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Hallmarks of Criticality in Neuronal Networks Depend on Cell Type and the Temporal Resolution of Neuronal Avalanches

KRISTINE HEINEY[1,2,*], VIBEKE DEVOLD VALDERHAUG[3], OLA HUSE RAMSTAD[3], IOANNA SANDVIG[3], AXEL SANDVIG[3,4,5] AND STEFANO NICHELE[1,6]

[1]*Department of Computer Science, Oslo Metropolitan University, Norway*
[2]*Department of Computer Science, Norwegian University of Science and Technology (NTNU), Norway*
[3]*Department of Neuromedicine and Movement Science, NTNU, Norway*
[4]*Department of Neurology, St. Olav's Hospital, Norway*
[5]*Division of Neuro, Head, and Neck, Umeå University Hospital, Sweden*
[6]*Department of Holistic Systems, Simula Metropolitan, Norway*

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The human brain has a remarkable capacity for computation, and it has been theorized that this capacity arises from the brain self-organizing into the critical state, a dynamical state poised between ordered and disordered behavior and widely considered to be well-suited for computation. Criticality is commonly identified in in vitro neuronal networks using an analytical approach based on the size distribution of cascades of activity called neuronal avalanches. In this study, criticality analysis was applied to different in vitro neuronal networks with two areas of focus: evaluating the effect of the size of the time bins used for neuronal avalanche detection and observation of the development of networks of neurons derived from human induced pluripotent stem cells. This preliminary study is expected to aid in the construction of models capable of emulating neuronal behaviors identified as well-suited for computation and ultimately inform the development of brain-inspired computing substrates that are better able to keep pace with increased demand for data storage and processing power.

Keywords: Bio-inspired computation, self-organized criticality, in vitro neuronal networks, neuronal avalanches

* Contact author: E-mail: kri@oslomet.no

1 INTRODUCTION

The human brain is a complex dynamical system that is able to process information with remarkable efficiency. It has been theorized that the brain lies in a dynamical regime poised between order and disorder, known as the critical state [1], and that this state of criticality is largely responsible for the brain's capacity for computation [2]. The functions of the brain arise from the interaction of many individual neurons communicating across dynamic networks to produce complex macroscopic behaviors. The study of individual neurons thus gives us little insight into the larger-scale activity of the brain, as the mechanisms governing local interactions lead to the generation of emergent complex behavior at a larger scale, a phenomenon known as self-organization that is commonly observed in complex systems [3, 4].

Criticality in dynamical systems can be considered analogous to the critical point observed in phase transitions in matter [5]. The interactions among the many microscopic constituent components of matter produce properties observable at the macroscale that simply do not exist at the microscale; for instance, the pressure of a gas, a macroscopic property, can only be considered meaningful when many gas molecules exist in a volume. This macroscopic behavior also exhibits transitions, known as phase transitions, when the system is subjected to different external conditions (e.g., temperature or pressure). The critical regime, lying between ordered and disordered phases, confers the distinct functional advantages of high sensitivity to a wide range of inputs and long-range information transmission, behaviors that are commonly considered to be characteristic of and beneficial in neural systems, and it has been theorized that the brain self-organizes into this state [6].

Thus, criticality may provide some insights into the mechanisms underlying the computational power of the brain. Such mechanisms have the potential to be harnessed in engineerable substrates toward the development of novel computing technology with greater data processing and storage capabilities than currently available devices. Current computing technology is based on the von Neumann architecture, in which tasks are performed sequentially and control, processing, and memory are each allocated to structurally distinct components. This approach to hardware construction cannot keep up with the rapidly rising amount of data generated in today's society. Furthermore, although recent advancements in machine learning methods have expanded our present data handling capabilities, processing continues to be performed on conventional hardware and thus requires huge amounts of training data, computational time, and computing power.

Turning to the brain for inspiration would enable the development of novel physical computing architectures that are capable of learning, scalable,

energy-efficient, and fault-tolerant. Behaviors we observe in the brain could be targeted for emulation in engineerable self-organizing substrates, which have an inherent capacity for information transmission, storage, and modification [1]. This would bring computation from the digital realm to the physical realm, enabling improved efficiency through the direct exploitation of material and physical processes for computation [7, 8]. The use of physical self-organizing substrates in computation has been explored in recent studies on reservoir computing, in which a computing substrate is exploited by training a readout layer to interpret the output of a system to a target problem [9, 10].

The present study is part of a larger project called Self-Organizing Computational substRATES (SOCRATES) [11, 12], the aim of which is to develop self-organizing computing hardware based in nanomagnetic substrates and biological in vitro neural networks, taking inspiration from the brain to guide the design. Criticality represents one important desirable behavior that can provide further insight into which mechanisms displayed by neuronal networks, such as mechanisms of functional connectivity and information storage and transmission, are most beneficial for computation. This approach is also expected to prove useful in the identification of perturbed networks, as it is expected that different perturbations, such as neuromodulation or electrical stimulation, may induce a deviation from the expected dynamical behavior as it relates to criticality. This will be applicable in the study of disease models and can also provide insight into how to ensure the robustness of brain-inspired computational systems against perturbations.

In the present study, criticality analysis based on the size distribution of neuronal avalanches was applied to electrophysiological data obtained from networks of dissociated neurons cultured atop microelectrode arrays (MEAs). The considered networks have been previously analyzed in related studies and were from two different types of neurons: primary rat cortical neurons [13] and dopaminergic neurons derived from human induced pluripotent stem cells (iPSCs) [14]. The results indicate that critical behavior is robustly identifiable with this classification method regardless of the time bin size. Additionally, preliminary investigation into the development of the iPSC-derived networks suggests that they may enter into an early stage of criticality and then deviate from criticality at later stages of their maturation.

The remainder of this paper is organized as follows. Section 2 presents related work using in vitro neuronal networks for computing and control, links criticality to computation, and gives some background on the fundamentals of the method used in this study to identify hallmarks of criticality in neuronal systems. Section 3 outlines the experimental and analytical methods used in this study. The results are presented and discussed in Section 4, and Section 5 concludes the paper.

2 RELATED WORK: COMPUTATION IN IN VITRO NEURONAL NETWORKS

Neural computation has long been a subject of interest among researchers from a wide variety of disciplines. The advent of MEA technology has provided researchers with the ability to record electrophysiological activity from populations of neurons over long time periods and to provide targeted inputs to the network via electrical stimulation [15]. This has opened the door to studying neural computation in a reduced in vitro model and using living neuronal networks for computational tasks. This section first gives some background on how neuronal networks may be interfaced with a physical or virtual environment or used as a computational "reservoir" to perform computing tasks. Then the concept of criticality in neural systems is introduced as a means to study the computational capacity of a neuronal network.

2.1 Embodiment of in vitro neuronal networks

Within the field of artificial intelligence, the concept of embodiment as put forth by Rodney Brooks states that any intelligence is in some regard dependent on a body to actualize itself [16]. That is, intelligence needs to be part of, or embodied as, an interacting agent within an environment (see Figure 1(a)). The conceptual and philosophical implications of this are far too vast to cover

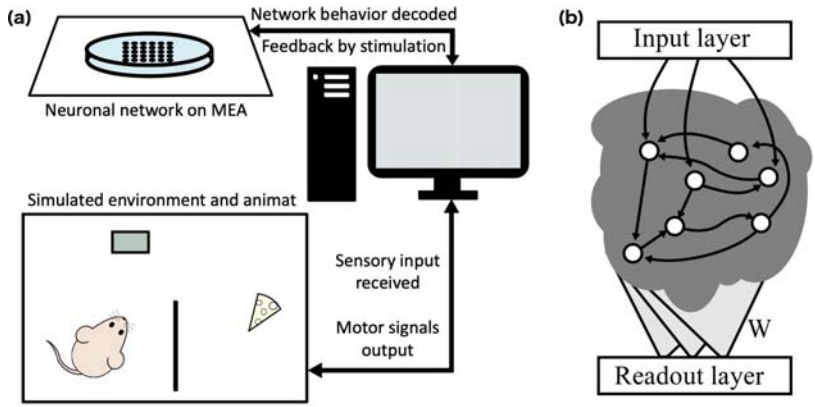


FIGURE 1
In vitro neuronal networks can be used for computational tasks. (a) Embodiment of a living in vitro neuronal network. The network activity is recorded by interfacing with an MEA and decoded to produce output behavior in the animat or other embodied agent. Sensory information from the animat is then fed back to the network via electrical stimulation. (b) Illustration of reservoir computing (reproduced from [21]). Inputs are encoded and fed into the reservoir, and outputs from the reservoir are decoded using a readout layer, which is a trained artificial neural network.

in any detail here, instead we will briefly consider how embodiment relates to the computational efficiency of an in vitro neuronal network. Hence, can the performance of an embodied network in an environment serve as a proxy for its computational capacity, and conversely, can alternate methods of estimating the computational capacity of a network be used to predict their embodied behavior?

Motor control, such as bipedal locomotion, still stands as a serious challenge within robotics where biological systems excel in comparison to artificial systems [16]. As a result, much of the focus within in vitro network embodiment has been geared towards sensory–motor circuits, utilizing the biological component as part of an adaptive control circuit for the robotic body, or "animat" [17]. As the in vitro network acts as part of the robot's motor control, the performance of the robot's interaction with the environment, such as its collision avoidance, provides a grounded and tangible readout for the network's computational ability.

The earliest implementations of this type of embodiment of in vitro networks stemmed from the Potter group at Georgia Tech [17–19]. As a proof-of-concept, an in vitro network of dissociated cortical neurons cultured on an MEA was embodied as a simplified flight controller with the objective of maintaining the pitch and roll of a virtual plane at constant values. Feedback control was implemented based on the activity recorded from the in vitro network at specific output electrodes of the MEA, and errors from the flight telemetry were fed back to the network by applying electrical stimulation at specific error feedback electrodes [20].

Despite being one of the first implementations of such an embodied system, this study remains one of the most complex and highlights many of the challenges with such embodied systems. These challenges can be briefly classified as follows: physical embodiment, closing the loop, providing input or feedback by stimulation, training, and network complexity. The first challenge is crucial to many reductionist approaches, as the control of a physical entity or robot is a difficult task. With a reduced virtual robot it is possible to abstract away the physical complications of actuators, energy supply, chassis, and environmental conditions while maintaining complete control over the sensors output to the encoders [17, 22, 23]. However, despite the complications, several working implementations of physically embodied cultures have been reported in the literature [18, 20, 23–26].

With both physical and virtual systems, it is necessary to consider the placement of the network within the circuit. In Demarse and Dockendorf's [20] virtually embodied flight controller, several input electrodes were designated to provide the encoded flight telemetry through electrical stimulation, closing the loop between input and output [19, 23, 27, 28]. Conversely in an open "output only" loop, networks receive no error feedback; this tends

to reduce performance, as neither the self-organizing nor plastic features of the cultures are utilized. A closed-loop system is closer to a truly embodied sensory–motor system; however, encoding and providing sensory input to the network brings with it several unique problems.

Biological neural networks are self-organizing and adaptive systems, which is a large component of their computational advantage compared to artificial systems [29–31]. However, the dynamics of these neural networks necessitate the researcher relying on either innate adaptation to stimuli, an adaptive encoder, or a training step to guide the network to a desired output state [20, 23, 25, 32–34]. Because of the immense complexity of neural dynamics, most previous studies on embodiment have relied on the network's plastic mechanisms in combination with some positive local or global reinforcement training, though some also utilize a form of adaptive encoder. As of today, the performance of input encoding and training remains the largest challenge in the embodiment of in vitro networks. Future studies promise to apply more realistic and sophisticated methods such as modular hierarchical networks and 3D or organoid cultures, but the input and training challenge will still remain with these methodologies—and likely at a greater scale than with the typical 2D monolayer cultures currently in use [22, 25, 35].

2.2 In vitro networks as computational reservoirs

Moving away from the embodiment to the related approach of reservoir computing [36], some of the major issues with embodiment can be bypassed, namely that of training and physical robotics, while still targeting the main question of how in vitro networks may be used for computational tasks.

Reservoir computing [37, 38] is a brain-inspired method of training recurrent neural networks, a notoriously difficult task, that maps a nonlinear input into a high-dimensional dynamical system—typically an untrained randomly connected recurrent artificial neural network—and trains only a simple linear readout layer to achieve the wanted computation (Figure 1(b)). Different substrates have been shown to possess the necessary rich dynamics to act as reservoirs, including a bucket of water [39] and different brain regions such as the primary visual cortex of anesthetized cats [40]. For a recent review of physical reservoir computing substrates see [41].

In an early approach to reservoir computing with in vitro neuronal networks [42], network responses were classified using a support vector machine. Additionally, a prototype closed-loop neuro-reservoir system, in which an in vitro network of iPSC-derived neurons is embodied in a virtual robot, provides insight into the link between embodiment and reservoir computing [12]. One important property of reservoir computers is the separation property, which represents the separability of output responses from the reservoir, and this property has been demonstrated in in vitro neuronal

networks after stimulation [43]. The echo state property of neuronal cell cultures has also recently been demonstrated using a high-density MEA [44]. In this preliminary work, the selected output nodes exhibited reproducible spike trains in response to identical driving stimuli, thus showing asymptotic properties of transient trajectories to driving signals.

Although work on the use of living neuronal networks as computational reservoirs is progressing, an important question remains unanswered: How can we predict the computational performance of neural systems and thereby select for those networks that are best suited as reservoirs? In many other dynamical systems, it has been shown that the critical state, also known as the "edge of chaos" [1], optimizes many properties related to the system's computational performance [45–53]. The study of criticality in neural systems has recently gained traction in the neuroscience community, and the ability to identify the dynamic state of neuronal networks offers great promise to the study of computation—not only in terms of the use of living neural systems in computational and embodiment applications but also in gaining inspiration from their self-organizing behaviors as we improve bio-inspired approaches to computation. In the next section, we review some fundamental approaches to studying criticality in in vitro neuronal networks.

2.3 Neuronal avalanches and criticality

Systems operating at or near the critical state have been shown to confer a number of computational advantages, maximizing various properties such as the dynamic range, the number of metastable states, and mutual information (reviewed in [54, 55]). This suggests that approaches to evaluating the dynamic state of neuronal networks may aid in predicting how well a network will perform at computational tasks and may also give insight into how the networks are able to efficiently process information. A number of methods to identify the dynamic state of in vitro neuronal networks have been developed; this work represents a first step in applying these methods with the goal of identifying networks well-suited for computation and studying their self-organizing principles.

A classification method based on the size distribution behavior of network-wide cascades of activity called neuronal avalanches was applied to data obtained from different in vitro networks of dissociated neurons. This analytical approach was developed by [56] based on the dynamical behavior observed in other similar physical systems. In neuronal information processing, individual neurons integrate inputs from many other neurons and redistribute their activity back to the network once a threshold is reached; this is similar to the integration and redistribution behavior observed in many other complex systems, such as earthquakes and forest fires [56]. With the activation of individual components of these systems comes a cascade of activity

that propagates through the network, known as an "avalanche." One characteristic of criticality is that dynamical systems in the critical state show scale-free behavior, with common behaviors traversing a wide range of scales [5].

With the method applied here, it can be determined whether the network is in the critical state at a given time point based on the results of fitting a power law to the probability distribution of the size of avalanches recorded in the network. In the critical state, the size distribution should follow a power law, meaning the probability of an avalanche occurring with a given size is proportional to the size raised to a constant power; in contrast, sub- and supercritical dynamics respectively yield exponential and bimodal size distributions. As stated in Section 1, systems in the critical state, poised between order and disorder, are considered well-suited for computation and have been shown to be sensitive to a wide range of inputs and capable of effectively storing and transmitting information [1]. In the case of neuronal systems, criticality rests at a transitional point between asynchronous and synchronous behavior, with many spikes setting off only tiny cascades of activity and few spikes eliciting full-network responses. The foundations and presumed benefits of the brain self-organizing into the critical state have been reviewed in recent papers [2, 6, 57].

The original study on neuronal avalanches and criticality focused on slice cultures [56], but work has been expanded to dissociated cultures as well (e.g., [58–60]). However, the focus of criticality analysis to date has been predominantly on cortical neurons, and little to no consideration has been given to the possibility of this behavior being observed in other types of neurons. Previous reports have demonstrated that avalanches of activity follow power-law scaling in both the spatial and temporal domains, and that this behavior is independent of the spatial sampling of the network (i.e., inter-electrode distance) and the selection of the time bin size [58].

One possible mechanism considered to be responsible for this critical behavior is the striking of an appropriate balance between excitation and inhibition [54, 61]. This balance appears to be reached only after a certain point in the maturation of dissociated networks, as they have been observed to tend to pass through an early subcritical stage followed by a supercritical stage before finally settling into the critical state; this has led to speculation about a mechanism of initial hyperconnectivity followed by pruning to achieve the appropriate balance of integration and segregation [58, 59]. Interestingly, a similar progression of synaptic density is seen in human development [62], indicating this behavior seen in some cases in vitro may be analogous to in vivo development. It has been demonstrated that criticality is associated with a large dynamic range [61] as well as high information capacity and transmission [54], further supporting the theory that criticality in the brain confers

functional benefits. Furthermore, modeling work has revealed that criticality may be associated with scale-free, small-world network architectures [63].

As stated previously, the data analyzed in the present study has been previously analyzed in earlier studies by the present authors on criticality in neuronal networks with induced Parkinson's-related proteinopathy [14] and on whether supercritical cortical networks can be manipulated into the critical state by increasing inhibition [13]. In the former study, the results indicated that proteinopathy can be associated with alterations in the normal developmental trajectory with respect to criticality. It was observed that the unperturbed (control) networks eventually deviated from criticality after an initial period consistent with criticality, whereas the networks with induced proteinopathy remained in a state consistent with critical behavior. In the latter study, the cortical networks were observed to mature over 51 days in vitro (DIVs), at which point they showed highly synchronized activity consistent with the supercritical state. Perturbation with γ-Aminobutyric acid (GABA) to increase inhibition was observed to break this synchrony and bring the networks into the critical state. Building on these previous works, this study delves more into the applied methodology and the implications this analysis has for future work. More focus is also given to observations of the normal course of maturation of the iPSC-derived networks in terms of their trajectory through different dynamic states.

3 METHODS

In this study, two types of neuronal networks were evaluated: primary rat cortical neurons and dopaminergic neurons derived from human iPSCs. This section first outlines the preparation of the networks and the setup of the electrophysiology experiments. The analytical methods used to classify the networks as critical or not critical are then described.

3.1 Preparation and electrophysiology of cortical and iPSC-derived networks

Two cortical networks (Networks cA and cB) and two iPSC-derived networks (Networks iA and iB) were analyzed in this study. The cortical networks are the same as those analyzed in a previous related study addressing whether supercritical cortical networks could be manipulated into the critical state by increasing inhibition in the networks [13]. The iPSC-derived networks are the two control networks in a previous study on the evaluation of criticality in networks with induced proteinopathy to investigate the associations between criticality and proteinopathy related to Parkinson's disease [14]. Detailed

preparation and electrophysiology methods can be found in these previous papers, and the main points are summarized here.

For the cortical networks, primary rat cortical neurons (Thermo Fisher) were seeded on a feeder layer of human astrocytes (Gibco, Thermo Fisher) and left to mature for 7 DIV prior to recording. The same MEA recording system was used to record the electrophysiological behavior of the netowrk every second day for 15 min. Microscope images of the two cortical networks are shown in Figure 2. At DIV 51, GABA (Sigma Aldrich) was added to the cortical networks at rising concentrations to disrupt the excitation-to-inhibition ratio by increasing network inhibition. After obtaining a baseline

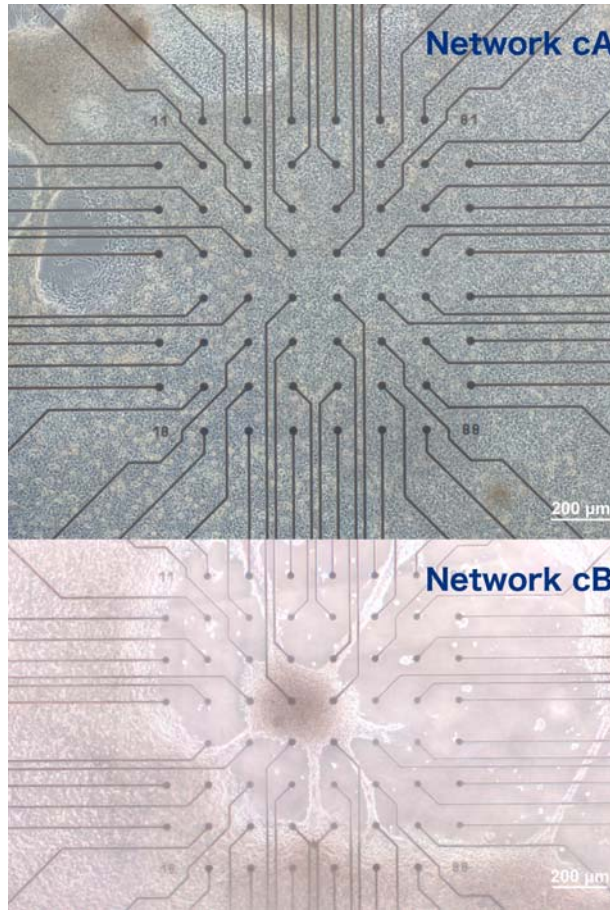


FIGURE 2
Microscope images of the primary cortical networks analyzed in this study. Network cA is shown at DIV 49, and Network cB is shown at DIV 51. Image reproduced from [13].

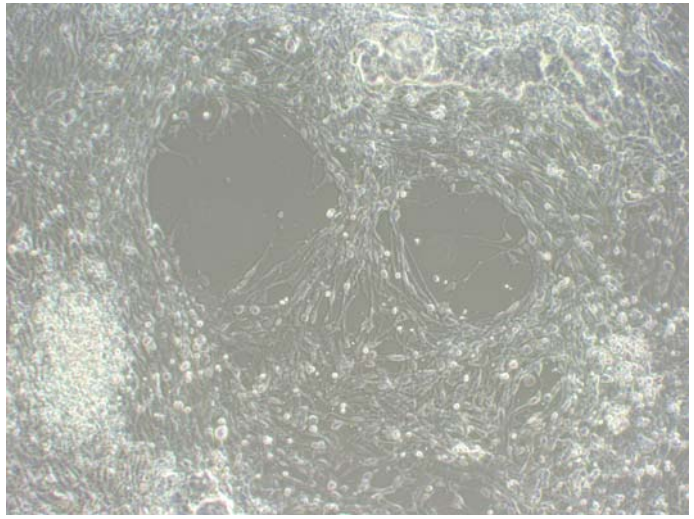


FIGURE 3
Representative example of an in vitro neuronal network derived from iPSCs.

recording, GABA was added directly to the culture media in microliter volumes, and recordings were taken immediately following perturbation. Different concentrations (10 and 50 μM for Network cA and 5, 10, and 25 μM for Network cB) were chosen to provide increasing degrees of perturbation; lower concentrations were used for Network cB because it showed lower levels of activity.

For the iPSC-derived networks, human iPSCs (ChiPSC18, Takara Bioscience) were reprogrammed using a protocol for midbrain dopaminergic neurons adapted from previous studies [64–66]. Reprogramming was concluded on day 16, at which point the cells were left to mature. A microscope image of a representative iPSC-derived network is shown in Figure 3. The spontaneous electrophysiological activity of the networks was recorded using a 60-electrode MEA together with the corresponding in vitro recording system (MEA2100-System, Multi Channel Systems) and software (Multi Channel Experimenter, Multi Channel Systems). Recordings of 6 min were taken starting after three weeks of maturation (DIV 21), starting from the date at which the reprogramming was concluded, and a total of 18 recordings taken over the period from DIV 21 to DIV 56 were analyzed for each network.

3.2 Criticality analysis

The criticality analysis was the same as that applied in the previous studies on these networks [13, 14]. The focus of the analysis in this study was twofold:

first, to demonstrate the effect of different time bin widths on the analysis results and discuss different motivations for time bin selection, and second, to present and discuss the observed course of maturation of the iPSC-derived networks in terms of their dynamic state.

The raw electrophysiological data obtained from each network at each time point was first bandpass-filtered with a passband from 300 Hz to 3 kHz. Spikes were detected using a simple thresholding approach using a threshold of a given number of standard deviations below the median of the signal* . The number of standard deviations for the threshold was set to 6 and 5 for the cortical and iPSC-derived networks, respectively. The threshold of 5 standard deviations was also used in the previous proteinopathy study [14]; as noted in this previous study, the spikes recorded from these networks had a small signal-to-noise ratio (SNR), which complicated the avalanche analysis if the threshold for detection was raised. In the previous study on the manipulation of the dynamic state of the cortical networks, multiple thresholds (6, 7, and 8 standard deviations below the median of the signal) were applied. Based on the results observed there, 6 standard deviations was selected to yield sufficiently high numbers of avalanches for a robust fitting; this is important for the post-perturbation recordings where criticality was observed, as GABA also has the effect of suppressing activity.

Avalanches were then detected according to the method developed by [56]. The detected spikes were binned in time using different time bin widths ranging from 0.2 to 16 ms to evaluate the effect of the bin width on the analysis results (see Figure 4), as has been done in previous studies to evaluate the robustness of the analysis against changes to the bin size and the effect of the bin size on the slope of the power law fitting (e.g., [56, 58]). Avalanches were detected as any number of consecutive time bins containing at least one spike on any recording channel, bounded before and after by empty time bins. The size of an avalanche is defined as the number of recording channels that are active over the course of the avalanche.

A power law fitting was then performed for each of the resulting avalanche size distributions. As in the previous cortical study [13], nonlinear regression (NLR) and maximum likelihood estimation (MLE) were applied as the fitting methods, and the results were compared. The power law for the fitting takes the form

$$P(s) \propto s^{-\alpha}, \tag{1}$$

where s is the avalanche size, $P(s)$ is the probability of an avalanche having size s, and α is the power of the fitted power law (slope of the fitted line in

* Code for spike detection is available at https://github.com/SocratesNFR/MCSspikedetection.

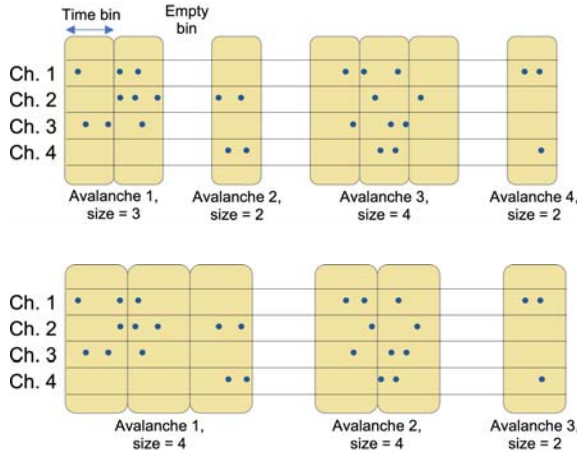


FIGURE 4
Definition of a neuronal avalanche and demonstration of the effect of different time bin widths on avalanche detection. Each dot represents a spike recorded on one of the recording channels (Chs. 1–4). A time bin is active when it contains at least one spike and empty when there are no spikes. An avalanche is defined as a sequence of consecutive active time bins preceded and followed by empty bins, and the size is the number of electrodes active during the avalanche. The use of larger time bins may result in spikes being collected into fewer avalanches with a greater average size.

log–log space). The fit was applied over the size range of $s = 2$ to 59 electrodes, following previous works [58, 63]. The goodness of fit was calculated using the method by Clauset et al. [67]. Synthetic datasets of avalanche sizes were generated from the fitted distribution, and the Kolmogorov–Smirnov (KS) distances of these datasets from the theoretical distribution were compared to the empirical KS distance. The fitting was rejected if the fraction p of synthetic KS distances that were greater than the empirical KS distance was less than 0.1 ($p < 0.1$)[†].

4 RESULTS AND DISCUSSION

This section first presents the effect that varying the time bin size had on the analysis results. Network cA is the focus of this type of analysis because it had the largest amount of activity, but similar results were observed across all four networks. The development of the iPSC-derived networks is then presented, with a focus on the development of Network iA. The section concludes with a plan for future work.

[†] Code for avalanche detection and goodness of fit evaluation is available at https://github.com/SocratesNFR/avalanche.

4.1 Impact of time bin size

The effect of the time bin width is presented here using the data from Network cA as a representative example among the networks, as it showed a higher mean firing rate and more active electrodes than the other networks, enabling better fittings over a wider range of bin sizes. However, similar trends were observed across all four considered networks when there were a sufficient number of avalanches ($N \gtrsim 1000$) to allow for robust fitting results.

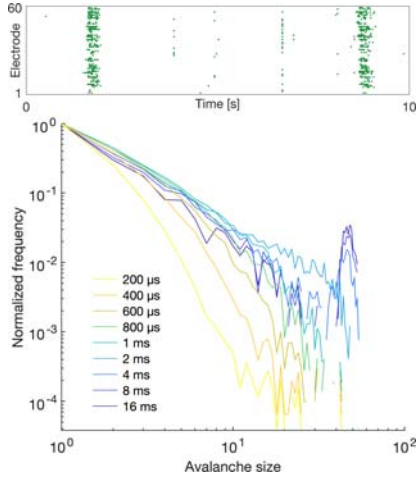
As stated in Section 3.2, the time bin width was set to different values ranging from 0.2 to 16 ms to evaluate the effect of the binning on the analysis results. The schematics in Figure 4 show how the use of larger bin sizes tends to cluster more spikes together into fewer avalanches of a larger size. It has been demonstrated by [58] that increasing the bin size to a certain point does not change the power-law scaling behavior of the avalanche size distributions that result; however, beyond a certain point, larger bin sizes may produce so many large avalanches that the network may appear supercritical.

Figure 5 shows the effect of the bin size on the avalanche size distribution functions normalized by the frequency of the smallest avalanche size, i.e., the number of detected avalanches of size 1. This choice of normalization makes it more clearly visible that there is a steeper drop-off in the avalanche size when smaller bins are used. Above each set of size distributions, raster plots showing the first 10 s of activity are shown to demonstrate the qualitative difference between the activity patterns displayed at the two time points.

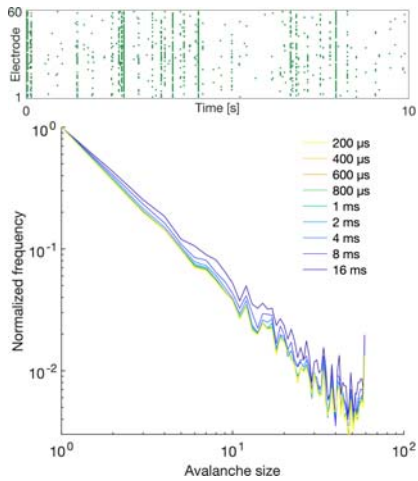
The two cases shown here are from two different recording time points of Network cA identified as corresponding to subcritical and critical behavior in the previous study [13], with the critical case corresponding behavior observed after the addition of GABA on DIV 51 to bring it from supercritical to critical. As stated in the previous paper, the analytical method applied here enables the classification of network dynamics as critical or not critical, and further classification of non-critical dynamics as sub- or supercritical was performed by visual inspection of the shape of the size distributions; rigorous classification of non-critical cases remains a task for future work.

In the non-critical case (Figure 5(a)), the use of smaller time bins (200 μs to 1 ms) produced rapidly decaying size distributions, consistent with the exponential decay expected when the dynamics are subcritical. Interestingly, larger bins (4 to 16 ms) produced the bimodal distributions typical of supercritical dynamics[‡]. This phenomenon arises because multiple avalanches detected with smaller bin sizes are grouped together when larger bin sizes are used (see Figure 4). Perhaps most noteworthy among the size distributions is the bin size of 2 ms. In this case, the power-law fitting results with

[‡] It should be noted that although the raster plot in this case (top of Figure 5(a)) appears to show rather synchronous behavior across the network, the synchrony is much looser than at later time points; for a more thorough comparison, see Figure 2 of [13].



(a)



(b)

FIGURE 5
Effect of varying the time bin size used for avalanche detection on the size distribution function in cases that have been identified as having (a) subcritical (DIV 21) and (b) critical (DIV 51 after perturbation by 50 μM GABA) dynamics. The avalanche size distributions are normalized by the frequency of the smallest avalanche size (size of 1 electrode). Both cases were obtained from Network cA.

the NLR method indicated a significant fit ($\alpha = 1.55,\ p = 0.216$), in contradiction with the size distributions obtained with the other bin sizes, all of which yield $p < 0.1$. This indicates that it may be possible to obtain spurious power-law fits if the bin size is not appropriately selected.

In contrast, in the critical case (Figure 5(b)), the power-law fitting results yielded a significant fit ($p > 0.1$) with the NLR method regardless of the bin size. As the bin size was increased, the value of α decreased ($\alpha = 1.4$ for 200 ms, $\alpha = 1.29$ for 16 ms), as larger bin sizes favor larger avalanches. This indicates that critical behavior may be identified robustly regardless of the time bin selection. Similar behavior was observed for Network cB on DIV 51 when it had been manipulated into the critical state with GABA, but as reported previously, the MLE fitting method rather than the NLR method was found to yield significant fits.

One interesting point to note is that the slopes obtained do not match with those indicated in previous studies using the average interval between successive events on different electrodes (inter-event interval, or IEI), considering only IEIs below a certain cutoff value (selected as, e.g., 65 ms in [58]). In the seminal study by [56], they proposed a binning method based on the average IEI that would produce a consistent power law exponent of $\alpha = 1.5$. However, the average IEI for IEIs below 65 ms in the case shown in Figure 5(b) is approximately 24 ms, and this choice of bin width yields $\alpha = 1.2$. This average IEI is much higher than other previously reported values, which tend to be on the order of 0.1 ms, because a great deal of activity has been curtailed by the addition of GABA. Thus, successive avalanches of activity that occur within less than 65 ms of each other skew the average toward a higher value. Constructing a histogram of the IEIs in this case reveals that the vast majority of IEI values fall below 1 ms and that the remaining IEIs are approximately uniformly distributed above 1 ms, providing further evidence for the above interpretation of the high mean IEI. This investigation indicates that the type of critical behavior that can be achieved by manipulating the ratio of excitation to inhibition in the network may differ in some way from that achieved through self-organization.

The above results and considerations beg the question of what constitutes an appropriate time bin size. As has been discussed by [58], the approximate speed of propagation of action potentials through the network, which is considered to lie in the range of approximately 30 to 300 mm/s, should be taken into consideration. It should also be noted that this speed is quite different from the propagation speed of local field potentials (LFPs), which are the type of activity monitored in the slice experiment by [56]. The aim of the avalanche analysis is to collect activity from different neurons in the network that arises from the same causal source. Thus, the time bin should be large enough that post-synaptic spikes are associated with the pre-synaptic spikes that contributed to activating them but small enough that successive spikes that are not causally related are not associated with each other. The activation of two neighboring electrodes (inter-electrode distance of 200 μm) given the above propagation speed range may range from approximately 0.5 to 5 ms.

This indicates that the cutoff value should likely be set closer to 20 ms for the present recording setup, as this value would correspond to the successive activation of two electrodes that are separated by a distance equal to four inter-electrode distances, roughly half of the maximal distance across the MEA active area, with a low propagation speed of 40 m/s. This cutoff applied to the present case shown in Figure 5(b) yields an average IEI of 6.2 ms and a power-law exponent of $\alpha = 1.4$.

4.2 Maturation of iPSC-derived neurons

The neuronal avalanche size distribution of two in vitro neuronal networks was observed as the network matured. This paper focuses largely on only one of these networks (Network iA), as the other frequently did not display enough avalanches for a reliable fitting. As stated previously, no rigorous analysis was yet applied to classify network as super- or subcritical; rather, only the goodness of fit of the size distribution to a power law was evaluated to assess whether the network was in a critical state during each analyzed recording. Preliminary classification of non-critical cases was performed by visual inspection. The same analysis was performed with different bin sizes, as described previously; however, some of the larger bin sizes yielded too few avalanches for a reliable fitting. The results presented here were obtained with a bin size of 1 ms. Additionally, it should be noted that the fitting results across different bin sizes were less consistent than in the case of the two cortical networks, and so further investigation would be of great value in confirming the observed behavior described herein.

The fitting results indicate that the network was already in the critical state when recordings began at DIV 21 (Figure 6(a)) and remained as such until DIV 42, with some brief deviations or periods of low activity. Many of these deviations from criticality were during the early recording period, and the network was stably in the critical state between DIVs 36 and 42. The mean exponent of the fitted power law distributions in the recordings where the network was in the critical state was $\alpha = 1.84 \pm 0.07$, which is higher than the value of 1.5 reported by [56]. From DIV 44 until the final recording on DIV 56, the network was no longer in the critical state, and a preliminary visual assessment of the avalanche size distributions indicate that activity progressed to supercritical (Figure 6(b)) and finally subcritical (Figure 6(c)) during the later recordings. Although no fittings were performed to rigorously assess whether the networks were in either of these states, the plots in Figure 6 appear to be consistent with this type of behavior.

The observed network did not ultimately settle into a critical state in the considered timeframe, though it did appear to pass through a period of relatively stable critical behavior. The deviation from criticality is likely due to the different cell types that arise during the reprogramming of iPSCs,

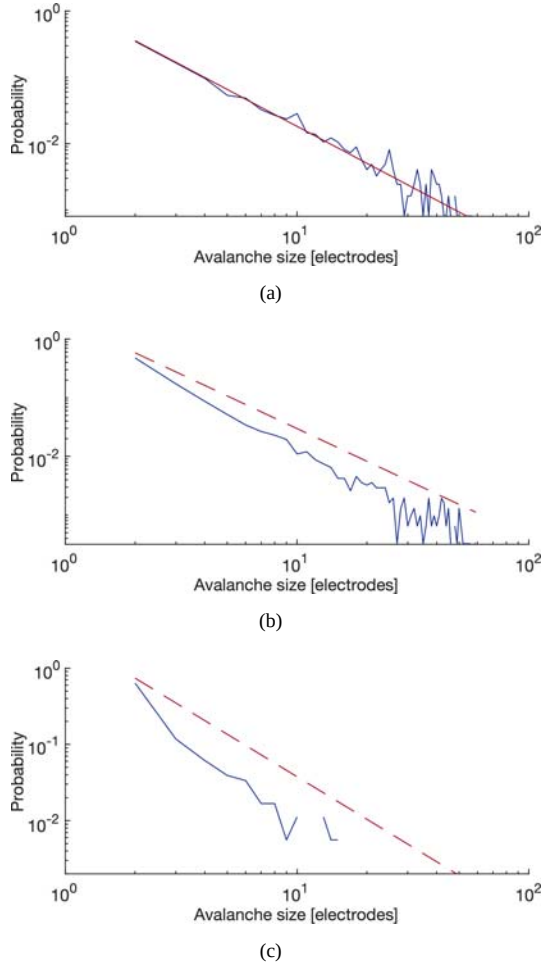


FIGURE 6
Probability distribution functions for three representative cases. (a) DIV 21: The fitting indicates the network is in a critical state, with $\alpha = 1.85$ ($p = 0.13$). The power-law fitting result is shown as a red line. (b) DIV 51: The network appears to be in a supercritical state with a bimodal distribution. (c) DIV 52: The network appears to be in a subcritical state with an exponential distribution. In (b) and (c), the dashed red lines correspond to $\alpha = 1.85$ for comparison with the distribution shown in (a).

particularly as the proliferation of these cells causes the composition of the culture to change over time. When differentiating iPSCs into a target cell type, the presence of other types of cells of the same lineage cannot be excluded; for example, glial cells are commonly present in iPSC-derived neuronal networks. This is different from networks that have been assessed in

previous studies on self-organized criticality in neuronal networks, as these have focused solely on primary cortical networks, which can be prepared with greater homogeneity. The heterogeneous and time-varying cellular composition likely produces changes to the signalling environment of the neurons, which may temporarily push the network away from criticality. Additionally, the neurons assessed here were dopaminergic neurons, which are likely to show a different course of maturation in terms of criticality than cortical neurons. It is possible that these types of networks show more complex oscillatory behaviors as they mature, or they may eventually settle into a critical state given enough time. Further work is necessary to capture the expected time course of the development of the criticality of such networks.

4.3 Future work

In future work, further investigations will be performed with networks manipulated into the critical state through the addition of GABA. This avalanche-based analysis will be applied in conjunction with other measures describing the fitness of a dynamic system for computation, including information and graph theoretical measures. In this line of investigation, it will also be observed how networks in different dynamical states respond to electrical stimulation. Such a multifaceted approach will provide better insight into the mechanisms driving neuronal communication and how certain environments may better leverage the computational capabilities of neuronal networks. The characteristics of the networks derived in this way will be used to inform the development of novel neural network models that more closely resemble their biological analog.

Further experiments will also be conducted to evaluate the development of different types of iPSC-derived networks. Thus far, criticality analysis has been confined to primary cortical networks; understanding how different parts of the brain behave in this context is expected to give a broader understanding of the overall behavior of the brain. This analysis will also be applied in future experiments on the classification of networks as perturbed or healthy, in a manner similar to our previous study on Parkinson's-related proteinopathy [14]. This type of analysis is expected to be a useful tool for this type of classification as well as opening doors to better understanding the mechanisms of action of neurological diseases.

5 CONCLUSION

In this study, a method of classifying neuronal networks as critical or non-critical was applied to electrophysiological data obtained from four in vitro neuronal networks, two primary rat cortical networks and two human

iPSC-derived dopaminergic networks. First, the effect of the time bin size on the classification results of neuronal avalanche analysis was evaluated. It was demonstrated that critical behavior is identifiable regardless of the time bin size, whereas the avalanche size distributions associated with non-critical behavior are strongly dependent on the size of the time bin. Following this investigation, the analytical framework was applied to iPSC-derived networks to determine whether they were critical or non-critical at different time points during the course of their development. The preliminary results reported here demonstrate emerging behavior that does not settle into criticality within the investigated time frame; further work is needed to better characterize the time course of the development of criticality in the networks studied in this work and characterize how this affects the network's suitability for computation. Future work will also involve further investigation into the behaviors of networks that make them well-suited for computation based on information and graph theoretical approaches to the data analysis. This work is expected to contribute to the development of models that can recapitulate the desired behaviors and will in turn inform the development of novel physical computing substrates.

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REFERENCES

[1] C. G. Langton. (1990). "Computation at the edge of chaos: Phase transitions and emergent computation," *Physica D*, 40, pp. 12–37.

[2] W. L. Shew and D. Plenz. (2013). "The functional benefits of criticality in the cortex," *The Neuroscientist*, 19(1):88–100.

[3] F. Heylighen. (1999). "The science of self-organization and adaptivity," in *in: Knowledge Management, Organizational Intelligence and Learning, and Complexity, in: The Encyclopedia of Life Support Systems, EOLSS*. Publishers Co. Ltd, 253–280.

[4] S. Camazine, J.-L. Deneubourg, N. R. Franks, J. Sneyd, E. Bonabeau, and G. Theraula. (2003). *Self-organization in biological systems*. Princeton university press.

[5] P. Bak, C. Tang, and K. Wiesenfeld. (Jul 1987). "Self-organized criticality: An explanation of the 1/f noise," *Physical Review Letters*, 59, 381–384.

[6] M. A. Muñoz. (Jul 2018). "Colloquium: Criticality and dynamical scaling in living systems," *Rev. Mod. Phys.*, 90, 031001.

[7] S. Stepney, S. Rasmussen, and M. Amos. (2018). *Computational Matter*, 1st ed. Springer Publishing Company, Incorporated.

[8] J. H. Jensen, E. Folven, and G. Tufte. (2018). "Computation in artificial spin ice," *The 2018 Conference on Artificial Life: A Hybrid of the European Conference on Artificial Life (ECAL) and the International Conference on the Synthesis and Simulation of Living Systems (ALIFE)*, 1(30):15–22.

[9] Z. Konkoli, S. Nichele, M. Dale, and S. Stepney. (2018). "Reservoir computing with computational matter," in *Computational Matter, Natural Computing Series*, S. Stepney, S. Rasmussen, and M. Amos, Eds. Springer, Cham, 269–293.

[10] B. Schrauwen, D. Verstraeten, and J. Van Campenhout. (2007). "An overview of reservoir computing: theory, applications and implementations," in *Proceedings of the 15th European Symposium on Artificial Neural Networks*, 471–482.

[11] Norwegian University of Science and Technology, "Socrates: Self-organizing computational substrates," https://www.ntnu.edu/socrates, 2018.

[12] P. Aaser, M. Knudsen, O. Huse Ramstad, R. van de Wijdeven, S. Nichele, I. Sandvig, G. Tufte, U. S. Bauer, Ø. Halaas, S. Hendseth *et al.* (2017). "Towards making a cyborg: A closed-loop reservoir-neuro system," in *Proceedings of the European Conference on Artificial Life 2017*. MIT Press.

[13] K. Heiney, O. H. Ramstad, I. Sandvig, A. Sandvig, and S. Nichele. (2019). "Assessment and manipulation of the computational capacity of in vitro neuronal networks through criticality in neuronal avalanches," in *2019 IEEE Symposium Series on Computational Intelligence (SSCI)*, 247–254.

[14] V. D. Valderhaug, K. Heiney, O. H. Ramstad, G. Bråthen, W.-L. Kuan, S. Nichele, A. Sandvig, and I. Sandvig. (2020). "Criticality as a measure of developing proteinopathy in engineered human neural networks," *bioRxiv*.

[15] M. E. J. Obien, K. Deligkaris, T. Bullmann, and D. J. Bakkum. (2015). "Revealing neuronal function through microelectrode array recordings," *Frontiers in Neuroscience*, 8, 1–30.

[16] R. A. Brooks. (1991). "Intelligence without representation," *Artificial Intelligence*, 47(1):139–159.

[17] T. B. DeMarse, D. A. Wagenaar, A. W. Blau, and S. M. Potter. (2001). "The neurally controlled animat: biological brains acting with simulated bodies," *Autonomous robots*, 11(3):305–310.

[18] D. J. Bakkum, Z. C. Chao, P. Gamblen, G. Ben-Ary, A. G. Shkolnik, T. B. DeMarse, and S. M. Potter. (2007). "Embodying cultured networks with a robotic drawing arm," in *2007 29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*. IEEE, 2996–2999.

[19] S. M. Potter, D. A. Wagenaar, and T. B. DeMarse. (2006). *Closing the Loop: Stimulation Feedback Systems for Embodied MEA Cultures*. Boston, MA: Springer US, 215–242.

[20] T. B. DeMarse and K. P. Dockendorf. (2005). "Adaptive flight control with living neuronal networks on microelectrode arrays," in *Proceedings. 2005 IEEE International Joint Conference on Neural Networks, 2005.*, 3. IEEE, 1548–1551.

[21] S. Nichele and A. Molund. (2017). "Deep learning with cellular automaton-based reservoir computing," *Complex Systems*, 26.

[22] J. Tessadori, M. Bisio, S. Martinoia, and M. Chiappalone. (2012). "Modular neuronal assemblies embodied in a closed-loop environment: toward future integration of brains and machines," *Frontiers in neural circuits*, 6, 99.

[23] J. Tessadori and M. Chiappalone. (2015). "Closed-loop neuro-robotic experiments to test computational properties of neuronal networks," *Journal of Visualized Experiments*.

[24] A. Novellino, P. D'Angelo, L. Cozzi, M. Chiappalone, V. Sanguineti, and S. Martinoia. (2007). "Connecting neurons to a mobile robot: an in vitro bidirectional neural interface," *Computational Intelligence and Neuroscience*.

[25] R. Pizzi, D. Rossetti, G. Cino, D. Marino, A. L. Vescovi, and W. Baer. (2009). "A cultured human neural network operates a robotic actuator," *Biosystems*, 95(2):137–144.

[26] K. Warwick, D. Xydas, S. J. Nasuto, V. M. Becerra, M. W. Hammond, J. Downes, S. Marshall, and B. J. Whalley. (2010). "Controlling a mobile robot with a biological brain," *Defence Science Journal*, 60(1):5–14.

[27] M. Kositsky, A. Karniel, S. Alford, K. M. Fleming, and F. A. Mussa-Ivaldi. (2003). "Dynamical dimension of a hybrid neurorobotic system," *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, 11(2):155–159.

[28] J. W. Pillow and M. Park. (2016). "Adaptive bayesian methods for closed-loop neurophysiology," in *Closed Loop Neuroscience*, A. El Hady, Ed. Elsevier, 3–18.

[29] A. Karniel, M. Kositsky, K. M. Fleming, M. Chiappalone, V. Sanguineti, S. T. Alford, and F. A. Mussa-Ivaldi. (sep 2005). "Computational analysis in vitro : dynamics and plasticity of a neuro-robotic system," *Journal of Neural Engineering*, 2(3):S250–S265.

[30] D. Poli, V. P. Pastore, and P. Massobrio. (2015). "Functional connectivity in in vitro neuronal assemblies," *Frontiers in Neural Circuits*, 9, 57.

[31] J. M. Wlfing, S. S. Kumar, J. Boedecker, M. Riedmiller, and U. Egert. (2019). "Adaptive long-term control of biological neural networks with deep reinforcement learning," *Neurocomputing*, 342, 66–74.

[32] C. M. Hales, J. D. Rolston, and S. M. Potter. (may 2010). "How to Culture, Record and Stimulate Neuronal Networks on Micro-electrode Arrays (MEAs)," *Journal of Visualized Experiments*, 39.

[33] D. Poli and P. Massobrio. (oct 2018). "High-frequency electrical stimulation promotes reshaping of the functional connections and synaptic plasticity in in vitro cortical networks," *Physical Biology*, 15(6):06LT01.

[34] F. Scarsi, J. Tessadori, M. Chiappalone, and V. Pasquale. (dec 2017). "Investigating the impact of electrical stimulation temporal distribution on cortical network responses," *BMC Neuroscience*, 18(1):49.

[35] Y. Li, R. Sun, B. Zhang, Y. Wang, and H. Li. (2015). "Application of hierarchical dissociated neural network in closed-loop hybrid system integrating biological and mechanical intelligence," *PloS one*, 10(5).

[36] H. Takahashi, S. Yasuda, Y. Yada, and R. Kanzaki. (2016). "Reservoir computing with dissociated neuronal culture," *Frontiers in Neuroscience*, 27.

[37] W. Maass, T. Natschläger, and H. Markram. (2002). "Real-time computing without stable states: A new framework for neural computation based on perturbations," *Neural computation*, 14(11):2531–2560.

[38] H. Jaeger. (2003). "Adaptive nonlinear system identification with echo state networks," in *Advances in neural information processing systems*, 609–616.

[39] C. Fernando and S. Sojakka. (2003). "Pattern recognition in a bucket," in *European conference on artificial life*. Springer, 588–597.

[40] D. Nikolić, S. Haeusler, W. Singer, and W. Maass. (2007). "Temporal dynamics of information content carried by neurons in the primary visual cortex," in *Advances in neural information processing systems*, 1041–1048.

[41] G. Tanaka, T. Yamane, J. B. Héroux, R. Nakane, N. Kanazawa, S. Takeda, H. Numata, D. Nakano, and A. Hirose. (2019). "Recent advances in physical reservoir computing: A review," *Neural Networks*.

[42] S. Hafizovic, F. Heer, T. Ugniwenko, U. Frey, A. Blau, C. Ziegler, and A. Hierlemann. (2007). "A cmos-based microelectrode array for interaction with neuronal cultures," *Journal of neuroscience methods*, 164(1):93–106.

[43] K. P. Dockendorf, I. Park, P. He, J. C. Príncipe, and T. B. DeMarse. (2009). "Liquid state machines and cultured cortical networks: The separation property," *Bio Systems*, 95(2):90–7.

[44] T. Kubota, K. Nakajima, and H. Takahashi. (2019). "Echo state property of neuronal cell cultures," in *ICANN*.

[45] L. M. van Kessenich, D. Berger, L. De Arcangelis, and H. J. Herrmann. (2019). "Pattern recognition with neuronal avalanche dynamics," *Physical Review E*, 99(1):010302.

[46] J. H. Jensen and G. Tufte. (2017). "Reservoir computing with a chaotic circuit," in *ECAL*.

[47] P. Barancok and I. Farkas. (2014). "Memory capacity of input-driven echo state networks at the edge of chaos," in *ICANN*.

[48] D. R. Snyder, A. Goudarzi, and C. Teuscher. (2013). "Computational capabilities of random automata networks for reservoir computing," *Physical review. E, Statistical, nonlinear, and soft matter physics*, 87(4):042808.

[49] H. Suetani. (2019). "Multiple pattern generations and chaotic itinerant dynamics in reservoir computing," in *ICANN*,

[50] T. L. Carroll. (2019). "Mutual information and the edge of chaos in reservoir computers," *ArXiv*, vol. abs/1906.03186.

[51] F. Matzner. (2017). "Neuroevolution on the edge of chaos," *Proceedings of the Genetic and Evolutionary Computation Conference*.

[52] J. Boedecker, O. Obst, J. T. Lizier, N. M. Mayer, and M. Asada. (2011). "Information processing in echo state networks at the edge of chaos," *Theory in Biosciences*, 131, 205–213.

[53] C. Gallicchio, A. Micheli, and P. Tiño. (2018). "Randomized recurrent neural networks," in *ESANN*.

[54] W. L. Shew, H. Yang, S. Yu, R. Roy, and D. Plenz. (2011). "Information capacity and transmission are maximized in balanced cortical networks with neuronal avalanches," *Journal of Neuroscience*, 31(1):55–63.

[55] J. Wilting and V. Priesemann. (2019). "25 years of criticality in neuroscience established results, open controversies, novel concepts," *Current Opinion in Neurobiology*, 58, 105–111, computational Neuroscience.

[56] J. M. Beggs and D. Plenz. (2003). "Neuronal avalanches in neocortical circuits," *The Journal of Neuroscience*, 23(35):11 167–11 177.

[57] J. Hesse and T. Gross. (2014). "Self-organized criticality as a fundamental property of neural systems," *Frontiers in Systems Neuroscience*, 8.

[58] V. Pasquale, P. Massobrio, L. L. Bologna, M. Chiappalone, and S. Martinoia. (2008). "Self-organization and neuronal avalanches in networks of dissociated cortical neurons," *Neuroscience*, 153, 1354–1369.

[59] Y. Yada, T. Mita, A. Sanada, R. Yano, D. J. Bakkum, A. Hierlemann, and H. Takahashi. (2017). "Development of neural population activity toward self-organized criticality," *Neuroscience*, 55–65.

[60] C. Tetzlaff, S. Okujeni, U. Egert, W org otter, and M. Butz. (2010). "Self-organized criticality in developing neuronal networks," *PLoS Computational Biology*, 6(12).

[61] W. L. Shew, H. Yang, T. Petermann, R. Roy, and D. Plenz. (2009). "Neuronal avalanches imply maximum dynamic range in cortical networks at criticality," *Journal of Neuroscience*, 29(49):15 595–15 600.

[62] H. P. R. (1979). "Synaptic density in human frontal cortex developmental changes and effects of aging," *Brain Research*, 163(2):195–205.

[63] P. Massobrio, V. Pasquale, and S. Martinoia. (2015). "Self-organized criticality in cortical assemblies occurs in concurrent scale-free and small-world networks," *Scientific Reports*.

[64] A. Kirkeby, J. Nelander, and M. Parmar. (2012). "Generating regionalized neuronal cells from pluripotency, a step-by-step protocol," *Frontiers in Cellular Neuroscience*, 6, 64.

[65] A. Kirkeby, S. Nolbrant, K. Tiklova, A. Heuer, N. Kee, T. Cardoso, D. R. Ottosson, M. J. Lelos, P. Rifes, S. B. Dunnett, S. Grealish, T. Perlmann, and M. Parmar. (2017). "Predictive markers guide differentiation to improve graft outcome in clinical translation of hesc-based therapy for parkinson's disease," *Cell Stem Cell*, 20(1):135–148.

[66] D. Doi, B. Samata, M. Katsukawa, T. Kikuchi, A. Morizane, Y. Ono, K. Sekiguchi, M. Nakagawa, M. Parmar, and J. Takahashi. (2014). "Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation," *Stem Cell Reports*, 2(3):337–350.

[67] A. Clauset, C. R. Shalizi, and M. E. J. Newman. (2009). "Power-law distributions in empirical data," *SIAM Review*, 51(4):661–703.