

Cultures of dissociated neurons display a variety of avalanche behaviours

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Abstract. Avalanche dynamics has been described in organotypic cultures and acute slices from rat cortex. Its distinctive feature is a statistical distribution of avalanche size and duration following a power law with specific exponents, corresponding to near-critical state. We asked whether the same dynamics is present in dissociated cultures from rat embryos, which are characterized by complete lack of anatomic structure and high, random synaptic connectivity. We indeed observed such dynamics in some, but not all, experimental preparations. We conclude that the variability found in the dynamics of dissociated cultures also affects general features like the criticality of avalanche behavior.

1 Introduction

Spontaneous activity recorded from dissociated neuronal populations is characterized by bursts of firing activity, which spread over whole population. Such propagation is not continuous or wave-like, but seems to be organized into finite avalanches.

An avalanche is a cascade of consecutive events, triggered by a small perturbation, such as a change in the state of a single element. A prototypical example of this behaviour is the sandpile model proposed by Bak, Tang and Wisenfeld [1]. Avalanche dynamics occurs frequently in complex systems and has been described in many natural phenomena [2-5]. Recently, avalanches have been observed in the spontaneous neural activity of rat cortical organotypic cultures and in acute slices, placed on micro-electrode arrays (MEAs) [6, 7]. Such behaviors display dynamical properties that are typical of self-organizing branching processes [8]. More specifically, the statistical distributions of avalanche size and life time follow a power-law, with exponents of, respectively, $-3/2$ and -2 , which correspond to a critical state [1]. In fact, the branching parameter, defined as the average ratio between the number of active sites in subsequent time bins (successors and ancestors) turned out to be roughly equal to 1.

Criticality has been associated to maximal information transmission and efficiency of information storage [6, 7, 9]. In a simple feed-forward neural network model with binary elements and stochastic connections, the branching parameter defines the overall probability that activation of one element propagates to the next layer. Simulations with different network sizes and stimulus sets have shown that the mutual information between output and input is maximized when the branching parameter is near 1. An intuitive interpretation is that a subcritical branching process would attenuate the input signal, whereas a supercritical one would activate most output units, thus hiding the dependence on the input [6]. Furthermore, recurrent patterns in avalanches were shown [7] to represent non-attractive metastable states,

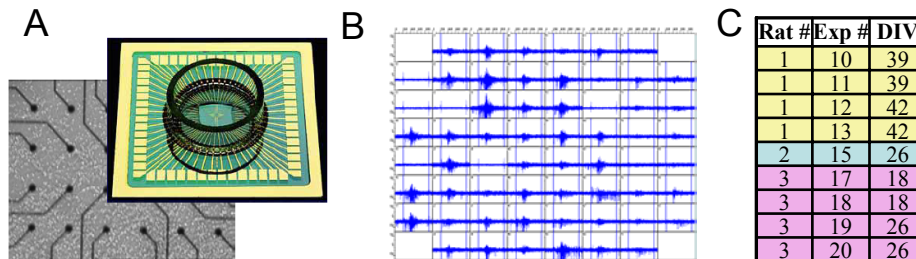


Fig. 1: A, (front) Micro-electrode array (MEA) device used for recording from dissociated cultures of neurons, layered in the center and (back) a microscope image of the neural preparation. B, Sample recording; each sub-panel displays the activity recorded from a different electrode. C, Details (animal, preparation and age of the culture) on the neural preparations analyzed here. DIV: days in-vitro

thus behaving as memory storage. A similar model, with a recurrent architecture[9], proved that near-critical systems maximize the number of these states.

Here we look at avalanches in cultures of dissociated cortical neurons. In these preparations, neurons rearrange into a two-dimensional, random network which lacks the organization found in organotypic cultures or acute slices. Our aim was to test whether and to what extent the spontaneous neural activity of dissociated cultures could be described in terms of critical self-organizing branching processes, with the aim of further investigating their efficiency in dealing with information transmission and storage.

2 Materials and Methods

Neural preparation. Dissociated cortical neurons, extracted from rat embryos (17–18 days), were gently plated on planar squared arrays of 60 TiN/SiN microelectrodes (MEA60, Multichannel Systems, Reutlingen, Germany), see Fig. 1, equipped with an integrated 60-channel pre-amplifier. The diameter of the electrodes and the inter-electrode spacing were, respectively, 30 μm and 200 μm . Neurons extracted from the developing brain can be kept in healthy conditions for a long time. They are spontaneously active and show complex patterns of spike and burst electrophysiological activity [10]. Our recordings started after 18–34 days in-vitro (DIV), i.e. when these preparations reportedly display maximum spontaneous activity [11].

Neural recordings and spike detection. Neural activity was recorded extracellularly from up to 60 microelectrodes (Fig. 1): items from each electrode could be constituted by single neuron activity as well as a mixture of signals coming from a few neurons (up to three) [12]. Because of the predominance of burst activity and our interest in collective behaviours, we did not attempt to perform spike sorting [13]. By operating on dissociated neurons culture, we were able to appreciate single spike temporal resolution, instead of organotypic culture and acute slice experiments where the distance between neurons in the slices and the electrodes, only allows registration of local field potentials (LFPs), which can result from the convolution of

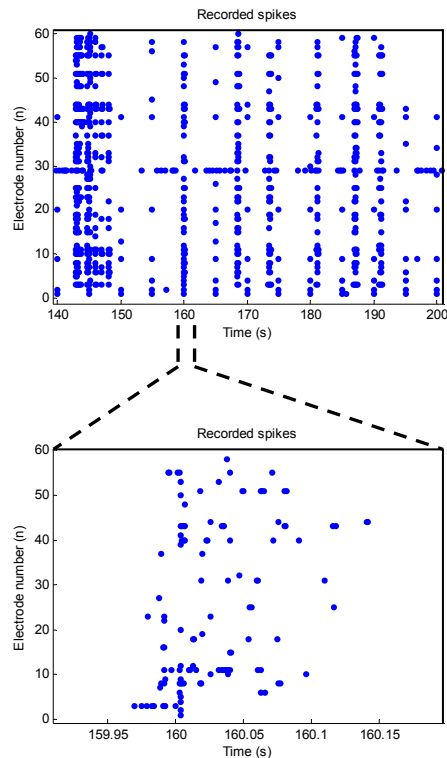


Fig. 2: Raster plot of activity from all electrodes shows correlated periods in which spikes do not appear on all electrodes at the same instant, but are scattered in time

more than one spike. Available data were records of 300 s each, sampled at 10 kHz. Spike detection was based on a threshold algorithm [14] within a 1 ms bin size.

Data analysis. Our starting point was the work of Beggs and Plenz on slice preparations [6, 7], based on analysis of LFPs. In our case, we were able to look at single spikes and therefore we could potentially look at the micro-structure of avalanches. However, it was necessary to formulate a suitable definition of avalanches and of avalanche sizes. To this purpose, we subdivided the recordings (spike trains from up to 60 electrodes) into time bins of length Δt ; the spatial pattern of activity from all electrodes in a time bin was called a frame. By defining an event as a single spike arising from any of the electrodes in a determinate instant, we defined an avalanche as the set of frames in which events occur, preceded and followed by at least one frame in which all electrodes are silent. We repeated the analysis with different bin sizes ($\Delta t = 1$ ms, 2 ms, 4 ms, 8 ms and 16 ms); however, an 'optimal' bin size can be identified as the ratio of the inter-electrode distance (200 μm) in our MEAs and the average propagation velocity of neural activity, estimated from cultured neurons on MEAs (~ 50 mm/s) [15]. The above rationale results into an ideal bin size of $\Delta t = 4$ ms [6]. Life time of an avalanche was simply defined as the temporal duration of the avalanche. The size of an avalanche could be defined in

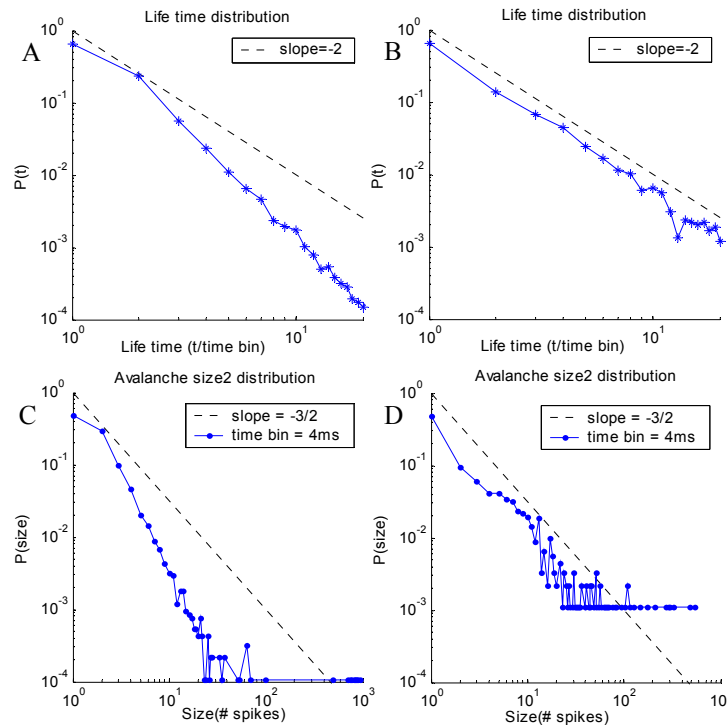


Fig. 3: Avalanche life time (top: A,B) and size (bottom: C,D) distribution for two different experiments. Left: slopes do not match those predicted by SOBP theory (broken lines). Right: slopes are very close to the critical values of -2 and -3/2 for, respectively, life time and size

different ways. We chose 3 definitions: (i) the maximum number of spikes recorded in one time bin internally at the avalanche (hereafter, size-1); (ii) the number of spikes of the entire avalanche (size-2); and (iii) the number of electrodes activated at least once in an avalanche (size-3). We analyzed the probability distributions of these different size parameters for all the avalanches detected in our recordings and fitted this distribution with a power law (i.e., a line in log-log scale).

3 Results

A preliminary analysis of the raster plots displaying the recorded spontaneous activity suggested a self-similar structure (see Fig. 2). What appeared to be simultaneous firing revealed, at a finer temporal resolution, a complex spatiotemporal pattern of firing. These patterns of activity were identified as avalanches according to the definition formulated in the Methods section. We identified avalanches from the 9 available experiments, using different time bins. In all the experiments, irrespective of the choice of the bin size, the probability distribution of avalanche life time and size displayed a close-to-linear relationship in log-log coordinates. Moreover, for each

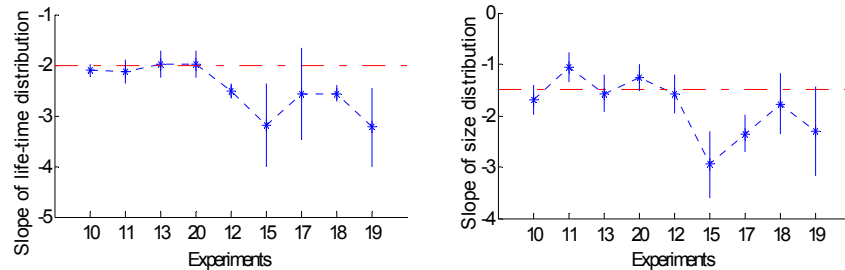


Fig. 4: Different experiments display a variety of avalanche behaviors. Power law exponents for avalanche life time (left) and size (right). Bin size: 4 ms. The size distribution refers to size-2. Only the first 4 experiments display exponents that are close to the critical values; in other experiments, exponents are greater

experiment the estimated slope did not depend on the particular definition of the avalanche 'size'. However, the slopes were not the same in all preparations. More specifically, in 4 preparations (out of 9) the slopes in the distributions of life time and size were similar to those found in experiments with slices and organotypic cultures and consistent with critical SOBP dynamics. However, the remaining 5 preparations displayed greater slopes (in absolute value), thus suggesting that large and long-living avalanches are less frequent. Fig. 3 shows the power law distributions of size and life time for two experiments that display different behaviors (bin size: 4 ms). The figure only displays the size-2 distribution, as size-1 and size-3 gave similar results. Fig. 4 summarizes the estimated power law exponents for all the 9 experiments.

4 Discussion and Conclusions

Cultures of dissociated neurons represent a reduced model system that can be used to investigate the emergent collective and functional properties of the nervous system in order to understand how the brain represents, stores and processes information. Avalanche dynamics seems to be a crucial property of the spontaneous activity of neuronal populations, as it has been related to efficient information processing and storage. The variety of power law exponents that were observed in the statistical distributions of avalanche size and life time in different dissociated cultures suggest that at least some of these preparations are fundamentally different from their acute or organotypic counterparts. In particular, they seem to exhibit a sub-critical behaviour (larger and long-living avalanches are less likely). This may be related to the lack of structure in the connectivity of dissociated cultures (in particular, the reduction of long-range connections). Other factors (e.g. age, density of the culture) could be implicated as well. These results also suggest relating the indicators of avalanche behaviours to other descriptors of the population behaviour, like the ability to encode information.

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