Rate Coding and Spike-Time Variability in Cortical Neurons With Two Types of Threshold Dynamics

T. Tateno and H.P.C. Robinson
Department of Physiology, University of Cambridge, Cambridge, United Kingdom
Submitted 30 June 2005; accepted in final form 22 December 2005

INTRODUCTION

Recent studies have shown that mammalian cortical neurons recorded in vivo exhibit highly variable responses to repeated presentation of the same stimulus (Britten et al. 1993; Snowden et al. 1992; Tolhurst et al. 1983; Tomko and Crapper 1974). In vivo recordings of conductance in cortical pyramidal cells indicate that cortical networks are typically highly active and induce a large fluctuating background synaptic conductance (Azouz and Gray 1999; Destexhe et al. 2003). Background presynaptic activity is thus an important source of unreliability and variability in spike timing of cortical cells in vivo. In contrast, in vitro recordings show that spike generation in cortical neurons can be precise and reproducible in response to repeated injections of an identical fast-fluctuating current (Mainen and Sejnowski 1995; Nowak et al. 1997) or conductance (Mainen and Sejnowski 1995; Nowak et al. 1997) curves, at several levels of random shunting inhibitory input. Second, using two statistics—the reliability of spike occurrence and the spike-timing jitter—we measured the firing variability of spike generation in the two types of threshold dynamics at several levels of inhibitory input and at different degrees of input synchrony or correlation. Third, we studied the sensitivity of firing variability to the relative timing of excitatory and inhibitory inputs. Finally, we discuss some possible functional roles of the two types of threshold dynamics, in cortical networks driven by fluctuating transient synaptic inputs.

METHODS

Slice preparation and recording

Transverse slices were prepared from somatosensory cortex of 15- to 21-day-old Wister rats using standard techniques (Sakmann and Stuart 1995). During slicing, tissue was kept in sodium-free solution that had the following composition (in mM): 25 sucrose, 2.5 KCl, 26 NaHCO3, 10 glucose, 1.25 NaH2PO4, 2 CaCl2, and 1 MgCl2. Slices (300 μm thickness) were cut on a vibrating slicer (Microslicer DTK-3000, D.S.K., Kyoto, Japan) and kept in Ringer solution at room temperature for ≥2 h before recording. The Ringer solution contained (in mM): 125 NaCl, 2.5 KCl, 25 NaHCO3, 25 glucose, 1.25 NaH2PO4, 2 CaCl2, and 1 MgCl2. Both slicing and recording solutions were maintained at 35°C.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
equilibrated with 95% O₂−5% CO₂ gas to a final pH of 7.4. Slices were viewed with an upright microscope (Olympus BW50WL, Olympus UK, London, UK) using infrared differential interference contrast optics. All experiments were performed at 34 ± 1°C. Whole cell patch-clamp recordings were made from the somas of neurons in layers 2/3. Putative RS cells were of pyramidal morphology, whereas putative FS cells were selected on the basis of a nonpyramidal shape and multipolar dendrites (Connors et al. 1982). During recording, the slices were perfused continuously with Ringer solution in which 10 M NaHCO₃, 10 μM CNQX, and 10 μM AP5 (Tocris Cookson, Bristol, UK) were included to block most intrinsic synaptic conductances. Somatic patch-pipette recordings were made from the somas of neurons in layers 2/3. The time constant values were as follows (ms): 2000; Koch 1999; Sacchi et al. 1998). The scaling factor values were as follows (pS): 0.5, 0.5, 0.5, and 0.5; for each cell, the mean was used. The primary Poisson process with a rate λ produces a nonstationary Poisson process, whose rate was modulated in a summation of exponentially decaying transients with a constant τₑ: the time-dependent rate λ(t) of the secondary process was described by

\[ \lambda(t) = S \sum_{i} h(t - \phi_i) \]  

where S is an initial peak rate, \{\phi_i\} is a time series of the primary events, and

\[ h(t) = \begin{cases} 0 & t < 0 \\ \exp(-u \tau_e) & t \geq 0 \end{cases} \]  

The mean rate of synaptic events is then given by \( \lambda = S \lambda_b \tau_e \). The intervals between synchronous bursts are random and exponentially distributed. To systematically vary the degree of synchrony or correlation in the input, we keep \( \lambda_b \) fixed, and vary \( S \) and \( \tau_e \) reciprocally, defining S, the amplitude of bursts in input rate, as the level of synchrony (see Fig. 5B). In practice, values of \( \lambda = 200 \) Hz and \( \lambda_b = 2.5 \) Hz were used for both excitatory and inhibitory conductance inputs when investigating the effect of synchrony on spike variability, and the ratio of \( \lambda \) values between excitation and inhibition was changed to investigate the effect of excitatory: inhibitory balance on spike timing (\( \lambda/\lambda_b = 0 \) to 1.2, in steps of 0.1 or 0.2). Similar doubly stochastic process models have been used to analyze bursting activity of lateral superior olive neurons (Turcott et al. 1994) and dissociated cortical neurons (Tateno et al. 2002). Test stimuli lasted for 2 or 5 s, and \( \geq 30 \) s of recovery time at the resting potential were allowed between successive stimuli. To study the effect of relative timing of inhibition and excitation, GABA\( \lambda \)-type inhibitory conductance input \( g_{I}(t) \) was produced by advancing or retarding the AMPA-type excitatory Poisson stimulus \( g_{E}(t) \) in 1-ms increments; i.e., \( g_{I}(t) = g_{I}(t - \text{delay}) \) and delay = 0, ±1, ±2, ±3, . . .(in ms).

**Spike statistics**

Spike times were measured as the times of upward zero crossing of the membrane potential. Instantaneous frequency (reciprocal of each interspike interval) was computed from trains of action potentials evoked by 600-ms-duration pulses for the first, second, fourth, and last interspike intervals. Steady-state (SS) firing frequency was computed as the average of instantaneous frequency for the last three intervals of a train. Current or conductance strength was usually progressively increased or decreased in small (10- or 20-pA) steps. Initial instantaneous frequency and steady-state firing rate were plotted as a function of the injected current strength, to construct frequency–current (f-I) relationships. The maximum firing rate of a neuron was computed from the number of spikes per trial at the highest current strength before depolarization block. The frequency adaptation properties of

**Point processes**

Once the parameters of the unitary synaptic input are fixed, the time series \( \{ T_j \} \) (j = E or I, k = 1, 2, . . .) completely determine the time course of conductance stimuli. As reported before (Harsch and Robinson 2000), we used two point-process models: I) Poisson and 2) doubly stochastic process models. Poisson stimulus trains were constructed by summing unitary events, such as AMPA- or GABA-type, at intervals denoted by a random variable \( t_i \), with the probability density

\[ p(t_i) = \lambda \exp(-\lambda t_i) \]  

where \( \lambda \) is the Poisson rate and \( \lambda \) is the excitatory input Poisson rate. The intensity of stimulation was varied by changing the Poisson rate \( \lambda \). The relationship between firing rate (f) and the excitatory input Poisson rate \( \lambda_e \) is afterward referred to as an f–r curve. To simulate correlated or synchronous firing of synaptic events, in addition, we used a doubly stochastic process model that mimics bursty presynaptic events. The primary Poisson process with a rate \( \lambda_e \) produces a nonstationary Poisson process, whose rate was modulated in a summation of exponentially decaying transients with a constant \( \tau_e \). The time-dependent rate \( \lambda(t) \) of the secondary process was described by

\[ \lambda(t) = S \sum_{i} h(t - \phi_i) \]  

where \( \lambda \) is the initial peak rate, \{\phi_i\} is a time series of the primary events, and

\[ h(t) = \begin{cases} 0 & t < 0 \\ \exp(-u \tau_e) & t \geq 0 \end{cases} \]  

The mean rate of synaptic events is then given by \( \lambda = S \lambda_b \tau_e \). The intervals between synchronous bursts are random and exponentially distributed. To systematically vary the degree of synchrony or correlation in the input, we keep \( \lambda_b \) fixed, and vary \( S \) and \( \tau_e \) reciprocally, defining S, the amplitude of bursts in input rate, as the level of synchrony (see Fig. 5B). In practice, values of \( \lambda = 200 \) Hz and \( \lambda_b = 2.5 \) Hz were used for both excitatory and inhibitory conductance inputs when investigating the effect of synchrony on spike variability, and the ratio of \( \lambda \) values between excitation and inhibition was changed to investigate the effect of excitatory: inhibitory balance on spike timing (\( \lambda/\lambda_b = 0 \) to 1.2, in steps of 0.1 or 0.2). Similar doubly stochastic process models have been used to analyze bursting activity of lateral superior olive neurons (Turcott et al. 1994) and dissociated cortical neurons (Tateno et al. 2002). Test stimuli lasted for 2 or 5 s, and \( \geq 30 \) s of recovery time at the resting potential were allowed between successive stimuli. To study the effect of relative timing of inhibition and excitation, GABA\( \lambda \)-type inhibitory conductance input \( g_{I}(t) \) was produced by advancing or retarding the AMPA-type excitatory Poisson stimulus \( g_{E}(t) \) in 1-ms increments; i.e., \( g_{I}(t) = g_{I}(t - \text{delay}) \) and delay = 0, ±1, ±2, ±3, . . .(in ms).

**Spike statistics**

Spike times were measured as the times of upward zero crossing of the membrane potential. Instantaneous frequency (reciprocal of each interspike interval) was computed from trains of action potentials evoked by 600-ms-duration pulses for the first, second, fourth, and last interspike intervals. Steady-state (SS) firing frequency was computed as the average of instantaneous frequency for the last three intervals of a train. Current or conductance strength was usually progressively increased or decreased in small (10- or 20-pA) steps. Initial instantaneous frequency and steady-state firing rate were plotted as a function of the injected current strength, to construct frequency–current (f-I) relationships. The maximum firing rate of a neuron was computed from the number of spikes per trial at the highest current strength before depolarization block. The frequency adaptation properties of
neurons were characterized by calculating the instantaneous firing rate as a function of time since the beginning of the 600-ms pulse. For each current intensity, the decay of firing rate was fitted to a single exponential function

\[ f = C_a \exp(-t/r_a) + F_a \]  

(7)

where \( f \) and \( t \) respectively represent the firing rate and time after the stimulus onset and \( C_a, r_a, \) and \( F_a \) are positive constant parameters. \( F_a \) represents the adapted firing rate. The strength of adaptation (adaptation index, \( A \)) was quantified as 100 × (1 – \( F_a/F_i \)), where \( F_i \) corresponds to the firing rate of the first interspike interval. Based on adaptation depended on the current intensity for any given neuron, we used the highest current level not producing depolarization block of spiking, to allow comparison among cells. For some cells, no adequate exponential fit could be obtained and, in these cases, \( F_a \) was calculated as the mean firing rate for the last 50 ms of the 600-ms current pulse and used to calculate the adaptation index. Results are reported as means ± SD. Membrane time constants were obtained by fitting a single exponential function to the initial part of more than 10 time-averaged voltage responses to small (±20 or ±10 pA), 600-ms-long hyperpolarizing current pulses. Input resistance was calculated from Ohm’s law by dividing the maximal average voltage deflection by the amplitude of the applied current pulses. Firing frequency \( (f) \) versus excitatory Poisson rate \( (f-r) \) curves were fitted with the function

\[ f = 1\left[\tau_0 + \tau \ln[(r-b)/(r-a)]\right] - c \]  

(8)

derived from a leaky integrate-and-fire model, where \( r \) is the excitatory Poisson firing rate in kilohertz, and \( \tau_0, \tau, a, b, \) and \( c \) are all constant parameters (Koch 1999). The parameter \( \tau_0 \) determines the slope of the initial rise and \( \tau \) determines the curvature and saturating value (i.e., the nonlinearity); the smaller the value of \( \tau \), the stronger the nonlinearity, although the nonlinearity depends on the parameter \( \tau_0 \) as well. All the parameters were estimated by minimizing mean-squared error between the model and the data. We report the values of \( \tau_0 \) and \( \tau \), which are the only parameters of interest here.

Data analysis

To calculate the firing statistics, the first 200 ms of responses were excluded to avoid the influence of adaptation. To characterize overall response variability, a statistical measure, the coefficient of variation (CV) of the interspike intervals, was used on ensembles of 20–35 successive trials, either 2 or 5 s in duration. CV = 1 for a stationary Poisson process. Each set of trials was termed a “session.” For each session of trials, the stimulus was resynthesized with new random numbers (i.e., different initial random seeds).

To characterize reproducibility of spike times, two additional measures, termed “jitter” and “reliability,” were used. For each session of 20–35 trials as described above, a peristimulus time histogram was constructed with a bin width of 1, 2, or 3 ms. The first 200 ms of responses were excluded to avoid the influence of adaptation. Repeatable spikes were defined by events in which firing occurred in the same time bin for ≥30% of trials and included spikes in these and the immediately adjacent time bins. Reliability was defined as the proportion of all spikes that were repeatable. Jitter was defined as the average value of the SD of spike times within each repeatable event. These measures of reproducibility are similar to those used by Mainen and Sejnowski (1995) and Nowak et al. (1997). Although varying the bin width shifted graphs of reliability and jitter values upward or downward, the bin width was not a critical factor in determining overall trends. Thus the results reported here use a bin width of 2 ms.

To analyze spike-timing variability in more detail during a decay-synaptic conductance, we also performed a finer analysis. In particular, we computed “individual” jitter and reliability values for the last three spikes during evoked burst responses. To find boundaries between two events, we used a discriminant analysis assuming that spike times in each event are normally distributed. In other words, if the mean spike times in events \( i \) and \( (i + 1) \) are \( t_i \) and \( t_{i+1} \), respectively, and their SDs (individual jitters) are \( \sigma_i \) and \( \sigma_{i+1} \), then the boundary between the two events is defined as \((\sigma_i t_i + \sigma_{i+1} t_{i+1})/(\sigma_i + \sigma_{i+1})\). In practice, once the boundary is determined, the elements belonging to each event may be rearranged. Thus we repeated this procedure iteratively until no further change occurred.

RESULTS

Cell types in the layer 2/3 of rat somatosensory cortex

Based on responses to injected step currents, cells recorded in layer 2 or 3 of somatosensory cortex were classified into three groups: regular-spiking (RS), fast-spiking (FS), and low-threshold–spiking (LTS) cells (Beierlein et al. 2003; Connors and Gutnick 1990; Kawaguchi and Kubota 1997). This study is based on recordings from 40 RS and 43 FS cells. Because we recorded only a small number of LTS neurons (seven cells), the analysis of firing variability below will be reported only for RS and FS cells. As previously reported (Tateno et al. 2004), we selected RS cells that had a typical pyramidal morphology under infrared differential interference contrast optics, whereas FS cells were selected on the basis of a nonpyramidal shape, with a round soma and multipolar dendrites. Figure 1, A and B shows typical action potential waveforms for an RS cell and an FS cell, respectively, at three levels of injected step current. RS cells had an average resting membrane potential of −72.5 ± 4.5 mV, input resistance of 419 ± 181 MΩ, membrane time constant of 39.2 ± 13.5 ms, maximum firing rate of 37.8 ± 8.5 Hz, adaptation index of 72.4 ± 8.9%, and adaptation decay time constant of 31.4 ± 10.4 ms. FS cells had an average resting membrane potential of −72.2 ± 3.5 mV, input resistance of 353 ± 143 MΩ, membrane time constant of 29.1 ± 5.8 ms, maximum firing rate of 91.0 ± 11.1 Hz, adaptation...
Firing frequency versus Poisson rate curves

To examine the effects of shunting inhibitory input on postsynaptic firing rate and interspike interval, cells were stimulated using the conductance injection technique (Robinson and Kawai 1993). Inhibitory GABA_A-type and excitatory AMPA-type Poisson conductance stimuli were synthesized and simultaneously injected to cells (see METHODS).

Figure 2Aa shows average firing frequency versus AMPA-type Poisson rate \( f-r \) relationships for an RS cell with four levels \([0 \text{ (control)}, 1, 2, \text{ and } 3 \text{ kHz}]\) of GABA_A-type Poisson conductance. As shown in the figure, for RS cells, \( f-r \) curves are nonlinear and inhibitory input shifts \( f-r \) curves to the right. For the control (zero inhibition), the parameter \( \tau_0 \) (see METHODS, Eq. 8) was estimated as \( 2.4 \pm 1.0 \times 10^{-2} \text{ ms} \) and \( \tau \) as \( 0.92 \pm 0.31 \text{ ms} \). With inhibitory Poisson input at a rate of \( 2 \text{ kHz} \), \( \tau_0 \) was \( 2.9 \pm 1.6 \times 10^{-2} \text{ ms} \) and \( \tau \) was \( 3.0 \pm 1.1 \text{ ms} \). Another common finding in the recordings was that \( f-r \) curves for several different levels of inhibition crossed at higher excitatory input rates, presumably because inhibitory inputs release action-potential depolarization block at higher levels of excitatory inputs, weakening the nonlinearity and increasing the maximum firing frequency. For the RS cell of Fig. 2Aa, the coefficient of variation (CV) of interspike intervals at the three levels of inhibitory input and in the control condition is shown in Fig. 2Ab. Above about \( 3 \text{ kHz} \), CV converges to around 0.45 and is similar for all levels of inhibition.

Figure 2Ba shows \( f-r \) relationships for an FS cell, at different levels \([0, 0.5, 1, 1.5, \text{ and } 2 \text{ kHz}]\) of shunting GABA-type Poisson conductance. Although inhibitory inputs shifted the curves rightward as for RS cells, their nonlinearity was weaker than that for RS cells, and curves did not cross at high-input rates. Figure 2Bb shows the CV of interspike intervals for the same cell as shown in Fig. 2Ab. In contrast to RS cells, increasing inhibitory input clearly causes an upward shift in CV-\( f \) curves. Parameters of the fits to \( f-r \) curves for the 21 cells examined (10 RS and 11 FS cells) are summarized in Table 2.

Spike-triggered average of conductance input

Figure 3Aa shows averaged action-potential shapes in an RS cell at four rates of Poisson excitatory conductance input \([2, 3, 4, \text{ and } 5 \text{ kHz}]\). The average spike properties such as spike width and amplitude strongly depended on the input rate as shown in Fig. 3Aa (see also de Polavieja et al. 2005). Spike-triggered averaging of the conductance stimulus (also known as “reverse correlation”) shows that for RS cells, spikes are typically associated with a rise in AMPA conductance lasting 2–8 ms.

Table 2. Summary of basic statistics on RS and FS cells

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RS</th>
<th>FS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>(-72.5 \pm 4.5)</td>
<td>(-72.2 \pm 3.5)</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>(419 \pm 181)</td>
<td>(352 \pm 143)</td>
</tr>
<tr>
<td>Maximum firing rate, spikes/s</td>
<td>(37.3 \pm 8.5)</td>
<td>(91.0 \pm 11.1)</td>
</tr>
<tr>
<td>Time constant, ms</td>
<td>(39.2 \pm 13.5)</td>
<td>(29.1 \pm 5.8)</td>
</tr>
<tr>
<td>Adaptation index, %</td>
<td>(72.4 \pm 8.9)</td>
<td>(40.2 \pm 6.9)</td>
</tr>
<tr>
<td>Adaptation decay time constant, ms</td>
<td>(31.4 \pm 10.4)</td>
<td>(57.2 \pm 31.5)</td>
</tr>
</tbody>
</table>

Values are means \pm SD. RS, regular spiking; FS, fast spiking.

index of \(40.2 \pm 6.9\)%, and adaptation decay time constant of \(57.2 \pm 31.5\) ms. These statistics are summarized in Table 1. To distinguish RS and FS cells more reliably, we also used several other measures as reported in Tato et al. (2004). In addition, it is notable that RS and FS cells characteristically have “type 1” and “type 2” threshold dynamics, respectively (see Tateno et al. 2004).

FIG. 2. Firing statistics during simultaneous excitatory (AMPA-type) and inhibitory (GABA-type) Poisson conductance inputs. A: an RS cell. a: firing frequency vs. excitatory Poisson rate \( f-r \) relationship at 3 different levels \([1.0, 2.0, \text{ and } 3.0 \text{ kHz}]\) of inhibitory Poisson conductance input. Control is without inhibitory conductance input. Addition of shunting conductance produces a rightward shift of \( f-r \) relationships. b: coefficient of variation (CV) as a function of excitatory Poisson rate at the three different levels of inhibitory Poisson input for the same cell in Aa. B: an FS cell. a: \( f-r \) relationship at 4 different levels \([0.5, 1.0, 1.5, \text{ and } 2.0 \text{ kHz}]\) of inhibitory Poisson input. Control is without inhibitory input. Similarly, addition of shunting conductance produces a rightward shift of \( f-r \) relationships. b: CV as a function of excitatory Poisson rate at the 4 different levels of inhibitory Poisson input, for the same cell as in Bb.
Effects of input correlation in time on spike-timing variability

Because firing of cortical neurons is typically correlated with population activity in the network, we examined how the clustering of conductance transients in time affects the measures of firing variability and the temporal fine structure of responses. For this purpose, we used a doubly stochastic process (DSP) model, composed of primary and secondary processes, to determine the input events (see METHODS). Briefly, the rate of the primary process in the DSP model is modulated in exponentially decaying transients (peak amplitude $S$ and decay time constant $\tau_R$), at intervals specified by a stationary Poisson process (rate $\lambda_p$) as shown in Fig. 5A. This allowed the degree of correlation in time to be varied, for any given $\lambda_p$, on the basis of the stationarity of the input correlation.

Fig. 5 shows examples of the effects of varying $\lambda_p$, which were 200 or 800 Hz for the RS and FS cells, respectively. For both cell types, increasing $\lambda_p$ from 200 to 800 Hz increased variability in the spike times and decreased the degree of correlation in the spike times, as shown in Fig. 5A (1st row) and B (1st row). Inhibitory conductance changes associated with spikes were also attenuated with increasing $\lambda_p$ (2nd row, 1 kHz), as shown in Fig. 5B (2nd row, 1 kHz). When the inhibitory conductance changes were at a maximum (4 kHz), the spike times were more correlated in the RS cell (3rd row, 4 kHz), as shown in Fig. 5B (3rd row, 4 kHz). However, for the FS cell, the spike times were less correlated in the RS cell (3rd row, 4 kHz), as shown in Fig. 5B (3rd row, 4 kHz). A similar pattern was observed for inhibitory conductance changes associated with spikes (4 kHz), as shown in Fig. 5B (3rd row, 4 kHz). A similar pattern was observed for inhibitory conductance changes associated with spikes (4 kHz), as shown in Fig. 5B (3rd row, 4 kHz).
RATE CODING AND SPIKE-TIMING VARIABILITY

To quantify the variability of postsynaptic spike timing evoked by conductance stimuli, we used two additional statistical measures: spike jitter and reliability (see METHODS). Although the CV describes the overall statistics of spike timing, it gives no insight into the reproducibility of responses to the same input. Therefore we examined spike timing in ensembles of responses to the same input, using the measures of spike jitter and reliability.

In an RS cell, at a specific level of inhibition (\(\lambda_{GABA}/\lambda_{AMPA} = 0.6\)), increasing synchrony decreased the firing rate in an approximately linear fashion as shown in Fig. 6Aa. As expected, greater synchrony increased CV of interspike intervals for the same cell (Fig. 6Ab). Figure 6, Ac and Ad shows that reliability and jitter both increased with the level of synchrony. FS cells (Fig. 6, Ba–Bd) showed a broadly similar pattern to RS cells in these measures, as a function of the synchrony parameter \(S\), except that firing frequency increased rather than decreased with increasing \(S\) and jitter showed no clear trend with \(S\). Table 3 summarizes the results for eight RS and nine FS cells that we analyzed.

Effects of inhibitory input on spike-timing variability

It is well established that CV measured in vivo is typically >1 (Gershon et al. 1998; Softky and Koch 1993). In contrast, for both RS and FS cells, CV during excitation by Poisson AMPA-type input is much lower than that in vivo firing as seen in the control cases of Fig. 2, Ab and Bb. However, simultaneous independent inhibition can increase spike-time variability by adding variance to the input (Fig. 2, Ab and Bb).

We investigated this effect in more detail, at high (Fig. 5Bc, \(S = 800\)) and low (Fig. 5Ba, \(S = 200\)) levels of correlation in the excitatory input.

In RS cells, independent random inhibition caused spikes to drop out, decreasing the firing rate at both low (\(S = 200\), Fig. 7Ad) and high (\(S = 800\), Fig. 7Ba) synchrony in the excitatory input. Setting the initial firing rate for \(\lambda_{GABA} = 0\) to be 13–20 spikes/s in this experiment, we found that increasing the \(\lambda_{GABA}/\lambda_{AMPA}\) ratio reduced firing frequency in an approximately linear fashion for both levels of synchrony. Inhibition decreased CV of interspike intervals at high synchrony (\(S = 800\), Fig. 7Bb), whereas it increased CV at low synchrony (\(S = 200\), Fig. 7Ab). Reliability (Fig. 7, Ac and Bc) and jitter (Fig. 7, Ad and Bd) increased as the proportion of the inhibition was increased. Results for all eight RS cells analyzed are given in Table 4.

In contrast with RS cells, FS cell reliability showed a clear maximum as illustrated in Fig. 8, Aa (\(S = 200\)) and Ba (\(S = 800\)), whereas spike timing jitter went through a clear minimum at about the similar level as shown Fig. 8, Ab (\(S = 200\)) and Bb (\(S = 800\)). Histograms of the optimal rate of inhibition for reliability and jitter respectively are shown in Fig. 8, Ac–Bd. Thus for FS neurons, there is an optimum level of shunting inhibition for achieving the most precise spike-time encoding, which is robust against changes in input synchrony. This result was observed over a range of different degrees of synchrony, or correlation (data not shown here). Firing rate and CV for FS cells showed a pattern similar to that shown in Fig.
FIG. 5. Doubly stochastic process (DSP) stimulus and evoked responses. A: schematic representation of the DSP model used to produce correlated or synchronized input timing. See METHODS for details. a: primary process with three parameters (S, \( \lambda_a \), and \( \tau_a \)). b: inhomogeneous Poisson point process. c: conductance input resulting from convolution of the point process and unitary synaptic event waveforms. B. a: response (top) to simultaneous, synchronous excitatory conductance input (middle), and inhibitory conductance input (bottom). For both excitatory and inhibitory conductance inputs, parameters: S = 200, \( \lambda_a = 2.5 \), and \( \tau_a = 0.4 \). b: S = 500, \( \lambda_b = 2.5 \), and \( \tau_b = 0.16 \). c: S = 800, \( \lambda_b = 2.5 \), and \( \tau_b = 0.10 \).

7 for RS cells (data not shown). All the results are summarized in Table 5.

Spike-time variability during a decaying synaptic conductance

Characteristic differences in the responses of RS and FS cells can be expected to emerge when they are stimulated by inputs close to their spike thresholds, arising from the difference of their threshold dynamics. We examined the consequences of this in responses of the two cell types to decaying synaptic input bursts, which pass through threshold with natural-like conductance fluctuations. As seen in the raster plots of Fig. 9, Ae and Be, spike times become considerably less precise with time during the response as a result of intrinsic noise in the spike-generating mechanism of the neuron. To quantify the irregularity, we used jitter and reliability of individual events rather than the overall averages (see METHODS). Figure 9, Ae and Be shows jitter as a function of spike time occurrence for RS and FS cells, respectively. In both cell types, jitter increases rapidly over the first 200 ms, approximately. The final stage is quite different between RS and FS cells. Whereas in RS cells jitter keeps rising (except for the final spike), in FS cells jitter consistently levels off or is even reduced, over the majority of the response. This effect is seen more clearly in jitter versus reliability plots for the last three spikes over all sessions (Fig. 10). Points in RS cells scattered over the jitter–reliability plane (Fig. 10A), whereas those in FS cells concentrated in the left side area (i.e., jitter < 4 ms; Fig. 10B). In other words, in comparison to RS cells, although FS cells are not necessarily reliable in producing spikes, the timing of spikes that are produced tends to be reliable.

Relative timing of inhibition and excitation and spike-time variability

Given the highly recurrent nature of the cortical columnar circuitry, a close temporal correlation among excitatory and inhibitory input to RS and FS cells is quite likely, and thus it is of interest to ask how spike-time reliability depends on the relative timing of inhibition and excitation. We examined jitter and reliability at a variety of delays between identically timed Poisson trains of inhibitory and excitatory events (Fig. 11A). In both types, results were broadly similar. Reliability showed a clear maximum when inhibition followed excitation by a delay of 2–3 ms as illustrated in Fig. 11, Ba and Ca, whereas spike timing jitter went through a clear minimum at about the same delay (Fig. 11, Bb and Cb). Histograms of optimal delay in 13 RS cells are shown in Fig. 11. Bc and Bd are for reliability and jitter, respectively. The optimal delay was 3.0 ± 1.1 ms for reliability and 2.9 ± 1.2 ms for jitter. In 14 FS cells, the optimal delay was 2.4 ± 1.2 ms for reliability (Fig. 11Cc) and 2.7 ± 1.1 ms for jitter (Fig. 11Cd).

DISCUSSION

RS cells in the cortex are well known to have "type 1" membrane excitability—i.e., continuous frequency versus steady current intensity (f–I) relationship—because they support extremely low frequency firing. In contrast, FS interneurons in the rat somatosensory cortex demonstrate "type 2" membrane excitability because FS cells begin repetitive firing with an "abrupt" onset at increasing levels of sustained current step stimuli, i.e., a discontinuous f–I relationship (Erisir et al. 1999; Kawaguchi 1995; Tateno et al. 2004).

Recently, the conductance injection or dynamic clamp technique has been increasingly used to understand spike generation during electrically realistic conductance input that mimics natural synaptic input (Chance et al. 2002; de Polavieja et al. 2005; Mitchell and Silver 2003; Suter and Jaeger 2004; for review, Destexhe et al. 2003). This approach is ideal for investigating reliability and spike-time variability because a complex fluctuating conductance input can be applied repeatedly and exactly. Here, our interest focuses especially on how the firing rate and spike-time variability can be modulated by simultaneous excitatory and inhibitory conductance stimulation in RS and FS cells, with their different types of threshold dynamics.

The firing frequency versus Poisson-input rate (f–r) curves at several levels of fluctuating inhibitory input showed that, in both RS and FS cells, random background inhibitory input shifted the f–r relationships rightward. This simple shift was...
independent of the type of threshold dynamics and differs from the multiplicative gain modulation by inhibition described in Chance et al. (2002) and Mitchell and Silver (2003), perhaps because of less dependency of the subthreshold membrane potential on the level of excitation in these cells and/or less difference from the reversal potential of inhibition. Furthermore, the extent of nonlinearity and the amount of the shift and linearization by inhibition differed between the two classes of cells (Fig. 2, Aa and Ba), with RS cells showing a depolarization block at high excitatory input rates, which could be relieved by background inhibition.

It is well appreciated that in a variety of in vitro preparations, stimuli with fast fluctuations resembling synaptic activity in vivo produce more reliable firing (Bryant and Segundo 1976; Harsh and Robinson 2000; Mainen and Sejnowski 1995; Nowak et al. 1997; Suter and Jaeger 2004; Tang et al. 1997). To compare with previous work, we used five statistical measures of spike variability averaged over hundreds of spikes arising from different trajectories of membrane potentials in different stimulation conditions.

In this study both RS and FS cells showed much less variability of spike intervals in response to steady Poisson AMPA excitation than that in vivo, where CV > 1 (Buracas et al. 1998; Stevens and Zador 1998). In vivo, however, clusters of spikes or bursts are driven by concerted activity in multiple nearby cells (Azouz and Gray 1999; Tsodyks et al. 1999) and bursting plays a significant role in sensory information transmission (Krahe and Gabbiani 2004). Therefore we introduced correlation in time or synchrony into excitatory and inhibitory conductance stimuli, using a nonstationary Poisson process as described (Harsch and Robinson 2000).

We found, in agreement with this previous study, that for RS cells, synchronous stimulation increased variability for the same mean input rate, and made it easy to increase variability to the in vivo levels, although we did not use N-methyl-D-aspartate (NMDA)--type conductance in this study. The novel
aspect of this study was to extend these results to FS cells. Variability and spike-time reliability increased in both RS and FS cells with increasing synchrony. The doubly stochastic input increased variability of spike trains, which exhibits two different time scales: within and between bursts. By changing the duration of clusters relative to their rate, we were able to systematically vary the degree of synchrony in the input. A linear relationship was found between each variability measure and the degree of synchrony in both types of single cells. This

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RS</th>
<th>FS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Firing frequency, Hz</td>
<td>$a$</td>
<td>$-5.47 \pm 2.11$</td>
</tr>
<tr>
<td></td>
<td>$b$</td>
<td>$14.4 \pm 3.3$</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>$a$</td>
<td>$1.78 \pm 0.22$</td>
</tr>
<tr>
<td></td>
<td>$b$</td>
<td>$0.379 \pm 0.236$</td>
</tr>
<tr>
<td>Reliability, %</td>
<td>$a$</td>
<td>$25.1 \pm 12.6$</td>
</tr>
<tr>
<td></td>
<td>$b$</td>
<td>$62.0 \pm 15.8$</td>
</tr>
<tr>
<td>Jitter, ms</td>
<td>$a$</td>
<td>$0.147 \pm 0.314$</td>
</tr>
<tr>
<td></td>
<td>$b$</td>
<td>$1.51 \pm 0.18$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Individual statistics ($y$) were approximated by the linear relationship $y = aS + b$, where $S$ denotes synchrony. Note that the ratio of rates of excitatory and inhibitory conductance inputs was fixed at 0.6.

TABLE 4. Summary of effects of inhibition on RS cell spike statistics

| Number of cells | 8     | 7       |
| Synchony ($S$)  | 200   | 800     |
| Firing frequency, Hz | $a$ | $-8.07 \pm 3.01$ | $-11.7 \pm 4.5$ |
|       | $b$ | $14.7 \pm 3.9$ | $19.2 \pm 4.6$ |
| Coefficient of variation | $a$ | $0.254 \pm 0.218$ | $-0.653 \pm 0.449$ |
|       | $b$ | $0.641 \pm 0.313$ | $1.68 \pm 0.449$ |
| Reliability, % | $a$ | $24.4 \pm 23.9$ | $20.1 \pm 22.6$ |
|       | $b$ | $58.9 \pm 23.2$ | $68.1 \pm 23.3$ |
| Jitter, ms | $a$ | $-0.366 \pm 0.219$ | $-0.434 \pm 0.165$ |
|       | $b$ | $1.75 \pm 0.19$ | $1.81 \pm 0.36$ |

Values are means ± SD. Individual statistics ($y$) were approximated by the linear relationship $y = ar + b$, where $r$ denotes the ratio ($\lambda_{GABA}/\lambda_{AMPA}$) between excitatory and inhibitory inputs.

FIG. 7. Effects of balance between excitatory (AMPA-type) and inhibitory (GABA-type) conductance inputs on firing statistics in an RS cell. $A$: at a low level of correlation in time or synchrony ($S = 200$). $a$: firing frequency vs. inhibitory ratio to excitatory input. $b$: coefficient of variation (CV) of interspike intervals vs. inhibitory ratio. $c$: reliability vs. inhibitory ratio. $d$: spike jitter vs. inhibitory ratio. $B$: at a high level of synchrony ($S = 800$). $a$: firing frequency vs. inhibitory ratio to excitatory input. $b$: coefficient of variation (CV) of interspike intervals vs. inhibitory ratio. $c$: reliability vs. inhibitory ratio. $d$: spike jitter vs. inhibitory ratio.
result indicates that variability is in part determined by input synchrony in RS and FS cells and that spike clusters or bursts may be more reliable codes in both cell types than are single spikes.

The high in vivo level of spiking variability of spiking has also been suggested to result from independent random inhibitory input in cortical neurons (Shadlen and Newsom 1998) and in cerebellar Purkinje cells and interneurons (Häusser and Clark 1997). However, in cortical neurons, injection of neither random inhibitory current (Stevens and Zador 1998) nor conductance (Harsch and Robinson 2000) was able to reproduce in vivo levels of variability. Here, we found that, in both RS and FS cells, increasing the level of inhibition had opposite effects on CV, depending on the level of synchrony; at low synchrony or with stationary Poisson stimulation, inhibition increased CV (Figs. 2, Ab and Bb, 7Ab, and 8Ab, S = 200), whereas at high synchrony, it decreased CV (Figs. 7Bb and 8Bb, S = 800). This supports the conclusion that high in vivo CV values do not solely arise from the additional variance of random inhibition.

For FS neurons, there appears to be an optimum level of shunting inhibition for achieving the most precise spike-time encoding. This was observed over a range of different degrees of synchrony in the excitatory and inhibitory input. FS neurons have an intrinsic lower limit of stable firing frequency at around 20–30 Hz (“type 2” threshold dynamics) as well as subthreshold oscillations at the similar frequency, whereas RS neurons do not (“type 1”) (Tateno et al. 2004). This optimization of FS cell firing precision might arise when the subthreshold membrane potential dynamics are tuned by the level of shunting inhibition so as to maximize subthreshold oscillation amplitude. This could have functional relevance in controlling the coherence of synchronous firing, which is believed to depend critically on the FS cell network.

In the cortex, RS and FS cells receive common thalamic input and FS cells inhibit local RS cells. It can thus be expected that many RS cells should receive inhibition whose timing is tightly coupled to excitation as, for example, reported in auditory cortex by Wehr and Zador (2003). Here, we showed that with identically timed inhibition and excitation, a delay of 2–3 ms between excitation and inhibition produces optimal reliability and jitter. This essentially occurs because inhibition

<table>
<thead>
<tr>
<th>Table 5. Summary of effects of inhibition on FS cell spike statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
</tr>
<tr>
<td>Synchrony (S)</td>
</tr>
<tr>
<td>Firing frequency, Hz</td>
</tr>
<tr>
<td>a</td>
</tr>
<tr>
<td>b</td>
</tr>
<tr>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>a</td>
</tr>
<tr>
<td>b</td>
</tr>
</tbody>
</table>

Values are means ± SD. Individual statistics (y) were approximated by the linear relationship \(y = ar + b\), where \(r\) denotes the ratio \((\lambda_{\text{GABA}}/\lambda_{\text{AMPA}})\) between excitatory and inhibitory inputs.
FIG. 9. Response to a synchronous burst of simultaneous AMPA-type and GABA-type conductance input in RS and FS cells. A: RS cell. a: an example membrane potential response at the soma. b: conductance inputs consisting of a train of unitary AMPA-type and GABA-type conductance transients, generated by a nonstationary Poisson process with an exponentially declining rate. AMPA-type and GABA-type conductance inputs are shown, respectively. Parameters: $S/Hz$ and $r_0 = 0.313 (s)$. c: raster display of 25 trials with the identical stimulus. d: peristimulus time histogram with 2-ms bin width. Events were discriminated by thresholding at 8 spikes per bin. e: SD against mean of successive spike times for each event. f: superimposed membrane potential trajectories for spikes of different precision, as indicated in a.

B: FS cell. As in A, but using a session of 28 trials, and a threshold of 9 spikes per bin. e: SD against mean of spike times for each event. f: Superimposed membrane potential trajectories for spikes of different precision, as indicated in a.

FIG. 10. Distribution of jitter and reliability in RS and FS cells for the final spikes of transient responses. A: for 14 RS cells, jitter and reliability of last 3 spike events are shown for 58 sessions. Last 3 spike events in each trial, i.e., events $N-1$, and $N-2$ are indicated respectively by filled circles (●), triangles (▲), and crosses (●+). B: 117 jitter-reliability pairs over 39 sessions are shown, from 11 FS cells. See also Table 6.
restricts the generation of spikes to brief time windows during stronger excitatory fluctuations and the effect is independent of threshold dynamical type.

In the neocortex, pyramidal RS neurons collect widely distributed inputs delivered in different layers and make excitatory synapses to other neurons. Because of relatively strong spike adaptation and type 1 threshold dynamics, the coherence of RS cell firing should fail relatively easily at weaker levels of input and during the decaying phase of transient inputs. Thus RS cells promote spike-time variability and are, in this sense, suited to rate coding and the transmission of transient information, rather than temporal coding and the maintenance of coherent rhythms (see Figs. 9A and 10A). In contrast, inhibitory interneurons of the same type (FS or LTS cells) are strongly interconnected not only by electrical synapses or gap junctions but also by chemical synapses, and are implicated in promoting synchronous firing (Beierlein et al. 2000; Galarreta and Hestrin 2001; Gibson et al. 1999). In addition, FS interneurons have type 2 threshold dynamics and little spike adaptation. The existence of subthreshold oscillations in FS cells means that the phase of rhythmic synchronous firing among FS cells can be kept stable even if input drops below the threshold level. Although, by virtue of their discontinuous \( f-I \) relationship, FS cells have a harder onset of spike initiation and are consequently less reliable in producing spikes than RS cells, FS cells concomitantly have little jitter in the late stage of a transient input (cf. Fig. 10, A and B). Thus threshold dynamics has far-reaching consequences on spike-time variability in RS and FS cells.

Simplified computational models of neural dynamics support this scenario (Ermentrout 1996; Gutkin et al. 2003; Robinson 2004; Robinson and Harsch 2002). For example, type 1 and type 2 Morris–Lecar (ML) models (Morris and Lecar 1981) capture the mathematical essence of thresholds (bifurcations) of regular firing, using just two variables, one representing voltage and \( \text{Ca}^{2+} \) channel activation and the other representing \( K^{+} \) channel activation. A small change in model parameters causes a shift from type 1 to type 2 behavior and profoundly changes the pattern of dispersion of the final spike times. This result can demonstrate how type 2 neurons intrinsically prefer to stay coherent or be silent, whereas type 1 neurons have a smooth transition between the two extremes. In addition, for the type 2 ML model, the dispersion of the final spikes is very robust against changes in the shape of the stimulus waveform, whereas the dispersion of spike times for the type 1 model is highly sensitive to the exact shape of the stimulus (data not shown). Thus the type of threshold dynamics should have a major impact on the reliability of spike generation and spike timing in the cortex. In the in vivo situation, neurons can receive a greater bombardment by synaptic input and are subject to control by neuromodulators, which could profoundly alter their discharge properties. Nevertheless, under controlled in vitro conditions, the threshold dynamical type.

### Table 6. Summary of spike-event statistics for decaying conductance stimulation in RS and FS cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>RS</th>
<th>FS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Number of sessions</td>
<td>58</td>
<td>39</td>
</tr>
<tr>
<td>Firing frequency, Hz</td>
<td>15.2 ± 4.9</td>
<td>16.6 ± 6.3</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.729 ± 0.149</td>
<td>0.724 ± 0.173</td>
</tr>
<tr>
<td>Event ( N ) Reliability, %</td>
<td>76.9 ± 21.9</td>
<td>52.9 ± 16.6</td>
</tr>
<tr>
<td>Jitter, ms</td>
<td>4.06 ± 2.87</td>
<td>5.82 ± 2.07</td>
</tr>
<tr>
<td>Event ( N-1 ) Reliability, %</td>
<td>82.6 ± 19.6</td>
<td>61.2 ± 19.2</td>
</tr>
<tr>
<td>Jitter, ms</td>
<td>3.71 ± 3.24</td>
<td>1.84 ± 2.04</td>
</tr>
<tr>
<td>Event ( N-2 ) Reliability, %</td>
<td>75.7 ± 21.1</td>
<td>63.5 ± 19.2</td>
</tr>
<tr>
<td>Jitter, ms</td>
<td>4.18 ± 2.39</td>
<td>2.13 ± 1.69</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( N \) is the number of spike events in each session ensemble.

![FIG. 11. Effects of delay between excitatory and inhibitory conductance inputs on spike-time variability.](http://jn.physiology.org/)

- **A**: schematic representation of excitatory and inhibitory conductance stimuli with delay.
- **B**: response to the compound stimulation.
- **C**: FS cells: \( a \): for 3 different cells, reliability vs. delay, \( b \): spike jitter vs. delay, \( c \) and \( d \): for total of 13 cells, histograms of the delays for \( c \) maximum reliability and \( d \) minimum jitter.

**RS cells**: \( a \): for 3 different cells, reliability vs. delay, \( b \): spike jitter vs. delay, \( c \) and \( d \): for total of 14 cells, histograms of the delays for \( c \) maximum reliability and \( d \) minimum spike jitter.
old dynamical type of RS and FS cells is a crucial feature of their distinctive patterns of reliability in responses to natural-like input.

In conclusion, in this study, we have compared the variability and reliability of responses of RS and FS cells to complex, natural-like stimuli. Both cell types showed the same pattern of increased CV of interspike intervals in response to higher input synchrony, and this effect is thus independent of the threshold dynamical type. However, there were striking differences between the two cell types in the precision and reliability of spiking during the processing of complex inputs, which may be understood as a consequence of the type 2 property of subthreshold oscillations in FS cells. In FS cells, we found that spike-time jitter is kept low even for late spikes during transient inputs, as expected for type 2 models. In FS cells, but not RS cells, an optimum was found in the level of inhibition required to achieve maximum precision, presumably a result of tuning membrane properties to maximize the amplitude of subthreshold oscillations. Finally, in contrast to RS neurons (de Polavieja et al. 2005), spike shape in FS neurons was hardly modified by the level of excitatory or inhibitory input conductance, meaning that significant spike shape encoding cannot occur. These differences between the two cell types are consistent with a role of RS neurons as input encoding integrators and a role of FS neurons as resonators controlling the coherence of synchronous firing.

ACKNOWLEDGMENTS

T. Tateno thanks Professor Taishin Nomura (Osaka University) for support and encouragement.

GRANTS

This work was supported in part by the Japanese Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Exploratory Research 17500885/2005, and by European Community Grant FP6.

REFERENCES


Kleppe IC and Robinson HP. Determining the activation time course of synaptic AMPA receptors from openings of colocalized NMDA receptors. Biophys J 77: 1418–1427, 1999.


