Single-Neuron Discharge Properties and Network Activity in Dissociated Cultures of Neocortex

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Giugliano, M., P. Darbon, M. Arsiero, H.-R. Lüscher, and J. Streit. Single-neuron discharge properties and network activity in dissociated cultures of neocortex. J Neurophysiol 92: 977–996, 2004. First published March 24, 2004; 10.1152/jn.00067.2004. Cultures of neurons from rat neocortex exhibit spontaneous, temporally patterned, network activity. Such a distributed activity in vitro constitutes a possible framework for combining theoretical and experimental approaches, linking the single-neuron discharge properties to network phenomena. In this work, we addressed the issue of closing the loop, from the identification of the single-cell discharge properties to the prediction of collective network phenomena. Thus, we compared these predictions with the spontaneously emerging network activity in vitro, detected by substrate arrays of microelectrodes. Therefore, we characterized the single-cell discharge properties to Gauss-distributed noisy currents, under pharmacological blockade of the synaptic transmission. Such stochastic currents emulate a realistic input from the network. The mean (m) and variance (s^2) of the injected current were varied independently, reminiscent of the extended mean-field description of a variety of possible presynaptic network organizations and mean activity levels, and the neuronal response was evaluated in terms of the steady-state mean firing rate (f). Experimental current-to-spike–rate responses f(m, s^2) were similar to those of neurons in brain slices, and could be quantitatively described by leaky integrate-and-fire (IF) point neurons. The identified model parameters were then used in numerical simulations of a network of IF neurons. Such a network reproduced a collective activity, matching the spontaneous irregular population bursting, observed in cultured networks. We finally interpret such a collective activity and its link with model details by the mean-field theory. We conclude that the IF model is an adequate minimal description of synaptic integration and neuronal excitability, when collective network activities are considered in vitro.

INTRODUCTION

In vivo neocortical neurons receive a continuous barrage of excitatory and inhibitory postsynaptic potentials (EPSPs/IPSPs) (Destexhe and Paré 1999). Such an intense background activity arises from the intracortical presynaptic neurons, spontaneously spiking at low rates, and it induces the postsynaptic membrane voltage to fluctuate as in a random walk, resulting in an irregular spike emission (Destexhe et al. 2003). Under such conditions, the biophysical properties of the neurons are substantially altered compared to those of a neuron at rest (see Bernander et al. 1991 and references therein; Destexhe et al. 2003). Such considerations define a different scenario with respect to traditional noise-free in vitro experimental paradigms, used either in the characterization of the integrative properties of single neurons or in the investigation of emerging network phenomena. As a consequence, observations carried out in vitro may not directly transfer to the in vivo situations (Steriade 2001).

Even more important, the characterization of the single-neuron response properties, performed under appropriate and realistic conditions, is a key element to predict and understand how a population of neurons collectively interacts and processes information in the intact brain. This view is supported by several theoretical studies (Amit and Tsodyks 1992; Brunel 2000; Mattia and Del Giudice 2002; Salinas 2003), where the single-neuron response properties were used to make predictions about the collective phenomena, such as the global spontaneous irregular activity (Amit and Brunel, 1997b), the emergence of network-driven oscillations (Brunel and Wang 2003; Fuhrmann et al. 2002), and of selective delay-activity states (Amit and Brunel 1997b; Wang 2001; Yakovlev et al. 1998).

Under these perspectives, novel electrophysiological paradigms were recently proposed by several experimenters, focusing on the integrative (Destexhe and Paré 1999, 2000; Poliakov et al. 1997; Rauch et al. 2003), computational (Chance et al. 2002; Mainen and Sejnowski 1995; Protopapas and Bower 2001), and adaptive properties of single neurons (Fuhrmann et al. 2002; Paninski and Bower 2003) and synapses (Froemke and Dan 2002; Sjöström et al. 2001). In particular, Rauch et al. (2003) proposed to mimic the conditions that neurons experience in the recurrent networks of the intact cortex. They reproduced a realistic background synaptic activity as a computer-synthesized noisy current that was injected into the soma, as shown in Fig. 1, A and B (see also Destexhe and Paré 2000). Under such conditions, layer V pyramidal neurons in acute neocortical slices respond as integrate-and-fire (IF) point neurons (Rauch et al. 2003). This suggests that an extremely simplified model neuron may be used to accurately describe the discharge properties of a single cell under simulated in vivo conditions and to predict and interpret collective emergent phenomena. Unfortunately, because it is not straightforward to observe, induce, or re-create coordinated network activity in neocortical slices (but see Giugliano and Lüscher 2003; Giugliano et al. 2003; and Shu et al. 2003), there was no attempt at validating any network-level prediction in vitro, resulting from the proposed minimal characterization of the single cell. In addition, a direct confirmation of the same predictions in awake and...
behaving animals is at present impossible or still requires extremely challenging technical issues to be overcome.

For such reasons, we decided to focus on the quantitative characterization of the discharge properties of neocortical cultured neurons, dissociated from the neocortex. Such a preparation has been widely used for investigating single-neuron properties as well as the network activity, which spontaneously emerges in vitro (see for a review Marom and Shahaf 2002). In particular, developing networks of synaptically connected cortical neurons can be grown and cultured on arrays of substrate microelectrodes (MEAs), so that the distributed electrical activity can be recorded for a long time, with a high spatial resolution (Potter 2001).

In this paper, we show that mature cultured neurons retain the features that characterize neurons in acute brain slices, investigated under the same experimental paradigm. Under these conditions, they respond as IF point neurons, not only with respect to the output mean firing rate but also to the subthreshold membrane voltage statistics, confirming and extending the work of Rauch et al. (2003). As a step toward the quantitative description of the mechanisms underlying population phenomena in cultured networks (Jimbo et al. 1999; Kamioka et al. 1996; Maeda et al. 1995; Marom and Shahaf, 2002), we computer-simulated the electrical activity of a network of model neurons, incorporating the single-neuron details identified in the experiments. We further interpret the results of our simulations by the extended mean-field theory (Renart et al. 2003), and we compare them with in vitro experimental recordings, using the MEAs.

METHODS

Cultures

Cultures of neocortical neurons were obtained from the somatosensory/motor cortex of newborn Wistar rats (P1-2), following standard procedures. Rats were anesthetized (0.4 ml Vetanacol) and killed by decapitation. Transverse brain slices, 225 µm thick, were exposed to a 0.3% trypsin solution for 3 min at 37°C for enzymatic digestion. Cells were mechanically dissociated and plated on substrate arrays of a 0.3% trypsin solution for 3 min at 37°C for enzymatic digestion.

Cells were microfabricated and plated on substrate arrays of planar microelectrodes (MEAs) or glass coverslips, at a density of 150,000 or 75,000/150 µl, respectively. MEAs were microfabricated as described previously (Tscherter et al. 2001) and coated for 1 h with diluted (1:50) Matrigel (Falcon/Biocat, Becton Dickinson AG, Basel, Switzerland). The glass coverslips were coated with polylysine (1 mg/ml overnight at 37°C or Matrigel (1:50). Cells were restricted to a small area (~50 mm²) using cloning glass cylinders attached to the MEAs or coverslips by silicone sealant. Cultures were incubated at 36.5°C in a 5% CO₂ air atmosphere and maintained in 150 µl nutrient medium. The medium contained MEM Eagle (Sigma) supplemented with fetal bovine serum 10%, glucose 0.2%, B27, and Glutamax (Gibco BRL, Life Technologies AG, Basel, Switzerland) for the cultures on glass coverslips, and serum-free Neurobasal medium supplemented with B27 and Glutamax (Gibco BRL) for the cultures on the MEAs. Half of the medium was changed weekly.

Recordings were made in a chamber mounted on the stage of an upright microscope (Nikon, Tokyo, Japan). Patch-clamp experiments were carried out on cells in which a few processes could be visually identified (e.g., Fig. 1E), from cultures of 4–7 wk of in vitro age. Within this period, no systematic change of single-neuron and network properties was detected, suggesting a complete maturation of the neurons and indicating that the cultures’ development had reached a steady state (Kamioka et al. 1996; Marom and Shahaf 2002).

Pharmacology

Several minutes before starting each recording session, the culture medium was replaced by an extracellular solution containing (in mM): NaCl 145, KCl 4, MgCl₂ 1, CaCl₂ 2, HEPES 5, Na-pyruvate 2, glucose 5, at pH 7.4. Recordings were performed either in the absence of a continuous flow of solution, with solution changes every 10–15 min, or in the presence of continuous superfusion at 1 ml/min. No differences were detected between these protocols. All recordings were made at room temperature (23–24°C).

As extensively described in the literature, mature cultures of dissociated neocortical neurons exhibit a spontaneous electrical activity, which results in simultaneous bursting network activity and mainly depends on the synaptic development and coupling between the neurons (Jimbo et al. 1999; Kamioka et al. 1996; Maeda et al. 1995; Marom and Shahaf 2002). In the patch-clamp experiments, given that our goal was to characterize the single-neuron response properties to a class of computer-synthesized stimulus waveforms, single-neuron recordings were performed while blocking glutamatergic synaptic transmission with D-APV (d-2-amino-5-phosphonovalerate 50 µM), a competitive antagonist of the NMDA (N-methyl-D-aspartate) receptors, and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, 10 µM) (both Sigma, Buchs, Switzerland), a competitive antagonist of non-NMDA receptors. These substances were bath applied and completely suppressed incoming synaptic activity and spontaneous spiking in the neurons. In the experiments involving multisite extracellular recordings of the neuronal electrical activity by the MEAs, an antagonist of GABAₐ receptors (bicuculline methochloride, 10 µM) (Tocris, Anawa Trading SA, Wangen, Switzerland) was bath applied to block synaptic inhibition, with the aim of focusing on the disinhibited pattern generation as well as establishing a more direct comparison to the computer-simulated networks, which included only excitatory model neurons.

Whole cell patch-clamp recording from single neurons

The patch-clamp technique was used, in the whole cell configuration (Hamill et al. 1981), by using an Axoclamp-2B amplifier (Axon Instruments, Union City, CA). Signals were recorded in current clamp, filtered at 1 kHz, sampled at 5 kHz, and digitized by a 12-bit A/D converter (Digidata 1200) and pClamp 8 software (Axon Instruments). Electrodes were pulled from filamented borosilicate glass capillaries (GC150F, Harvard Apparatus GmbH, March-Hugstetten, Germany) on a horizontal puller (DMZ, Zeitz Instrumente GmbH, Munich, Germany) and their resistance was 5.5 ± 0.6 MΩ. Electrodes were filled with a solution containing (in mM): K-glucate 100, KCl 20, HEPES 10, Mg-ATP 4, Na₂-GTP 0.3, Na₇ phosphocreatine 10; pH 7.3, 290 mOsm. Other (standard) pipette solutions were reported not to alter significantly the response properties of the cells, under the very same experimental protocol (Rauch et al. 2003). High-resistance seals (2–4 GΩ) were formed and the whole cell configuration was achieved by the application of a negative pressure pulse. The bath application of D-APV and CNQX always followed the establishment of the whole cell patch configuration.

Conventional procedures, consisting of repetitive hyperpolarizing step currents, were used to obtain a direct estimate of the passive (cable) properties of cultured neurons (Iansek and Redman 1973). Such estimates are related to the time constant τm and capacitance Cm of an equivalent lumped RC compartment (Abbott and Dayan 2001).

Emulating a stationary realistic input from the network

Independent realizations of the stochastic Ornstein–Uhlenbeck process were computer-synthesized and injected under current clamp (see Fig. 1C). (Interactive stimuli-synthesis and analysis software tools were developed and are available on request.) Such nondeterministic current stimuli mimic a realistic barrage of excitatory/inhibitory

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postsynaptic currents (EPSCs/IPSCs) for a cell embedded in a large in vivo network, spontaneously and randomly active at low rates (Destexhe et al. 2003), as well as in a cultured network where spontaneous neurotransmitter release at individual synapses, as well as other sources of inhomogeneity and randomness determine an irregular background synaptic noise in vitro (Maeda et al. 1995). Although a conductance injection would have appeared more appropriate (e.g., by means of the dynamic-clamp technique; see Destexhe and Pare 2000), it has been proven that noisy conductance-driven and noisy current-driven stimulations are equivalent with respect to the evoked neuronal steady-state mean firing rates, apart from 2 stimulus-independent scaling factors, for the input mean and variance (La Camera et al. 2003; Rauch et al. 2003). More important, the comparison of the experimental data to the predictions of mathematical model neurons has been carried out under the same current-driven conditions. Under these hypotheses and because the impact of a single EPSC/IPSC on the postsynaptic membrane voltage is weak in evoking suprathreshold responses in vitro (Nakanishi and Kukita 1998; Nakanishi et al. 1999), the overall current experienced by a generic postsynaptic neuron can be approximated as a diffusion stochastic process (Destexhe and Paré 2000; Fourcaud and Brunel 2002). Therefore, under extended mean-field hypotheses on the interactions between neurons in a population (Amit and Brunel 1997b), such noisy stimuli may statistically account for a wide range of feedforward/recurrent network architectures and regimes. For instance, by indicating with $N_{ex}$ the number of excitatory/inhibitory afferents with stationary mean activation rates $f_{ex}$, under the hypothesis that synaptic inputs are approximately independent, the distribution of the resulting postsynaptic somatic current amplitude becomes Gaussian (i.e., by the central-limit theorem), with steady-state mean $m$ and variance $s^2$ given by the expressions reported below (Amit and Brunel 1997b)

$$m = N_{ex}f_{ex} \tau_e - N_{in}f_{in} \tau_i,$$

$$s^2 = N_{ex}f_{ex} \tau_e^2/2 + N_{in}f_{in} \tau_i^2/2,$$

where $\tau_e$ and $\tau_i$ are the effective peak-amplitude and decay time constant at the soma, for individual excitatory and inhibitory postsynaptic currents, respectively. Throughout this work, we set $\tau_e = \tau_i = \tau_f \in \{1; 5\} \text{ ms}$, thereby mimicking (AMPA- and GABA$_A$-mediated) fast synaptic currents (Destexhe et al. 1994).

We note that the features of the incoming synaptic current arising from the particular spike-timing precision and reliability of individual presynaptic neurons were not explicitly investigated (Mainen and Sejnowski 1995; Jolivet et al. 2004). Consistently, the neuronal response to such noisy current input was routinely analyzed by characterizing the output mean spike rate at the steady state. However, provided that the hypothesis on the statistical independence of the presynaptic activity holds, the impact of neuronal precision and reliability is expected to weakly contribute to the network activity we discuss in the present work. Spontaneous synaptic release and other sources of network randomness will in fact introduce uncorrelated stochastic components to the overall synaptic inputs to any neuron. Therefore, under such an hypothesis, the overall resulting current incoming to a generic postsynaptic neuron of the network is still Gaussian-distributed and completely characterized by mean $m$ and variance $s^2$.

**Noisy current-clamp protocol**

The stimulation protocol consisted of the repeated somatic injection of independent current realizations $I(t)$, each lasting 20 s and interleaved by 30–60 s of recovery time. For any pair $(m, s^2)$, the following iterative expression was used to synthesize a realization $I(t)$ of the process (Cox and Miller 1965)

$$I(t + dt) = I(t) + \left[ m - R(t) \right] \frac{dr}{\tau_f} + s \sqrt{ \frac{2dr}{\tau_f} } \xi,$$

where $\xi$ is a unitary Gauss-distributed random variable (Press et al. 1992), updated at every time step at a rate of 5 kHz (i.e., $dt = 0.2$ ms) (Fig. 1C). The exploration of the plane $(m, s^2)$ typically requested 30–45 min to be completed and it was carried out in a shuffled order, randomly repeating each pair twice. During such a time interval, the
lack of spontaneous synaptic events, the stability of the resting membrane potential, and the cell input resistance were continuously monitored. The entire procedure was stopped in case of drifts in any of these observables.

Analysis of the single-neuron response properties

Collected data consisted of the voltage responses to each noisy stimulus, lasting 20 s and associated with the pair \((m, s^2)\) (see Fig. 1D). To account for current-clamp offsets, the actual injected current was also monitored and the actual value of \((m, s^2)\) directly estimated from it. Raw voltage traces were processed in Matlab (The MathWorks, Natick, MA) by a peak-detection algorithm to extract individual spike times and shape. When no substantial change in the shape of the individual action potentials, or in the instantaneous firing rate at the beginning and at the end of the elicited spike train occurred (representative of possible nonstationarities), the trial was accepted. The steady-state mean spiking frequency \(f\) was estimated, discarding an initial transient (i.e., 2–5 s) and averaging across the 2 available repetitions. After a successful completion of the stimulation protocol, the experimental curve \(f_s = f_s(m_n, s_n), n = 1, 2, \ldots , M\) (for at least 3 distinct values of \(s\) and typically \(M \sim 20\) was plotted and compared to the theoretical responses of 2 model neurons, driven by the same current statistics (Fig. 2). The recordings were further compared to the data available for pyramidal layer V neurons in acute slices, collected under the same experimental conditions (Rauch et al. 2003).

Mathematical model of the single-neuron response

The mathematical modeling and the procedures for the model fit to the collected response data are standard and closely followed Rauch et al. (2003). Briefly, 2 single-compartment standard mathematical descriptions of neuronal excitability were considered: the Lapicque’s or leaky integrate-and-fire neuron LIF (Abbott and Dayan 2001; Tuckwell 1988) and the constant leakage integrate-and-fire neuron with a floor (CLIFF) (Fusi and Mattia 1999; Mongillo and Amit 2001; Rauch et al. 2003) (see Fig. 2). These models have been studied in depth (Fourcaud and Brunel 2002; Mattia and Del Giudice 2002) and widely used in simulations of large-scale networks (Mattia and Del Giudice 2000; Reutimann et al. 2003) and hardware implementations (Chicca et al. 2003). As opposed to the biophysically realistic conductance-based models (Abbott and Dayan 2001), these descriptions are characterized by a single state variable \(V\) (i.e., the membrane potential) and by a reduced set of effective constant parameters: the absolute refractory period \(\tau_{\text{ref}}\) and reset voltage \(H\), the membrane capacitance \(C\), the voltage threshold for spike emission \(\theta\), and the subthreshold voltage decay rate \(-C/(E - V)/\tau\) for the LIF (\(\tau\) being the membrane effective time constant and \(E\) the resting potential) or \(-\lambda\) for the CLIFF model. Finally, both models incorporated a simplified spike-frequency–dependent adaptation, modeling the contribution of intracellular calcium- and/or sodium-activated outward currents to the net membrane current, modulated by a constant factor \(a\) and implemented as described in Liu and Wang (2001) and van Vreeswijk and Hansel (2001). The stationary effect of such an adaptation is to reduce the gain of the frequency–current curve by a factor that does not depend on the adaptation time constant \(\tau_a\) (see Eqs. 2 and 3). We refer as aLIF

![Diagram of single-compartment models](http://jn.physiology.org/)

**Fig. 2.** A: Sketch of the electrical equivalent circuits of the single-compartment models considered for the fit of the experimental responses: for the sake of simplicity neither the absolute refractoriness nor the spike-frequency–dependent adaptation was represented. The condition \(V(t) = \theta\) corresponds to the emission of an action potential at time \(t\). For both models, under the current-driven stimulation protocol (see Methods), a description of the steady-state discharge properties can be analytically derived. B: the response functions (Eqs. 2 and 3) were plotted for increasing fluctuations amplitude \(s\), including (i.e., aLIF, aCLIFF) or not including (i.e., LIF, CLIFF) the spike-frequency–dependent adaptation. Adaptation generally decreases the spike rates, while preserving the sensitivity to fluctuations \((s^2)\), as discussed by La Camera et al. (2002). The plots reveal a different dependency on \(s\) in the two models, evident at small and large \(s\) (50–800 pA, step 50 pA).
and aCLIFF, to the LIF and CLIFF models incorporating the spike-frequency–dependent adaptation, respectively (see Fig. 2B).

Model parameters fit to the experimental data

The full analysis of the response of the spiking neuron models to the input current, specified by Eq. 1 (i.e., a colored noise), can be performed only under approximate treatments (Fourcaud and Brunel 2002) but it was not considered in the present work. Instead, it is considerably easier to analyze the models’ response to an idealized “equivalent” white current (i.e., δ-correlated), characterized by the infinitesimal mean μ and variance σ² determining an asymptotically equivalent effect on the subthreshold membrane voltage V (Rauch et al. 2003). The last is a satisfactory approximation, when the evoked mean interspike interval is much larger than τₚ as verified by computer simulations. Under these hypotheses, the steady-state current-to-rate response function Φ(t, s²) can be analytically determined (Fusi and Mattia 1999; Tuckwell 1988) (Fig. 2B) and the corresponding parameter space may be searched for the best fit to the stationary data points \{f₉(mₙ, sₙ), h = 1, 2, ..., M\}, collected for each cell. The implicit formulas corresponding to the mean firing rate ΦₐLIF/₁ᵣₐₚ/CLIFF under noisy current stimulation are

\[
\Phi_{\text{aLIF}} = \left[ \tau_{\text{sp}} + \tau \sqrt{\pi} \int_0^{\infty} e^{-r^2}[1 + \text{erf}(s)]dr \right]^{-1}
\]

\[
\Phi_{\text{aCLIFF}} = \left[ \tau_{\text{sp}} + \left( \frac{(\theta - \bar{H}) + \omega(\sigma^2 e^{-\mu} - e^{-\mu\omega})}{\mu} \right) \right]^{-1}
\]

indicating for the aLIF model

\[
\hat{H} = (H - \mu)/(\sigma \sqrt{\pi}), \quad \hat{\theta} = (\theta - \mu)/(\sigma \sqrt{\pi})
\]

\[
\mu = (m - \alpha \Phi_{\text{aLIF}})/(C), \quad \sigma = (\sqrt{\pi})/\tau \quad \text{with} \quad \tau = RC \quad \text{membrane time constant (Fig. 2A), whereas for the aCLIFF model}
\]

\[
\mu = (m - \lambda - \alpha \Phi_{\text{aCLIFF}})/(C), \quad \sigma = (\sqrt{\pi})/\tau \quad \text{with} \quad \tau = (m + \sigma^2) \mu
\]

Without loss of generality, all the voltages were referred to 0 mV and the spike-emission threshold \(\theta\) was kept fixed at 20 mV, with respect to the resting membrane potential (i.e., \(E = 0\) mV and \(\theta = E + 20\) mV).

For each cell, simulated-annealing optimization techniques (Press et al. 1992) were used to fit the above reported theoretical response functions to the data, by minimizing the following mean-square error with respect to the model parameters

\[
\chi^2 = \sum_{h=1}^{M} \left[ \frac{f_{\text{exp}}(m, s)}{\hat{f}_h} - \Phi(m_h, s_h) \right]^2
\]

Each term of the sum is weighted by the inverse of an accuracy interval, estimated by using a simplified phenomenological binomial model of the spike-emission processes (Rauch et al. 2003). Briefly, by specifying a desired confidence level \(q\) on the spike rate estimate \(f_{\text{exp}}\), \(h = 1, 2, ..., M\) and indicating with \(N_{\text{sp}, h}\) the number of spikes emitted in the time interval \(T\) as a realization of a binomial random variable, the mean rate \(f\) and its experimental estimate \(f_{\text{exp}} = N_{\text{sp}, h}/T\) satisfy the following relationship

\[
\text{Prob} \{ f_{\text{exp}} - \delta_{\text{e}, h} \leq f < f_{\text{exp}} + \delta_{\text{e}, h} \} \geq q
\]

with

\[
\delta_{\text{e}, h} = \left\| 0.5K \pm K \sqrt{N_{\text{sp}, h} + 0.25K^2}/T \right\| \quad \text{and} \quad q = 1 - \text{erfc}(K/\sqrt{2})
\]

The last relationships imply that, by setting \(K \geq 1\), the resulting accuracy interval \(\delta_{\text{e}, h}\) for \(f_{\text{exp}}\) corresponds approximately to a confidence of at least 68%.

Finally, \(\chi^2\) and its minimum \(\chi_{\text{min}}\) are random variables, known to be approximately distributed according to a \(\chi^2\) distribution (Press et al. 1992). This makes it possible to refer to the probability Prob \(\chi^2 > \chi_{\text{min}}\) as a standard indication of the goodness of the fit. Model fits were accepted when such a probability was >0.1. The minimal value of \(K\) of successful \(\chi^2\)-test was chosen as a quantitative analogue measure of the quality of the fit, comparing the performances of the aLIF, LIF, aCLIFF, and CLIFF models over the entire data set. Finally, the parameters search was repeated twice for each experiment: under free-search conditions and introducing additive quadratic cost-penalty constraints to the simulated annealing energy (Press et al. 1992). This aimed at discouraging the exploration of the parameter space in a limit-valued region (i.e., \(H \rightarrow \theta\) and \(\tau_{\text{ap}} \rightarrow +\infty\)). We refer to these conditions as free search, or no-penalty best fit, and as penalty best fit.

MEA recording and analysis of the network activity

In some experiments, MEAs were used as a substrate for the cultured networks, so that neuronal somata as well as axons were allowed to develop close to the individual MEA microelectrodes. Such a proximity makes possible the extracellular detection of the emission of action potentials by one or more neighboring cells (Streit et al. 2001). MEAs contained 68 platinum planar electrodes, spaced at 200-μm intervals and laying out in the form of a rectangle. Recording channels showing activity were selected by eye and their recordings digitized at 6 kHz per channel and stored on a hard disk. The detection of extracellularly recorded action potentials (i.e., fast voltage transients) and further analysis were performed off-line in IGOR. (WaveMetrics, Lake Oswego, OR), as described previously (Streit et al. 2001; Tschirter et al. 2001). No attempt was made to sort spikes detected by the same MEA electrode. The electrical noise of individual channels was very small. The selectivity of event detection was routinely checked by recordings obtained in the presence of tetrodotoxin (TTX, 1.5 μM, Sigma), as a “zero” reference.

The spontaneous network activity, detected by the MEA, consisted of asynchronous activity and population bursts (PBs). The asynchronous activity was defined as one or a few spikes detected by a single or several electrodes, whereas the PBs, consisting of episodes of activity occurring simultaneously across several recording channels, spread over the network. The processed network activity was visualized in the form of event raster plots and of the instantaneous population mean firing rate (see Fig. 5A). The last was computed by counting the total number of detected events from all recording channels, within a sliding time window of 10 ms. With the aim of summarizing the features of the detected network activity, the mean and the coefficient of variation (CV) of the interburst intervals (IBI) distribution and of the burst durations (PBd) distribution were estimated over at least 10 min of continuous recording (15 PBd minimum).

Mathematical model of a network of IF neurons

We studied and computer-simulated the collective electrical activity of small networks of \(N_c = 100–1,000\) interacting excitatory aLIF identical neurons (van Vreeswijk and Hansel 2001), using the single-neuron effective parameters identified in the experiments (Table 1). We chose to neglect network inhomogeneities to keep the interpretation of the results as simple as possible. Anyway, our interpretative framework can be extended to cover a similar situation, following the approach described in Amit and Brunel (1997a).
In the model, each excitatory neuron is characterized at time $t$ by its membrane potential $V_i(t)$ and its adaptation current $X_i(t)$. These quantities follow the dynamical equations

$$\begin{align*}
CdV_i &= -C_iV_i + I_i(t) + m_0 + \xi_i \\
dX_i &= -X_i dt/	au_a
\end{align*}$$

indicating with $r_i^t$ the times of emission of a spike by the $i$th neuron [i.e., $V_i(t) = \theta$], the previous equations are complemented by the conditions

$$\begin{align*}
V_i(t) &\to H \quad t \in [r_i^t, r_i^{t+}] \\
X_i(t) &\to X_i(r_i^{t+}) + \tau_a^{-1}
\end{align*}$$

whereas, indicating with $C_p$ the connectivity matrix and by $\Theta(t)$ the unitary step function (i.e., $\Theta(t) = 0, t < 0$ and $\Theta(t) = 1, t > 0$), the total synaptic current $I_i(t)$ into the neuron $i$th is given by

$$I_i(t) = \sum_{j=1}^{N} J_{ij} C_p e^{-|r_j^t - r_i^t|}/r_i^t - \vartheta(t - r_j^t - \delta)$$

Focusing on cultured neocortical networks, we chose an unstructured connection topology (Marom and Shahaf 2002) with a probability $C_p$ of synaptic connection between any 2 neurons of 0.3–0.4 (i.e. Prob $\{C_{ij} = 1 | i \neq j\} = C_p$) (Nakanishi and Kukita 1998; Nakanishi et al. 2001). In agreement with the hypotheses underlying the stimuli injected in the experiments, the synaptic interactions were described by currents rather than conductance changes. The adaptation currents were also described as current changes, as well. Describing these variables by conductance changes does not qualitatively affect the results reported. Synaptic interactions between 2 connected neurons were triggered by the presynaptic emission of action potentials, after an effective delay $\delta$ of 1.5 ms, which included the axonal propagation delay and synaptic release latency (Nakanishi and Kukita 1998). The resulting individual postsynaptic currents were characterized by an instantaneous rise to $J_s$ and by an exponential decay with a time constant $\tau_a = 5$ ms, and no activity-dependent short- or long-term change. The effect of spontaneous synaptic release and of other sources of randomness (Maeda et al. 1995) was incorporated into an activity-independent additional random synaptic drive. The effective value of $\alpha$ unavoidably included the superimposed stationary contribution of both fast and slower adaptation mechanisms, estimated in the patch-clamp experiments over 20 s of stimulation time. With the aim of focusing on the time scales characterizing the network bursting (i.e., $<0.1–1$s), sometimes we decreased the value of $\alpha$ that affected the PBd and not the IBIs statistics. For the sake of simplicity, the intrinsic cumulative inactivation experimentally measured was not included in the simulations (but see Giugliano et al. 2002).

Under the same extended mean-field hypotheses that underlie the noisy currents that were injected into real neurons, the collective activity of the simulated network can be fully predicted in terms of its statistical mean firing rate $f$, by the knowledge of $\Phi_{d,1}(m, s^2)$ and the details of the synaptic connectivity (Amit and Brunel 1997b). In the present case, the statistics of the total recurrent synaptic current experienced by a generic neuron of the network is approximately Gauss-distributed and it can be fully described by its mean and variance

$$m(f) = N C_p J_s f \tau_a + m_0 \quad \text{and} \quad \sigma^2(f) = N C_p J_s^2 \tau_a/2 + s_0^2$$

where $f$ is the network mean firing rate and $J_s$ is the effective peak amplitude for the individual postsynaptic currents, uniform across all the synapses of the network. $m_0$ and $s_0^2$ are 2 fixed parameters, corresponding to the spontaneous neurotransmitter release and other sources of randomness, independent of $f$.

### Statistics

The Pearson’s $r$ linear correlation as well as the Kendall’s Tau nonparametric (rank-order) test (Press et al. 1992) were used to assess statistical correlations. The last provides a correlation measure together with an estimated significance level $p_X$, which corresponds to the probability of obtaining the same correlation from statistically independent samples. Averages are expressed as means ± SE.

### Results

#### Cultured neurons respond as simple IF neurons

As opposed to a DC stimulation (see Fig. 1A), under noisy current injection, the neuronal membrane voltage evolves in time as in a random walk, leading to irregular spike emission (see Fig. 1, A and B). Its subthreshold amplitude distribution becomes bell-shaped, with mean and SD increasing with the steady-state mean $m$ and variance $s^2$ of the injected current, respectively (see Figs. 1D and 4).

With respect to the steady-state spiking frequency, cultured neocortical neurons respond as simple IF neurons (Fig. 2), similarly to pyramidal neurons (Rauch et al. 2003) and with a...
similar response gain. Actually, the curves $f(m, s^2)$, collected for each cell, were quantitatively fitted with high accuracy by the IF model response functions, in the frequency range where the experimental response was stationary (Table 1). Spike-frequency–dependent adaptation ($\alpha$) was indeed required by the models to fit the data points at the steady state (La Camera et al. 2002).

No difference was found in the response properties of the neurons to noisy currents characterized by an autocorrelation time length $\tau_1 = 1$ ms ($n = 17$) and 5 ms ($n = 18$). Thus, in both cases the responses could be captured by the white-noise input approximation, mentioned under METHODS.

However, collected data resembled the aLIF and not the aCLIFF responses (Fig. 3), as opposed to pyramidal cells in acute slices, where both models were reported to describe the data set equally well (Rauch et al. 2003). The aLIF model was substantially better in fitting the responses of the cells over the entire set of experiments ($n = 35$) (see Fig. 3 and Table 1).

Moreover, cultured neurons showed very similar response properties compared to neurons from acute slices, when repetitively stimulated by the same noise realization. This was shown to yield a much higher precision in the timing of individual spikes, compared to a DC stimulus (Mainen and

![Graphs showing responses of cultured neurons](http://jn.physiology.org/)

**Fig. 3.** The plots report the results from 6 different experiments. Current-to-rate response of cultured neurons, dissociated from the rat neocortex, can be captured remarkably well by the integrate-and-fire (IF) dynamics. The responses were evaluated at the steady state as a function of $m$ (markers), for increasing values of $s$ (i.e., $s_1 < s_2 < s_3$, estimated from the injected current realizations) and compared to the corresponding best-fit prediction (lines), provided by the aLIF (left panels) and the aCLIFF models (right panels) (see also Fig. 2B). The aLIF model reproduced the responses of the cultured neurons with high accuracy, whereas the aCLIFF model routinely failed in passing the fit-confidence test. In both cases of free search and penalty best-fit search, the aLIF showed superior performances and a lower $K$ (see METHODS) (e.g., in the free-search best fit, $K = 1.31 \pm 0.09$ for the aLIF; $K = 2.03 \pm 0.12$ for the aCLIFF model). We note however that, even when the $\chi^2$-test gave low performances for both models, the average absolute discrepancy on the predicted frequency was always well below 2 Hz (i.e., 0.75 $\pm$ 0.07 Hz for the aLIF and 1.2 $\pm$ 0.11 Hz for the aCLIFF).
Sejnowski 1995), it was confirmed in a set of experiments \([n = 5, 26–43 \text{ days in vitro (DIVs), not shown}]\), and it suggests that the somatic spike emission mechanism of cultured neurons is not intrinsically noisy.

**Best-fit effective parameters and passive membrane properties**

As mentioned in METHODS, the passive membrane properties \((\tau_m \text{ and } C_m)\) of the patched neurons were routinely measured. As opposed to the previous report by Rauch et al. (2003), the passive properties and the corresponding best-fit effective parameters of the IF models showed a significant cross-correlation, both for the best-fit free search and the penalty search. We report the results of the Pearson’s \(r\)-test and, between parentheses, those of the Kendall’s Tau test. In the best-fit free search, between \(C_m\) and \(C\) the correlation coefficient was 0.66 (0.54) for the aLIF and 0.73 for the aCLIFF model, whereas between \(\tau_m\) and \(r\) it was 0.77 (0.57) for the aLIF. In the best-fit search with penalties, between \(C_m\) and \(C\) the coefficient was 0.56 (0.44) for the aLIF and 0.68 for the aCLIFF model, whereas between \(\tau_m\) and \(r\) it was 0.81 (0.61) for the aLIF. Such results were validated by a very high significance of the nonparametric test (i.e., \(p_K < 10^{-5}\); see METHODS).

**Interspike interval variability and subthreshold voltage distribution**

As stated in the previous sections, by using the set of best-fit effective parameters, the steady-state response of the aLIF model accurately matches the corresponding experimental mean firing rates, in the plane \((m, s^2)\). In layer V pyramidal neurons the best-fit parameters could sometimes account for the CV of the interspike intervals distribution as well (Rauch et al. 2003), although the fit criterion involved the mean firing rates only. Carrying out a similar analysis on the aLIF model in cultured cells, it turned out that model responses poorly matched the CV, experimentally estimated at the steady state (not shown).

However, when the steady-state subthreshold distribution of the membrane voltage, recorded in the experiments, was estimated and compared to the aLIF model prediction, a good agreement was observed over the available set of pairs \((m, s^2)\) (see Fig. 4). To quantitatively compare the aLIF model behavior to the experimental voltage traces, individual action potentials were clipped and a unique offset and a scaling factor were required for the model internal state-variable \(V\) to optimally match the voltage distribution (i.e., \(V \rightarrow \beta V - E\)). For each cell, these additional 2 parameters do not affect the current-to-rate response function and they were the same over the pairs \((m, s^2)\) (Fig. 4).

---

**FIG. 4.** The steady-state amplitude distribution of the subthreshold membrane voltage (open circles) was in good agreement with the aLIF model prediction (continuous thick line), although its parameters were tuned to match the mean firing rates only. A–D: the panels indicate the results from 4 different experiments, and in each subplot the voltage amplitude density distribution is plotted for increasing values of \(m\), from left to right, and of \(s\) from top to bottom, within the same panel. For each cell, the scaling factors (i.e., \(V \rightarrow \beta V - E\)) were fixed across the \((m, s^2)\) plane and their values were: \(A\): \(E = 53.14 \text{ mV}, \beta = 0.36\); \(B\): \(E = 49.27 \text{ mV}, \beta = 0.51\); \(C\): \(E = 49.02 \text{ mV}, \beta = 0.64\); and \(D\): \(E = 55.32 \text{ mV}, \beta = 0.67\). The values of \(E\) weakly correlated to the experimentally measured resting membrane potentials, estimated as: \(A\): \(E = -59.24 \text{ mV}\); \(B\): \(E = -56.25 \text{ mV}\); \(C\): \(E = -62.19 \text{ mV}\); and \(D\): \(E = -55.9 \text{ mV}\) (\(r = 0.47\); Tau = 0.67; \(p_K = 0.17\)).
Slow/cumulative inactivation and the stationary spike frequency

As indicated in METHODS, special care was taken in assessing the stationarity of the neuronal responses, with the aim of direct comparison with the model predictions, available at the steady state. Nevertheless the temporal dynamics of the output firing rate was characterized by fast (frequency-dependent) adaptation components, occurring over a time scale of several hundreds of milliseconds (see Fig. 1D), and by a slower component, occurring over a time scale of several seconds. Although the steady-state effect of both adaptation processes was captured by the model (i.e., by $\alpha$; see METHODS), a cumulative (inactivation-related) component contributed to set an upper limit to the maximal stationary response frequency, sustained by the neuron over the entire duration of the stimulation (Fleidervish et al. 1996; Powers et al. 1999; Rauch et al. 2003; Sanchez-Vives et al. 2000; Sawczuk et al. 1997). For instance, the injection of a noisy stimulus current for 20 s with $m > 200–300$ pA or more, depending on the cell input resistance, resulted in a slowly decaying instantaneous firing rate, eventually turning into a cumulative inactivation of the action-potential generation (not shown). This was reminiscent of the slow cumulative sodium-current inactivation characterized by Fleidervish et al. (1996), and made it impossible to quantify the steady-state responses above about 30 Hz, with the current stimulation protocol.

The presence of such a cumulative inactivation was explicitly tested ($n = 8$, 21–43 DIVs) by extending the protocol described in (Fleidervish et al. 1996; Schwindt et al. 1989), consisting in a repeated-pulse stimulation, lasting 1 s, with a very short recovery time. Under noisy current injection, by using the same current realization for each repetition, the same phenomenon occurred, although the voltage fluctuations, induced by the nondeterministic stimulus waveform, delayed the onset of the inactivation at parity of $m$, and sometimes tran- siently reversed the inactivation for a few tens of milliseconds, compared to DC stimulii. As expected, larger values of $\tau_f$ induced slow modulations on the membrane voltage trajectory (see Svirskis and Rinzel 2000), making the episodes of partial transient recovery more frequent, at parity of $m$ and $s$ (not shown).

The spontaneous emergence of patterned network activity in vitro

Networks of neocortical dissociated neurons show spontaneous collective patterned activity (Fig. 5A). Such an activity starts as asynchronous and spatially uncorrelated firing, toward the end of the first week in culture, and evolves into nonperi- odic, synchronized, population bursting after 3–4 wk (Kamioka et al. 1996). Such an activity is reported to stay unchanged for more than 8 wk and thus represents the mature state of the network (Marom and Shahaf 2002). During such a period, single cells emit rare and irregular spikes or bursts of action potential, superimposed on spontaneous voltage fluctuations around a membrane potential of about $-60$ mV (Nakanishi and Kukita 1998). A similar spontaneous network-driven activity constitutes a typical feature of dissociated neuronal networks (Marom and Shahaf 2002) and was also observed in adult neocortex (Sanchez-Vives and McCormick 2000).

The recording of the network activity by means of MEAs led to a quantitative characterization of the distributions of the interburst interval (IBI) and of the population burst durations (PBd), in 7 cortical cultures. Under control extracellular me- dium, the IBIs were characterized by a mean ranging from 4.6 ± 0.4 to 30.3 ± 4.8 s, and by a CV ranging from 44 to 70%. The PBs were characterized by a mean ranging from 54 ± 8 to 146 ± 8.5 ms, and by a CV ranging from 26 to 80%. Under pharmacological disinhibition (see METHODS), the PBs became longer with a mean ranging from 600 ± 70 ms to 1.7 ± 0.053 s, and a CV ranging from 7 to 30%, whereas IBIs were characterized by a mean ranging from 12.7 ± 1.3 to 51.8 ± 11.1 s, and by a CV ranging from 31 to 96%. Figure 5D summarizes graphically the results from 4 experiments, under bicuculline.

Simulations of networks of aLIF model neurons

By using the single-neuron effective parameters, we computer-simulated the collective electrical activity, emerging from a small homogeneous population of excitatory aLIF neurons (see METHODS). We considered estimates for the synap- tic connectivity available from the literature (i.e., $C_m$; see METHODS), and we set $(m_0, s_0)$ in Eq. 4 to match the spontaneous low-rate asynchronous background activity observed in the MEAs experiments ($\approx 1$ Hz).

Similarly to previous theoretical reports (Segev et al. 2001; Tateno 2002; van Vreeswijk and Hansel 2001; Wiedemann and Lüthi 2003), but with a stronger original motivation for the use of the aLIF model, and a quantitative goal to compare computer simulations to the available MEAs recordings, we found that the simulated network activity consisted of asynchronous activity and of population bursts.

Although the individual neurons of the simulated network were not intrinsic burster cells and no pacemaker mechanisms had been introduced in the simulations, the emerging activity evolved into a collective network-driven bursting, depending on the strength of synaptic coupling $J_c$ (see Fig. 8). In agreement with the experiments of Maeda et al. (1995), the spatial origin of PBs varied randomly with each burst, consistent with the conclusions about the lack of a unique pacemaker mecha- nism, driving the network.

On a first approximation, the simulated IBIs had a lower bound set by the time scale of the recovery processes of adaptation $\tau_a$, which was set in the simulations in the range of 0.7–2 s (see Fig. 5, B and C, gray shading). Transiently and following each PB, the network activity was almost completely suppressed by the adaptation currents in each neuron and it later recovered. On the other hand, the synaptic coupling as well as the strength of the spontaneous synaptic release or other sources of randomness (i.e., $m_0$ and $s_0$) were correlated to the bursting frequency.

Three different global regimes were observed. The first one corresponds to a situation in which the excitatory synaptic interactions between neurons are very weak or the network connectivity is very low. Under such conditions, the network of model neurons was exclusively dominated by low-rate asyn- chronous activity ($\approx 1$ Hz). No PB occurred either spontaneously or evoked by any brief depolarizing stimulus (as opposed to Fig. 7A). Actually, in such a regime the global dynamics was dominated by a single low-rate stable state. These conditions
The dotted horizontal gray lines represent the prediction from the mean-field theory for the intraburst statistics. Computer simulations of the network of distribution and of the duration of the population bursts (PBd). Numbers associated with each marker help to identify the same experiments under bicuculline were plotted, by simultaneously studying the CV and the mean of the interburst intervals (IBIs) activity, simultaneously matching the IBIs and the PBd, while sweeping $J_{e}$, determined by $\text{Ne}_{e}/\text{H11005}$. Determined by the random fluctuations in the network collective activity, represent an effective mechanism to considerably slow down oscillation frequency to a time scale that is much longer than the recovery time constants $\tau_{a}$. To compare quantitatively the simulation results to real network data, the results from 4 MEA experiments under bicuculline were plotted, by simultaneously studying the CV and the mean of the interburst intervals (IBIs) distribution and of the duration of the population bursts (PBd). Numbers associated with each marker help to identify the same experiment in both plots and indicate the number of PBs, collected in 10 min of continuous recording and considered for the statistics. Computer simulations of the network of dLIF model neurons reproduced the same features of the spontaneous patterned activity, simultaneously matching the IBIs and the PBd, while sweeping $J_{e}$ from 12 to 18 pA (indicated by the arrows). Interestingly, the statistics of the simulated IBs resembled those of a Poisson point process with dead time, whose functional relationship relating coefficient of variation and mean of the distribution, is reported as $CV_{\text{IBIs}} = \frac{\text{mean}_{\text{IBIs}} - T_{\text{dead}}/\text{mean}_{\text{IBIs}}}{\text{mean}_{\text{IBIs}}}$. Parameters used were: $N_{e} = 100$, $C_{e} = 0.38$, $\tau_{e} = 5 \text{ ms}$, $\tau_{a} = 2.1 \text{ s}$, $\alpha = 0.75 \text{ pA/s}$, $m_{0} = 25.1 \text{ pA}$, $s_{0} = 92 \text{ pA}$, $T_{\text{dead}} = 6.1 \text{ s}$; $B$, $J_{c} = 13 \text{ pA}$; and $C$, $J_{e} = 16 \text{ pA}$.

FIG. 5. The spontaneous emergence of patterned network activity: microelectrode array (MEA) recordings and computer simulations. A: disinhibited network activity, emerging in dissociated cultures of neocortical neurons, was detected by the MEAs. The raster plot indicates the time of occurrence of the events extracellularly detected by 7 substrate electrodes, and the resulting population mean firing rate (bottom panel) was estimated as described in Methods (scale bars: 60 s, 10 Hz). Both B and C contain the raster plot (top panels) of the spikes emitted by a subset of the neurons of a simulated IF network, together with the population mean firing rate (bottom panels). The gray shading reports the instantaneous adaptation level, averaged over all the model neurons. The dotted horizontal gray lines represent the prediction from the mean-field theory for the intraburst firing rate $f_{\text{intra}}$ and the resting activity $f_{\text{rest}}$ (see Fig. 8B) (scale bars: 60 s, 30 Hz). Depending on the synaptic coupling between neurons, the simulated network bursting occurs at a frequency of about 0.016 Hz ($B$), determined by fluctuations and by the probability of spontaneous network transitions (see Fig. 7 and the Results), or at a frequency of 0.05 Hz ($C$), imposed by the relaxation of the adaptation mechanisms ($\tau_{a}$). Spontaneous synaptic release or other sources of randomness, together with the finite-size fluctuations in the network collective activity, represent an effective mechanism to considerably slow down oscillation frequency to a time scale that is much longer than the recovery time constants $\tau_{a}$. D: to compare quantitatively the simulation results to real network data, the results from 4 MEA experiments under bicuculline were plotted, by simultaneously studying the CV and the mean of the interburst intervals (IBIs) distribution and of the duration of the population bursts (PBd). Numbers associated with each marker help to identify the same experiment in both plots and indicate the number of PBs, collected in 10 min of continuous recording and considered for the statistics. Computer simulations of the network of dLIF model neurons reproduced the same features of the spontaneous patterned activity, simultaneously matching the IBIs and the PBd, while sweeping $J_{e}$ from 12 to 18 pA (indicated by the arrows). Interestingly, the statistics of the simulated IBs resembled those of a Poisson point process with dead time, whose functional relationship relating coefficient of variation and mean of the distribution, is reported as $CV_{\text{IBIs}} = \frac{\text{mean}_{\text{IBIs}} - T_{\text{dead}}/\text{mean}_{\text{IBIs}}}{\text{mean}_{\text{IBIs}}}$. Parameters used were: $N_{e} = 100$, $C_{e} = 0.38$, $\tau_{e} = 5 \text{ ms}$, $\tau_{a} = 2.1 \text{ s}$, $\alpha = 0.75 \text{ pA/s}$, $m_{0} = 25.1 \text{ pA}$, $s_{0} = 92 \text{ pA}$, $T_{\text{dead}} = 6.1 \text{ s}$; $B$, $J_{c} = 13 \text{ pA}$; and $C$, $J_{e} = 16 \text{ pA}$.

The second regime corresponds to an increased strength of excitatory synaptic interactions between neurons. It has been shown that as a real network matures (from 3 to 40 days), the frequency and propagation velocity of the PBs markedly increases (Maeda et al. 1995), suggesting an increase in the number of synaptic contacts as well as in the synaptic efficacy. Therefore, this situation might be regarded as an evolution of the previous regime, determined by neurite outgrowth, synaptic development, and by homeostatic and activity-dependent plasticities (Desai et al. 1999; Turrigiano et al. 1998). While the low-rate asynchronous activity was still characterizing the spontaneous electrical activity of the network at the same frequency, the occurrence of spontaneous PBs was possible with a probability dependent on the average synaptic strength and, inversely, on the size of the network. The last dependency is determined by the random fluctuations in the population firing rate, which are related to finite-size effects (Mattia and Del Giudice 2002). The features of spontaneous bursting were also determined by the same factors, qualitatively resulting in a rare and unpredictable occurrence of short and irregular PBs, or in a frequent and more regular occurrence of longer and more regular PBs. Under such conditions, a brief external triggering stimulus could successfully recruit the neurons to transiently sustain an evoked PBs, whose duration is inversely determined by $\alpha$. Actually, in such a regime the global dynamics of the network is transiently bistable and once a PB is started, the adaptation slowly redefines the location and the existence of the stable states for the network dynamics, until there is suddenly only a single stable state at 0 Hz. This somehow corresponds to a reset for the collective network activity. In details, the adaptation hyperpolarizing contribution,
which starts to build up in every neuron recruited by the PB, decreases the output firing rate until the recurrent synaptic inputs to any neuron stop.

In such a regime, the statistics of the simulated IBIs and PBd matched the experiments, performed under pharmacological disinhibition (see Fig. 5, B–D).

The third and last regime occurs for stronger excitatory synaptic interactions in the network. Under such conditions, the network is characterized by a high-rate asynchronous regime (≈55–80 Hz). In this regime, the firing of the individual neurons is more regular than in the previous ones. Moreover, any attempt at transiently silencing the activity of the network, by hyperpolarizing a large fraction of the neurons of the population, would not prevent the network to later recover its global state (as opposed to the simulations reported in Fig. 7). Although, under physiological conditions, it is probably not possible for a cortical network to sustain such a firing regime for a long time, an interesting phenomenon was observed for an intermediate synaptic interactions strength: occasional population breaks. This appears as rare and unpredictable simultaneous short interruptions of the global activity and is determined by the finite-size fluctuations of the activity.

The single-neuron response and the mean-field theory interpretation

In the present section, we show that the quantitative knowledge of the single-neuron response function, identified in the previous experiments, is very relevant to predict and interpret the emergence of the network activity described above. As widely discussed in the literature, a homogeneous network of synaptically interacting excitatory neurons may be regarded as a single dynamical system. Its stationary states can then be predicted and interpreted, in the limit of an infinite number of neurons $N_e \to \infty$, by using the mean-field Eq. 4 and studying $\Phi_{aLIF}$ as a function of $f$ (Amit and Brunel 1997b).

We first consider the collective activity in the absence of spike-frequency–dependent adaptation (i.e., $\alpha = 0$). Under such hypotheses, because of the simultaneous dependency of $\Phi_{aLIF}$ on $m$ and $c^2$, the collective firing rate of the network may be characterized by 2 stable dynamic-equilibrium states, in a small range of average synaptic coupling $J_e$ (Figs. 6A and 8, left panel) (Amit and Brunel 1997b; Fusi and Mattia 1999).

Such global activity configurations correspond to the solutions $f^*$ of the following self-consistent network equation, further satisfying a stability condition

$$f^* = \Phi_{aLIF}^0(f^*, s(f^*)) \quad \frac{d_s}{df_s} \Phi_{aLIF}^0(f^*) < 1$$

These solutions have been graphically identified as the intersection points of $\Phi_{aLIF}^0(f)$ with the unitary-slope line (see Figs. 6A and 7, insets).

In such dynamic equilibria, the population activity is the result of reverberating interactions between the neurons, which are sustained by excitatory synapses. The transitions from one state to the other can be triggered by an external brief stimulus...
affecting each neuron (i.e., \( m_0 \rightarrow m_0 \pm \Delta m_0 \)) and resulting into an intrinsically bistable network activity (Fig. 7A). This has been already described as a possible neuronal correlate of the selective delay-activity states in vivo (Yakovlev et al. 1998) and UP-states in vitro (Compte et al. 2003). Interestingly, such transitions can even occur spontaneously in small networks (Fig. 6B), as a result of the fluctuations induced by finite-size effects (Mattia and Del Giudice 2002).

**The mechanisms of a PB**

Considering the full network model, where individual neurons keep adapting their output rate as a function of the activity, it is possible to carry out a simple approximate analysis. Provided that the mechanisms responsible for the excitability reduction (e.g., the adaptation) act on a time scale \( (\tau_a) \) that is longer compared to the single-neuron dynamics \( (\tau) \), an analysis of the quasi-stationary equilibria may tell us a lot about the collective activity of the network. In other words, by assuming that adaptation is delayed and transiently uncoupled from the neuronal dynamics, we may consider it as frozen and determine it as frozen and determined by the previous global regime.

Let us consider the situation of weak excitatory synaptic interactions between the model neurons: a low-rate stable regime \( f_{\text{rest}} \) characterizes the network dynamics because it can be immediately determined from \( \Phi_{\text{LIF}} \). By making explicit the dependency on \( m \) and \( s \), as well as on \( \alpha \) (see METHODS and Eqs. 2 and 4), we can write the following self-consistent equation and numerically find the stable solution \( f_{\text{rest}} \)

\[
f_{\text{rest}} = \Phi_{\text{ext}}[m(f_{\text{rest}}) - \alpha f_{\text{new}} s(f_{\text{rest}})]
\]

Such a solution is approximately located at 1 Hz and the instantaneous network firing rate \( f(t) \) fluctuates around it, as a result of finite-size network effects. However, every neuron of the network will instantaneously respond to the synaptic input drive according to

\[
\Phi_{\text{LIF}}(m - \alpha f_{\text{rest}} s)
\]

with \( f_{\text{rest}} \) a constant. In other words, the network is not immediately experiencing the impact of adaptation. Therefore, assuming that the network was at “rest,” we may study and determine any additional stable dynamical attractor \( f^* \), which may exist even though transiently. This is done by numerically solving

\[
f^* = \Phi_{\text{ext}}[m(f^*) - \alpha f_{\text{new}} s(f^*)]
\]

The last equation is satisfied at least by \( f^* = f_{\text{rest}} \), and for increasing synaptic coupling, \( f^* = f_{\text{burst}} \) is also a solution \( (f_{\text{burst}} > f_{\text{rest}}; \text{see Figs. 6B and 8, right panel}) \). Because of the profile of the single-neuron response function, under these circumstances there always exists an unstable equilibrium \( f_0^* \) \( (f_{\text{rest}} < f_0 < f_{\text{burst}}) \), separating the 2 basins of attraction (Fig. 6).

From the last considerations, it follows that when a fluctuations in the global activity is large enough to overcome the distance \( \Delta_0 = (f_0 - f_{\text{rest}}) \), the stability of \( f_{\text{rest}} \) may be (transiently) lost and the entire network synchronously shifts to a new regime where \( f(t) = f_{\text{burst}} \). Therefore, the unstable state acts as a kind of threshold or a no-return point, as the network

**FIG. 7.** The comparison between the numerical computer simulations and the predictions of the extended mean-field analysis shows a remarkable agreement, in the case of no adaptation (i.e., \( \alpha = 0 \)); parameters as in Fig. 5). A: In the top panels, the theoretical analysis of the stationary network activity has been carried out under 3 different conditions: \( m_0 = 25.1 + \Delta m_0 \) pA, \( m_0 = 25.1 \) pA, and \( m_0 = 25.1 - \Delta m_0 \) pA and the stable (unstable) equilibrium states have been evaluated and graphically represented by filled circles (star), as done in Fig. 6A. The remaining traces report (from the top to the bottom) the temporal evolution of \( m_0 \), the membrane voltage of 3 IF neurons taken by chance, the raster plot of the spikes emitted by a subset of the neurons, and the resulting population mean firing rate, overlapped to the predicted steady-state regimes (gray dotted lines). B: the same simulation was repeated after increasing the excitatory synaptic coupling between neurons, without any depolarizing external stimulation. Network finite-size effects may induce a spontaneous transition from one stable state to the other (A, \( J_e = 10.75 \) pA; B, \( J_e = 11.5 \) pA; scale bars; 200 ms and 25 Hz/50 mV).
FIG. 8. In the absence of adaptation, as extensively discussed in the literature, network bistability is possible in a homogeneous excitatory population, in a small range of synaptic coupling \( J_e \) (A, \( \alpha = 0 \)), as reported by the phase diagram. This is a direct consequence of the profile of the network response function, as plotted in Fig. 6A. Under the hypotheses of a separation between time scales (see RESULTS), a similar phase diagram can be considered for the full model network, including adaptation (B). By studying the quasi-stationary/instantaneous stable states, it is possible to make quantitative predictions on the mean firing rates during asynchronous regimes as well as during population bursts. Inserts: actual traces of the simulated network activity, for different synaptic coupling, and comparison of the actual resting and bursting activity levels to the predictions of the theory (black and gray dotted lines). In both panels, black continuous and dashed traces indicate stationary and quasi-stationary stable states, respectively. Dashed gray lines report the location of unstable stationary and quasi-stationary equilibria, whose distance from \( f_{\text{rest}} \) determines the regular/irregular character of network oscillations. Markers represent the network mean firing rates, measured in the computer simulations under different regimes. Although the simulated network considered was very small, \( N_e = 100 \), the agreement with the theory is remarkable. Parameters as in Fig. 5.

In the absence of adaptation, as extensively discussed in the literature, network bistability is possible in a homogeneous excitatory population, in a small range of synaptic coupling \( J_e \) (A, \( \alpha = 0 \)), as reported by the phase diagram. This is a direct consequence of the profile of the network response function, as plotted in Fig. 6A. Under the hypotheses of a separation between time scales (see RESULTS), a similar phase diagram can be considered for the full model network, including adaptation (B). By studying the quasi-stationary/instantaneous stable states, it is possible to make quantitative predictions on the mean firing rates during asynchronous regimes as well as during population bursts. Inserts: actual traces of the simulated network activity, for different synaptic coupling, and comparison of the actual resting and bursting activity levels to the predictions of the theory (black and gray dotted lines). In both panels, black continuous and dashed traces indicate stationary and quasi-stationary stable states, respectively. Dashed gray lines report the location of unstable stationary and quasi-stationary equilibria, whose distance from \( f_{\text{rest}} \) determines the regular/irregular character of network oscillations. Markers represent the network mean firing rates, measured in the computer simulations under different regimes. Although the simulated network considered was very small, \( N_e = 100 \), the agreement with the theory is remarkable. Parameters as in Fig. 5.

Population breaks

As summarized in Fig. 8, adaptation changes the phase diagram of the network, creating a region of transient network-driven bistability. Interestingly, for stronger synaptic coupling, \( f_{\text{rest}} \) is no longer a stable solution of

\[
\frac{df}{dt} = \Phi_{\text{diss}}[m(f^*) - \alpha f_{\text{burst}}, s(f^*)]
\]

Instead, such an equation is satisfied by \( f^* = f_H = 0 \) Hz, with \( f_H < f_{\text{burst}} \) (see Figs. 6B and 8, right panel). Therefore, while adaptation progressively builds up in individual neurons, the network activity decreases as the locations of the \( f_{\text{burst}} \) and \( f_0 \) tend to become coincident, until their existence is lost (Fig. 6B). As soon as this happens, the steady-state network dynamics suddenly converges to \( f_H \) and the neurons generally stop firing, reminiscent of the hyperpolarization experienced by the membrane voltage after an action potential. This accounts quantitatively for the generation of PBs, as tested by network simulations (Fig. 8, markers). The amount of time spent in the PB is therefore related to the distance \( \Delta = f_{\text{burst}} - f_0 \) and to the time requested by adaptation for the full buildup (i.e., \( \alpha \) and \( \tau_a \)). Qualitatively, it can be concluded that for an increasing \( J_e \), \( \Delta \) decreases while \( \Delta' \) increases; thus the mean IBI decreases and the PB duration increases.

Similarly to the previous situation, such a new regime cannot be sustained indefinitely because \( f_H \) is not a solution of

\[
\frac{df}{dt} = \Phi_{\text{diss}}[m(f^*) - \alpha f_{\text{burst}}, s(f^*)]
\]

Because \( f_H < f_{\text{rest}} \), the network will recover the resting activity level \( f_{\text{rest}} \), as described at the beginning.

As summarized in Fig. 8, adaptation changes the phase diagram of the network, creating a region of transient network-driven bistability. Interestingly, for stronger synaptic coupling, \( f_{\text{rest}} \) is no longer a stable solution of

\[
\frac{df}{dt} = \Phi_{\text{diss}}[m(f^*) - \alpha f_{\text{burst}}, s(f^*)]
\]

Instead, such an equation is satisfied by \( f^* = f_H = 0 \) Hz, with \( f_H < f_{\text{burst}} \) (see Figs. 6B and 8, right panel). Therefore, while adaptation progressively builds up in individual neurons, the network activity decreases as the locations of the \( f_{\text{burst}} \) and \( f_0 \) tend to become coincident, until their existence is lost (Fig. 6B). As soon as this happens, the steady-state network dynamics suddenly converges to \( f_H \) and the neurons generally stop firing, reminiscent of the hyperpolarization experienced by the membrane voltage after an action potential. This accounts quantitatively for the generation of PBs, as tested by network simulations (Fig. 8, markers). The amount of time spent in the PB is therefore related to the distance \( \Delta = f_{\text{burst}} - f_0 \) and to the time requested by adaptation for the full buildup (i.e., \( \alpha \) and \( \tau_a \)). Qualitatively, it can be concluded that for an increasing \( J_e \), \( \Delta \) decreases while \( \Delta' \) increases; thus the mean IBI decreases and the PB duration increases.

Similarly to the previous situation, such a new regime cannot be sustained indefinitely because \( f_H \) is not a solution of

\[
\frac{df}{dt} = \Phi_{\text{diss}}[m(f^*) - \alpha f_{\text{burst}}, s(f^*)]
\]

Because \( f_H < f_{\text{rest}} \), the network will recover the resting activity level \( f_{\text{rest}} \), as described at the beginning.
The variability of the IBIs distribution

From the previous considerations, it is possible to predict and interpret the regular/irregular character of the IBIs. In the last paragraphs, an analogy between PBs and action potentials occurring in an excitable membrane was proposed. In fact, similarly to the temporal evolution of the membrane potential in the single-neuron IF dynamics discussed previously (see METHODS), the activity of a network randomly fluctuates as a result of synaptic noise. Occasionally these fluctuations may be large enough to overcome an excitability threshold (i.e., $\Delta_{f_e}^{\text{thick}}$ and later the activity is strongly refractory to any further generation of PBs ($f \approx f_{\text{rest}}$). Our proposal is to regard the generation of PBs as similar to the generation of an action potential, in a model of integration of a noisy input. Although the finite-size fluctuations are quite different from an Ornstein–Uhlenbeck process, because no drift is present and its variance increases with the mean network activity (Mattia and Del Giudice 2002) and because the network dynamics is more complicated than a linear integration of inputs (Gerstner 2000), a comparison to an IF-like model may be indeed proposed. By mapping the mean firing rate $f$ into the membrane voltage of an abstract LIF model neuron, we make the previous comparisons explicit, setting the resting membrane voltage to $f_{\text{rest}}$, the spike threshold to $f_{\text{th}}$, the reset potential to $f_{\text{reset}}$, and the absolute refractory period proportional to $\tau_e$.

By increasing the network synaptic coupling $I_e$, and thus decreasing the distance $f_{\text{th}} - f_{\text{reset}}$ (see Fig. 8), the rate of threshold crossings is expected to monotonically increase, while preserving an irregular character. Such predictions were confirmed by the simulated network activity, where IBIs statistics are approximated very well by a Poisson process with a refractory time (Gerstner and Kistler 2002) (Fig. 5D, dotted thick line). This is reminiscent of a well-known balanced (i.e., drift-free) integration process (see Shadlen and Newsome 1998), where the threshold crossings are determined by subthreshold fluctuations only, in a noise-dominated regime.

DISCUSSION

In this work, we quantitatively evaluated the single-neuron discharge properties in dissociated cultures of neocortex. We proved that a simplified point-neuron IF model is an adequate description when the network mean firing rates are considered. Under noisy current input, the spike response properties of cultured neocortical neurons qualitatively resemble those of cells in acute slice preparations. This is of interest given that dissociated neurons undergo a different development of intrinsic biophysical properties, compared to the neurons in vivo.

Then, we presented and analyzed the collective activity arising in a simulated network of interacting excitatory model neurons in terms and on the basis of the network-response properties, emerging from $f = \Phi(m, s)$. The matching with the MEAs recording is satisfactory (Fig. 5, B–D), indicating that the discharge response properties to noisy current stimuli and the experimentally characterized spike-frequency adaptation are sufficient to account for the emerging collective activity, observed in the experiments. Although the theoretical approach that we used holds in the limit of an infinite number of neurons $N_e \to \infty$, the quantitative agreement between the approximate theoretical predictions and the numerical simulations is remarkable (Figs. 7 and 8), even with small-sized networks (e.g., $N_e \sim 100$) and incorporating the effect of adaptation. This further supports the validity of the mean-field hypotheses on the neuronal interactions in a recurrent network and the motivation for the noisy current-clamp protocol.

Cultured neurons respond as aLIF neurons at steady state

One of the results of the present work is that cultured neurons, dissociated from rat neocortex, show qualitatively similar response properties as those of pyramidal cells of acute brain slices, under noisy current clamp. Further supporting the observations of Rauch et al. (2003), our results imply that the IF model is an appropriate description of the spike emission process under a realistic network input drive. As a consequence, when neurons are studied under a similar nondeterministic experimental paradigm, the morphological complexity, the biophysical intrinsic details, and the nonlinearities of the active ion currents may collapse to a reduced effective point-neuron dynamics (see also Rudolph and Destexhe 2003).

More data and experiments are indeed needed to interpret and understand why the predictive power of the IF model sometimes extend to the interspike intervals’ higher-order statistics, such as the coefficient of variation, or to the subthreshold voltage distribution (Jolivet et al. 2004). However, under the assumption of the extended mean-field theory of a network of recurrently interacting neurons, higher-order features of the evoked spike trains and the subthreshold voltage dynamics do not affect the collective population activity, as reflected in the statistics of $I(t)$ (see Eq. 4) (Amit and Brunel 1997b). Instead, these might play an important role in the context of synaptic plasticity (Buonomano and Merzenich 1998). Thus, our results suggest that the aLIF model, incorporating the effective parameters, may be adequate to simulate not only the collective activity but also long-term plasticities at the network level (Del Giudice and Mattia 2001; Fusi 2003).

The spontaneous emergence of patterned network activity

Among the global activity regimes, predicted by the theory and tested by numerical simulations, the first two regimes are the most physiological. Importantly, they further quantitatively reproduce the results obtained in our experiments (see Fig. 5D), as well as those corresponding to early developmental stages, in which the effective coupling between neurons is weak (Kamioka et al. 1996).

In our simulations, the synchronization across the neurons during PBs arises as a consequence of a global modulation of the population activity. This is a consequence of the alternating and opposing interplay of two mechanisms. The first is represented by the probability of spontaneous transitions, which occasionally recruit enough recurrent connections to trigger and sustain a persistent global spiking regime. The second is represented by the build-up of an activity-dependent mechanism, able to decrease the network excitability. The quasi-stationary analysis presented in the previous sections, to quantitatively interpret the collective activity of the network simulations, relies on the assumption that the refractory mechanisms responsible for an activity-dependent reduction of network excitability act on a time scale that is longer compared to
to that of the single-neuron dynamics. Because the experimental single-neuron response properties are mainly affected by spike-frequency adaptation, in our analysis we examined such an intrinsic neuronal refractory mechanism, but the proposed procedure might be extended to other (coexisting) mechanisms. However, the hypothesis of separation among dynamical time scales might not be always satisfied, and the consequences include the overestimation of the actual mean frequencies characterizing the PB regimes (i.e., \( f_{\text{burst}} \)). This might play a minor role in the case of slow/cumulative inactivation, as mathematically described by Fleidervish et al. (1996) or by Giugliano et al. (2002), or it could be more severe in the case of short-term synaptic depression (Tabak et al. 2000; Tsodyks et al. 2000; Wiedemann and Lüthi 2003), phenomenologically modeled following Tsodyks et al. (1998) because the related time constant changes as the presynaptic frequency changes.

The framework we discussed in our work may explain, in quantitative terms, the spontaneous periodic synchronized bursting activity (Fig. 5) observed in mature neuronal cultures and experimentally correlated to the in vitro synaptogenesis and development (Kamioka et al. 1996). Consistent with the in vitro connectivity pattern that is restricted to spatially neighboring sites, a cultured network could be thought as a homogeneous chain of weakly synaptically connected subpopulations of a few hundreds of neurons each, as described here. Each population would then have equal probability to start a PB, spreading to the entire culture by means of the longer range sparse excitatory connectivity, and characterize by the same properties of PBs occurring in an isolated subpopulation. Physical network sectioning experiments of Maeda et al. (1995), and of Nakanishi and Kukita (1998), provide evidence for such a global network architecture, consistent with the hypothesis that synchronized bursting is emerging from synaptic activity rather than by way of gap junctions and/or diffusible factors.

These considerations greatly expand the relevance of the experimental protocol we described and may contribute to a full understanding of the spontaneous dynamics emerging in in vitro preparations. As opposed to previous computational studies, in which extensive numerical computer simulations have been used, a unifying theory was considered. The same theory inspired the single-neuron experimental protocol and it allowed us to gain a deeper insight on the way a particular type of refractory mechanism might affect the global dynamics. Different kinds of refractoriness have a different impact, at the steady state and at quasi-steady states on the statistical properties of input currents: in terms of a very slowly changing hyperpolarizing membrane current, the spike-frequency adaptation affects the mean input current only, whereas synaptic depression induces a change to both the input mean and variance, through a change in \( J_e \) (see Eq. 4). Intuitively, this would transiently deform the profile of the network response function (Fig. 6B), modifying the location of the intersections with the unitary-slope line, in a peculiar way.

Spontaneous activity and synaptic release are not just triggering such episodes; they also determine the configuration of the collective dynamics, as proven by the dependency on \( s^2 \) of the single-cell discharge properties.

A limit of our study is that we did not take into explicit consideration the impact of NMDA glutamatergic receptors (Maeda et al. 1995). Although a similar mathematical approach could be possible (Brunel and Sergi 1998; Fourcaud and Brunel 2002), in the present work we ignored the longer time constant associated with the synaptic currents mediated by the NMDA receptors and we implicitly regarded it as contributing to the overall mean synaptic efficacies (i.e., \( \eta_e \)). However, Wang (1999) already extensively showed that in a similar network architecture, synaptic components mediated by NMDA receptors support and greatly enhance network bistability.

Finally, a major limitation of the LIF model is constituted by the stereotyped description of the spike-initiation mechanisms. Real neurons have a nonlinear voltage–current relationship near threshold, where the activation of the fast sodium currents becomes relevant. This is not the case for the LIF, where the integration of the incoming synaptic inputs is always voltage independent. By comparing the response of biophysically realistic conductance-based and LIF model neurons to weakly modulated oscillatory input currents, it has been recently shown that the oversimplifications of the last affect the response properties to high-frequency input components (i.e., >50–100 Hz) and, in the same frequency range, they lead to an unphysiological dependency on the temporal correlations of the background synaptic noise (i.e., on \( \tau_s \)) (Fourcaud-Trocme et al. 2003; H. Köndgen, C. Geisler, S. Fusi, X. J., Wang, H.-R. Lüscher, and M. Giugliano, unpublished observations).

These considerations are unlikely to qualitatively change the results described in the present work, given that the frequency of the PBs is generally well below 10 Hz in cultured networks. However, the prediction of the responsiveness of individual neurons to a sudden increase in their net synaptic input might be slightly affected, resulting in a wrong estimate of the slope of the rise and decay of each simulated PB. However, the work of Fourcaud-Trocme et al. (2003) did not systematically take into consideration the contribution of adaptation, which may behave as a high-pass filter at low frequencies (Fuhrmann et al. 2002), thus compensating for the loss in responsiveness.

Finally, by extending the class of IF models to incorporate a nonlinear behavior at the threshold (Fourcaud-Trocme et al. 2003), it is possible to recover the correct low- and high-frequency neuronal response properties as well as to reproduce the timing of individual spikes (Jolivet et al. 2004). Because the steady-state current-to-rate response function \( \Phi(m, s^2) \) can be still analytically determined for such extended IF models, an experimental identification of the model parameters may be devised, similar to what has been described here.

**Network activity and single-neuron model details**

An additional important issue to be discussed is represented by the quantitative link between model details and network activity. However, to determine a strong correlation between the measured single-neuron properties and the emerging network activity in vitro, the availability of additional detailed information about the precise neuronal and synaptic heterogeneity, coupling, topology, and density would be required. In fact, throughout our analysis, a few degrees of freedom still characterized the definition of the network model. These are represented by the synaptic coupling and by the extent of the neuronal ensembles participating in the population bursting. By making realistic assumptions and compensating for such a lack of experimental details by data reported in the literature...
(Nakanishi and Kukita 1998; Nakanishi et al. 1999), we could
a posteriori confirm the consistency of some results in terms of
the realistic range for the excitatory synaptic coupling \( J_e \) in
vitro. However, we further considered the systematic study of
some aspects of the network activity, emerging in the computer
simulations, as a function of the single-neuron parameters (see
Fig. 9). This kind of analysis might improve our understanding
of which features are indeed required for the network-patterned
activation and it would be considerably difficult by using a
more detailed neuronal model, characterized by several param-
eters and kinetic mechanisms.

From such an analysis, it appears that the most important
parameter determining the emergence of PBs is \( \alpha \) (Fig. 9, A
and B). As already discussed, only when \( \alpha > 0 \) a PB may occur
(see Fig. 8). For increasing values of \( \alpha \in (0; 15] \) pA·s, the
range of \( J_e \) for the emergence of irregular PBs increases (i.e.,
\( \Delta \theta > 0 \)), and the regimes of regular and frequent PBs extend to
larger \( J_e \) (Fig. 9A). The emergence of population breaks is
affected by a change in \( \alpha \) only in terms of an offset on the
minimal synaptic coupling \( J_e \), because the slope of \( \Delta \theta \) remains
unchanged (Fig. 9A). For a given synaptic coupling the ampli-
tude of the population bursting (i.e., the intraburst spiking rate)
\( f_{\text{burst}} \) is insensitive to \( \alpha \) (Fig. 9B), although the burst’s duration
decreases with \( \alpha \) (not shown).

The effective parameters \( \tau_{\text{arp}} \) and \( H \) in the aLIF are related
to the strength and kinetics of the outward membrane currents
that are activated after each spike. By definition, the absolute
refractory period \( \tau_{\text{arp}} \) is mainly limiting the maximal output
firing rate of the single neurons of the network. From Fig. 9, C
and D, it can be concluded that the regular/irregular character
of the PBs (i.e., \( \Delta \theta \)) is only weakly affected by increasing
\( \tau_{\text{arp}} \in [0.5; 30] \) ms, which shrinks the range of \( J_e \) where PBs
may occur and increases the probability of population breaks
(i.e., \( \Delta \theta \) decreases). As expected, the amplitude of the PB
strongly depends on \( \tau_{\text{arp}} \) (Fig. 9D).

The reset voltage \( H \in [0; 19] \) mV, following the emission of
each action potential, does not substantially affect the ampli-
tude and the probability of PB and population breaks, unless it
approaches the excitability threshold \( \theta \) (Fig. 9, E and F). Under
these conditions, with the distance to cross the spiking thresh-
old reduced in each neuron, the PBs occur for weaker synaptic
coupling \( J_e \), whereas the probability of population breaks is
considerably reduced.

Finally, it is important to underline that, provided that the
essential features of the current–frequency response properties
are retained, alternative mathematical models selected to quan-
titatively fit the same experimental response under noisy cur-
cent clamp are expected to perform in a qualitatively similar
way when their network activity is considered. We investigated
whether this was indeed the case for the aCLIFF model, which
routinely did not pass the fit tests. Instead of considering
and comparing the aLIF and the aCLIFF characterized by average
values for the parameters, we decided to look at the 2 param-
eter sets obtained for the same cell. The resulting simulated
network activity is very similar (Fig. 9, G and H), with the
exception of a different threshold for the emergence of the
population bursts and a slightly reduced dynamic range for the
synaptic coupling \( J_e \), corresponding to irregular PBs. Therefore
a network of aCLIFF, or even conductance-based model neu-
rons matching the experimental current-to-firing rate response
function and the dependency on the amplitude of the fluctua-
tions \( s \), would perform very similarly.

**Summary of the results and predictions**

With the aim of summarizing the results discussed so far and
comparing our predictions to additional in vitro experiments,
we report a list of the most important issues. Most of the
suggested experimental protocols described below were indeed
tested and the predicted resulting network transitions were
confirmed by several investigators.

1. Temporally patterned aperiodic synchronization of the
electrical activity may emerge in a network, without ad hoc
single-cell pacemaker mechanisms (Maeda et al. 1995) or ad
hoc active membrane conductances.

2. Because the predictions about the simulated collective
dynamics are in good agreement with in vitro data, the protocol
used for identifying single-neuron response properties was
adequate to perform the reduction leading to IF models.

3. As long as the network activity is of concern, on the basis
of the extended mean-field hypotheses, only single-neuron
firing rates are determining the in vitro collective activity.

4. The glutamatergic synapses, the sporadic irregular firing
of individual neurons, and the finite-size activity fluctuations
are sufficient to account for the mechanisms of PBs initiation,
which can be assimilated to a noise-dominated first-passage
time with dead time.

5. The mechanism of PBs termination is consistent with the
temporal development of intrinsic neuronal adaptation (Rob-
inson et al. 1993) and, together with the location of stable and
unstable activity configurations (Fig. 8), determines the dura-
tion of the PBs and the statistics of the IBI distribution.

6. Any pharmacological/ionic enhancement of synaptic
transmission mediated by AMPA and NMDA receptors (e.g.,
by removing AMPA-R inactivation by cyclothiazide; see
Chiappalone et al. 2003), decreasing (increasing) the extracel-
lular concentration of Mg\(^{2+}\) (Ca\(^{2+}\), etc.), is expected to shift
the network activity from asynchronous activity toward a more
regular bursting, with longer-lasting PBs (Canepari et al. 1997;

7. Any pharmacological/ionic suppression of the AMPA-,
and NMDA-mediated synaptic transmission (e.g., by CNQX,
D-APV, increasing the extracellular concentration of Mg\(^{2+}\)
or decreasing Ca\(^{2+}\)) is expected to shift the network activity back
to asynchronous activity and irregular rare bursting, charac-
terized by shorter PBs and a lower intraburst spike rate (Canepari

8. Developmental synaptogenesis (Kamioka et al. 1996) as
well as the use of a repeated electrical stimulation to induce
activity-dependent long-term potentiation (LTP) of glutama-
teric synapses, correspond to an increase in \( J_e \) (Maeda et al.
1998). This is expected to change the spontaneous network
activity from an asynchronous regime, or from rare PBs com-
posed by a few spikes, to more frequent PBs composed by a
higher number of intraburst spikes (see Fig. 8B). The same is
expected to hold for electrically evoked PBs, and finally a
graded modification of synaptic strength would correspond to
a graded increase in PBs frequency and in its amplitude (i.e.,
the intraburst frequency), according to Fig. 8.

9. A brief distributed electrical stimulation is expected to
elicit a PB, even in a silent culture (Jimbo et al. 2000). The
FIG. 9. The analysis of Fig. 8B was repeated by keeping all the model parameters fixed (see Table 1), while changing $\alpha$ (A–B), $\tau_{arb}$ (C–D), and $H$ (E–F), respectively. With the aim of outlining the distinct dependency of some features of the network activity on the single-neuron parameters, the most relevant quantities, $\Delta_a^+$ and $\Delta_a^-$ (left column), as well as the interburst firing rate during the PBs and for stronger synaptic coupling (right column), have been plotted as a function of the synaptic efficacy $J_e$. Finally, by selecting the experimental data from a single cell and comparing the corresponding best-fit parameter sets for the aLIF and the aCLIFF model (G–H), it is possible to predict that a network of aCLIFF neurons would perform very similarly to a network of aLIF units, apart from an offset in the synaptic efficacy $J_e$ and a slightly different sensitivity in $\Delta_a^+$ and $\Delta_a^-$, and not in $f_{burst}$ and $f_{rest}$. Parameters used were: $N_e = 100$, $C_m = 0.38$, $\tau = 5$ ms, $m_0 = 25.1$ pA, $s_0 = 92$ pA; (aLIF): $\alpha = 3.18$ pA s, $\tau = 20.13$ ms, $C = 26.12$ pF, $H = 16.4$ mV, $\tau_{arb} = 47.7$ ms; and (aCLIFF) $\alpha = 3.0$ pA s, $\lambda = 4.2617$ mV ms$^{-1}$, $C = 88.6$ pF, $H = 18.49$ mV, $\tau_{arb} = 48.21$ ms.
existence and location of stable network states could be unmasked in such a way.

II) By evoking PBs of increasing amplitudes, an indirect evidence for the role of Δf (see RESULTS) might be provided by an analysis of the evoked PB duration, expected to be longer for those stimuli that evoke more intraburst spikes (Maeda et al. 1998).

II) An increase in the single-cell excitability, by raising the extracellular potassium concentration [K+]0 would correspond to a permanent upregulation of the network response function (i.e., like m0 → m0 + Δm0; see Figs. 6 and 7). This is expected to increase frest and to decrease Δf, making the bursting network activity more regular and frequent (Canepari et al. 1997).

In this work we investigated the single-neuron response properties that are relevant for the quasi-stationary collective network activity in vitro. We provided a minimal quantitative description of the experimental data set, summarizing the current-to-firing rate response properties of cultured neocortical neurons by just 5 parameters. Although accurate in accounting for the mean firing rate output of a neuron, under realistic re-created network inputs, such a description is simple enough to provide analytical predictions on the spontaneous network activity, emerging in the numerical computer simulations of a population of interacting neurons.

Because such collective activity regimes match the activity experimentally observed in the cultures of dissociated neurons in vitro, our results attempt at answering a more general question: among the richness of biological details, which features should a model of neuronal excitability retain, when the electrical activity, emerging from the synaptic interactions of individual cells, is of concern?

Under such a perspective, we are convinced that many other recent modeling studies, using simplified model neurons (Segev et al. 2001; Tal et al. 1998; Tato et al. 2002; Tsodyks et al. 1998, 2000; Wiedemann and Lütthi 2003), will greatly benefit from the availability of the effective model parameters, identified in our work, as well as from the observations of intrinsic neuronal properties such as the spike-frequency adaptation and the spike-emission slow cumulative inactivation, to perform large-scale computer simulation with increased realism.

Finally, it is interesting to note that the reduction driven by the experimental data at the single-neuron level did not compromise the richness of phenomena occurring at the level of a population. In particular, the network activity investigated here depends only quantitatively on the model details, provided that the experimental current-to-firing rate response function is reproduced and that the sensitivity to the input fluctuations retained.

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