I.2. Neuronal Physiology

I.2.1 Levels of Organization and Cellular Physiology

The function of the nervous system can be examined at a number of levels of organization (see fig. I.2) and in such a perspective, a highly interdisciplinary approach is required. For instance, *Biochemistry* and *Molecular Biology* investigate the properties of molecules that perform tasks important for neuronal function (e.g. the membrane ionic-permeability, the ligand-receptor dynamics, etc.). *Physiology* studies the characteristics of individual neurons (e.g. the features of signal propagation and attenuation of neuritic branch points, etc.) as well as of collections of cells that are functionally related (e.g. reflex arcs, chemical and electric synaptic interactions, etc.). *Behavioral Psychology* explores patterns of behavior and its modification-learning in experimental animals ranging from lower invertebrates to humans. *Computational Neuroscience* and *Bioengineering* attempt to put it all together, to model higher brain functions in terms of the known properties of molecules, cells, or collections of cells. Throughout the present thesis, major emphasis will be given to *signaling between* nerve cells (i.e. *inter-cellular* information transfer). This kind of cell communication is essential for an organism to *sense information about its environment*, to *import this information into its brain where it can be processed*, and to *generate an appropriate behavioral response* (Levitan and Kaczmarek, 1997).
**Figure I.2:** Levels of organization of the structure and function of the CNS: (from top to bottom) level of molecules, level of individual cells, level of pairs of neurons connected by synapses, level of network of interacting neurons, level of systems in the brain that regulate behavior and level of behavior (adapted from Levitan and Kaczmarek, 1997).

Although the main focus of this thesis is on the modeling and simulation of cellular and molecular phenomena, it must be emphasized that *Cellular* and *Molecular Neurobiology* do not exist in a vacuum. Actually, most of the cellular organelles and sub-cellular mechanisms in the context of the nervous system have their counterparts in other cell types, so that there is an emerging awareness of a satisfying unity in *Cell Biology*. Moreover, it is becoming increasingly evident that the understanding the nervous system requires a study at all the levels of organization depicted in figure...
I.2, from behaving animals to single cells and to molecules that regulate cellular processes. As already stated, no single level is inherently more important than any other, and for even a rudimentary quantitative account of physiological and pathological phenomena in the CNS, information and accurate experimental details from all the levels are necessary.

Since the mid of the nineteenth century, it was evident that discrete entities were the basic architectural units constituting a living tissue. However, only fifty years later the same principle was accepted in the context of the organization of the nervous system, as the passionate argument between the two neuro-anatomists Santiago Ramón y Cajal and Camillo Golgi, about whether the brain consists of enormous numbers of discrete cells (i.e. the cellular hypothesis or neuron doctrine), or is a continuous syncytium of tissue (i.e. the reticular theory), came to a successful end.

The answer to such a question was of enormous impact for understanding how signals spread from one part of the nervous system to another. Thanks to a tissue staining technique, fortuitously discovered by Golgi, Cajal managed to make individual neurons show up clearly in tissue sections that actually contain a large number of neurons (see fig. I.3). In fact, by using other staining methods that stain all the neurons, the same tissue sections would only have looked like tangled thickets under the optic microscope. Eventually, those discrete entities were correctly identified as individual nerve cells, although Golgi never accepted such an interpretation and continued to put forward his idea of a continuous meshwork. One of the reasons for the long debate over this issue is the complexity of brain tissue: a huge number of different cell types composes the nervous tissues, and many of these cells have a complex asymmetric three-dimensional morphologies that makes it extremely difficult to ascertain where one cell ends and the next begins.

As it was already stressed, the essence of nervous system function and of its cellular components is signaling, both intra-cellularly (i.e. from one part of a cell to another) and inter-cellularly (i.e. between cells). This is a fundamental premise for the following considerations, as most relevant advances in the neurosciences came by investigating those specialized aspects of neuronal morphology and structure contributing to the information transfer, to the mechanisms of intra-cellular neuronal communications, to the patterns of neuronal connectivity and sub-cellular mechanisms of intra-cellular signaling, to the relationship of various patterns of neuronal connectivity to different behaviors, and finally to the ways in which neurons and their connections can be modified by experience, in an activity-dependent fashion.

In particular, it can be further stated that each of the three unique sub-cellular structures characterizing neuronal cells, being the axon (i.e. specialized for intracellular information transfer),
the dendrites (i.e. the sites at which information is received from other neurons) and the synapses (i.e. the points of information transfer between neurons) are highly devoted to signaling and communication.

Figure I.3: Micrograph of a Golgi-stained single pyramidal neuron in the hippocampus: apical dendrites (top), the soma and basal dendrites (middle - low) can be optically resolved through staining (adapted from Levitan and Kaczmarek, 1997).

The axon is a thin tube-like process, arising from the neuronal cell soma and traveling for distances ranging from micrometers (e.g. in the retina) to meters (e.g. in the spinal-cord). Specialized proteins interleaved in the axonal membrane constitute the key mechanism allowing the neuron to rapidly
transmit electrical signals along the axon length, from soma to the terminals (see the Methods). The axon originates at a thickening on the cell body called the *axon hillock*, and it is often unbranched until just before it terminates, where it may branch many times. Its diameter is approximately the same throughout its length and structure, like the dendrite, and it is formed and maintained by the *cytoskeleton*, a cellular scaffolding that is present in all cell types, however exhibiting unique properties in unusually shaped cells such as neurons.

*Dendrites* are neuronal processes that tend to be thicker and much shorter compared to the axon, and often highly branched, constituting a dense network of processes known as the *dendritic tree* (see figure I.3). Moreover, dendrites, whose cytoskeleton differs from that of axons, often originate from the cell body, but in some neurons (e.g. in invertebrates) they arise even from the proximal regions of the axon. Three-dimensional computer reconstruction from images taken by means of a confocal microscope, often reveal the presence of numerous finger-like small projections or thickenings on the dendrites of some neurons. These projections, called *dendritic spines*, arise from the main shaft of the dendrite (see fig. I.4) and on first approximation represent the synaptic input sites at which the neuron receives information from another cell, although not necessarily all the connections among neurons involve such structures.

![Figure I.4: Three-dimensional computer reconstruction of a “spiny” dendrite from electron scanning microscope sections.](image-url)
Like the axonal membrane, the plasma membrane of dendrites contains specific proteins that allows the dendrite to receive and integrate information from other nerve cells, by affecting the membrane ionic permeability. The physiological role of the dendrite does not however consist exclusively in gathering signals from other cells as, in a few cases, dendrites share with axons the ability to actively transmit electrical signals (e.g. dendritic calcium spikes), and in many nerve cells both input and output of electrical signals occur on the same set of dendrite-like fine processes.

The primary difference between neurons and most of other cell types (e.g. liver cells) is that neurons can generate and transmit either electrical or chemical signals, being the messengers used by the nervous system for all its functions. It is therefore of paramount importance to understand the principles and mechanisms of neuronal signaling and communication. Despite the extraordinary diversity and complexity of neuronal morphology and connectivity, a number of basic principles of signaling for all neurons and synapses is adopted throughout the nervous system, collectively referred to as excitability properties. In neurons or other excitable cells (e.g. muscle cells and pancreatic β-cells), electrical signals are carried primarily by transmembrane ion currents, and result in changes in transmembrane voltage. Four ion species are mainly involved in such currents: sodium (Na$^+$), potassium (K$^+$), calcium (Ca$^{2+}$) and chloride (Cl$^-$), with the first three carrying positive charges (i.e. cations) and the fourth carrying negative charges (i.e. anions). The flows of these ions across the membrane are governed by physical laws and molecular mechanisms, later reviewed, discussed and quantitatively modeled in the Method section of the present thesis, and whose main energy source, ensuring ion movements, comes from ionic concentration gradients between the cytoplasm and the extracellular environment. These gradients are indefinitely maintained against thermodynamic equilibrium by active transport mechanisms called ion pumps, whose energy is derived from the hydrolysis of ATP molecules (i.e. adenosinetriphosphate). The concentration gradients set up the electrochemical potential across the membrane, which drives ion flow in accordance with the laws of diffusion and drift (i.e. at a first approximation on the basis of the Ohm’s law). Although the energy sources and ion species involved in electrical signals are relatively simple, the gating mechanisms modulating the passage of ions across the membrane and determining the ionic membrane permeability, can be quite complicated. Actually, ions flow across the membrane through aqueous pores formed by transmembrane protein molecules, also know as the ion channels. These molecules may undergo three-dimensional conformational changes that, under certain conditions, allow ion passage (i.e. gate in the open state) but under other conditions...
deny ion passage (i.e. gate in the closed state), depending on the transmembrane local electric field as well as on the chemical interaction with ligand molecules. The quantitative description of ion permeability and channel gating in biological membranes (i.e. both voltage-dependent and ligand-gated protein channels) will be examined in the next chapters, because such phenomena are very important for understanding the cellular bases of the electrophysiological collective behavior of a in vivo as well as in vitro neuronal networks, representing the cornerstones of neuronal signaling in the nervous system.

1.2.2 Intercellular Communication

In the previous paragraph, it was outlined the relevance of the intercellular communication, resulting in the information transfer from one part of the nervous system to others, and being the essence of nervous system function. It is just such a feature of an active information transfer that distinguishes the nervous system from other organs. For such a reason, it is not surprising that the neuron evolved a unique and highly specialized subcellular structure, the synapse, to carry out this task (see fig. I.5).