RAFAEL LORENTE de NÓ (1902-1990): THE PIONEER OF PHYSIOLOGYCAL NEUROANATOMY

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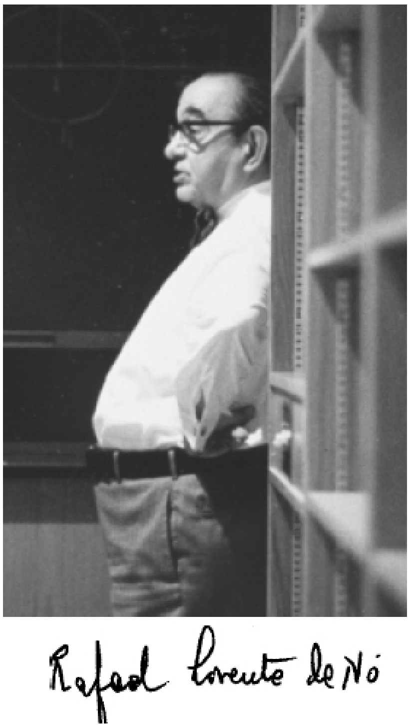

The Origins

Rafael Lorente de Nó, son of Francisco Lorente and Maria de Nó was born on 8th of April 1902 in Zaragoza, the capital of Aragon, a land prolific in artists and scientists; Francisco Goya, Luis Buniel, Miguel Servet and Santjago Ramon y Cajal all came from there. For the rest of his life, friends, colleagues and students called him Don Rafael, Lorente or Dr. Lorente [1, 2].

Rafael Lorente de Nó had a very early start in science, matriculating in the medical School of Zaragoza in 1917, when he was only 15 years old [1, 2]. At that same age he published his first research manuscript entitled “Temperatura” that was dedicated to a mathematical analysis of thermodynamics [1, 3]. When reading medicine in Zaragoza he began studying the nervous system under the guidance of Pedro Ramón y Cajal, professor of obstetrics and gynaecology, former histology teacher and brother of Santiago Ramón y Cajal [1, 2, 4]. At that time he performed several independent studies focussing on the neurite outgrowth and regeneration after starch injections into the central canal of the spinal cord of amphibians. Results of these investigations were published in 1921 [5].

Rafael Lorente de Nó was an exceptional student indeed, and in 1920 (when he was only 18 years old) Pedro Ramón y Cajal encouraged him to visit and talk to Santiago Ramón y Cajal in Madrid; thus young Lorente came to Madrid and presented his histological studies [4, 6, 7]. After this initial meeting Santiago Ramón y Cajal invited Rafael Lorente de Nó to work in his Laboratory, as a fellow of the JAE (Junta para la Ampliación de Estudios e Investigaciones Científicas – Comission for Advanced Studies). In parallel Lorente continued and eventually completed studies in Medicine – he became a Doctor of Medicine in 1923 when he was only 21! [1, 2, 4, 6-8]

The Neuroanatomist

After moving to Madrid Rafael Lorente de Nó commenced a prolific career in neurohistology and neuroanatomy under guidance of Ramón y Cajal. Lorente de Nó thoroughly perused Cajal’s “Textura del Sistema Nervioso” and was fascinated about all the achievements of his teacher in the fine anatomy of the brain [2, 8-10]. However, at the very beginning of relationship with Santiago Ramón y Cajal, Lorente de Nó already showed his boldness. Responding to a somewhat singular question of Santiago Ramón y Cajal: “What do you think about Cajal?” He answered: “Which one, the Master who described the texture of the nervous system, winning the Nobel Prize or the one that has done little afterwards?" [2, 10, 11]. Luckily, for Rafael Lorente de Nó, Cajal was rather amused by this remark, regarding it as a sign of youngster audaciousness and ambition [2, 10]. Anyway, a long master/disciple relationship ensued; a relationship that extended until the very last moments of Cajal's live. This was reflected by the fact that the last letter Cajal's wrote to Rafael Lorente de Nó was dated by 15th of October 1934; that is it was sent just two days before Cajal's death. In this letter indefatigable Cajal made some comments regarding Lorente de Nó's last work "Studies on the structure of the cerebral cortex II. Continuation of study of the ammonic system" [8, 12, 13].

When Rafael Lorente de Nó joined Cajal's Laboratory in Madrid he became the youngest of Santiago Ramón y Cajal's pupils. At that time Cajal's laboratory was populated by the very best members of the famous Spanish school of Neuroscience and Neurology [2, 6 10], including Pío Del Río Ortega, Jorge Francisco Tello, Fernando de Castro and Nicolás Achúcarro [2, 6 10]. Beside Santiago Ramón y Cajal, young Rafael Lorente de Nó was profoundly influenced by other neuroscientists such as Oskar Vogt, Hermann von Helmholtz and Camillo Golgi [2, 10]. When Rafael Lorente de Nó started his work in Cajal's Laboratory, he partially put aside his interest in plasticity of amphibians and reptiles spinal cord and initiated studies in the mammalian cerebral cortex and vestibulo-ocular system [2, 4, 6]. For this initial work on the mouse cortical organization he used the well-established method of Golgi staining, producing impressive results that astonished Cajal at the time [8]. This pioneering work in the primary somatosensory cortex of the mouse [14] followed by other classical studies [13, 15, 16], which demonstrated structural evidence that the cortical areas of mammals are organized in a columnar manner rather than in horizontal layers, thus articulating for the first time the basic features of the columnar organization of the cerebral cortex [8, 17]. This also corroborated de No's beliefs, that the evolution of cerebral cortex in mammalian is not linear [8, 13, 15, 16]. In these papers Rafael Lorente de Nó described more than 60 defined types of nerve cells with precise laminar position and/or extent of their axonal and dendritic distributions that account for cortical functionality [8, 13, 15]. These studies also resulted in the first rose description of the fundamentally important concept of the "internuncial neurones"; which we know nowadays as interneurones [2, 8, 18]. These were defined and described by Rafael Lorente de Nó as follows: "The role of cortical neurones cannot be any other than to regulate the discharge of the efferent fibres. The pyramidal cells will carry the impulses further, and the internuncial neurones have to regulate the discharge of the efferent ones" [18]. In parallel with his studies of the cerebral cortex, Rafael Lorente de Nó also focused part of his research on the vestibule-ocular reflexes as well as on the organisation of the vestibular nuclei of rabbits. This he did while visiting the University of Uppsala (1924-1927) where he worked with Professor Robert Bárány; the latter won the Nobel Prize on 1914 for research on the pathology and physiology of the vestibular apparatus [1]. At those times it was thought that the vestibular information controlled the ocular reflexes by direct projection to the oculomotor nuclei [2]. However, Rafael Lorente de Nó demonstrated, that after lesioning the pontine and medullar reticular formation rabbits were unable to display nystagmus whereas they preserved the slow component of the ocular reflex. Thus for the first time functional role of the reticular nuclei in the vestibulo-ocular reflex was demonstrated [2, 19, 20]. When staying in Sweden de No briefly sojourned in the Laboratory of Oskar and Cécile Vogt, at the Berlin Brain Research Institute, where he immersed himself in the study of the architecture of human cerebral cortex [1, 2, 8]. This period at the Vogt's Laboratory was fundamental for the later publications on the cellular architecture and organization of both the entorhinal cortex and the hippocampal formation [13, 14]; these were extremely important for the understanding of neuronal networks and cortical function. In fact, the hippocampal subdivision, based on connectivity patterns in "cornu ammonis fields" (CA1-CA4) [13] is still up to date and has resisted the passage of time.

From the Neurone Doctrine to the Functional Synapse

After a brief passage through Spain (1927-1931), when he was working in Madrid and Santander, in the fall of 1931 Lorente de Nó and his wife moved to the United States where he started working in St. Louis (Missouri) at the Central Institute for the Deaf (CID) as Research Director [1, 2], after independent recommendations by Robert Bárány and Oskar and Cécile Vogt. He stayed in the CID for 5 years before moving to the Rockefeller Institute in New York, by invitation of Herbert S. Gasser (who together with Joseph Erlanger won the Nobel Prize in 1944 for their discoveries of the highly differentiated function of single nerve fibers) where he spent the rest of his career until his official retirement in 1970 [1, 2, 4, 21]. This period represents a clear change in the direction of Lorente de Nó research towards physiology and electrophysiology, the area that he begun to be interested during his stay in Uppsala [2, 19, 20]; but without neglecting his neuroanatomical work. As he explained to his master, Ramón y Cajal, in a letter in April 1934: “Just now I dedicate half of my time to physiological experiments…since structure is illuminated by function” [1]. Thus, first in the CID and then in the Rockefeller Institute he focused his studies in neuronal activation, nerve conduction and synaptic transmission [1, 21]. By using the cathode–ray oscillograph and with the development of microelectrodes for nerve recording he was able to record electrical potentials from neurones, dendrites and axons, which allowed him to study not only the impulse conduction but also to establish the elementary units and circuits of the cortex as well as to understand how neurones and synapses operate in the cortex and in other systems such as the cochlear nuclei and the spinal cord (motorneurones) [1, 2, 8, 16, 18, 21-26]. In the early years at CID he extended studies on the vestibulo-ocular system expanding and implementing Sherrington principles on synaptic activation and more specifically on the concepts of temporal and spatial summation and synaptic delay [1]. His first 15 years of neurophysiological research in the USA contributed to the publication of a two-volume monograph entitled “A Study of Nerve Physiology” which appeared in 1947 [27, 28], being called by the students “the telephone book”. Part of the contents was already presented in three papers in the first volume of the *Journal in Neurophysiology* in 1938 in which, for the first time, he considered synaptic transmission in motoneurons [25, 26]. Furthermore he also managed to combine theoretically his previous results with his knowledge in cortical anatomy and electrophysiology [18]; which we can define as the first example of functional neuroanatomy. Regarding the synaptic transmission in motoneurones, Lorente de Nó detected the existing delays in motoneurone synapses and considered that a very small variation in synaptic delays sets necessary limits for establishing a theory of synaptic transmission [21, 25]; whilst on the second work he advanced for the first time the idea that the motoneurone stimulation is directly depended from all the synapses located on a given area of the motoneurone (concurrent activation) [21, 26].

As we just mentioned, the combined effort of his neuroanatomical techniques together with the electrophysiological approach produced seminal description of the cortical organization [8, 18, 21]:

“All the neurons in the central nervous system are reciprocally connected by numerous pathways, some having great and others lesser degrees of complexity”…”The number and complexity of central pathways are best described by saying that with but few exceptions, at least one pathway can be found connecting any two neurons in a manner so that the impulse may be conducted from one to the other neuron”.

Based on these assumptions and on his previous works on the cerebral cortex [13, 15] and in the vestibulo-ocular system (which he made in Uppsala), Lorente de Nó conceived that the cortical circuits work as an ensemble of reflex arcs [8, 18] extending the observations of Cajal based on Sherrington principles. Based on this affirmation he conceived that a cortical circuit would be a reflex arc in which the cortical neurones (both projection and interneurones) are the regulators of neuronal chains (i.e. neuronal networks) [8, 13, 15, 18, 21]. Therefore, the cortical physiological unit is formed by an efferent cortical neurone with its fibre together with the interneurones which regulate its activity;

all of them forming a kind of cylindrical unit [8, 13, 15, 18]. Lorente de Nó, like Sherrington, considered that dendrites receive the impulse and axons carried it forward [23, 29], thus excitation being unidirectional and having graded levels in the cell somata and dendrites. These synapses could be of two types, excitatory and inhibitory [8]. However, Sherrington made these entire hypothesis for motoneurons, where integration only happens in the cell body [29]; whereas in the cortex dendrites are the major element that receives the synapses (several thousand on a single pyramidal neurone) thus the summation of all this impulses gives rise to the final response [8, 29]. Therefore, in order to define a cortical circuit Lorente de Nó considered two types of cells according of their capability of being or not being excited by the afferent fibres. The neurones that are excited would receive direct contacts while the other cells can only generate action potentials due to the intracortical activity [8, 18]. This is clearly summarised in his definition of two fundamental elementary circuits of internuncial neurones: the multiple (M) type of chain of neurones and the closed (C) type of chain [8,16,18,21], which can be of any length and comprise multiple parallel pathways. The M chains are based in the plurality of connections and follow Cajal's ideas of the avalanche conduction [8,16,18,21], in which a weak stimulus might undergo "amplification" to attain a given cognitive threshold resulting in the activation of a large number of neurones; whereas the second one illustrates the principle of reciprocity of connections [8,16,18,21], that what we know presently as feed-back loops.

The Master and his Legacy

Lorente de Nó, like many scientists of his time was extremely independent and energetic, and may be for that he never created a large laboratory [1]. Nevertheless, he trained and collaborated with the best neuroscientist of his time including Nobel Prize winners Santiago Ramón y Cajal, Robert Bárány and Herbert S. Gasser [1, 2, 4]. Among his first pupils and collaborators we have to name James Lee O'Leary whom he trained on the Golgi impregnation methods achieving remarkable results on the cat visual system [30, 31]. Later, when at the Rockefeller Institute he collaborated with T.P. Feng on the action of barium in the rhythmical activity of nerves, A. Gallego on the role of monovalent ions in nerve conduction, Y. Laporte on synaptic function in sympathetic ganglia and G.A. Condouris on decremental conduction in peripheral nerves [1]. Finally, he supervised anatomical studies of K.E. Åstroms on the cranial nerve nuclei in the mouse brain stem, which resulted in publication of extensive series of experimental observations and theoretical aspects of nerve transmission with V. Honrubia [1, 2]. Lorente de Nó has been one of those men, ahead of his time leaving a scientific legacy that is still used and valid in our days and will remain valid in the future. During his studies on the impulse conduction in vertebrate, Lorente de Nó synthesised tetramethylammonium (TEA), which now is widely used as a standard pharmacological blocker of potassium channels [32]. In addition, Lorente de Nó (similarly to his teacher Cajal) was also a consummate artist and drawer being specifically intersted in detail and perspective. In this sense, (and contrary to Cajal, who preferred to make drawing from his recollections after the microscope experiment) Lorente de Nó developed a system to exactly project microscope images from the eyepiece to the table work; the technique which we know as camera lucida [2]. Finally, one of his ideas had a major influence for Hebb's work "The organization of behaviour" (1949) [32], and in consequence in what we know as the Hebb synapse and Hebbian plasticity, which is key to the processes of learning and memory including long-term potentiation (LTP) [21, 32].

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