Input synchrony and the irregular firing of cortical neurons

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Cortical neurons in the waking brain fire highly irregular, seemingly random, spike trains in response to constant sensory stimulation, whereas *in vitro* they fire regularly in response to constant current injection. To test whether, as has been suggested, this high *in vivo* variability could be due to the postsynaptic currents generated by independent synaptic inputs, we injected synthetic synaptic current into neocortical neurons in brain slices. We report that independent inputs cannot account for this high variability, but this variability can be explained by a simple alternative model of the synaptic drive in which inputs arrive synchronously. Our results suggest that synchrony may be important in the neural code by providing a means for encoding signals with high temporal fidelity over a population of neurons.

Cortical neurons in the waking brain fire highly irregular spike trains that have more in common with the ticking of a Geiger counter than of a clock. What is the source of this irregular firing? Softky and Koch[1] noted a theoretical conundrum posed by the irregularity of cortical neurons firing at a constant average rate *in vivo*. If each cortical neuron were providing independent input to the other similar cortical neurons it contacts, then the input to any neuron would just be a shower of statistically independent excitatory postsynaptic potentials (EPSPs) with a constant mean rate. Yet such an input, when tested on a theoretical model of a cortical neuron, gave rise to a firing variability much less than that observed *in vivo*. What, then, is the source of the unexpectedly large variability in spike timing that characterizes *in vivo* firing behavior? Is the high variability due to some subtle interplay between the noise resulting from randomly timed synaptic inputs and the nonlinear spike-generating mechanism, or instead due to unexpectedly large fluctuations in the synaptic input itself? Although most earlier workers have argued that the large variability arises from the interaction between this noisy synaptic input and the spike-generating mechanism, we will conclude that large fluctuations in the synaptic drive, such as might arise from the synchronous arrival of inputs from many neurons, are necessary to account for the high *in vivo* variability.

The neuronal spike generator converts input current into output spike trains with high fidelity[2,3]. Irregular firing must, then, reflect fluctuations in the currents that drive the spike generator, rather than some intrinsic noise in the spike-generating mechanism itself[4]. Most previous attempts to identify the source of irregular firing have focused on the details of the spike-generating mechanism and on the importance of the inhibitory synaptic noise[5–7] (Tsodyks *et al.*, *Soc. Neurosci. Abstr.*, 20, 1527, 1994). These workers have concluded that unstructured synaptic noise processed by a model spike generator can produce the unexpected variability in spike timing. However, most of these proposals have relied on theoretical models of the neuronal spike generator (but see ref. 8), and thus one may question the extent to which such models can be relied upon to provide a sufficiently realistic representation of spike generation.

We therefore set out to determine experimentally whether the high variability observed *in vivo* could arise from the superposition of independent excitatory and inhibitory inputs. Because reconciliation of the *in vivo* variability with the synaptic drive depends critically on the details of the spike generation mechanism, we have used a direct approach rather than rely on theoretical models of the spike generator. We have injected 'synthetic' synaptic currents through a somatic electrode to drive neurons in neocortical slices to fire. With this method, we can assess the output variability in response to any input ensemble. We have complemented this approach by testing the response to miniature excitatory postsynaptic currents (EPSCs) whose rate of release was elevated at many synapses independently through the local application of hypertonic solution.

We find that when neocortical neurons are driven by a population of independent inputs, the spike variability is consistently lower than that observed *in vivo*. We thus cannot confirm the earlier conclusions that unstructured synaptic noise interacting with the spike-generating mechanism can account for the unexpectedly large variability of neuronal activity in the neocortex. However, when a population of transiently synchronous inputs is added to the background of independent inputs, the observed firing variability is within the range observed *in vivo*. The high variability observed *in vivo* is therefore inconsistent with the activity of independent excitatory and inhibitory inputs, but could arise from large rapid fluctuations in the synaptic drive, such as would result from the nearly synchronous firing of subpopulations of afferents.

Results

The results are organized as follows. First we show that, in response to a synthetic synaptic input corresponding to an ensemble of purely excitatory inputs firing at a constant rate, the coefficient of variation (CV) of the interspike interval distribution is well below that observed *in vivo*. Next we show that CV increases but remains below the *in vivo* level when the input consists of a steady mix of excitatory and inhibitory

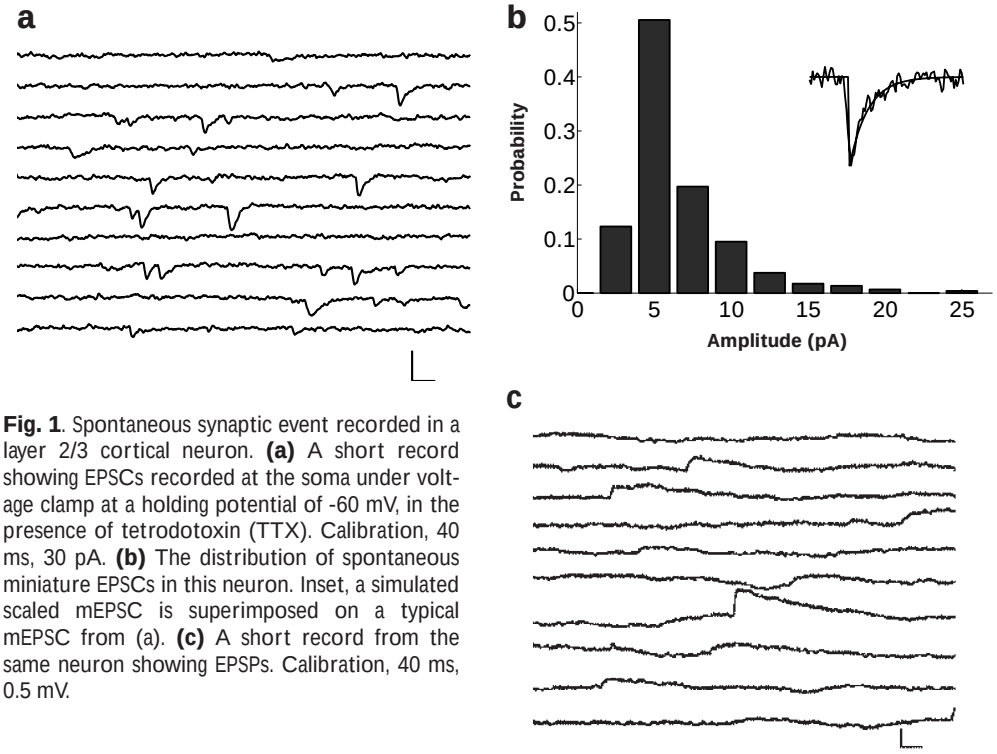

Fig. 1. Spontaneous synaptic event recorded in a layer 2/3 cortical neuron. **(a)** A short record showing EPSCs recorded at the soma under voltage clamp at a holding potential of -60 mV, in the presence of tetrodotoxin (TTX). Calibration, 40 ms, 30 pA. **(b)** The distribution of spontaneous miniature EPSCs in this neuron. Inset, a simulated scaled mEPSC is superimposed on a typical mEPSC from (a). **(c)** A short record from the same neuron showing EPSPs. Calibration, 40 ms, 0.5 mV.

inputs. We then repeat the analysis of the response variability for a complementary measure, the Fano factor. We then confirm that the spiking variability in response to miniature EPSCs evoked by hypertonic solution is still below the *in vivo* levels. Finally, we demonstrate that input synchrony can yield *in vivo* levels of variability.

Purely excitatory input

Cortical neurons *in vivo* are driven to fire by a shower of EPSCs. An estimate of the size of the unitary EPSCs that comprise this shower was obtained by recording spontaneous EPSCs from pyramidal neurons in rat neocortical slices under voltage-clamp conditions (**Fig. 1a**). As previously observed[9–11], spontaneous EPSCs varied in size, with a mean amplitude of 6.4 ± 3.4 pA (mean ± standard deviation) and a skewed distribution (**Fig. 1b**). Under slice recording conditions, this spontaneous release rate was too low to cause fluctuations of more than about a millivolt (**Fig. 1c**), and was never sufficient to cause spontaneous spiking.

We therefore generated synthetic synaptic currents to test whether such EPSCs, arriving at a sufficiently high rate, could account for the variability observed *in vivo*. In order to simulate this drive in a neocortical slice, synthetic synaptic currents were generated online and injected through a somatic patch electrode. Chemical synaptic inputs were pharmacologically silenced, so that all the drive was supplied by the electrode. The synthetic currents were constructed to mimic the current that would be generated at the soma by a population of excitatory inputs, each firing independently according to a Poisson process with a constant mean rate. These currents have the advantage that the synthetic synaptic noise can be created with known statistical structure. The unitary event used to construct the synthetic synaptic inputs, 30 pA, was chosen to be at the extreme high end of the observed range. Because the sum of independent Poisson processes is itself a Poisson process, the input statistics were determined solely by the net rate at which excitatory inputs showered onto the soma. We made no explicit assumption about the number of synapses or the rate of neurotransmitter release from each synapse, so that, for example, 100 synapses each generating 20 EPSCs per second would be indistinguishable from 200 synapses generating 10 EPSCs per second.

Figure 2a shows the results of a typical experiment in which

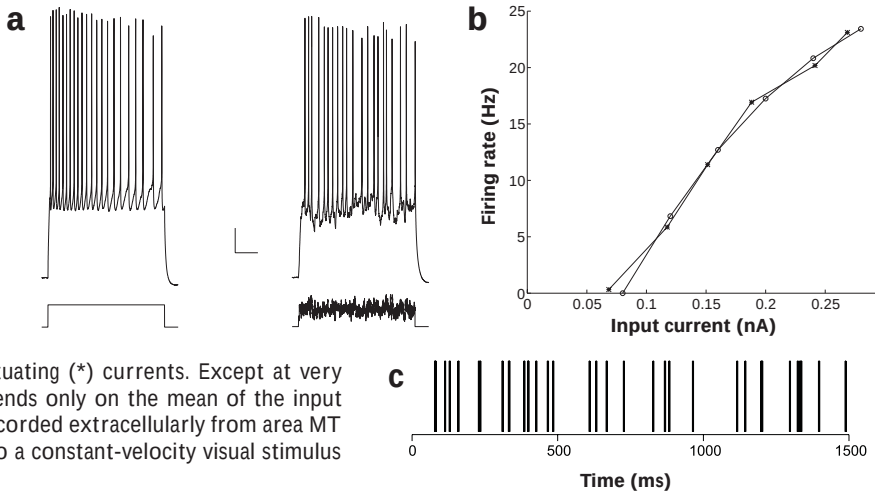

Fig. 2. Fluctuating currents affect the fine structure of the spike train but not the mean rate. **(a)** Sample traces show typical responses of a layer 2/3 cortical neuron to a constant current (left), and a fluctuating current (right) consisting of the sum of independent Poisson EPSCs firing at a population rate of 2.4 per ms. Calibration, 200 ms, 10 mV, 0.3 nA. **(b)** The average number of spikes in a one second trial is plotted as a function of the mean input for the constant (o) and fluctuating (*) currents. Except at very low firing rates, the mean spike rate depends only on the mean of the input current. **(c)** An example of a spike train recorded extracellularly from area MT of an alert macaque monkey in response to a constant-velocity visual stimulus (for details, see ref. 16).

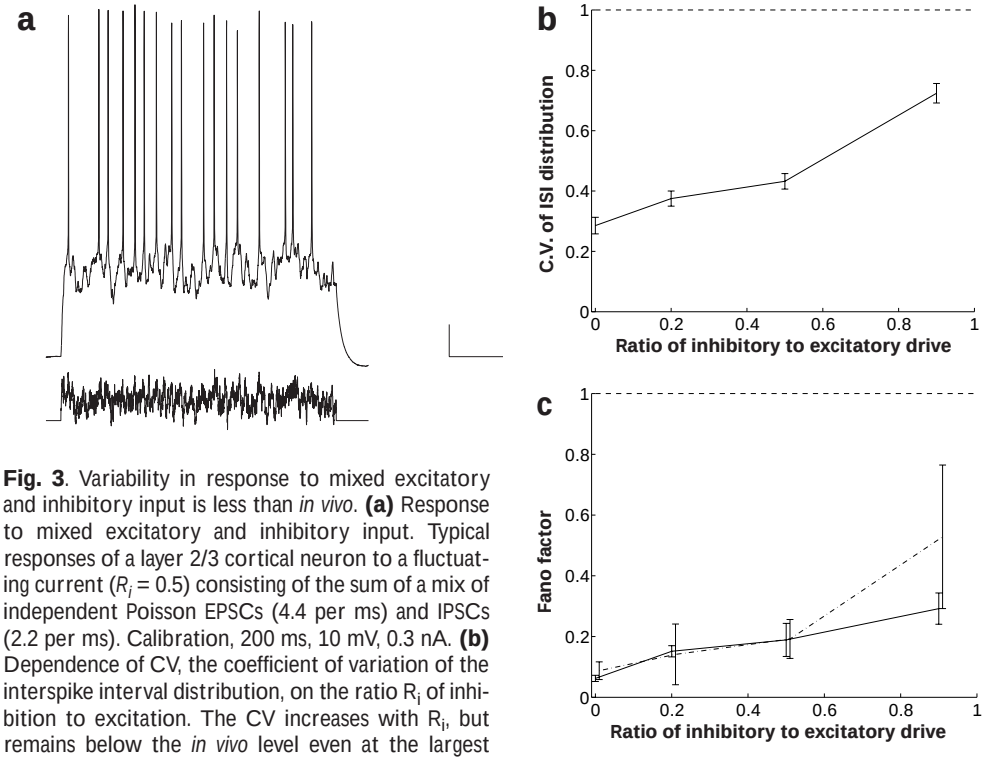

Fig. 3. Variability in response to mixed excitatory and inhibitory input is less than *in vivo*. **(a)** Response to mixed excitatory and inhibitory input. Typical responses of a layer 2/3 cortical neuron to a fluctuating current (R_i = 0.5) consisting of the sum of a mix of independent Poisson EPSCs (4.4 per ms) and IPSCs (2.2 per ms). Calibration, 200 ms, 10 mV, 0.3 nA. **(b)** Dependence of CV, the coefficient of variation of the interspike interval distribution, on the ratio R_i of inhibition to excitation. The CV increases with R_i, but remains below the *in vivo* level even at the largest value of R_i tested. The dotted line indicates the CV of a Poisson process. The error bars indicate the standard error. **(c)** Dependence of the Fano factor F (the variance divided by the mean of the spike count) on the ratio R_i. The solid line shows the actual Fano factor, whereas the dashed line shows the Fano factor predicted from $F = CV^2$. Even for high values of R_i, the Fano factor of the response to synthetic synaptic currents remains far below that observed *in vivo*. The dotted line indicates the Fano factor of a Poisson process. The error bars indicate the standard error.

a neuron from layer 2/3 of rat neocortex was driven to fire with synthetic synaptic currents. In this example, the input rate of 2.4 EPSCs per millisecond yielded a firing rate of 21 Hz, indicating that about 115 EPSCs were required for each action potential. For comparison, the response to an injection of constant current is also shown. The striking difference between the responses in Fig. 2a is in the fine temporal structure of the spike trains. In response to a constant input, spikes arrive at regular intervals (which become longer during the one-second stimulus as a result of spike adaptation), whereas in response to the synthetic synaptic current, the interspike intervals are much more irregular.

Although the fine temporal structure of the spike trains generated by the synthetic synaptic current was very different from that generated by the constant stimulus, the mean output firing rate in response to this ensemble depended only on the mean input. The f–I curves (the firing frequency f, averaged over the one second stimulus, as a function of the input current I) for the two stimulus ensembles are fairly linear, showing some slight saturation at high firing frequencies, and are in close agreement over the range of currents tested (**Fig. 2b**).

Is the irregularity of the spike train in Fig. 2a as high as that seen *in vivo*? One approach to quantifying spike irregularity is based on the coefficient of variation (CV) of the distribution of interspike intervals (ISIs). The CV is defined as the standard deviation σ_{isi} divided by the mean μ_{isi} of the ISI distribution, $CV = \sigma_{isi}/\mu_{isi}$. For a Poisson process, CV is one[12], but the converse is not true: one cannot conclude that a process is Poisson simply because CV is one.

The CV of spike trains from cortical neurons recorded *in vivo* are generally near or above unity[1,13–15]. The CV depends on a number of factors, including the firing rate and the degree of adaptation during a response. In order to provide a specific benchmark against which to compare the present *in vitro* results, we computed the CV of responses of neurons in the middle temporal (MT) cortex of alert macaque monkeys driven with constant-motion stimuli[16]. An example of one such response, emphasizing the markedly irregular activity of neocortical sensory neurons *in vivo*, is depicted in Fig. 2c. To minimize the effect of adaptation within a trial, only responses after the first 500 ms were considered, and to minimize any effect of slow trial-to-trial drift, the CV from each trial was computed separately and then these CVs were averaged. The mean CV was 1.1 ± 0.16 (range, 0.91 to 1.4; n = 10) for neurons with a mean firing rate of μ = 17 ± 6.7 Hz (range, 11 to 33 Hz). Based on these data, it is reasonable to consider a CV of 0.8 as a lower limit for the *in vivo* range at the firing rates considered here.

The spike trains from the neuron shown in Fig. 2 had a CV of 0.28. Similar results were obtained in all other neurons for which similar stimuli were tested (CV = 0.29 ± 0.09, μ = 21 ± 2.4 spikes per s; n = 9). These values are much lower than the CV value of 1.1 observed in MT cortical neurons *in vivo*. In other experiments, we examined a range of synthetic synaptic

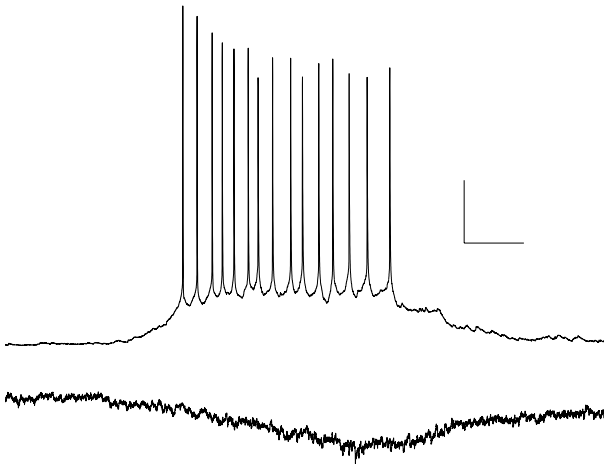

Fig. 4. Responses elicited by hypertonic solution evoked increases in the rate of miniature EPSC release. **(a)** A regular train of action potentials driven by miniature EPSCs elicited by a one-second application of hypertonic solution from a puffer pipette positioned about 40 μm from the soma. The variability of these responses (*CV* = 0.26, Fano factor *F* = 0.05) was close to that elicited by comparable synthetic synaptic currents. **(b)** The synaptic current elicited by hypertonic solution in the same neuron. Calibration, 300 ms, 20 mV (top), 100 pA (bottom). The bath solution contained standard Ringer's with 100 μM picrotoxin, and 30 μM cadmium added to prevent recurrent activity. The hypertonic solution consisted of the bath solution plus 0.5 mM sucrose.

currents and found that, as expected, the CV was smaller for currents constructed from smaller synthetic EPSCs. For this reason, we chose to test an EPSC amplitude at the upper limit of the spontaneous responses recorded in slice (30 pA), because any smaller EPSC would have yielded a lower CV even more inconsistent with the *in vivo* range. We also examined higher firing rates and found that, as expected, the CV decreased as the firing rate increased. The CV reported here, then, can be considered an upper limit on the CV likely to be generated by the random superposition of independent inputs. Thus a purely excitatory steady drive from independent inputs does not account for the observed irregularity of cortical spike trains.

Mixed IPSPs and EPSPs

The synaptic drive to cortical neurons *in vivo* contains a substantial inhibitory component mediated by GABA receptors[17,18]. It has been suggested that the irregularity observed *in vivo* might arise in part from added fluctuations in the drive caused by inhibitory inputs[6,7]. Inhibition increases the output variability by increasing the variance in the input drive associated with a given mean. To test whether the inclusion of inhibitory inputs could account for the irregularity of spike trains *in vivo*, we synthesized synaptic currents consisting of a mixture of excitatory and inhibitory currents. Figure 3a shows the response of a neuron to such an input. In this example, the total inhibitory current R_i was half the excitatory current ($R_i = 0.5$); the excitatory drive (the average rate of EPSC occurrence) was increased in order to maintain a high firing rate. As expected, the addition of this inhibitory component increased the irregularity, to CV of 0.44. Similar results were obtained in all other neurons for which similar stimuli were tested ($CV = 0.43 \pm 0.09$, $\mu = 20.7 \pm 2.4$ spikes per s; $n = 9$). Thus although this input does increase spike irregularity, the resultant values are still substantially below the CV value of 1.1 observed *in vivo*.

The fraction of the total input to a cortical neuron provided by inhibition has not been measured *in vivo*. Could a higher inhibitory-to-excitatory ratio R_i boost the CV into the *in vivo* range? The dependence of the CV on R_i is shown in Fig. 3b. As expected, the CV increased with increasing R_i, but remained well below unity for the highest values tested ($CV = 0.72 \pm 0.07$, $\mu = 19.2 \pm 1.2$ spikes per s; $n = 5$ for $R_i = 0.9$). Thus at the highest levels of R_i tested, the CV approached, but remained below, the values observed *in vivo*. Because ratios of inhibition to excitation are unlikely to exceed 0.9 (see ref. 17), we conclude that these experiments represent an approximate upper limit on the variability that can arise from synaptic noise from uncorrelated sources, and that such noise thus cannot account for the high CV observed *in vivo*.

Trial-to-trial variability

The CV is one of several standard measures of firing variability. A high CV may reflect irregularity in the fine structure of the spike train, but it may arise from changes arising on a time scale longer than a typical interspike interval, such as those induced by spike adaptation. Indeed, the response to the constant stimulus shown in Fig. 2 has a CV of 0.43, which is about the same as that for the mixed excitatory/inhibitory input with R_i of 0.5. The response to the purely excitatory synthetic synaptic input also shows strong adaptation.

We therefore also considered a second measure of variability, the Fano factor. The Fano factor F is defined as the variance divided by the mean of the spike count N, $F = \sigma_N^2/\mu_N$ where the spike count N is the number of spikes generated on a (typically one-second) particular trial. The Fano factor is insensitive to slow changes such as adaptation that occur within a single trial, as long as they occur consistently from one trial to the next. For any Poisson process (including both homogeneous and rate-modulated Poisson processes), the Fano factor is exactly one; spike trains from cortical neurons *in vivo* are consistently found to have a Fano factor near or above one[16,19]. For example, neurons from the middle temporal (MT) area of alert monkeys were reported to have a Fano factor of 1.3 (ref. 16).

In certain limiting cases, the Fano factor and the CV are related by $F = CV^2$. The main requirement is that the spike train be a stationary renewal process[12], that is, a process in which each interspike interval (ISI) is statistically independent of every other ISI. Although there are many ways in which a spike train could deviate from a renewal process, in the present context deviations are most likely to occur if successive (or neighboring) ISIs are statistically dependent (for example, neurons show some degree of bursting or short ISIs tended to be followed by long ISIs), or if the spike train adapts, for instance slows down, during the one-second stimulus. Because the response both to the constant input and to the synthetic synaptic drive can show considerable adaptation, at least the possibility of adaptation during the stimulus must be considered. Thus the Fano factor can provide additional and independent information about how well the responses to the synthetic currents mimic the variability observed *in vivo*.

The Fano factor of the response to the constant stimulus was only 0.02, compared with the Fano factor ($\hat{F} = 0.43^2 = 0.18$) pre-

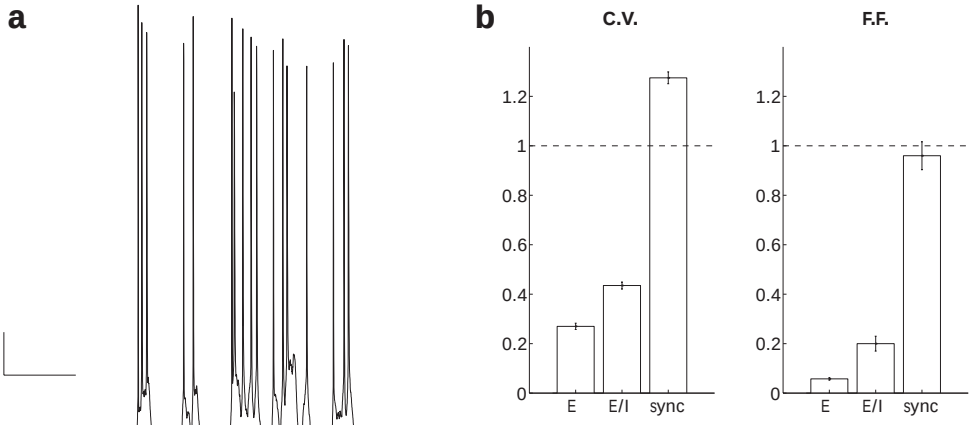

Fig. 5. Input synchrony yields *in vivo* variability. **(a)** Response to an input drive consisting primarily of occasional large brief (30–50 ms) excitatory events, randomly distributed in time, each consisting of the nearly synchronous arrival of on average 100–200 EPSCs, yielding transient peak currents as high as 1 nA or more. Calibration, 200 ms, 10 mV (top), 0.1 nA (bottom). **(b)** Summary of CV and Fano factor. Left, CV for purely excitatory (E), mixed excitatory/inhibitory (E/I), and synchronous (sync), inputs. Right, same statistics for the Fano factor. For both graphs, the error bars indicate standard errors. The dotted lines indicate the value of unity expected for both CV and Fano factor for a perfect Poisson process and are near the values typically observed from cortical spike trains *in vivo*.

dicted from the renewal assumption (where $\hat{F}$ rather than F is used to denote the Fano factor predicted from the CV). The large discrepancy indicates that for a constant stimulus the renewal assumption is not satisfied: marked adaptation within a single trial leads to a large CV that is not reflected in the trial-to-trial measure F. The Fano factor for the purely excitatory input was only slightly higher than for the constant current ($F = 0.06 \pm 0.03$; $n = 9$), and close to that predicted from the renewal assumption ($\hat{F} = 0.29^2 = 0.08$). However, for the highest inhibitory/excitatory ratios tested ($R_i = 0.9$), the observed value ($F = 0.29 \pm 0.12$; $n = 5$) was substantially less than the predicted value ($\hat{F} = 0.72^2 = 0.52$) and well below the *in vivo* levels of one or above (**Fig. 3c**). The finding that the *in vitro* Fano factors, even for responses driven by inputs with a strong inhibitory component, were consistently lower than those observed *in vivo* reinforces the previous conclusion that independent inputs cannot account for the high variability observed *in vivo*.

Asynchronous EPSC-evoked action potentials

The results presented so far have relied on current injected through a somatic electrode as a model for the synaptic currents that drive neurons *in vivo*. Injected current may not, however, mimic synaptic current in all respects. For example, injected current implicitly treats each synapse as a current source, whereas in fact a synapse is more properly considered as a conductance in series with the driving potential at the subsynaptic membrane. (The 'dynamic clamp'[20] can only partially compensate for this error, as most excitatory synapses onto cortical neurons are onto dendrites that are electrotonically remote from the soma and whose subsynaptic voltage cannot be controlled by a somatic electrode.) We therefore developed a more direct approach to study the spiking variability in response to synaptic stimulation.

Because conventional extracellular stimulation triggers a large increase in the rate of transmitter release from all synaptic terminals simultaneously, it is not a good model for the asynchronous and independent barrage of synaptic stimulation under consideration here. By contrast, local perfusion with hypertonic solution elevates the rate of miniature EPSC release from many terminals independently and thereby generates a slowly varying average current. The mechanism of this sucrose-evoked transmitter release is independent of presynaptic action potentials and calcium influx[11,21,22]. The miniature EPSCs released under these conditions therefore provide a much closer approximation of the drive to a cortical neuron expected if all inputs were independent.

Figure 4 shows the response of a layer 2/3 neuron to a hypertonic solution-evoked increase in the miniature EPSC rate. The spike train was very regular, with CV of 0.26 and Fano factor of 0.05. These results are in very close agreement with values obtained for the purely synthetic current ($CV = 0.29$ and $F = 0.06$; see **Fig. 2**). Thus the hypertonic solution-evoked responses showed much less variability than observed *in vivo*.

We were unable to elicit spiking in the absence of blockers of (inhibitory) GABAergic inputs ($n = 4$), presumably because the hypertonic solution did not activate a sufficiently high ratio of excitatory to inhibitory input. This may be due in part to the positioning of the puffer pipette near the soma, where the density of GABAergic terminals may be higher. We were therefore unable to use hypertonic solution to validate the variability measurements for the synthetic inputs that mixed excitation and inhibition. Nevertheless, the close agreement between the synthetic and hypertonic solution-evoked variability for the purely excitatory input suggests that currents injected at the soma can adequately mimic synaptic inputs.

Input synchrony yields *in vivo* firing variability

Because a steady input consisting of independent EPSCs and IPSCs fails to drive firing that is as irregular as is observed *in vivo*, we tested the possibility that some degree of input synchrony might be required. We considered a simple model in which the input consisted primarily of occasional large brief (30–50 ms) excitatory events, randomly distributed in time (average inter-event interval, 100 ms), each consisting of the nearly synchronous (30–50 ms) arrival of on average about 100 to 200 EPSCs, yielding peak currents of 1 to 2 nA (Methods). Evidence from a variety of sources suggests the importance of correlated firing in the neocortex[9,16,23–30].

Figure 5a shows a typical experiment in which the syn-

chronous input was synthesized according to this simple model. The irregularity of the resulting spike train was much higher, with both the CV and the Fano factor in the *in vivo* range in all neurons tested ($CV = 1.3 \pm 0.11$; $F = 0.94 \pm 0.2$, $\mu = 19.8 \pm 1.0$ spikes per s; $n = 5$). The data (**Fig. 5b**) demonstrate that input correlations could account for the high degree of variability observed *in vivo*.

Discussion

The observations[1] on the paradox posed by the irregularity of *in vivo* cortical spike trains have sparked considerable debate on the underlying mechanism[5–7,32–34]. Although Softky and Koch[1] raised the possibility that the unexpectedly large variability might arise from input synchrony, later workers have argued that statistically stationary synaptic noise, interacting with the spike-encoding mechanism, can account for the large variability. All of these previous studies have, however, relied on simulations of the spike encoder, and the results are sensitive to assumptions about the encoder properties.

Our results do not rely on any theoretical model of the spike encoder. Rather, we have used the actual spike encoder of neurons *in vitro* to test directly whether the high variability observed *in vivo* could arise from a constant shower of statistically independent synaptic inputs. We conclude that it could not, and propose that the high *in-vivo* variability arises from correlations among the inputs[28,29]. We illustrate this by matching the *in-vivo* variability with a simple model of the input drive, in which occasional large brief synchronous events are superimposed on a tonic background.

Our conclusions rest on two main assumptions. First, we have assumed that somatic current injection represents a reasonable model of synaptic drive. Although the close agreement between the somatic current injection and hypertonic solution-evoked miniature EPSC results (**Fig. 4**) supports this notion, it is possible that the agreement would not be as good when the inhibitory drive was very strong. Second, we have assumed that the *in-vitro* biophysical properties of neocortical neurons can be used to make inferences about their *in-vivo* behavior. This supposes, for example, that the spontaneous miniature EPSCs recorded in slices accurately reflect the *in-vivo* synaptic amplitude distribution. In addition, the input–output transformation might be different *in vivo*, because of neuromodulatory influences[35] for example, or the activation of large nonlinear conductances[36].

Mechanism underlying spiking variability *in vivo*

Irregular firing *in vivo* might in principle arise from input variability or from noise in the spike-generating mechanism. Because the neuronal spike generator is very reliable, input variability is likely to be the primary source[2,3]. In spinal neurons, 'synaptic noise' (fluctuations in membrane potential arising from a barrage of EPSPs) fully accounts for output variability[4]. In the neocortex, the *in-vivo* synaptic drive inferred from current clamp recordings could, when injected into neocortical neurons *in vitro*, reproduce their basic firing statistics[8]. These latter experiments did not interpret the injected currents in terms of the underlying synaptic properties and correlational structure of the inputs, nor did they establish the properties of the drive necessary or sufficient to account for the high variability.

In the present study, we therefore began with the assumption that output variability reflects fluctuations in the input current and asked what form the synaptic noise must take to give rise to the observed spiking variability. The conundrum is that the fluctuations in the input drive generated by independent excitatory afferents seem too small to account for the high variability of *in-vivo* cortical firing. The initial report[1] emphasized the difficulty in accounting for the high variability at very high firing rates (over 100 Hz). In our experiments, we found that even at more moderate firing rates (15–25 Hz), independent EPSCs cannot account for the variability observed *in vivo*; at higher firing rates, independent EPSCs induced even lower output variability.

Previously proposed resolutions fall into two main classes. First, simulations suggested that inhibitory inputs increase the input fluctuations and thereby the output variability sufficiently to account for the *in vivo* responses[5–7]. Our experimental results, however, demonstrate that the output variability in response even to a drive with a very strong inhibitory component remains well below the *in vivo* levels, indicating that some alternative mechanism must be responsible.

The second class of explanation invokes the details of the spike-generating mechanism. In one proposal[7], the reset voltage following an action potential was used to match the slope or 'gain' of the firing-intensity curve for the first interspike interval in an integrate-and-fire model. In simulations, the output variability of responses generated by this model was near that found *in vivo*. This model failed, however, to predict the responses of cortical neurons to the synthetic synaptic currents we used (C.S. and A.Z., unpublished results). The initial report[1] proposed a more radical modification of the spike generator. Here it was suggested that the coincident arrival of just a few EPSPs might activate powerful nonlinear dendritic conductances that would then trigger a somatic spike. Although our experiments cannot rule out this mechanism (and indeed, the large events shown in Fig. 5 could in principle arise from the activation of dendritic nonlinearities), we favor the notion that the dynamics of cortical networks, rather than the properties of single neurons, underlie the requisite large fluctuations in the input drive.

The model of synaptic drive that we have used is by no means unique in its ability to account for the variability observed *in vivo*; countless other input ensembles would certainly have done as well. The key requirement is that the input current contain very large fluctuations, much larger than would be expected if all the synapses were releasing quanta independently and at a constant rate. These fluctuations could arise from the brief and coordinated increase in the firing rate of a large number of excitatory, or perhaps inhibitory[37], synapses. Moreover, if a small number of presynaptic neurons each made dozens of powerful synaptic contacts onto a single postsynaptic target[38], then the requisite postsynaptic fluctuations might arise from correlated activity in just this smaller subpopulation of neurons. Our experiments do not address the specific network mechanisms that might give rise to this correlated synaptic activity. Nevertheless, the present results indicate that, however the input current is generated, some kind of correlation among the synaptic inputs is likely to play a critical role in generating the high degree of variability observed *in vivo*.

Synchrony and the neural code

The irregularity of cortical spike trains has led to two rather different models of how cortical circuitry operates. In the conventional model, this irregularity represents 'noise' around the 'signal', a perturbation around a mean firing rate that is

obtained by averaging over some period (such as one second) that is much longer than a typical ISI. In this view, the precise timing of individual spikes conveys little information, because it reflects only the noisy activity of other neurons attempting to signal their own mean rate. This model follows readily from the idea that the drive to cortical neurons is composed of the uncorrelated activity of its synaptic inputs; it would be hard to imagine how the precise timing of spikes could represent more than noise in this model, because adding or removing just a few EPSPs might perturb the spike timing appreciably.

In the second model, the irregular spikes reflect modulation on a fine time scale of the neuron's output rate. It is now established that under some conditions, the fine temporal structure of the spike train (the precise location of each spike) can carry information[16,39,40]. For example, when visual stimuli have fine temporal structure, the timing of spikes from neurons in MT cortex can be tightly (2–5 ms) locked to the stimulus[16,41]. Recordings of local field potentials suggest that this high fidelity of spike timing is achieved by rapid comodulation of the rates of the input neurons[16]; under these conditions, the input correlations encode temporal edges within the stimulus. However, our results suggest that even when the sensory stimulus does not have fine temporal structure, spikes in cortical neurons may nevertheless arise from large events in the input drive that represent the correlated activity of many neurons. Such large events are presumably more robust to noise than the small fluctuations that are posited to drive firing in the first model. Thus even when the sensory stimulus is devoid of fine temporal structure, spikes may be encoding something with high temporal fidelity.

Methods

SLICE PREPARATION. Brain slices were prepared from Long-Evans rats (postnatal day 14–28) that were deeply anesthetized with metofane and then decapitated. The skull was rapidly opened and the brain placed in ice-cold Ringer solution. The cooled brain was glued with cyanoacrylate to the stage of a vibratome and 400-µm slices were cut. Slices were transferred to a holding chamber and incubated for at least one hour at room temperature in a solution continuously bubbled with a mixture of 95% O_2 and 5% CO_2, and then placed in a recording chamber.

PATCH-CLAMP RECORDING. Whole-cell, patch-clamp recordings were made from neurons in the sensory neocortex visualized through an upright microscope equipped with infrared light and differential interference optics[42]. Recordings were performed at 33–35°C. Slices were continuously perfused with a Ringer's solution containing (in mM) NaCl 120, KCl 3.5, $CaCl_2$ 2.6, $MgCl_2$ 1.3, NaH_2PO_4 1.25, $NaHCO_3$ 26, glucose 10, pH 7.35. Unless otherwise indicated, recordings were obtained in the presence of the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 µM). Recording pipettes were filled with an internal solution consisting of (in mM) K gluconate, 170; HEPES, 10; NaCl, 10; $MgCl_2$, 2; EGTA, 1.33; $CaCl_2$, 0.133; MgATP, 3.5; GTP, 1.0, pH 7.2 and 290–300 mOsm. Resistance to bath was 3–5 MΩ before seal formation.

In experiments in which the rate of miniature EPSCs was elevated by locally perfusing with hypertonic solution, the bath Ringer's contained 100 µM picrotoxin (to block γ-aminobutyric acid type A receptor responses), 30 μM Cd^{+2} to block synaptic responses that could cause recurrent activity and no CNQX. To elicit hypertonic-solution-evoked miniature EPSCs, visual guidance was used to position a 2 µm puffer pipette (containing bath Ringer's to which 0.5 mM sucrose had been added) close to dendrites about 30–50 µm from the somatic recording electrode. A picospritzer II was then used to apply 1–2 second pulses (3–6 psi) of the pipette solution.

Recordings were obtained using an Axopatch 200A or 200B (Axon Instruments). Most recordings were obtained in the current-clamp mode, without series-resistance compensation. Traces were filtered at 2 KHz and digitized at 4 KHz. Series resistance, monitored prior to each trace, was typically in the range of 8–20 MΩ, and never more than 30 MΩ. To compensate for any slow drift in membrane potential, prior to each trace sufficient current was injected to return the membrane to a value determined at the start of the experiment (between -60 and -75 mV); if the actual rest potential decreased by more than 5 mV from this level, the experiment was terminated. The response to constant current injection (an 'F–I curve') was also periodically monitored to assess washout, and if the spike rate at the maximum intensity decreased by more than 30%, the experiment was terminated (usually 30–60 minutes).

Forty-two regular spiking neurons[43] from cortical layers 2/3 were analyzed. In addition, results from two layer 5 neurons were consistent with the other results and so were included in the averaged data. Current stimulation was usually applied for one second every five or ten seconds; the long interstimulus rest period helped to reduce the influence of one trial on the next. For the analysis, only neurons whose average firing rate was between 15 and 25 Hz were used. At higher sustained firing rates, recording stability tended to degrade more quickly.

Data were acquired using a National Instruments AT-MIO-16-F-5 A/D card on a 120 MHz Pentium-based computer under the Window NT (Microsoft) operating system. Software written in Labview (National Instruments) with Dynamic Data Exchange links to Matlab (Mathworks) allowed convenient online synthesis and injection of arbitrary synthetic current waveforms.

SYNTHETIC SYNAPTIC CURRENTS. Neurons were driven to fire through currents injected through a somatic patch electrode. Several models of correlated synaptic inputs were tested. In the independent EPSC model, the synaptic drive was given by $I_e = P(r_e) * I_{ampa}$, where $P(r_e)$ is a sequence of independent Poisson points arriving at a rate r_e, $I_{ampa} = W_e\, e^{-t/\tau e}$ is the waveform of the basic EPSC (W_e = 30 pA, τ_e = 3 ms), and * indicates convolution. The firing rate under these conditions is determined by a single free parameter, the rate of synaptic impulses r_e.

In the mixed model, an inhibitory drive given by $I_i = P(r_i) * I_{gaba}$ was added to the excitatory drive, where as above $P(r_i)$ is a sequence of independent Poisson points arriving at a rate r_i, $I_{gaba} = W_i\, e^{-t/\tau e}$ is the waveform of the basic IPSC (W_i = 30 pA, τ_i = 6 ms), and * indicates convolution. The total input under these conditions was given by $I_b = I_i + I_e$. In this scenario, the firing rate depended both on r_i and r_e. The value of r_i was chosen to keep the ratio R_i of total inhibitory and excitatory currents fixed, $R_i = \kappa\, r_i / r_e$ where $\kappa = (W_e\, \tau_e)/(W_m\, \tau_m)$ takes into account differences in the synaptic waveforms.

Finally, we examined the effect of synchronous excitatory inputs, $I_s = C(t) * I_{ampa}$, where C(t) was the stochastic function that describes the occurrence of synchronous events. C(t) consisted of 30–50-ms periods of elevated input activity; these periods occurred in a random (Poisson) fashion at a rate of 10 Hz. During each 30–50-ms period of elevated activity, about 200 EPSCs were distributed in 2–8 shorter events. The synchronous input I_s was added to I_e and I_i.

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