual stimulation varied by at least a factor of 2, and sometimes up to a factor of 10, in almost all cells. For such data to be usable, these changes in rate must be taken into account. In a previous paper (Softky and Koch 1993), the mean firing rate of the cell was estimated from poststimulus time histograms and the C_V was computed separately for each firing rate. Here we used a simpler technique that does not require knowledge of the poststimulus time histogram.

To measure the intrinsic variability of the spiking process, intervals of time corresponding to different firing rates should not be directly compared. Our new measure C_{V2} avoids this difficulty by comparing only adjacent intervals.

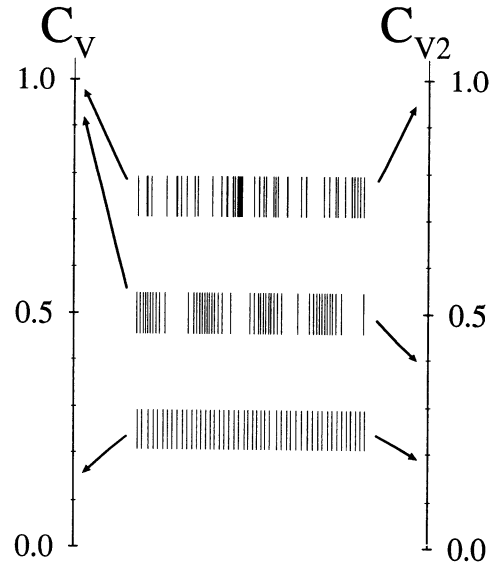


FIG. 1. New method for measuring the intrinsic variability of a spike train. One traditional method for measuring spiking variability, C_V (left), evaluates the spread of interspike interval (ISI) values relative to the mean ISI for the entire train, so that random spikes (top: a Poisson process) yield a C_V value of 1.0 and regular trains (bottom: ISIs drawn from a gamma distribution of order 50) yield a low C_V value; however, regular trains whose firing rates are slowly modulated (middle: ISIs drawn from a gamma distribution of order 50, with the mean rate sinusoidally modulated) yield a high C_V value, masking their true spike-to-spike regularity. Our new measure, C_{V2} (right), compares the relative difference of adjacent intervals, so that only truly random or intrinsically variable trains (top) will give a high C_{V2} value. Both measures are dimensionless, reflecting the structure of the trains without depending on their absolute firing rates. (To obtain a single number for C_{V2} here, we averaged the C_{V2} values over the whole train. Ordinarily a spike train gives many C_{V2} values; see METHODS.)

To obtain C_{V2} for spike i we compute the standard deviation of two adjacent ISIs (thus the subscript “2”), divide the result by their mean, and multiply by $\sqrt{2}$ (so that C_{V2} has a mean of 1 for a Poisson process; see below)

$$C_{V2} = \frac{2|\Delta t_{i+1} - \Delta t_i|}{\Delta t_{i+1} + \Delta t_i} \quad (4)$$

An average of C_{V2} over i estimates the intrinsic variability of a spike train, nearly independent of slow variations in average rate, by comparing adjacent intervals and not widely separated intervals (Fig. 1). The mean value of C_{V2} is approximately equal to C_V for a variety of different spike trains (see APPENDIX). C_{V2} will be high for a train with a high interval-to-interval variability, but it will be lower for a train in which the variation takes place at time scales much longer than the typical ISI.

Neurons cannot fire as variably at high rates as at low rates because of their refractory period and time constant. Therefore it is usually not possible to characterize a cell’s firing variability by a single number. Some previous investigators have plotted C_V versus mean ISI (Softky and Koch 1993; Tuckwell 1989; Werner and Mountcastle 1963). For the reasons presented above, we want to avoid comparing periods when the cell is firing slowly with periods when the cell is firing fast, so we do not compute the mean ISI or the mean C_{V2} over the whole train. Instead, C_{V2} for each pair of adjacent ISIs is plotted against the mean of those two ISIs. For a Poisson process with a refractory period t_r , C_{V2} is

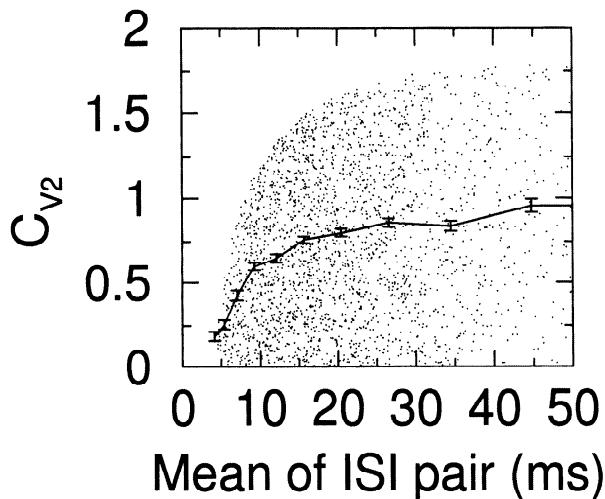


FIG. 2. Application of C_{V2} to a Poisson process with a refractory period. The boundary curve [$C_{V2} = 2(1 - 4/(\Delta t))$] is determined by the refractory period (here set to 4 ms). Note that C_{V2} values are uniformly distributed between 0 and the upper bounding curve. The lines on the plot are mean C_{V2} values in logarithmically spaced bins (ratio between bin boundaries was 1.3). Logarithmic binning was chosen because the upper limit of the data at short ISIs changes much more rapidly than the upper limit at longer ISIs. Error bars: SE. Simulations lasted 5 s and the mean firing rate was 50 Hz.

uniformly distributed between 0 and $2(1 - t_r/(\Delta t))$, and thus has a mean of $1 - t_r/\bar{T}$ (see APPENDIX for PROOF); a sample scatter plot is shown in Fig. 2. Dealing with a cloud of data points is inconvenient, so we compute mean C_{V2} values for different values of the ISI (lines with error bars in the figure). This average makes it possible to see small differences in C_{V2} .

The measure C_{V2} can be generalized to $C_{V2,j}$ by comparing interval pairs that are not adjacent, i.e., using Δt_{i+j} in place of Δt_{i+1} in Eq. 4. A plot of the modified measure $C_{V2,j}$ versus j can show the time scale (in units of the mean ISI) at which irregularity appears in spike trains. Such an analysis works best for trains with a low intrinsic variability and slowly but strongly varying average rate (unlike the cortical spike trains explored here).

C_{V2} for data from slice and from the intact animal

Sample traces from one cell in a cat visual cortex slice (*p1c3.s91*) and one cell from the anesthetized cat (*p3c2.e16*) are shown at the top of Fig. 3. In the slice data, it was possible to classify the cells with the use of the scheme of earlier investigators (Agmon and Connors 1992; Connors et al. 1982; McCormick et al. 1985); most of the cells were regular spiking, 10 were regular spiking but had stronger adaptation (RS_2), 1 was a fast-spiking cell, and 1 was intrinsically bursting (IB).

Clearly the cell in Fig. 3 in the intact animal fires much more irregularly than the cell in vitro, even when the same experiment is performed (passing current through the electrode). To quantify the variability, we used the C_{V2} measure. The bottom of Fig. 3 is a scatterplot of C_{V2} against the mean of the two intervals compared for each of 21 cells for which we had both current injection and visual stimulation data. There is one point on the graph for each pair of adjacent ISIs from each cell.

We specifically examined all the slice trains that had average C_{V2} values >0.3 and found that they fell into one of two categories. First, in a number of cases (the RS_2 cells), adaptation was incomplete by 50 ms, the cutoff at which we started to use the data; for some of these cells, the ISI was lengthening significantly for a few of the intervals considered here. This can significantly affect C_{V2} (although less than C_V ; see above). Second, one cell (type IB) fired clusters (bursts) of two or more spikes. These two sources account for essentially all of the high C_{V2} values in Fig. 3A. Adaptation is present to some extent in almost all of the traces, so these C_{V2} values are upper bounds on the variability of the data in slice.

Obviously the variability in slice is much less than the variability in vivo. We were surprised at the high variability in response to constant current injection in vivo. However, there is a small but significant difference between the current injection and visual stimulation case, especially at ISIs <30 ms, which is analyzed in more detail below.

Figure 4 shows for 16 cells in vivo the variability in response to current and visual stimulation. We found three different kinds of behavior. In some cases (*top 2 rows*), there was a noticeable difference between current injection and visual stimulation variability. This is mostly caused by a difference in “burstiness” of the visual stimulation data. An example of this is shown in Fig. 5A. “Bursts” here are defined as an excess of isolated short ISIs. On a C_{V2} plot, a short interval followed by a longer one (or vice versa) will appear as a point near the upper boundary curve. Notice how in Fig. 5A there is clearly a large density of points near the upper boundary for visual stimulation, but not for current injection. For a Poisson process, there will be a uniform distribution of points between $C_{V2} = 0$ and the upper boundary of the curve (Fig. 2); in Fig. 5A, there is an excess of points near the boundary. These bursts are not present in the current injection traces. In response to visual stimulation, this cell is more bursty than is expected from a Poisson process with a refractory period in response to current injection. We also observed cells for which the degree of burstiness changed over the course of the impalement (not shown).

A second result of current injection is a downward shift of the whole cloud of C_{V2} values; this shift is more prominent in nonbursty cells (e.g., Fig. 5B). This is intuitively sensible, because the sustained injected current should overwhelm the noise source, thereby reducing variability. In a number of cases (e.g., see Fig. 4, *3rd row*), a difference between current injection and visual stimulation was apparent only for short ISIs. For most of these cells, there was no obvious difference between the burstiness of current and visual stimulation. A larger difference at short ISIs is expected on the basis of a simple integrate-and-fire model (see DISCUSSION). For some cells, there appeared to be a combination of the two effects.

In a few other cases, no difference at all was perceptible (Fig. 4, *bottom row*). These cells tended to be bursty in response to both current and visual stimulation (not shown).

DISCUSSION

We measured the variability of visual cortical neurons in response to constant current injection in intact animals and

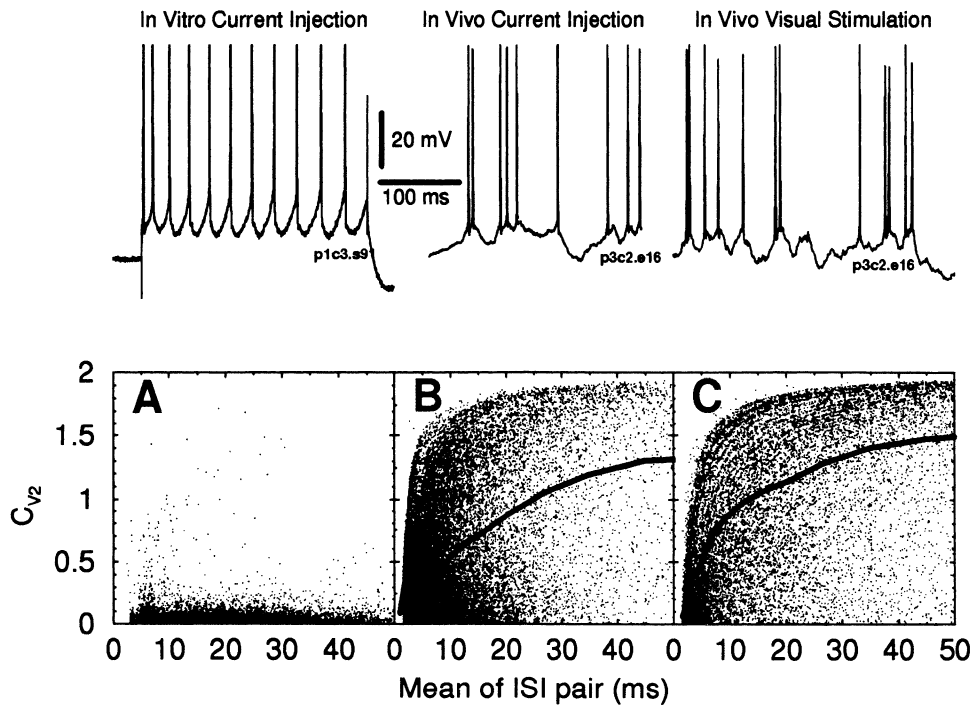


FIG. 3. Comparison of primary visual cortex cells from adult cats in slice and in vivo. *Top*: sample traces from 2 pyramidal cells, 1 from a slice (of the regular spiking type; *left*) and 1 from an intact animal on the boundary between layers II and III that was stimulated by current injection (*middle*) and by a bar moving across the receptive field (*right*). The lack of a large difference between spiking variability in response to current or visual stimulation is intriguing. *Bottom*: C_{V2} values for each interval pair from all cells. *A*: C_{V2} values for 89 cells recorded from slices. *B*: C_{V2} values for 21 cells in vivo in response to constant current passed through the electrode. *C*: C_{V2} values for the same 21 cells in vivo responding to moving light or dark bars across their receptive field. The banded appearance is a discretization artifact; it was not present with current injection because of the higher sampling rate. Heavy lines for *B* and *C* are mean C_{V2} values. Standard errors on the estimate of the C_{V2} value are roughly the width of the thick lines. The shape of the upper limit on C_{V2} values is determined by the cells' refractory period t_r ; a simple prediction of the form $C_{V2} = 2(1 - t_r/\langle\Delta t\rangle)$ fits the data well (not shown).

in slice, and the variability in response to visual stimulation. We used a new measure of variability, called C_{V2} , which is less sensitive to rate fluctuations than C_V (the coefficient of variation of ISIs). For point processes with gamma ISI distributions, however, C_{V2} is approximately equal to C_V . C_{V2} values are very low for spike trains from cells in slice but quite high for cells from anesthetized cats (Fig. 3). In all of the in vivo visual cortical data we have analyzed, we have never found a cell with an average C_{V2} (or C_V) value <0.3 , whereas essentially all nonbursting cells in slice have average C_{V2} (and C_V) values <0.3 . Furthermore, because C_{V2} compares only adjacent ISIs, the high variability measurements in vivo cannot come from a relatively regular process with a mean rate that varies on the time scale longer than an ISI.

One possible explanation of the high irregularity of cells in vivo is that the spiking mechanism is unreliable or stochastic. This seems unlikely, because other kinds of neurons, such as α -motoneurons, are capable of firing much more regularly even at much lower rates (Calvin and Stevens 1967, 1968). Furthermore, the same kind of neurons in slice fire very regularly (see Fig. 3A). In fact, the timing of the spikes in neocortical slice is highly reproducible from trial to trial if the current stimulus has significant temporal structure (Mainen and Sejnowski 1995).

Our favored explanation is random synaptic background activity in the whole animal that is absent in the slice. In

visual cortex, even in the anesthetized animal, a large amount of spontaneous firing is present; our cells from cat area 17 discharge spontaneously at rates between 0 and 15 Hz in the absence of any stimulus, and others have measured spontaneous firing rates up to 20 Hz (Gilbert 1977). This spontaneous firing recirculates within the cell population as synaptic input, and thus should affect each cell's membrane voltage. Although no clear excitatory postsynaptic potentials are visible in the intracellular voltage traces (Fig. 3, *top*), the large fluctuations in membrane potential are presumably caused by synaptic input. If, as we suggest, the background synaptic activity is responsible for the high firing irregularity during current injection, then the variability in response to current should diminish in the intact animal if the spontaneous activity is markedly reduced pharmacologically or by other means.

If the noise is due to synaptic activity or some other source of constant background noise (as opposed to a stochastic spike trigger mechanism), injecting current into the cell should make it fire more regularly, because the sustained current input through the electrode will be larger relative to the amplitude of the background fluctuations. To understand this, consider an integrate-and-fire neuron with no refractory period, whose mean ISI in response to spontaneous synaptic background activity is $\langle\Delta t\rangle_0$ (i.e., its mean spontaneous firing rate is $1/\langle\Delta t\rangle_0$). If a sustained current step is added to its input, decreasing the mean ISI to $\langle\Delta t\rangle$, then the associated variability will be reduced to

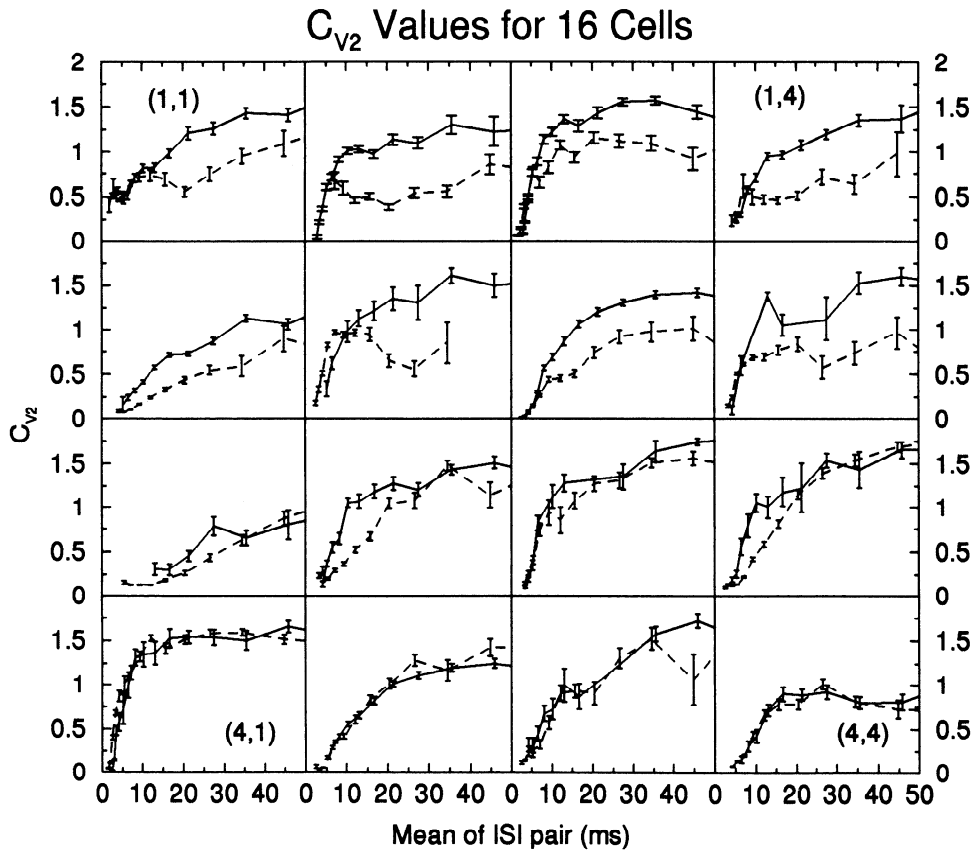


FIG. 4. Visual (—) and current stimulation (---) variability for 16 cells from the visual cortex of anesthetized cats. Error bars: SE on C_{V2} values in the bins. To distinguish the error bars, the visual injection curve was displaced 1 ms to the right. As in Figs. 2 and 3, C_{V2} values were calculated in logarithmically spaced bins. *Top 2 rows*: cells in which there is a difference between the current injection and visual stimulation variability, especially at large ISIs. Most of these cells are more “bursty” in response to visual stimulation than to current injection. *Third row*: cells that show a difference between current and visual stimulation only at short ISIs. A difference at short ISIs is what is expected on the basis of an integrate-and-fire model (see DISCUSSION). *Bottom*: cells that do not show a difference between current and visual stimulation variability.

$$C_V = C_{V,0} \sqrt{\frac{\langle \Delta t \rangle}{\langle \Delta t \rangle_0}} \quad (5)$$

where $C_{V,0}$ is the variability of spontaneous firing caused by background activity in the absence of current input. This reduction occurs because the additional constant current will lead to an increase in the number of spikes triggered without any change in the variance in the number of spikes (see APPENDIX for details).

On the other hand, increasing the firing rate of a cell by visual stimulation should not reduce the variability if visual stimulation simply changes the rate of synaptic input without changing the statistics or the correlation structure. (This argument assumes that dynamic processes with slow time constants, such as I_{AHP} or γ -aminobutyric acid receptor type B, are not important in determining variability. Also, the refractory period of the cell will limit variability at high firing rates.) Even including these effects, the variability of the model cell driven by noisy synapses should be higher than when driven to the same rate by constant current.

Because $C_V \propto \sqrt{\langle \Delta t \rangle}$ for injection of constant current into an integrate-and-fire unit, neither C_V nor C_{V2} should change rapidly with $\overline{\Delta t}$. Particularly when there is some uncertainty in measuring C_V or C_{V2} , we would not necessarily expect to see a difference between visual and current stimulation except when the cell is firing rapidly. For example, if the standard error in measuring C_{V2} is 0.1 and the mean C_{V2} value for spontaneous activity is 1, the firing rate will have to change by a factor of ~ 2 for the difference between visual stimulation and current injection to be statistically significant.

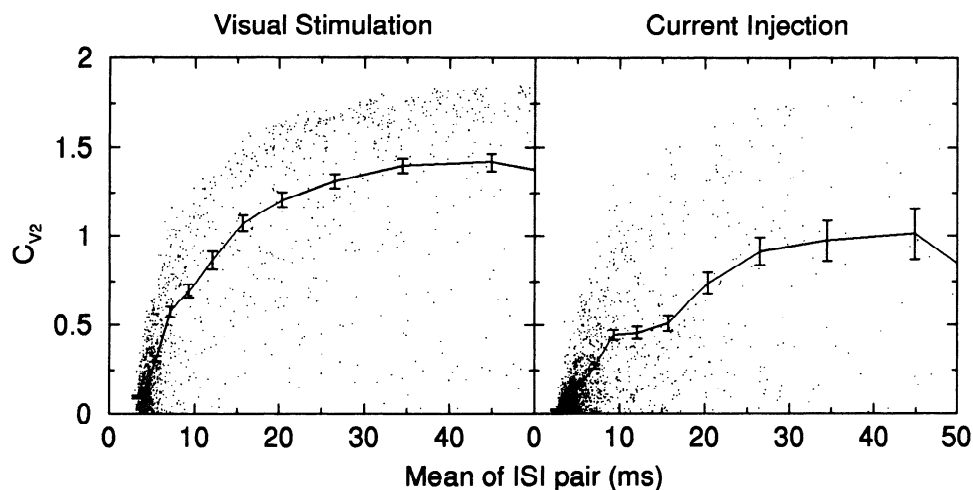
These calculations are based on an integrate-and-fire model, a severe simplification of a real neuron. One improvement to the model is for the membrane voltage to decay over time, causing the cell to forget its inputs. The resulting variability of such a cell model will be higher than of an integrate-and-fire model. Furthermore, the time constant of that decay will change with the level of synaptic input (Bernander et al. 1991). These effects are not easily amenable to analysis, although much work has been done studying leaky integrators (see reviews in Holden 1976; Sampath and Srinivasan 1977; Tuckwell 1989). Nevertheless we expect that results from the simple model may be qualitatively correct.

A difference between current and visual stimulation is seen for the majority of neurons (Fig. 4), although in some cases it is visible only for short ISIs. Part of the difference appears to be due to the general lowering of C_{V2} values (e.g., Fig. 5B) predicted by this simple analysis.

There is also a second effect. Frequently cells respond in a more bursty fashion to visual stimulation than to current injection (Fig. 5A): the band of high C_{V2} values in Fig. 5A simply disappears (or is reduced in density). A possible origin of the difference in burstiness is that current injection stimulates the cell at or near the soma, whereas visual stimulation arrives at the dendrites. Dendritic stimulation might better trigger calcium spikes (e.g., Amitai et al. 1993; Bernander et al. 1994; Kim and Connors 1993; Markram and Sakmann 1994; Yuste et al. 1994), which may underlie burst generation.

However, we do not believe that such bursting accounts

A. Difference in Burstiness



B. General lowering of C_{V2}

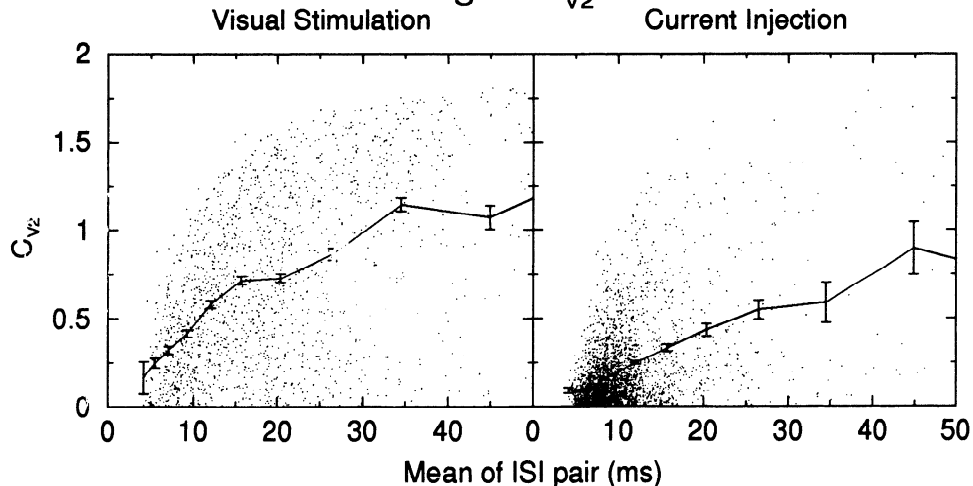


FIG. 5. *A*: C_{V2} values for a cell that is more bursty in response to visual stimulation than to current injection. As before, each dot represents 1 pair of ISIs; the lines are mean C_{V2} values and the error bars are SE. Note that there is a higher density of points near the upper boundary in the visual stimulation panel than in the current injection panel. A short ISI followed by a long one (or vice versa) will have a C_{V2} value near the upper boundary. This band disappears in the C_{V2} plot for current injection. This is from cell (2,3) in Fig. 4. *B*: C_{V2} values for a cell that is not bursty. In this case, the C_{V2} values have simply shifted downward when current was applied. Cell (2,1) in Fig. 4.

for all of the high firing variability of neocortical cells (Wilbur and Rinzel 1983), because 1) some cells do not seem to burst very much, and they still fire irregularly; 2) when bursts are replaced by single spikes with the use of the numerical algorithm from Bair et al. (1994), the variability is still high (not shown); 3) during current injection trials, when the bursts are absent or less common, variability is still high.

If the background synaptic activity were lower, presumably the cells would fire much more regularly in response to constant currents. Cells in visual cortex have unusually high unstimulated firing rates compared with some other cortical areas. On the basis of published figures, firing variability in response to current injection appears to be lower in other cortical areas (e.g., Nuñez et al. 1993 in association cortex, and Baranyi et al. 1993a,b in motor cortex). This variability difference might be due to differences in the amount and type of synaptic background activity across areas.

Other investigators (Bernander et al. 1991; Rapp et al. 1992) have shown that background synaptic activity can strongly influence the cable properties of a neuron, and thus

how synaptic efficacy depends on time and on distance from the soma. Here we provide evidence that background activity may also influence firing irregularity. With the same current injection stimulus, the same cell type fires very differently in vivo and in slice. For this reason, data from slices, although essential to our understanding of neuronal function, must be interpreted with caution.

This study raises a number of further questions. What is the correlation structure of the background synaptic activity that gives rise to the firing variability? Are dendritic nonlinearities important in amplifying fluctuations (Softky 1995), or does inhibition approximately balance excitation (Shadlen and Newsome 1994)? Most importantly, why is it useful or necessary for these neurons to fire irregularly (Knight 1972; Softky and Koch 1993)? That is, what is the computational function (if any) of this high variability?

APPENDIX

Probability distribution of C_{V2} for a Poisson process

If two ISIs are drawn independently from a known probability distribution, then one can compute the distribution of the random

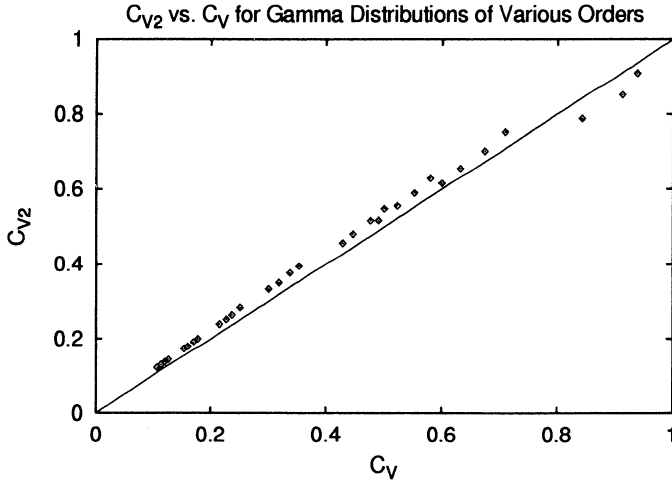


FIG. A1. C_{V2} and C_V give similar values for simple stationary (ISI) distributions. C_V and the mean value of C_{V2} were calculated for spike trains with gamma-distributed ISIs of orders 1–20, to which various refractory periods (0–3 ms) were added (the fact that all trains had mean rate of 50 Hz is irrelevant to the calculation, because both measures are dimensionless). Points exactly on the diagonal would correspond to $C_{V2} = C_V$. The 2 measures differ by up to 10% of the C_V value; the largest fractional differences are for high-order gamma distributions (low C_V values), which are not found for cortical cells.

variable C_{V2} . In the case of a Poisson process with no refractory period, the ISIs are distributed as

$$p(\Delta t)d\Delta t = \lambda \exp(-\lambda\Delta t)d\Delta t$$

where λ is the mean rate of the Poisson process. We define the new random variable X

$$X = \frac{2(\Delta t_1 - \Delta t_2)}{\Delta t_1 + \Delta t_2}$$

(Note that C_{V2} of Δt_1 and Δt_2 is just $|X|$.) The distribution of X can be computed as follows

$$\begin{aligned} \Delta t_2 &= \Delta t_1 \frac{2 - X}{2 + X} \\ P(X > X_0) &= \int_0^\infty P\left[\Delta t_2 < \frac{\tau(2 - X_0)}{2 + X_0}\right] p(\Delta t_1 = \tau) d\tau \\ &= \int_0^\infty \left[1 - \exp\left(-\lambda\tau \frac{2 - X_0}{2 + X_0}\right)\right] \lambda \exp(-\lambda\tau) d\tau \\ &= \int_0^\infty \lambda \exp(-\lambda\tau) d\tau - \int_0^\infty \lambda \exp\left(-\lambda\tau \frac{4}{2 + X_0}\right) d\tau \\ &= 1 - \frac{2 + X_0}{4} \\ &= \frac{2 - X_0}{4} \end{aligned}$$

The probability density of X is just the derivative of this, or $1/4$. Therefore X is uniformly distributed between -2 and 2 . Thus $C_{V2} = |X|$ is uniformly distributed between 0 and 2 .

For a Poisson process with a refractory period t_r , the maximum C_{V2} value for a given mean ISI $\overline{\Delta t}$ would occur when one of the ISIs $\Delta t_2 = t_r$

$$\overline{\Delta t} = \frac{\Delta t_1 + t_r}{2}$$

$$\Delta t_1 = 2\overline{\Delta t} - t_r$$

$$\begin{aligned} C_{V2,\max} &= \frac{2(\Delta t_1 - t_r)}{\Delta t} \\ &= \frac{2(2\overline{\Delta t} - t_r - t_r)}{t_1 + t_r} \\ &= 2(1 - t_r/\overline{\Delta t}) \end{aligned}$$

C_{V2} will now be uniformly distributed between 0 and this maximum, because adding a refractory period to a Poisson process is equivalent to discarding ISIs shorter than the refractory period.

Numerical relationship to C_V

The traditional variability measure C_V is similar to C_{V2} in some respects. A perfectly regular train has mean C_{V2} and C_V values of 0 , and a Poisson process has mean C_{V2} and C_V values of 1 . We have found by simulations that the mean value of C_{V2} is very close to C_V for stationary gamma distributions of various orders and refractory periods (Fig. A1).

Effect of constant current on C_V

Understanding variability requires knowing how input fluctuations are transformed into output fluctuations. A number of studies have attempted to address this question in different systems with the use of a variety of mathematical techniques or simulations (e.g., Gerstein and Mandelbrot 1964; Segundo et al. 1968; Shapley 1971a,b; Stein 1965, 1967; Tuckwell et al. 1984; see reviews in Holden 1976, 1983; Moore et al. 1966; Sampath and Srinivasan 1977; Tuckwell 1989). The evolution of a neuron's membrane potential is typically regarded as a bounded random walk, and the probability distribution of the time to the first threshold crossing (1st passage time) can be computed if the input form is simple enough. There is, however, an analytical technique that can predict an integrate-and-fire neuron's variability for a wide variety of inputs.

C_V is related to another measure of variability that is easier in some cases to analyze but not as easy to estimate from real data. This measure is the ratio of the variance of the number of spikes in time T to the mean number of spikes in the same time period

$$S = \frac{\text{Var } N}{\langle N \rangle} \quad (\text{A1})$$

This measure has been used by other experimenters (e.g., Barlow and Levick 1969; Dean 1981). We have found that it is difficult to estimate S directly from our data because it requires a large number of trials with the same stimulus, whereas C_V or C_{V2} can be estimated from a single sufficiently long train. However, if the firing is a renewal process, the expected value of S is simply related to the expected value of C_V (for details, see Cox 1962, p. 45–60)

$$S = C_V^2 \quad (\text{A2})$$

in the limit as the length of the train $T \rightarrow \infty$. This is not a new result but a property that seems not to be widely appreciated in the study of neuronal variability. Cortical cell firing is not exactly a renewal process (unpublished data; see also Holden 1976) but the serial correlations are usually weak unless the cell has well-defined bursts.

It is simple to predict the variability, in terms of the S measure, of an integrate-and-fire neuron with no refractory period and no leak conductance. Such a neuron sums up its input until the sum exceeds a threshold, when it generates a spike and resets the membrane potential. The number of spikes it fires in some time T will be proportional to the total charge

$$N = \alpha \int_0^T I(t) dt \quad (\text{A3})$$

where α is the slope of the frequency-current curve. Actually, N must always be an integer, but if the trains are long enough so that $N \gg 1$, the difference will not matter. This is only valid when excitation exceeds inhibition on average. Because of random fluctuations, even if there is more inhibition than excitation, the cell will still fire occasionally, although the ISI will be very large (Gerstein and Mandelbrot 1964; Stein 1965).

The variance and mean of N are given by

$$\begin{aligned} \langle N \rangle &= \alpha T \langle I \rangle \\ \text{Var } N &= \alpha^2 \text{Var} \left[\int_0^T I(t) dt \right] \end{aligned} \quad (\text{A4})$$

We can predict the S value if we can evaluate the above expressions.

A constant current I_{dc} superimposed onto the synaptic background activity $i_{syn}(t)$ will change the mean number of spikes without changing the variance

$$\begin{aligned} N &= \alpha \left\{ \int_0^T [I_{dc} + i_{syn}(t)] dt \right\} \\ \langle N \rangle &= \alpha T (I_{dc} + \langle i_{syn} \rangle) \\ \text{Var } N &= \alpha^2 \text{Var} \left[\int_0^T I_{dc} dt + \int_0^T i_{syn}(t) dt \right] \\ &= \alpha^2 \text{Var} \left[\int_0^T i_{syn}(t) dt \right] \end{aligned}$$

because for any independent random variables X and Y , $\text{Var } X + Y = \text{Var } X + \text{Var } Y$, and $\text{Var} \int_0^T I_{dc} dt = 0$

$$\begin{aligned} S &= \alpha \frac{\text{Var} \int_0^T i_{syn}(t) dt}{T(I_{dc} + \langle i_{syn} \rangle)} \\ &= \alpha \frac{\text{Var} \int_0^T i_{syn}(t) dt}{T \langle i_{syn} \rangle} \frac{T \langle i_{syn} \rangle}{T(I_{dc} + \langle i_{syn} \rangle)} \\ &= S_0 \frac{\langle i_{syn} \rangle}{(I_{dc} + \langle i_{syn} \rangle)} \end{aligned}$$

where S_0 is the firing variability due to the synaptic background activity. The same result can be expressed in terms of the change in firing frequency caused by the current injection

$$\begin{aligned} S &= S_0 \frac{f_0}{f} \\ C_v &= C_{v,0} \sqrt{\frac{f_0}{f}} \\ &= C_{v,0} \sqrt{\frac{\langle \Delta t \rangle}{\langle \Delta t_0 \rangle}} \end{aligned} \quad (\text{A5})$$

where $f_0 = 1/\langle \Delta t_0 \rangle$ is the original firing rate without current injection and $f = 1/\langle \Delta t \rangle$ is the new firing rate. This analysis was used by Barlow and Levick (1969) to study the effects of surround inhibition (modeled as a constant current) on retinal ganglion cell variability, but it is also applicable to depolarizing currents.

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