Chapter 1
Motivation: the brain challenge

The huge variety of human behaviours relies on an elaborate array of sensory receptors connected to an extraordinarily plastic organ, the brain, which actively organizes incoming sensory signals. Perception inputs are in part stored in memory for future reference, and in part converted into immediate behavioural responses. All these processes are managed by a network of interconnected neurons, which are the structural and functional units of the nervous system. Starting from embryonic neuronal progenitor cells which, through millions of years of evolution, have been equipped with the potency to produce every neuron in the nervous system, the adult human brain counts approximately 10^{11} neurons and as many non-neural cells (Azevedo et al., 2009). This enormous number of cells takes part in constituting circuits with precise functions, in a network that is thought to count more than 10^{15} synapses (Pakkenberg et al., 2003). The complexity of this system arises not only from the number of elements constituting it but also from their diversity, both in terms of shapes and functions. In fact, neurons of the human brain can be classified, according to gene expression profiling, in up to 10^4 different cell sub-types (Muotri and Gage, 2006), spanning from elongated retinal cone photoreceptors involved in visual perception, to arborized cerebellar Purkinje cells coordinating motor function, to the hypothalamic osmoreceptor neurons secreting antidiuretic hormone (ADH), to cite just a few. This extraordinary diversity exists even between cells within the same neuronal subtype. Indeed, even neurons with similar morphology can differ in important molecular details (e.g. expression of different combination of membrane ion channels, providing neurons with diverse excitation thresholds and/or distinctive firing patterns), highlighting the importance of single neurons in the network. How and when the outstanding neuronal diversity is generated in the course of neurogenesis, remains however still unclear. Since during embryonic neurogenesis, proliferative precursor neuroblasts migrate to form the adult grey matter and in this process only 15-40% of post migratory cells survive (Finlay and Slattery, 1983;Ferrer et al., 1992), it has been hypothesized that this putative selection process could be responsible for the generation of neuronal diversity (Oppenheim, 1991), through a DNA recombination similar to that seen in the immune system with V(D)J mechanism (Jones and Gellert, 2004). In addition to this process, has been demonstrated the existence of activity-dependent neuronal diversity in postmitotic neurons driven by environmental stimuli that dynamically refine neuronal networks, as it happens, for example, in hippocampal “place cells” (Burgess and O'Keefe, 2003). Yet, to make things even more complex, both neurons and their connections are modified by

Lapo Turrini, *Development of optical methods for real-time whole-brain functional imaging of zebrafish neuronal activity*, © 2020 Author(s), content CC BY 4.0 International, metadata CC0 1.0 Universal, published by Firenze University Press (www.fupress.com), ISSN 2612-8020 (online), ISBN 978-88-5518-070-2 (PDF), DOI 10.36253/978-88-5518-070-2

experience. The outstanding structural and functional plasticity of this vast interconnected system accounts for the fact that during all the course of our lives we keep storing new memories and learning new skills, as a child that begins to walk or a man that learns to play the piano.

Our understanding of the functioning of neural cells, as well of the fine mechanisms underlying neuronal synapses, has greatly advanced in recent years. By comparison, our knowledge of neuronal structural and functional connectivity in the brain is far behind. The staggering computational abilities, of which this organ is capable, is a perfect example of what the theory of complex systems calls *emergent behaviour*[1], a phenomenon typical of biological systems. Indeed, the deep comprehension of the interactions occurring between all the parts of this system requires an intense multilevel approach, ultimately necessitating the possibility to access and image the intact working organ in its entirety at a nanometric and sub-millisecond scale, sufficient to "see" neurotransmitters vesicles released in the synaptic cleft yet simultaneously in the whole organ. The task is absolutely out of reach, other than obvious ethical and practical reasons, because of the peculiar multi-scale structure of the human brain itself. In fact, this organ (tens of cm) is composed by several lobes and nuclei (cm), which are constituted by millions of highly packed cells arranged in clusters (mm). Each neuronal soma (tens of μm) project very small neurites (even < 100 nm), extending over large distances (cm) and taking contact with thousands of other cells. For these reasons, a whole-brain approach with high spatio-temporal resolution results unfeasible, at list with present technology.

Indeed, analysis on brain-wide scale are typically achieved by fMRI (functional magnetic resonance imaging) and EEG (electroencephalography). Despite extremely useful, in particular in a clinical environment, both these techniques suffer from limited temporal and/or spatial resolution[2].

To perform investigation of the brain with cellular and high temporal resolution was thus necessary to turn our attention on organisms having a smaller and "simpler"[3] CNS. The most common mammalian model used in neuroscience is the mouse, which, despite providing an essential benchmark in research, because of the presence of the bone skull (like most vertebrates), does not allow optical access to the whole encephalon, but only limited cortical or sub-cortical access, through *ad hoc* cranial windows (Holtmaat et al., 2009) or implanted optical fibres (Pisanello et al., 2017), respectively. In the last decades, the use of zebrafish has been increasingly popular in this field. A small teleostean fish, zebrafish detains a number of attributes that make it the vertebrate model of election to perform whole-brain functional imaging. Indeed,

[1] The set of properties of a system that does not depend on its individual part, but on their relationships to one another. Typically, life is considered as an emergent behaviour since it cannot be explained only considering the properties of cellular components.

[2] In particular, fMRI, despite offering a spatial resolution of 2-4 mm, sufficient to investigate the brain on a neuronal-cluster scale, it achieves temporal resolution of only 1-4 s, which is not enough to follow the rapid communication between neurons. In contrast, the EEG offers a millisecond-range temporal resolution but poor spatial resolution (cm range).

[3] The term "simpler" is to be intended relatively to the human brain. Suffice it to say that the genome of the worm *Caernorabditis elegans* contains sequences for 80 different types of potassium-selective ion channels, 90 ligand-gated receptors, and around 1000 G protein-linked receptors (Bargmann, 1998). The combinatorial possibilities are astonishing for a nervous system with only 302 neurons.