Cortical neurons are often classified by current–frequency relationship. Such a static description is inadequate to interpret neuronal responses to time-varying stimuli. Theoretical studies suggested that single-cell dynamical response properties are necessary to interpret ensemble responses to fast input transients. Further, it was shown that input-noise linearizes and boosts the response bandwidth, and that the interplay between the barrage of noisy synaptic currents and the spike-initiation mechanisms determines the dynamical properties of the firing rate. To test these model predictions, we estimated the linear response properties of layer 5 pyramidal cells by injecting a superposition of a small-amplitude sinusoidal wave and a background noise. We characterized the evoked firing probability across many stimulation trials and a range of oscillation frequencies (1–1000 Hz), quantifying response amplitude and phase-shift while changing noise statistics. We found that neurons track unexpectedly fast transients, as their response amplitude has no attenuation up to 200 Hz. This cut-off frequency is higher than the limits set by passive membrane properties (~50 Hz) and average firing rate (~20 Hz) and is not affected by the rate of change of the input. Finally, above 200 Hz, the response amplitude decays as a power-law with an exponent that is independent of voltage fluctuations induced by the background noise.

Keywords: dynamics, frequency response, noise, oscillations, pyramidal cell, somatosensory cortex

Introduction

The response of a single neuron to a changing input is limited by the neuron’s maximal spike frequency. Inputs which vary faster can only be encoded in the collective activity of a population. This can be observed in cortical rhythms when individual cells fire irregularly and at much lower spiking rate than the population rhythm revealed through local field potentials (Buzsaki and Draguhn 2004). Individual cells tend to fire more often at the peak of the oscillation but cannot emit a spike for every cycle. However, whereas 1 cell is in the refractory period another 1 may fire during the next cycle, so that the population can globally sustain fast rhythms. It is therefore of central importance to investigate how neurons respond to time-varying inputs and to identify the impact of synaptic background noise (Paré et al. 1998; Shadlen and Newsome 1998, Steriade 2001).

By investigating how the instantaneous firing rate is modulated by a noisy input with a small sinusoidal component, we experimentally estimated the linear response properties of layer 5 pyramidal cells of the rat somatosensory cortex, over a wide frequency range of input oscillations (i.e., 1–1000 Hz). We evaluated the extent of response linearity, tested the ability of cells to track temporally varying inputs, and investigated the impact of background noise. In the limit of small input amplitude, this allows one to predict the spiking activity of a population of weakly interacting neurons, on the basis of the single-cell responses to elementary sinusoidally modulated currents. This also allows to study how neurons take part in collective rhythms, inferring the preferred global frequency in recurrent networks (Fuhrmann et al. 2002; Brunel and Wang 2003; Wang 2003; Geisler et al. 2005) where each cell responds to a correlated foreground rhythm (i.e., the signal) while experiencing a distinct synaptic background activity.

Although the response properties of cortical neurons to stationary fluctuating inputs have been previously characterized (Chance et al. 2002; Rauch et al. 2003; Giugliano et al. 2004; Higgs et al. 2006; La Camera et al. 2006; Arsigny et al. 2007), this is the 1st time that the response of cortical neurons to temporally modulated inputs is investigated over a wide range of input frequencies and through analysis of the background noise.

Materials and Methods

Experimental Preparation and Recordings

Tissue preparation was as described in Rauch et al. (2003). Briefly, neocortical slices (sagittal, 300 µm thick) were prepared from 14- to 52-days-old Wistar rats. Large layer 5 (15), regular-spiking pyramidal cells (McCormick et al. 1985) of the somatosensory cortex with a thick apical dendrite were visualized by differential interference contrast microscopy. Some neurons were filled with biocytin and stained (Hsu et al. 1981), to check that the entire neuronal apical dendrite is indeed in the plane of the slice, which was always the case. Whole-cell patch-clamp recordings were made at 32 °C from the soma (10–20 MΩ access resistance) with extracellular solution containing (in mM): 125 NaCl, 25 NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, 25 glucose,
bubbled with 95% O₂, 5% CO₂, perfused at a minimal rate of 1 mL/min. Electrode resistance and capacitance were 6.97 ± 0.18 MΩ and 23.73 ± 1.11 pF, respectively, when filled with an intracellular solution containing (in mM): 115 K-glucuron, 20 KCl, 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 4 adenosine triphosphate-Mg, 0.3 Na₂-guanosine triphosphate, 10 Na₃-phosphocreatine, pH adjusted to 7.3 with KOH. All the chemicals were from Sigma or Merck (Switzerland). Other pipette solutions were reported not to alter significantly the response properties of the cells under very similar experimental conditions (Rauch et al. 2003). A BVC-700A bridge amplifier (Dagan Corporation, MN) was used in current-clamp mode and bridge balance and capacitance neutralization were routinely applied. Hyperpolarizing current steps and linear swept sine waves (ZAP) were injected to obtain estimates of the passive properties of patched neurons, such as the total membrane capacitance \( C_m \) and apparent input resistance \( R_m \) (Lanese and Redman 1973), as well as the membrane impedance amplitude profile (Hutcheon et al. 1996). Signals were low-pass filtered at 2.5 kHz, sampled at 5–15 kHz, and captured on the computer.

Finally, care was taken to ensure that the neuronal response was consistent and reproducible throughout the whole recording session (see Fig. 1b). The total whole-cell resistance \( R_m \) and the resting membrane voltage \( E_m \) were continuously monitored (during \( T_2 \) and \( T_1 \), respectively, located as in Fig. 1). Data collection began after these observables attained stable values and the experiment was stopped in case of any drift.

The results reported here represent data from L5 pyramidal cells (\( n = 67 \)) of the somatosensory cortex. The average resting membrane potential was \( E_m = -66 ± 4.4 \text{ mV} \), the apparent input resistance (\( R_m \)) was \( 45 ± 2.6 \text{ MΩ} \), the membrane time-constant (\( \tau_m \)) was 18.32 ± 0.8 ms. The total capacitance \( C_m \) was estimated as 448 ± 19 pF. Liquid junction potentials were left uncorrected.

**Injection of Sinusoidal Noisy Currents**

To probe the response dynamics of pyramidal cells under in vivo-like conditions, independent realizations of a noisy current were computer-synthesized and injected somatically in current-clamp configuration (see Fig. 1a). Each experiment consisted of the repeated injection of current stimuli \( I(t) \), lasting \( T = 10–30 \text{ s} \) each, interleaved by a recovery \( T_{rec} \) of 30 s. A deterministic sinusoidally oscillating current with frequency \( f \) was superimposed to the noisy current component and injected (Fig. 2c,d), so that

\[
I(t) = I_0 + I_1 \sin(2\pi ft) + I_{\text{noise}}(t).
\]

\( \tau \) denotes the time constant of the recording conditions on a trial-by-trial basis. Panel b shows a typical experimental session, plotting over time the whole-cell resistance \( R_m \) (estimated during \( T_2 \), \( b \)—upper panel), the resting membrane potential \( E_m \) (averaged during \( T_1 \), \( b \)—middle panel), as well as the reproducibility of the cell discharge rate \( r_{nx} \), evaluated in response to a stationary noise, characterized by fixed statistics (\( s, \tau_{fix} \), during \( T_2 \)). Continuous lines in (b) represent average values of each observable across the whole experiment, whereas the gray shading in (b)—lower panel) indicates a confidence level of approximately 68%, which describes the variance allowed for the data. The middle panel shows a layer V pyramidal cell of the somatosensory cortex of the rat stained with Biocytin.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** In vivo-like stimulation protocol and the stability of in vitro recording conditions. In vivo irregular background synaptic inputs were emulated in vitro by injection of noisy currents under current-clamp. Specifically, gaussian currents characterized by mean \( I_0 \), standard deviation \( \sigma \) and correlation time \( \tau \), were injected into the soma of layer 5 pyramidal cells. A deterministic sinusoidally oscillating waveform of amplitude \( I_1 \) and modulation frequency \( f \) was then superimposed to the background noise \( I_0 \); \( a \)—lower trace, and the stimulation trials were interleaved by a recovery interval \( T_{rec} \). The initial segments of each stimulus (i.e. lasting \( T_1 \), \( T_2 \), and \( T_3 \)) were used to monitor the stability of the recording conditions on a trial-by-trial basis. Panel b shows a typical experimental session, plotting over time the whole-cell resistance \( R_m \) (estimated during \( T_2 \), \( b \)—upper panel), the resting membrane potential \( E_m \) (averaged during \( T_1 \), \( b \)—middle panel), as well as the reproducibility of the cell discharge rate \( r_{nx} \), evaluated in response to a stationary noise, characterized by fixed statistics (\( s, \tau_{fix} \), during \( T_2 \)). Continuous lines in (b) represent average values of each observable across the whole experiment, whereas the gray shading in (b)—lower panel) indicates a confidence level of approximately 68%, which describes the variance allowed for the data. The middle panel shows a layer V pyramidal cell of the somatosensory cortex of the rat stained with Biocytin.

\[ I_{\text{noise}}(t) = I_0 + I(t) + I_{\text{noise}}(t). \]
Data Analysis

The membrane voltage was recorded in response to each noisy current stimulation. Data analysis was performed by the peristimulus time histograms (PSTHs) (bars) across repeated trials and successive input cycles, and quantified by the mean firing rate $r(t)$ (black thick line). For the sake of comparison, the sinusoidal component of $i(t)$ (lower panels) was plotted in red and superimposed to the actual injected waveform. Although the mean firing rate $r_0$ remains constant, its modulation $r_1$ and phase-shift $\Phi$ depend on the input frequency $f$.

Phenomenological Model

Along the lines of phenomenological “cascade” predictive models of neural response properties (French 1976; Victor and Shapley 1979c; Carandini et al. 1996; Kim and Rieke 2001; Slee et al. 2005), in closer analogy to classic Fourier System Identification (Brogan 1991), we considered an input-output relationship based on linear ordinary differential equations (i.e., a linear filter, eq. 5), similarly to Powers et al. (2005). Unlike that approach, we focused on the transformation of the input signal component (i.e., sinusoids or $i(t)$) into firing rates $r(t)$. Thus, the identification of these transformations depended on the statistics of the background noise (i.e., $I_b$, $s$, and $\tau$). Instead of the time-domain, the linear filtering was operator-specific and identified in the frequency-domain (eq. 6). This allowed us to consider a reduced number of free parameters.

In detail, the input is first fed into a threshold-linear element $H(x)$ (see Fig. 7a):

$$H(t) = H(t_1(t)) = \begin{cases} i_1(t) - P_1, & i_1(t) > P_1, \\ 0, & i_1(t) < P_1. \end{cases}$$

where $i_1(t)$ is the input signal measured in nA. Then $H(i_1(t))$ is transformed into $r(t)$ according to the following equation,

$$a_0 d^n y/dt^n + \ldots + a_1 dy/dt + a_0 y = b_0 d^m H/dt^m + \ldots + b_3 dH/dt + b_0 H,$$

where $n > m$ (Brogan 1991). The filter model alone as employed in Figures 5 and 6, can simply be obtained by setting $H(x) = x$ in equation...
(5). Under periodic regimes, equation (5) is equivalent to the product $\hat{y}(t) = \hat{X}(f) \hat{H}(f)$ where $\hat{y}(t)$ and $\hat{H}(f)$ are the (discrete) Fourier transforms of $y(t)$ and $h(t)$, respectively, and $\hat{X}(f)$ can be written as

$$\hat{X}(f) = G_0 \left[ \frac{(f + z_1)(f + z_2) \ldots (f + z_m)}{(f + \pi_1)(f + \pi_2) \ldots (f + \pi_n)} \right] \frac{z_1 \pi_1 \ldots z_n \pi_n}{z_2 \pi_2 \ldots z_m \pi_m} \tag{6}$$

where $j = \sqrt{-1}$ and $G_0$ is a real number that represents the low-frequency gain. $\{z_i\}$ and $\{\pi_i\}$ are the roots of the polynomials with coefficients $\{b_i\}$ and $\{a_i\}$ and as act the lower or upper cut-off frequencies of elementary high-pass or low-pass filters, respectively, arranged in cascade and have the physical meaning of the inverse of intrinsic time-constants. The filter input-output gain and phase-shift across input modulation frequencies $f$ are fully specified by $G_0$ and by the number of distinct $\{z_i\}$ and $\{\pi_i\}$ (i.e., $m$ and $n$) and their values. For instance, equation (6) accounts for the high-frequency ($f \to + \infty$) power-law $\angle \hat{X}(f) \approx f^4$ observed in our experiments, with $\alpha = n - m$, strictly integer. Identical input-output relationships are commonly employed to describe electrical filters, composed of linear resistors, capacitors and inductors (Horowitz and Hill 1989). Finally, a constant propagation delay $\Delta t$ was further included, together with an output offset, so that

$$r(t) = \gamma_1 (1 - \Delta t) + P_2, \text{ or equivalently } \angle \hat{X}(f) \to \angle \hat{X}(f) - 360 \cdot f \Delta t, \tag{7}$$

where the phase of $\angle \hat{X}(f)$ was indicated by $\angle$ and expressed in degrees.

In summary, equations (5-7) describe a linear transformation preceded by a static, or no-memory, threshold-linear stage (eq. 4). The cascade ordering "nonlinear-linear" was preferred to "linear-nonlinear" for slightly better fit performances. All the parameters (i.e., $P_1, P_2, G_0, \{z_i\}, \{\pi_i\}, \Delta t$) were adjusted to minimize the discrepancies between actual data and model predictions, employing Simulated Annealing techniques (Press et al. 1992). The chosen cost-function to minimize was represented by the $\chi^2$ that quantified the mean quadratic discrepancy between actual data and model prediction, weighted by the confidence interval (Press et al. 1992). Large deviations are therefore weighted on the basis of the confidence on these data estimates. For the identification of the full cascade model in the time-domain, $\chi^2$ was complemented by 1st-derivative mean discrepancies.

Statistics

Ninety-five percent confidence accuracy intervals on the nonlinear least-square parameter estimates were determined for $n_0, n_1(f), \Phi(f)$ by the Levenberg-Marquardt fit algorithm, providing error bars in the plots of Figures 5 and 6 as in Fourcaud-Trocme et al. (2003). For Figure 1b(lower panel) and Figure 6b, the gray shading represents the asymmetric 68% confidence accuracy interval (i.e., corresponding to 1 standard deviation) for the mean firing rate $n_0$, as in Rauch et al. (2005).

In the case of identification of the phenomenological filter models, the $\chi^2$-test was used to evaluate the quality of the fits (Press et al. 1992), implicitly taking into account the number of free parameters. Kendall's Tau nonparametric (rank-order) test (Press et al. 1992) was finally employed to assess correlations among spike-shape features and stimulation parameters, providing a measure $c$ of correlation together with its significance level $P$, which represents the probability of obtaining the same value for $c$ from statistically independent samples (i.e., false positive).

Results

The Linear Response to Time-Varying Noisy Inputs

Due to irregular spontaneous activity and the high degree of convergence, cortical neurons receive a continuous barrage of excitatory and inhibitory potentials in the intact brain. At the same time cortical cells participate in a variety of oscillations, whose frequency spans several orders of magnitude (e.g., 0.05–500 Hz) during distinct behavioral states (Buzsaki and Draguhn 2004). What is the impact of the background activity on neuronal responsiveness and on collective oscillations? We approached these issues by studying the linear response properties of single neurons characterizing their instantaneous discharge rate $\rho(t)$ in response to a noisy background current with a small sinusoidal component, hereafter referred to as the “signal.” This allowed us not only to investigate how cortical neurons participate in an oscillatory regime, but especially how cells track temporally varying inputs under distinct background conditions (Fig. 1). We systematically varied the input oscillation frequency $f$, its amplitude $I_0$ and offset $I_0$, as well as the statistics $(\delta, \tau)$ of the background noise (eqs. 1 and 2). Because no correlation between the shape of the action potentials and these stimulation parameters was found, we restricted our analysis to the timing of each spike. However, very small correlations $c$ exist between $(I_0, I_1, \delta)$ and the maximal upstroke velocity and spike duration $(\delta \leq 0.1; P < 10^{-5})$, but they are consequence of nonideal bridge-balancing. Weak correlations $c$ were instead found between the rat postnatal day and the spike upstroke velocity $(\delta = 0.21; P < 10^{-12})$, downstroke velocity $(\delta = -0.23; P < 10^{-14})$, and spike duration $(\delta = -0.27; P < 10^{-19})$, as observed by many investigators.

The firing rate $\rho(t)$ was estimated from the peristimulus time histograms (PSTHs) of the spike times over hundreds of cycles of the input current and over several stimulation trials. It was interpreted as the instantaneous discharge probability or, equivalently, as the firing rate of a cortical population composed of independent neurons.

In the limit of small-signal input amplitude $I_1$, $\rho(t)$ could be well approximated by a sine wave oscillating at the same frequency $f$ as the input current (Fig. 2c,d, upper panels):

$$\rho(t) = r_0 + r_1(f) \sin[2\pi ft + \Phi(f)]. \tag{8}$$

$r(t)$ is fully described in terms of mean firing rate $r_0$, modulation amplitude $r_1(f)$, and phase-shift $\Phi(f)$ relative to the input current, as well as in dynamical transformations. At the beginning of each experiment, the stimulation parameters were selected in a way that $r_0$ was in the range 10–20 Hz, the membrane voltage fluctuations induced by the noise were 1–5 mV, and the discharge modulation amplitude $r_1$ was $0 < r_1 < r_0$. Figure 2 reports typical spike responses evoked by input modulations at $f = 10$ Hz and at $f = 250$ Hz, recorded in the same cell. Individual firing times across successive input cycles and trial repetitions showed high variability (Fig. 2a,b, lower panels), as a consequence of the noise component uncorrelated with the sinusoidal signal oscillations.

Scaling the input amplitude $I_1$ in the range 20–200 pA while keeping $I_0, \delta, f$ fixed resulted in a linear scaling of the output amplitude $r_1$ (n = 3, not shown). However, for large input modulation depth (i.e. $I_1 > 0.3 I_0$), the amplitudes of output superimposed sinusoidal oscillations characterized by multiple frequencies of $f$ (i.e., higher harmonic components) increased (n = 3), revealing the presence of input-output distortions as the limit of small input amplitude was exceeded. Thus, in most of the experiments we employed $I_1$ smaller than 30% of $I_0$ to fulfill the validity of the linear approximation where higher harmonics in the output could be neglected. Although similar values of $I_1$ are not infinitesimal with respect to $I_0$, this choice was confirmed to be reasonable by studying and predicting the neuronal discharge in response to more complex inputs signals across a wide range of firing rates, as discussed in the experiments of Figure 7.
Consistent with the hypothesis of linearity, no significant difference between the sum of the responses to individual sinusoids and the response to the sum of multiple sinusoids injected simultaneously was observed (Movshon et al. 1978; Victor 1979; Carandini et al. 1996) \((n=20, \text{not shown})\).

**Cortical Neurons Track Fast Inputs**

Our experimental characterization aimed at identifying the linear neuronal response properties and at studying the way background noise affects them (Sakai 1992; Chichilnisky 2001; Fourcaud-Trocme et al. 2003; Naundorf et al. 2005; Apfaltrer et al. 2006). In the framework of classic Fourier decomposition of any input signal to a neuron, \(r(f)\) and \(\Phi(f)\) give quantitative information on how the neuronal encoding differentially attenuates and delays each frequency component \(f\) of the input, in the limit of small-signal amplitude.

Figure 3 summarizes population data and reports the unexpectedly wide bandwidth of the output temporal modulation depth \(r_1/r_0\) and output phase-shift \(\Phi\). Although \(r_0\) was unaffected by \(f\), \(r_1\) decreased significantly only for \(f > 100-200\) Hz, regardless of the intensity and temporal correlations of the background noise. The profile of \(r_1(f)\) across frequencies did not match the membrane impedance, which was dominated by voltage-dependent resonances in the low-frequency range (i.e., 5–10 Hz—previously related to \(b\)-currents and \(M\)-currents) and by a low-pass behavior at high frequencies (not shown) with strong attenuation above 50 Hz (Gutfreund et al. 1995; Hutcheon et al. 1996).

Above 200 Hz the output modulation depth decayed as a negative power-law, which appears as a straight (dashed) line in the double-logarithmic plot of Figure 3. The power-law exponent estimated by linear regression through the population data of Figure 3 was close to 2 \((\alpha = -1.80)\) and it matched the value obtained by averaging the exponents estimated in single experiments \((\alpha = -1.81 \pm 0.31, n = 6—\text{see Fig. 4a})\). A similar qualitative dependence, induced by system linearization, was anticipated by theoretical studies (Gerstner 2000; Knight 1972a) and could be replicated quantitatively in the case of integer power-law exponents through canonical phase oscillator models (Naundorf et al. 2005), nonlinear integrate-and-fire models (Fourcaud-Trocme et al. 2003), and conductance-based neuronal modeling (Fourcaud-Trocme et al. 2003). Integer values of \(\alpha\) also relate to the number of best-fit free parameters of the phenomenological band-pass filters used in Figures 5–7 (see the Methods—eq. 6), introduced to fit the experimental data as discussed in the following sections.

Even though the inspection of Figure 3 seems to indicate that the points at highest frequencies can be fitted by \(1/f^2\), Figure 4a supports the conclusion that \(1/f^2\) is a more precise characterization. Nevertheless, numerical simulations showed that the high-frequency asymptotic behavior might be reached at frequencies which are much higher than the cut-off frequency (Fourcaud-Trocme et al. 2003), so that assessing the precise value of \(\alpha\) might not be conclusive on the basis of our observations.

As opposed to typical linear systems, the phase-shift at high frequencies did not saturate but decreased linearly with \(f\) (see Fig. 4b). This is reminiscent of the presence of a constant time

**Figure 3.** Modulation depth \(r_1/r_0\) and phase-shift \(\Phi\) of the response to a noisy oscillatory input. The instantaneous firing rate \(r(t)\) evoked by small sinusoidal currents over a noisy background revealed sinusoidal oscillations with amplitude \(r_0\) and phase-shift \(\Phi\), around a mean \(r_0\) (quantified as in Fig. 2c,d). Surprisingly, pyramidal neurons can relay fast input modulations, up to several hundred cycles per second. The high-frequency response behavior matches a power-law relationship (i.e., \(r_1 - f\)) with a linear phase-shift (i.e., \(\Phi - \Phi\)). These plots were obtained for 67 cells, averaging across available repetitions and distinguishing between offset-currents and \(\Delta t\) and phase-shifts of the corresponding cell (as in Fig. 5). Data points corresponding to distinct input modulation frequencies were pooled together in nonoverlapping bins with size 0.1–10 Hz (low frequencies) and 100–200 Hz (high frequencies). Error bars represent the SE across the data points available (32 ± 25) for each bin. Markers shape and color identify the suprathreshold or weak-noise regime (black) and the subthreshold or strong-noise regime (red), characterized by distinct values for \(I_0\) and \(s\), adapted to yield a similar mean rate \(r_0 = 20\) Hz (i.e., 19.7 ± 1.5 Hz).

**Figure 4.** The high-frequency dynamical response properties of a typical cortical neuron, plotted in linear scale. The modulation amplitude (a) \(r_1/f\), elicited by noisy oscillatory inputs, shows a power-law behavior (see also Fig. 3) captured by \(1/f^\alpha\), with \(\alpha = 2\), whereas the phase \(\Phi\) of the response (b) decreases linearly with increasing frequencies \(f\) (i.e., \(\Phi \rightarrow -360^\circ/f\cdot\Delta t\)). Stimulation parameters \((V, I, s) = (400, 150, 500)\) μA and \(\tau = 5\) ms.
Figure 5. The intensity of background fluctuations affects the dynamical response of cortical neurons. The impact of the noise variance \( \sigma^2 \) was examined across a wide range of input frequencies \( f \), in 4 distinct cells (a–d), under the same conditions of Figure 3. Strong background noise smoothes \( r_f(f) \) at intermediate frequencies, as in a programmable equalizer. Linear instead of logarithmic scale has been employed here for the y-axis. Each subpanel (top to bottom) reports \( r(f) \) and \( \Phi(f) \), identifying the suprathreshold or weak-noise regime ("supra"—black markers) and the subthreshold or strong-noise regime ("sub"—red markers) by different marker shapes and colors. Each regime is characterized by distinct values for \( I_0 \) and \( s^2 \), adapted to yield a similar mean rate \( n_0 \) to 20 Hz. Experimental data points (markers) have been plotted together with the best-fit predictions from a phenomenological filter model (continuous traces). For these cells, band-pass 2nd-order filters (i.e., \( n = 2 \)—eq. 6) were found to describe the experimental data with high significance (see Supplemental Table S1). Error bars represent the 95% confidence intervals, obtained by the Levenberg-Marquardt fit algorithm. High-frequency error bars were sometimes much larger than the "threshold-to-peak" change of 10 mV/ms, and it was in the range of 0.3–0.5 ms. As "old" as the membrane voltage corresponding to a rate of each action potential, upon conventional definition of "threshold" (i.e., "subthreshold"). Thus, the mean firing rate \( n_0 \) can be brought to spiking even when its average input is below fluctuations are induced in the membrane voltage, the neuron only when its input current surpasses a certain threshold (i.e., the rheobase current). When the input current is noisy and \( \Delta t \) between input and output. This delay was in the range 0.3–1.1 ms, sometimes much larger than the "threshold-to-peak voltage" lag \( \tau_{sp} \) during a spike. \( \tau_{sp} \) quantifies the rising phase of each action potential, upon conventional definition of "threshold" as the membrane voltage corresponding to a rate of change of 10 mV/ms, and it was in the range of 0.3–0.5 ms. As expected from the previous report (Fourcaud-Trocme et al. 2003), \( \Delta t_{model} \) was always equal to \( \tau_{sp} \) in single-compartmental computer simulations (not shown). However, the mismatch between \( \tau_{sp} \) and \( \Delta t \) observed in some cells might be explained in terms of relevant additional axo-somatic and somato-axonic propagation latencies of about 0.2 ms each. This was measured directly by Palmer and Stuart (2006), who reported that cortical cells initiate action potentials at the distal end of the initial axon segment (see also Shu et al. 2006).

The Background Noise Affects the Neuronal Dynamical Response at Intermediate Frequencies

In the absence of background fluctuations, a neuron discharges only when its input current surpasses a certain threshold (i.e., the rheobase current). When the input current is noisy and \( \Delta t \) between input and output. This delay was in the range 0.3–1.1 ms, sometimes much larger than the "threshold-to-peak voltage" lag \( \tau_{sp} \) during a spike. \( \tau_{sp} \) quantifies the rising phase of each action potential, upon conventional definition of "threshold" (i.e., "subthreshold"). Thus, the mean firing rate of the neuron \( n_0 \) is determined by both the mean current \( I_0 \) and the standard deviation \( s \) of the noise. At the beginning of each experiment, \( I_0 \) and \( s \) were tuned to obtain the same mean firing rate \( n_0 \), chosen in the range 10–20 Hz. This allowed us to evoke 2 different discharge regimes, reflected in the degree of the irregular firing; the suprathreshold or weak-noise regime and the subthreshold or strong-noise regime. In the weak-noise regime, the background input fluctuation amplitude \( s \) was set to 20–50 pA and its mean \( I_0 \) was chosen above rheobase. Conversely, in the strong-noise regime, \( I_0 \) was set below rheobase, and \( s \) was increased until \( n_0 \) matched the value obtained in the suprathreshold regime.

Figure 5 summarizes the results of these experiments, reporting the responses of 4 typical cells (see also Fig. 3). It shows that the intensity \( s \) of the background noise, mimicking synapnic firing as well as presynaptic background cross-correlations (Rudolph and Destexhe 2004), differentially affects the neuronal response. This occurs especially at intermediate frequencies, flattening the response profile, and smoothening resonances as predicted in theoretical studies (Knight 1977a; Brunel et al. 2001; Fourcaud-Trocme et al. 2003; Richardson et al. 2003). The modulation of the neuronal discharge does not appear significantly attenuated at frequencies lower than 100–300 Hz in both regimes (see also Fig. 2), as for Figure 3 but plotted in linear instead of logarithmic scale for the vertical axis. At low input frequencies \( f \) (1–20 Hz), an increase in \( n_0 \) and a phase-advance were always observed (see Figs 5 and 6). These effects are apparent when analyzing single-cell responses rather than population averages (compare Figs 3 and 5).
In general, uniform and dense sampling of the frequency axis was not practicable, given the limited time window for stability and reproducibility of the neuronal response in typical recordings (see the Methods). This resulted in privileging high frequencies in some experiments (e.g., see Fig. 4) while neglecting intermediate frequencies in others, and vice versa (e.g., Fig. 6). This prompted us to test a posteriori whether data points collected simultaneously on the response magnitude $r_1$ and phase $\Phi$ were consistent with the hypothesis of linearity, while providing meaningful interpolations between samples (see Fig. 5d). In fact, the mutual relationship between $r_1$ and phase $\Phi$ cannot be arbitrary in a linear system. Therefore, a filter model (eqs. 6 and 7, see the Methods) was routinely employed to fit the data from each experiment. This model captured the neuronal response to the input signal component and its best-fit attenuation and phase-shift were plotted in Figures 5 and 6 as thick continuous lines. As in electrical filters made of linear resistors, capacitors and inductors (Horowitz and Hill 1989), the number and location of the model intrinsic time-constants account for integer power-law behavior and for low frequencies resonances and phase-advance, while matching the profiles of $r_1$ and $\Phi$ simultaneously. Changing the background noise level (black and red colors in Figs 3 and 5) resulted only in a shift in the best-fit values of the intrinsic time-constants of the model and required no modification of their number. This shift was smaller for faster time-constants (i.e., less than $\pm$ 30%, for time constants below $\sim$ 3 ms—see Supplemental Table S1), indicating that the high-frequency response of the neuron was generally unaffected by the noise intensity.

**Background Temporal Correlations Do Not Speed up Neuronal Reaction Times**

The timescale of background fluctuations (i.e., the “color” of the noise) was systematically varied in our experiments (Fig. 6). This is set by the correlation time $\tau$ of the noise (eq. 2) that mimics the decay time-constant of synaptic currents. In previous theoretical studies, the dependence of $\Phi$ on $\tau$ was emphasized (Brunel et al. 2001), suggesting that synaptic noise might have an impact on the reaction times to fast inputs transients reducing the response phase-lag to zero and removing amplitude attenuations (Knight 1972a; Gerstner 2000). Here, we explored the effect of changing the values of $\tau$ in the range 5–100 ms, thereby mimicking the contribution of fast (AMPA- and GABA$_A$-mediated), slow (NMDA- and GABA$_B$-mediated) synaptic currents. Both $\Phi$ and $r_1$ showed sensitivity to $\tau$ for intermediate frequencies, but not in the high-frequency regime, as plotted in Figure 6 for 4 typical cells. This is consistent with the results of the simulations of a conductance-based model neuron (not shown), and with the predictions of Fourcaud-Trocmé et al. (2003).

As discussed in the previous section and shown in Figure 5, $r_1(f)$ and $\Phi(f)$ could be simultaneously fit by the frequency response of a linear filter model. A change of the noise time-constant $\tau$ shifted the best-fit parameters, but required no modification of their number. The shift was smaller for faster
weakly interacting with each other and receiving the same input signal. In Figure 7b, a sample waveform of the broadband input was plotted, together with the raster-plots of the spikes evoked across hundreds of cycles and repetitions. In analogy to the analysis shown in Figure 2, the peristimulus time histograms (PSTHs) computed from the raster-plot was used to estimate the instantaneous firing rate \( r(t) \).

Although instantaneous input amplitudes were not small compared with \( \theta \), the phenomenological filter employed in Figures 5 and 6 could predict the time-varying neuronal response with satisfying accuracy over a wide range of output firing rates (Fig. 7), tracking fast input transients. However, to account for large negative input amplitudes that occasionally occur, a minimal current-threshold was needed in cascade to the linear filter (eqs. 4, 6, and 7). Without it, the correct dynamical range of the response could not be replicated and the fitting procedure led to low prediction performances. The order “nonlinear-linear”, sketched in Figure 7a, was preferred to the “linear-nonlinear” (Sakai 1992) as it systematically led to slightly superior fit performances, as well as to a possible interpretation as the neuronal rheobase.

**Discussion**

In the present work we studied the basic questions of how neurons encode time-varying inputs into spike trains, how efficiently they achieve it and what the impact of the background noise is. This is of central importance to understand network activities like network-driven persistent oscillatory regimes, which depend on the single-cell dynamical response properties and on recurrent connectivity.

Previous studies used deterministic oscillating inputs in invertebrate (Knight 1972b; French et al. 2001) and vertebrate neurons in hippocampus and entorhinal cortex (Schreiber et al. 2004), in thalamocortical neurons (Smith et al. 2000), in spinal interneurons and motoneurons (Baldissera et al. 1984), in the vestibular system (du Lac and Lisberger 1995; Ris et al. 2001), in the auditory (Liu et al. 2006), and visual systems (Victor and Shapley 1979a, 1979b; Sakai 1992; Carandini et al. 1996; Nowak et al. 1997), with emphasis on spike timing and reliability (Fellous et al. 2001; Schaette et al. 2005) and synchronization (Gutkin et al. 2005). Our results extend those studies in 2 ways: 1) by examining the contribution of background fluctuations and 2) by systematically exploring the dynamical response properties up to the high-frequency range (1 kHz).

By the interpretation of the instantaneous firing rate as a population activity, our analysis suggests that cortical ensembles are extremely efficient in tracking transients that are much faster than the membrane time-constant (~20 ms—see the Methods) and the average interspike interval (~1/\( \tau_0 \) ~ 50 ms) of individual cells. This finding was anticipated by many theoretical studies and it correlates with the previous observations that single cortical neurons (Mainen and Sejnowski 1995) and hypoglossal motoneurons (Powers et al. 2005) may have phase-locked firing responses to fast-varying current inputs, as well as with the study of Bair and Koch (1996), who observed large cut-off frequencies in the power spectra of the responses of middle temporal cortical neurons to in vivo random visual stimulation. However, our results extend the previous studies to the case of high-frequency phase-locking of the population firing rates, under noisy background. Although this is not unexpected (Knight 1972), our findings disprove
that the noise and its temporal correlations make a neuronal population respond instantaneously to an input (Knight 1972a; Gerstner 2000; Brunel et al. 2001; Silberberg et al. 2004). In fact, both noise intensity and correlation time modulate the neuronal response only at low and intermediate input frequencies and do not affect the low-pass filtering profile of the response, in agreement with Fourcaud-Trocme et al. (2003) and with Naundorf et al. (2005).

The location of the observed cut-off frequency was higher than the predictions from single-compartmental conductance-based model neurons (Fourcaud-Trocme et al. 2003). In those studies, the cut-off was of the order of \( r_0 \) and increased with the increasing sharpness of the action potentials. Similarly, Naundorf et al. (2005) observed an increase in the neuronal response at input frequencies much higher than \( r_0 \) (i.e. up to 200 Hz) for increasing action potential onset speed, while studying a phase-oscillator point neuron model. We propose that the effective spike sharpness could be higher than what was previously modeled at the soma. We speculate that a multicompartamental description that incorporates the details of axonic spike initiation (McCormick et al. 2007; Shu et al. 2007) might quantitatively support our experimental observations.

We observed a phase-advance at low input frequencies that was previously interpreted mechanistically on the basis of ion currents responsible for spike-frequency adaptation (Fleidervish et al. 1996; Ahmed et al. 1998; Fuhrmann et al. 2002; Compte et al. 2003; Paninski et al. 2003), as well as of resonances of the membrane impedance (Brunel et al. 2003; Richardson et al. 2003). These hypotheses are consistent with the input membrane impedance (Brunel et al. 2003; Richardson et al. 2003), as well as of resonances of the location of the cut-off frequency as well as of the resonances due to subthreshold resonances (Richardson et al. 2003). These hypotheses are consistent with the input range we investigated.

Finally, the local slope (i.e., gain) of the static \( f-I \) curve affects neuronal responses regardless of the input modulation frequencies (Fourcaud-Trocme et al. 2003). Previously reported gain-modulations induced by background noise (Chance et al. 2002; Higgs et al. 2006) are qualitatively distinct than the effects shown in Figures 5 and 6, as they act by scaling the firing rate output of the neurons across all the input frequency-bands.

Our experimental results then suggest that the action potential is a major evolutionary breakthrough, not only for making possible long-distance propagation of signals, but more importantly because it represents a powerful large-bandwidth digital intercellular communication channel, through population coding. In fact, our work shows that population coding with spikes has no significant attenuation in the range 0–200 Hz, while it compensates the heavy drawbacks of the analog intracellular membrane properties, which filter out input frequencies faster than ~50 Hz.

**Relations to Reverse-Correlation Methods**

Neural coding and the dynamical characterization of the input-output transformation operated by neurons, have been previously addressed by using methods of stimulus reconstruction (Bialek et al. 1991; Rieke et al. 1995) or reverse correlation (de Boer and Kuyper 1968; Gerstner and Kistler 2002). The last identifies the typical input current preceding a spike. Such a procedure estimates the 1st-order Wiener kernel and thus the linear component of the system (Kroll (1992) even though underlying nonlinearities might be present "in cascade" (Chichilnisky 2001). The reverse correlation kernel is proportional to the impulse response of the linear response of the neuron and it characterizes the "meaning" of each spike (Kroll 1992). The frequency-domain characterization that we considered so far directly relates to such an impulse response upon Fourier transform, although here we focused only on the encoding of the input signal (and not of the overall waveform) into the output. We thus generalized the previous experimental investigations to include the effect of background noise.

Consistently with the weak impact of background noise at high input frequencies that we reported, one might expect that similar cut-off frequencies (i.e. 100–200 Hz) were quantitatively observed by previous investigators, although they might have not included any background noise. For instance, the "stimulus kernel" identified by Powers et al. (2005) in motoneurons by injecting stationary "white"-noise inputs, appears to be dominated by a single decay time-constant in the order of 5–10 ms, indeed matching the 100–200 Hz cut-off frequencies of our data. Similarly, the low-noise phase-advance properties we observed (e.g., Fig. 2a) and the filter model intrinsic (high-pass) time-constants identified in our experiments, quantitatively correlate with the "feed-back" kernel computed by the same...
authors after selecting short and long interspike-intervals to unveil the effect of spike-frequency adaptation.

Finally, with the aim of further exploring the relationship of our approach with the previous ones, we directly computed the spike-triggered average (STA) of the input current preceding a spike, in 3 experiments where a broadband signal \( I(t) \) was injected. In Figure 8, we compare the Fourier transform of the STA to the frequency response of the best-fit linear filter model optimized to match the instantaneous firing rate (as in Fig. 7). As expected interpreting the STA as the 1st-order kernel reveals striking similarities between the 2 approaches, especially for frequencies higher than 200 Hz.

**The Phenomenological Filter Model**

Linear response properties are relevant to predict the response to complex noisy waveforms, even though the hypothesis of small input amplitude was not strictly respected by \( t_o(t) \). As discussed by Carandini et al. (1996), our experiments support the idea that in vivo membrane potential fluctuations linearize the response to stimulus-related input components (Masuda et al. 2005). Consistently, the neuronal response \( r(t) \) could not be captured by employing a static nonlinearity alone (not shown), even though for stationary noisy stimuli a similar description is appropriate (Rauch et al. 2003; Giugliano et al. 2004; La Camera et al. 2006; Arsiero et al. 2007). The additional cascade threshold-linear element simply relates to the presence of a minimal input threshold. It is interesting to note that the piecewise-linear profile of such nonlinearity reflects the minor role played by distortions and harmonics in our experiments.

Summarizing, a simple “cascade” model could quantitatively capture the time course of the instantaneous discharge rate (see also Shelley et al. 2002; Gutkin et al. 2005; Schaeffer et al. 2005), although it neglected the precise firing times. On the other hand, these can be captured by spiking neuron models, as in Jolivet et al. (2006) and Paninski (2006), identifying the parameters of an exponential (or quadratic) integrate-and-fire including spike-frequency adaptation as in Brette and Gerstner (2005).

**Cortical Rhythms**

Slow inputs produced a phase-advance of the output response whereas fast inputs a phase-lag, relative to the input modulation (Fuhrmann et al. 2002). This has been proposed to have important consequences for emerging population dynamics in recurrent networks, as the signals propagation between pre- and postsynaptic spikes does not only depend on the synaptic delays but also on the (oscillation frequency-dependent) delay introduced by the postsynaptic neuron itself. The fact that spike timing depends on \( f \) is particularly relevant for the emergence of population rhythms including fast ripples (Buzsaki et al. 1992; Csicsvari et al. 1999; Grenier et al. 2003; Buzsaki and Draguhn 2004; Buzsaki et al. 2004). In fact, the spikes of a presynaptic neuron, which is engaged in network-driven oscillations, generate periodic synaptic currents. Then the postsynaptic neuron experiences the periodic maxima of these currents after a synaptic delay, and responds to such a current signal reaching the maximum of its firing rate with an additional delay \( \Phi(\tau) \) and attenuation \( r_i/\tau_i \). If both presynaptic and postsynaptic neurons are participating in the same global rhythm, the overall delay between the pre- and postsynaptic spikes must be consistent with the period of the global oscillation and no strong attenuation should occur at that frequency, as shown in computer simulations by Fuhrmann et al. (2002), Brunel and Wang (2003), and Geisler et al. (2005). Therefore, not every oscillation frequency \( f \) is compatible with a given recurrent network architecture, synaptic coupling and firing regime.

We showed that the phase-shift and response amplitude of L5 pyramidal cells depends on the background fluctuations (Figs 5 and 6). This suggests that the frequency of emerging rhythms can be modulated by a background network embedding those neurons, as the phase of single-cell response is affected. More general, any network activity that relies on the timing of recurrent spikes is governed not only by the synaptic dynamics but is also controlled by the response properties (\( \Phi(\tau), r_i \)) of single cells.

**Supplementary Material**

Supplementary material can be found at: [http://www.cercor.oxfordjournals.org/](http://www.cercor.oxfordjournals.org/)

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