Balance between Excitation and Inhibition Controls the Temporal Organization of Neuronal Avalanches

F. Lombardi,[1] H. J. Herrmann,[1,2] C. Perrone-Capano,[3] D. Plenz,[4] and L. de Arcangelis[5]

[1]*Institute Computational Physics for Engineering Materials, ETH, Zürich, Switzerland*
[2]*Departamento de Física, Universidade Federal do Ceará, 60451-970 Fortaleza, Ceará, Brazil*
[3]*Biological Sciences Department, University of Naples Federico II and IGB-CNR, Napoli, Italy*
[4]*Section on Critical Brain Dynamics, NIH, Bethesda, Maryland 20892, USA*
[5]*Department of Information Engineering, Second University of Naples, Aversa (CE), Italy*

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Neuronal avalanches, measured *in vitro* and *in vivo*, exhibit a robust critical behavior. Their temporal organization hides the presence of correlations. Here we present experimental measurements of the waiting time distribution between successive avalanches in the rat cortex *in vitro*. This exhibits a nonmonotonic behavior not usually found in other natural processes. Numerical simulations provide evidence that this behavior is a consequence of the alternation between states of high and low activity, named up and down states, leading to a balance between excitation and inhibition controlled by a single parameter. During these periods, both the single neuron state and the network excitability level, keeping memory of past activity, are tuned by homeostatic mechanisms.

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Spontaneous neuronal activity can exhibit slow oscillations between bursty periods, or up states, followed by substantially quiet periods. Bursts can last from a few to several hundreds of milliseconds and, if analyzed at a finer temporal scale, have often shown a complex structure in terms of neuronal avalanches. *In vitro* experiments record avalanche activity [1,2] from mature organotypic cultures of rat somatosensory cortex where they spontaneously emerge in superficial layers. The size and duration of neuronal avalanches follow power law distributions with stable exponents, which is a typical feature of a system in a critical state, where large fluctuations are present and the response does not have a characteristic size. The same critical behavior has been measured *in vivo* from rat cortical layers during early postnatal development [3], from the cortex of awake adult rhesus monkeys [4] using microelectrode array recordings, as well as for dissociated neurons from rat hippocampus [5,6] or leech ganglia [5]. *In vitro*, quiet periods measured between bursts, also called down states, can last up to several seconds. The emergence of these down states can be attributed to various mechanisms: a decrease in the neurotransmitter released, either due to the exhaustion of available synaptic vesicles or to the increase of a factor inhibiting the release [7], such as the nucleoside adenosine [8], the blockade of receptor channels by the presence of external magnesium [9], or else spike adaptation [10]. A down state is then characterized by a *disfacilitation*; i.e., the absence of synaptic activity of a large number of neurons causing long-lasting returns to resting potentials [11]. Recently, it was shown analytically and numerically that critical behavior [12] characterizes up states, whereas down states are subcritical [13].

Whereas action potentials are rare during down states, small amplitude depolarizing potentials, reminiscent of miniature potentials from spontaneous synaptic release, occur at higher frequencies. The nonlinear amplification of small amplitude signals contributes to the generation of larger depolarizing events bringing the system back into the up state, as observed in cortical slabs [14], dissociated cultures [15], and slice cultures [16]. The analysis of the amount of time striatal spiny neurons [17,18] and cortical pyramidal neurons [19] spend at each value of the membrane potential shows that both cell types toggle between two preferred values [20]: a very negative one in the down state and a more positive, depolarized one in the up state. The up state being just a few millivolts from the action potential threshold suggests that during the up state neurons respond faster and more selectively to synaptic inputs. For cortical neurons the up state would be a metastable state; i.e., the membrane potential would soon decay down to the resting potential value if network mechanisms would not sustain the activity. The up state has, therefore, network, rather than cellular, properties.

Here we focus on the temporal organization of neuronal avalanches both in organotypic cultures and neuronal networks simulations. Each avalanche i is characterized by its starting and ending times, t_i^i and t_i^f. The temporal organization is analyzed by evaluating the distribution of waiting times $\Delta t_i = t_{i+1}^i - t_i^f$. This is a fundamental property of stochastic processes, widely investigated for natural phenomena and able to discriminate between a simple Poisson and a correlated process. Indeed, in the first case the distribution is exponential, whereas it exhibits a more complex behavior with a power law regime if correlations are present. For a wide variety of phenomena, e.g., earthquakes and solar flares [21], human dynamics [22], biological systems [23], etc., this distribution always shows a monotonic behavior. Recent results on freely behaving rats provide a lognormal size distribution and a monotonic waiting time distribution uniquely controlled by the average occurrence rate [24]. Anesthetized rats, conversely, exhibit a more heavy-tailed size distribution and no universal scaling for the waiting time distribution. Here we show that the waiting time distribution for neuronal avalanches *in vitro* has an unusual nonmonotonic behavior. Numerical simulations on neuronal networks suggest that this is controlled by the slow alternation of up and down states, which determines both the network and the single neuron behavior.

Experiments were performed on coronal slices from rat dorsolateral cortex (postnatal day 0–2; 350 μm thick) attached to a poly-D-lysine coated 60-microelectrode array (MEA; Multichannelsystems, Germany) and grown at 35.5 °C in normal atmosphere and standard culture medium without antibiotics for 4–6 weeks before recording. Avalanche activity was measured from cortex-striatum-substantia nigra triple cultures or single cortex cultures as reported previously [1]. Spontaneous avalanche activity is recorded outside the incubator in standard artificial cerebrospinal fluid (laminar flow of 1 ml/ min) under stationary conditions for up to 10 h. The spontaneous local field potential (LFP) is sampled continuously at 1 kHz at each electrode and low-pass filtered at 50 Hz. Negative deflections in the LFP (nLFP) are detected by crossing a noise threshold of -3 SD (~3–5 μV) followed by negative peak detection within 20 ms. nLFP times and nLFP amplitudes are measured. Neuronal avalanches are defined as spatiotemporal clusters of nLFPs on the MEA [25]. A neuronal avalanche consists of a consecutive series of time bins of width δt that contain at least one nLFP on any of the electrodes. Each avalanche is preceded and ended by at least one time bin with no activity. The waiting time Δt is simply given by the number of empty bins between two successive avalanches times δt. Without loss of generality, the present analysis is done with δt estimated for each culture as the average inter-nLFP interval on the array and ranged between 3–6 ms for all cultures.

In Fig. 1 we show the waiting time distribution for different cultures of rat cortex slices. The curves exhibit a complex nonmonotonic behavior with common features: an initial power law regime and a local minimum followed by a more or less pronounced maximum. The presence of a power law implies that avalanche occurrence is not a pure Poisson process; namely, successive avalanches are temporally correlated [26]. Moreover, the nonmonotonic behavior is not usually observed in natural phenomena. In order to investigate the origin of this behavior, we simulate avalanche activity by a neuronal network model [27–29], which is able to reproduce the scaling properties of neuronal avalanches. Here we question whether and how the complex temporal organization of avalanches can be caused by the slow alternation between up states and down states. The basic idea is that after a large avalanche the involved neurons become hyperpolarized and the system goes into a down state. Conversely, after a small avalanche active neurons remain depolarized and the system stays in an up state.

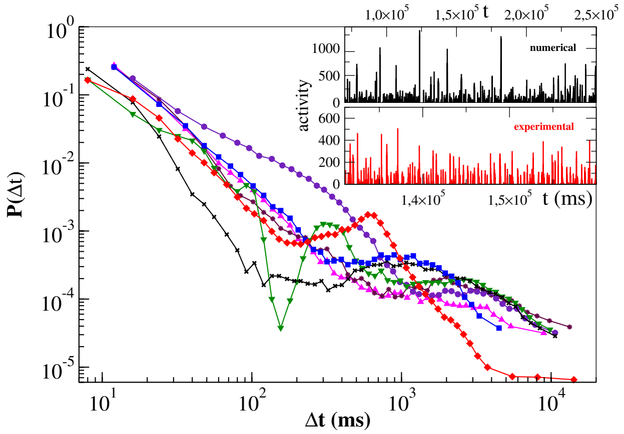

FIG. 1 (color online). The distribution of waiting times for seven different slices of rat cortex. All curves show an initial power law regime between 10 and about 200 ms, with an average exponent of 2.15 ± 0.32. For $\Delta t > 200$ ms, curves can become quite different with the common characteristics of a local minimum located at 200 ms $< \Delta t_{\mathrm{min}} < 1$ s, followed by a more or less pronounced maximum at $\Delta t \simeq 1$–2 s. In the insets: two temporal sequences of neuronal activity for numerical (sum of potential variations) and experimental (μV) data.

We consider N neurons at random positions, characterized by their potential v_i. Neurons are connected by a classical scale-free network [30], where a neuron i has an outgoing connectivity degree k_{out_i}. Once the network of output connections is established, we identify the resulting degree of in connections k_{in} for each neuron. To each synaptic connection we assign an initial random strength g_{ij}, with $g_{ij} \neq g_{ji}$, and to each neuron an excitatory or inhibitory character with 10% inhibitory synapses. Whenever at a given time the value of the potential at a site i is above a certain threshold, $v_i \geq v_{\max}$, the neuron sends action potentials which arrive to each of the k_{out_i} presynaptic buttons. As a consequence, the total charge entering the connected neurons is $q_i \propto v_i k_{\mathrm{out}_i}$, as in a firing rate based charge distribution. Each neuron receives charge in proportion to the synaptic strength g_{ij}, $v_j(t+1) = v_j(t) \pm \frac{q_i(t)}{k_{\mathrm{in}_j}} \frac{g_{ij}(t)}{\sum_k g_{ik}(t)}$, where the sum is on all outgoing connections of i. Here the membrane potential variation is obtained by dividing the received charge by the surface area of the soma of the postsynaptic neuron, proportional to the number of ingoing terminals k_{in_j}. The plus or minus sign is for excitatory or inhibitory g_{ij}, respectively. After firing, a neuron is set in a refractory state lasting 1 time step (about 10 ms), during which it is unable to receive or transmit any charge. At the end of an avalanche, we implement

Hebbian plasticity rules: the strength of the used connections between active neurons is increased proportionally to the activity of the synapse [31]; namely, the membrane potential variation of the postsynaptic neuron, $g_{ij}(t+1) = g_{ij}(t) + (v_j(t+1) - v_j(t))/v_{\max}$. Conversely, the strength of all inactive synapses is reduced by the average strength increase per bond, $\Delta g = \sum_{ij,t} \delta g_{ij}(t)/N_b$, where N_b is the number of bonds. The presence of both strengthening and weakening rules implements a homeostatic regulatory mechanism for synaptic strengths, which underlies the system's critical behavior. An external stimulus triggers further activity in the system: at the end of each avalanche the potential of a random neuron is increased by a small amount until another neuron arrives at threshold and starts an avalanche. We implement the plasticity rules during a series of stimuli in order to modify the synaptic strengths, initially random. Previous studies have verified that the critical behavior of avalanche distributions does not depend on parameter values or network properties and that this model reproduces quantitatively the background spectrum of measured EEG signals [27–29]. The implementation of a scale-free network of connections in the present study is motivated by numerical convenience in terms of CPU time.

In order to implement the alternation between up and down states, at the end of each avalanche we measure its size in terms of the sum of depolarizations δv_i of all active neurons, $s_{\Delta v} = \sum \delta v_i$. If the last avalanche is larger than a threshold, $s_{\Delta v} > s_{\Delta v}^{\min}$, the system transitions into a down state and neurons active in the last avalanche become hyperpolarized proportionally to their previous activity; namely, we reset

$$v_i = v_i - h \delta v_i, \tag{1}$$

where $h > 0$. This rule introduces a short range memory at the level of a single neuron and models the local inhibition experienced by a neuron, due to spike adaptation, adenosine accumulation, synaptic vesicle depletion, etc.

Conversely, if the avalanche just ended has a size $s_{\Delta v} \leq s_{\Delta v}^{\min}$, the system either will remain in or will transition into an up state. All neurons firing in the previous avalanche are set to the depolarized value

$$v_i = v_{\max}(1 - s_{\Delta v}/s_{\Delta v}^{\min}). \tag{2}$$

The neuron potential depends on the response of the whole network via $s_{\Delta v}$, in agreement with measurements of the neuronal membrane potential, which remains close to the firing threshold in the up state. $s_{\Delta v}^{\min}$ controls the extension of the up state and, therefore, the level of excitability of the system. The high activity in the up state must be sustained by collective effects in the network; otherwise, the depolarized potentials would soon decay to zero, and therefore, the random stimulation in the up state has an amplitude that depends on past activity. Equations (1) and (2) each depend on a single parameter, h and $s_{\Delta v}^{\min}$, which introduces a memory effect at the level of single neuron activity and the entire system, respectively. In order to reproduce the behavior observed experimentally, the parameters $s_{\Delta v}^{\min}$ and h are controlled separately. Our simulations will show that the ratio $R = h/s_{\Delta v}^{\min}$ is the only relevant quantity controlling the temporal organization of avalanches.

Numerical simulations show that the system indeed switches between up and down states with different temporal durations (insets of Fig. 1). The numerical waiting time distributions (Fig. 2) exhibit the nonmonotonic behavior of the experimental curves, where the position of the minimum is controlled by the value of $s_{\Delta v}^{\min}$, and the power law regime scales with the same exponent ~ -2 as experimental data. The agreement between the numerical and the experimental distribution is confirmed by the Kolmogorov-Smirnov test at a $p = 0.05$ significance level. Both distributions pass the statistical test with $p = 0.99$ (bottom panel) and $p = 0.68$ (top panel). The different contribution from the two states is reflected in the activity temporal scale (insets of Fig. 2). The up state generates

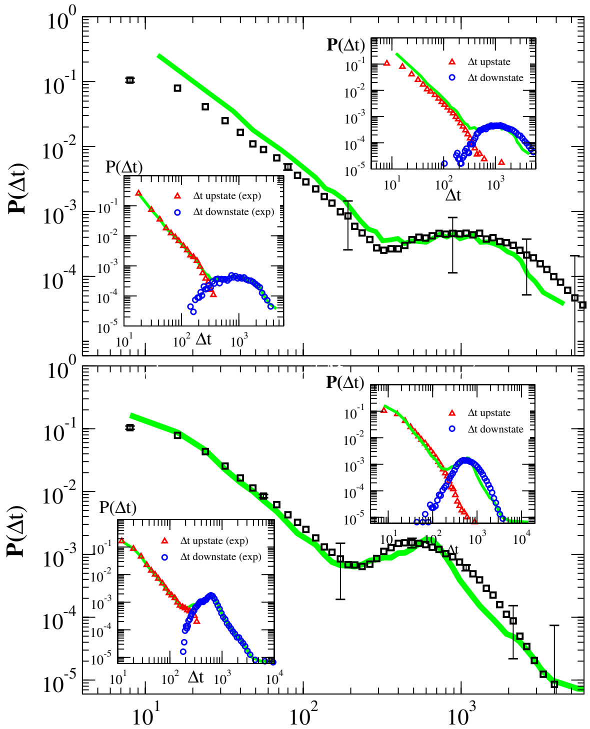

FIG. 2 (color online). Waiting time distributions measured experimentally are compared with the average numerical distributions for 100 networks with $N = 64\,000$ neurons. Top: numerical curve ($s_{\Delta v}^{\min} = 140$ and $h = 0.017$) fitting the experimental curve with blue squares in Fig. 1; bottom: numerical curve ($s_{\Delta v}^{\min} = 110$ and $h = 0.02$) fitting the experimental curve with red diamonds in Fig. 1. In the insets: the waiting time distribution evaluated separately in the up and down state for the numerical (upper insets) and the experimental curves (lower insets). For the numerical curves, statistical error bars not shown are comparable to the symbol size.

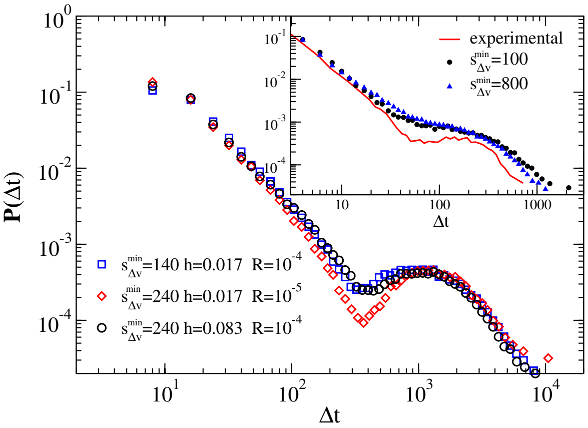

FIG. 3 (color online). Waiting time distribution measured numerically for 100 networks of $N = 16000$ neurons with different $s_{\Delta v}^{\min}$ and h. The best agreement is obtained for $R \simeq 10^{-4}$. In the inset: waiting time distributions obtained with different stimulations in the up and down states and without the single neuron state behavior ($h = 0$).

strongly clustered avalanches, originating at the power law regime of the waiting time distribution, whose extension depends on $s_{\Delta v}^{\min}$. Large Δt between avalanches generated in the up state are observed with a very small probability, which increases with decreasing h. Conversely, the waiting time distribution evaluated in the down state has a bell-shaped behavior centered at large intertimes, which depends on h; i.e., for a larger disfacilitation of the network the probability to observe intermediate waiting times decreases in favor of long Δt.

The presence of the minimum and the height of the relative maximum are sample dependent (Fig. 1) and for each sample the agreement between numerical and experimental data depends on the subtle balance between excitation and inhibition. For different samples, optimal agreement is realized when the ratio $R = h/s_{\Delta v}^{\min} \simeq 10^{-4}$. For instance, enhancing excitation, by increasing the threshold value $s_{\Delta v}^{\min}$, clearly produces a major shift in the data (Fig. 3). Increasing inhibition, by increasing the parameter h, generates the opposite effect, recovering the good agreement with experimental data. Interestingly, the avalanche size and duration distributions also reproduce the experimental scaling behavior for the parameter values, expressing the balance between excitatory and inhibitory components. The abrupt transition between the up and down state, controlled by a threshold mechanism, generates the minimum observed experimentally. Simulations of up states and down states only in terms of different external drives, without the single neuron state dependent behavior [Eqs. (1) and (2)], provide a monotonic waiting time distribution (inset Fig. 3).

This complex nonmonotonic behavior, controlled by the system balance level between excitation and inhibition expressed by the parameter R, does not simply depend on the occurrence rate. The different behavior with respect to alive rats [24] could be attributed to a larger separation in characteristic temporal scales between up and down states. Indeed, long-lasting down states in our case originate from waiting times 1 order of magnitude longer than for awake rats. Avalanches are temporally correlated in the up state, whereas down states are long term recovery periods where memory of past activity is erased. A detailed analysis of power spectra may shed further light on the temporal features of this alternation. The good agreement with experimental data indicates that the transition from an up state to a down state has a high degree of synchronization. Moreover it confirms that alternation between up and down states is the expression of a homeostatic regulation which, during periods of high activity, is activated to control the excitability of the system and avoid pathological behavior. The model suggests that the crucial feature of this temporal evolution is the different single neuron behavior in the two phases. These collective effects must be supported by the single neuron behavior which toggles between two preferential states: a depolarized one in the up state and a hyperpolarized one in the down state. The model suggests that the depolarized neuron state is a network effect: the avalanche activity itself determines how close to the firing threshold a neuron stays in the up state. Conversely, the hyperpolarized state is a form of temporal autocorrelation in the neuron activity. The critical state realizes the correct balance between excitation and inhibition via these self-regulating mechanisms.

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