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"Brains on a chip": Towards engineered neural networks

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ABSTRACT

The fundamental mechanisms of complex neural computation remain largely unknown, especially in respect to the characteristics of distinct neural circuits within the mammalian brain. The bottom-up approach of building well-defined neural networks with controlled topology has immense promise for improved reproducibility and increased target selectivity and response of drug action, along with hopes to unravel the relationships between functional connectivity and its imprinted physiological and pathological functions. In this review, we summarize the different approaches available for engineering neural networks treated analogously to a mathematical graph consisting of cell bodies and axons as nodes and edges, respectively. After discussing the advances and limitations of the current techniques in terms of cell placement to the nodes and guiding the growth of axons to connect them, the basic properties of patterned networks are analyzed in respect to cell survival and activity dynamics, and compared to that of in vivo and random in vitro cultures. Besides the fundamental scientific interest and relevance to drug and toxicology tests, we also visualize the possible applications of such engineered networks. The review concludes by comparing the possibilities and limitations of the different methods for realizing in vitro engineered neural networks in 2D.

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1. Introduction

Understanding how the human brain stores and processes information is undoubtedly one of the grand challenges of this century,

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underlined also by the biggest collaborative research projects around the globe [1, 2]. Unraveling the basic concepts could lead to a better understanding of neural diseases, provide the key for improved brain-machine interfaces, and revolutionize the domain of machine-learning. From the technological point of view, advances in neurosciences may also induce a brain-oriented paradigm shift, which could open a new era of cluster-computing orders of magnitude more power efficient than that of today [3].

The two major approaches in studying the brain are termed bottom-up and top-down. The latter starts with the nervous system as a whole and moves down to smaller scales by looking for examples of stimulation-based behavioral changes, while the bottom-up approach attempts to extrapolate the elementary functions of single neurons and small circuits to higher-level systems. An intermediate technique, that is also heavily used in drug discovery and neurotoxicology, is to use brain slices to observe and manipulate synaptic connectivity of maintained networks under stable physiological conditions; however, the advantage of accessibility is counterbalanced by the partially destroyed circuits.

In spite of the collaborative efforts studying neuronal networks at all levels, there is still little known about how geometry affects functional expression. Top-down approaches have to deal with the extreme complexity of the whole brain, as well as the lack of control on the topological and developmental diversity. Bottom-up approaches, on the other hand, have a higher level of flexibility, but the artificial nature of the applied geometrical and other constraints brings significant consequences and limitations on network growth, maturation, and survival. Existing studies mostly focus on either experimental or analytical tools to create engineered networks or analyze the results of hypothetical models, respectively, and so far there has been little success to combine the two. Constructing experimental setups targeting basic network properties, which then can be evaluated by simple theoretical models to test and fine tune them, could lead to a higher level of understanding of functional networks.

The small networks of the bottom-up approach are primarily cultured in vitro and often tailored to fit on electrode arrays that are used to both stimulate the neuronal cultures and record their spatiotemporal activity. The precise geometry of these cultures following the fixed position of the electrodes can result in better reproducibility compared to recordings from random cultures. Direct access to a single layer of neurons and the possibility to record from multiple sites at the same time have made the in vitro recording systems an efficient tool for exploring the pharmacological and toxicological effects of numerous compounds [4]. Although random networks already provide valuable information for these studies, most of the previous experimental results were based on observing only global, network-wide properties of the neuronal activity. In addition, neurological diseases always manifest in highly complex network behavior, and the currently used random cultures cannot serve as realistic models of such conditions.

To further improve the analytical aspects, constraints on the connectivity can be added to gain control over the network topography, reaching improved neurocomputational models in a deterministic rather than statistical way. While such controlled networks are difficult to classify in the brain due to its complexity, a recent work has shown that tailored small cultures exhibit properties similar to those of their large, brain-scale counterparts [5], and in this respect, these engineered networks are valid investigative tools to study the fundamental mechanisms of the brain. The targeted stimulation of single neurons within a defined network can help to gain more focused information on the cellular and molecular changes induced, and questions regarding the information processing capabilities of the network as a whole, such as changes in synaptic strengths and network plasticity under the influence of different drugs can then be addressed. The expected increase in target selectivity of drug action would result in fewer side effects, and furthermore, studying the network response to an acute mechanism of action would help to better understand the therapeutic effect of certain drugs.

A systematic and constructive study following the bottom-up approach could start with creating and analyzing the simplest neuronal networks that have feedforward topology or loops, similar to those depicted in Fig. 1. Analogous to a mathematical graph, a neuronal culture with controlled topology can be represented as a collection of nodes and edges that symbolize the cell bodies and extended neurites, respectively. As a first step, cell bodies have to be delivered to and localized at the nodes of the desired graph. Once the cells have settled and adhered, neuronal processes grow and connect the cells to each other. During this step, one of the extending neurites of each neuron, usually the longest [6, 7], becomes an axon while the others form the dendritic tree, defining the direction and input of information flow, respectively. This polarization, characteristic to the neural networks and depicted by the direction of the edges in the graph representation, is an essential component towards understanding the brain. Therefore, methods to reliably incorporate axon guidance into the different culturing protocols are necessary

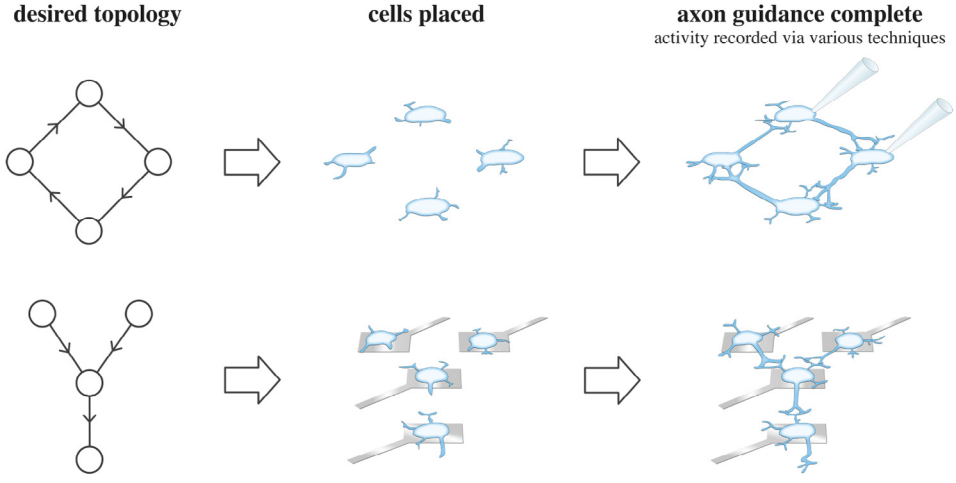

Fig. 1. Steps towards engineered neuronal networks with controlled topology. Cultures are schematized with a directed graph, where nodes and edges represent the neurons and axons, respectively. In order to create the desired topology, a method to direct axons must be implemented following cell delivery and localization. The two most common techniques for recording neuronal activity, multi-electrode arrays and patch clamping, are depicted in the figure.