Review

Cajal and Lorente de Nó on cortical interneurons: Coincidences and progress

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ABSTRACT

This essay explores the contributions to the organization of neuronal microcircuits in the cerebral cortex by Rafael Lorente de Nó, a renowned disciple of Santiago Ramón y Cajal. Lorente de Nó was impressed by the advances in functional parcellation of the cerebral cortex, and wished to find an anatomical correlate, not in cytoarchitectonic charts but in the fine details of neurons and (soon) of neuronal circuits within a cortical locale. His early analysis culminated in two major papers in 1933 and 1934: he introduced a hypothetical frame in which to integrate circuit anatomical complexity with the ideas on the physiology of the neuron prevalent at the time. In an interlude (1934–1938), Lorente embarked in studies of neuron physiology that inclined him to a reductionist interpretation of the axon as the main functionally relevant entity of neurons. This essay describes my attempts at tracing the links between the master’s tradition, the minutiae in the early Golgi studies by Lorente and his concepts of neurophysiology. These are the bases to approach his final synthesis: The cerebral cortex: architecture, intracortical connections and motor projections, published as an invited chapter in J.F. Fulton’s Physiology of the Nervous System in 1938. © 2007 Elsevier B.V. All rights reserved.

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1. Introduction

In the early 1920s, Rafael Lorente de Nó (1902–1990) was a brilliant medical student in Zaragoza, Spain. He had thoroughly perused Cajal’s *Textura del Sistema Nervioso* (Río-Hortega, 1986; Fairén, 1993) and was fascinated by Cajal’s achievements on the fine anatomy of the brain (Gallego, 1991; Kruger and Woolsey, 1990; Woolsey, 2000; Larriva-Sahd, 2002, 2005). He was going to be the latest disciple of Cajal. While still a medical student, in 1920 he joined Cajal’s laboratory at the Facultad de Medicina in Madrid and initiated studies of cortical organization in the mouse, using the Golgi method. Cajal had culminated his Golgi-based studies on human cerebral cortex in 1904 but at that time, when he was 68 years old, he embarked newly on Golgi studies of defined cortical areas: the visual cortex in cat (Cajal, 1921) and the retrosplenial cortex in mouse (Cajal, 1922).

Lorente’s work in Cajal’s lab was interspersed with stages abroad, mainly in Uppsala (with Robert Bárány), hence his perdurable interest in acoustic and vestibular neuroscience. All these stages were supported by the Junta de Ampliación de Estudios (a Commission for Advanced Studies) created in 1907 and presided over by Cajal. After returning to Spain in the late twenties, Lorente’s sought-after desire was professional stability. He found it in a laboratory and clinic created for him at Casa de Salud Valdecilla in Santander, in northern Spain. Although the laboratory seemed to be well equipped, overwhelming clinical duties deterred Lorente of performing any experimental work there. This inclined Lorente to accept an invitation from the Central Institute for the Deaf in Saint Louis, in 1930, to head a neuroanatomical laboratory. Cajal was deeply disappointed, overtly because he interpreted this outcome as an important failure of the Junta’s objectives. Indeed, on Cajal’s advice, one of the major aims of the *Junta de Ampliación de Estudios* had been to finance the training of the most promising young scientists abroad to favor the surge of science in Spain after their return. Unfortunately, if not enough measures were implemented to ensure an adequate scientific environment to the returnees, an undesired companion to that kind of science policies has been and still is brain drain.

No much reference has been available so far about the scientific interaction between Cajal and Lorente. Fortunately, RodríguezQuiroga (2002) has published a well documented series of letters crossed between Cajal and Lorente (1930–1934) that reflect unknown aspects of the relationships between master and disciple along Cajal’s last years, precisely when most of Lorente’s work on cerebral cortex was reaching completion. The scientific influence of Cajal was repeatedly recognized by Lorente in his papers. However, it is perhaps less known that Lorente seemed to have always sought a more tangible collaboration with Cajal along the lines he admired, the neuronal organization of the nervous system. In one of the letters, dated 10 April 1934, he expressed how he had caressed the idea of updating Cajal’s *Textura*:

“...along the fourteen or fifteen years of serious dedication to the nervous system, I have explored practically all its regions and observed new facts everywhere; those, together with the many [facts] that you describe[d] would make a very complete book.”

Perhaps the time was not yet ripe for that endeavor, for he hastened to add:

“...anatomical investigations bring glory and fame only when the physiologists find their [functional] application—a good example are your investigations on the cerebral cortex, which I am bringing back to light now.” ... “That is why I am devoting half of my time to physiological experiments.”

In the last letter in RodríguezQuiroga compilation (2002), Lorente answered certain queries of Cajal over his paper on entorhinal cortex (Lorente de Nó, 1933a,b). Lorente had sent Cajal not the manuscript, but a reprint of the published paper. In his letter, Lorente indicated that he had sent to Cajal the galley proofs of his paper on the Ammon’s horn (Lorente de Nó, 1934). What possibly could be the very last letter from Cajal was addressed to Lorente (VázquezTapioles, 2005). It was dated 15 October 1935, exactly 2 days before his death. In it, Cajal raised some criticisms, most possibly over certain aspects of this last paper. Because of the interest of that correspondence, I shall return to these letters later, since they illuminate how Lorente developed his ideas on the cortex and the nervous system generally along those times.

2. The first paper on the cerebral cortex (Lorente de Nó, 1922): the formulation of a research project

This initial work was extremely well done so as to have impressed Cajal—it still impresses us today. The publication contains extremely detailed tracings of many types of neurons in what we know now is the primary somatosensory cortex and, as I will summarize below, contains much new information. In addition, the paper introduces the main questions about the nervous system that Lorente imagined at the beginning of his career. Anybody perusing “The cerebral cortex of the mouse” will perceive the remarkably originality of these questions.

Perhaps the most functionally relevant question in this early writing is the concern, how could “sensory excitation” be localized in the brain in a very precise manner, “What is the mechanism by which sensory impressions are restricted to a specific cerebral region?” in spite of “the numerous relay stations of sensory excitation” and of its dispersion in the cerebral cortex, where “…it is unlikely that one single cell is not influenced by a part of such excitation.” This is a question that still could not find an answer, and so in this paper there is no conclusion whatsoever. This was a recurrent theme in Lorente’s cortical research. We have to wait until his following papers on the cerebral cortex (Lorente de Nó, 1933b, 1934) for a further intellectual elaboration of such an initial question based on Sherrington ideas of graded events in neuronal somata, and a few years later (Lorente de Nó, 1938a,b) for a more personal alternative. Along the time, he had developed a real interest to think of his anatomical diagrams in functional terms.
Probably after seeing his first successful Golgi impregnations, Lorente did not think the microanatomy revealed by the Golgi method was to be simple (and boring) in mice:

“We do not believe in the “simplification” of the cortex [in mice]. Indeed, a glance at the illustrations that accompany this article should be enough to become convinced of the enormous complexity of the Cortex cerebri in the mouse.” (Lorente de Nó, 1922).

By choosing the mouse as an animal model, Lorente formulated an interesting question on mammalian evolution. It is true that Cajal had recommended earlier (Cajal, 1893) to use animal models to study the organization of the cortex but, later when he had studied the human cerebral cortex (see Cajal, 1917), he became so enthusiastic about it that he left little room for alternative observational approaches in “lower species.” It sounds nicely provocative to hear from a novice researcher what his purpose was, as he referred retrospectively: “the problem was to ascertain whether the cerebral cortex of the lower mammals has the same plan of structure as the human cortex.” (Lorente de Nó, 1933b).

For Lorente, the evolution of the cerebral cortex in the mammalian scale was not linear:

“That by descending the zoological scale the number of cerebral “fields” diminishes is a fact irrefutably settled, above all, by the research of Brodmann and his disciples. However, it is also certain that this process of reduction does not extend uniformly over all the [cortical] “areas.” The reduction and disappearance of some [fields] is accompanied by the expansion of others and perhaps by the appearance of new fields related to the [specialized] cerebral functions the animal possesses.”

At that time, cytoarchitectonics pervaded most research on cortical structure and function. Lorente believed that cell clustering was not functionally relevant. For him, the study of neuronal form was useful only insofar it might indicate the modes of synaptic connectivity. At his very early period, no better a tool than the Golgi method could give a clue to understanding the functional anatomy of the cortex. If this was the driving force in Lorente’s mind (it seems the case), this was unattainable, for he evolved not to see as many differences as similarities among the different cortices he described, the tone of generalization in his final cortical report of 1938 (Lorente de Nó, 1938b). Moreover, this report was based on neuronal circuits, which he arrived to consider as the functional unit in the nervous system, with neglect of other neuronal properties.

Short of adequate information at that time (1922), Lorente could not identify the cortex under scrutiny as the somatosensory cortex (Fairén, 1993). Indeed, he was describing what today we call the barrel cortex, the cortex that contains barreis, the visible representation of the large mystacial vibrissae in the animal’s snout. So, this is the first description of neurons that arborize their dendrites and axons in the special context of the barrels. Fig. 1 (taken from Lorente de Nó, 1922, Fig. 8) shows different categories of Golgi-stained neurons in two adjacent barrels. Most are spiny stellate cells, some are pyramids, and still there are in this figure some nonspiny stellate cells. Lorente explained:

“...in those sections where the chrome-argentie reaction took place preferentially in layer IV, leaving layers II and V free [of staining], layer IV appears composed as clusters of neurons, separated by pale vertical bands, as is shown in Fig. 8 [i.e., Fig. 1 here]. The cellular masses correspond to the pale territories of the Nissel method, and the bands to the columns shown by the same method.”

It is obvious to the modern reader that he is talking about the hollows and the septa of barrels. He found these clusters similar to glomeruli in the olfactory bulb, and attributed to them that same name. These are the barrels (Woolsey and Van der Loos, 1970; Van der Loos and Woolsey, 1973).

This paper contains an unsurpassed database of neuron types in the mouse cortex based upon Golgi stains. As Lorente thought (Lorente de Nó, 1933b), this description “contains more than sixty perfectly defined types of nerve cells.” This is an assertion with a positive tone: also in the 1933 paper there is an extraordinary database of neuron types of the entorhinal cortex. For today standards, this is exaggerated but it obviously depends on the criteria used for classification and, at the time of the original publications, criteria were forcefully limited. As a matter of fact, the precise laminar position or the extent of the axonal and dendritic distributions was taken into account as classification criteria, and he suggested how these could influence function (Lorente de Nó, 1933b, 1934). Perusing these texts makes me think that any individual neuron of these composite drawings such as that reproduced here (Figs. 2 and 3) was a representative of a neuron class he conceived on these grounds. As with taxonomists, authors dealing with neurons as classifiable objects could be either lumpers or splitters. When dealing with microanatomy, Lorente seemed here a prime example of a splitter. However, as I will show later, he was able to synthesize such intricate classifications when elaborating his
hypothetical interpretations of neuronal circuits. He departed further from detailed anatomy when proposed the organization of the nervous systems as gigantic ensembles of reverberating neuronal chains (Lorente de Nó, 1938a,b).

It is of interest to remark how Lorente depicted certain types of short axon cells in relation to barrels. That relationship manifested as neuronal forms that are not been seen elsewhere, not even in further Golgi studies along the times (review: Fairén, 1984). In my belief, this has caused certain reluctance toward the neuron database offered by the paper. Lorente carefully depicted the neurons he saw in the Golgi preparations using a camera lucida, trying to represent all the fine details of architecture and the exact forms of the presumed synaptic contacts. In Fig. 2, I reproduce Fig. 13 of Lorente de Nó (1922). This composite drawing contains two examples (cells D and E) of interneurons commonly found in rodent cortex (Peters and Fairén, 1978). Two interneurons (A and C) bear relationships with barrels and have part of their dendrites and their axonal ramification encased within barrels. For the form of such neurons, we have not seen a match in any more recent Golgi preparation or in any Golgi study of the rodent cortex (Fairén, 1984) but we have seen their possible counterparts among glutamate decarboxylase positive neurons, and proposed naming them Lorente de Nó cells (Cobas et al., 1987). The idea that the drawings by Lorente of cells A and C were faithful representations of reality came to me as indirect evidence. One of the most recent approaches to analyze the phenotypes of interneurons born at specific places and times during prenatal development is that pioneered by Gord Fishell and Arturo Álvarez-Buylla (Wichterle et al., 2001; Butt et al., 2005). Figs. 1–3 show examples of alkaline-phosphatase tagged interneurons in adult mice, whose precursors were transplanted into the medial ganglionic eminence at embryonic day E12.5. Both seem like modern illustrations of cells A and C as drawn by Lorente. Another useful comparison came from certain transgenic mice. Transgenic mice may reveal in a Golgi-like fashion Lorette and proposed naming them Lorente de Nó cells (Cobas et al., 1987). The idea that the drawings by Lorente of cells A and C were faithful representations of reality came to me as indirect evidence. One of the most recent approaches to analyze the phenotypes of interneurons born at specific places and times during prenatal development is that pioneered by Gord Fishell and Arturo Álvarez-Buylla (Wichterle et al., 2001; Butt et al., 2005). Figs. 1–3 show examples of alkaline-phosphatase tagged interneurons in adult mice, whose precursors were transplanted into the medial ganglionic eminence at embryonic day E12.5. Both seem like modern illustrations of cells A and C as drawn by Lorente. Another useful comparison came from certain transgenic mice. Transgenic mice may reveal in a Golgi-like fashion Lorette and proposed naming them Lorente de Nó cells (Cobas et al., 1987).

3. The elementary unit of the cerebral cortex

When Lorente wrote the next two cortical papers (Lorente de Nó, 1933b, 1934), he was at the onset of an important phase of the development of his ideas on the nervous system that came from his experiments using the cathode-ray oscillograph (Lorente de Nó, 1935a,c, 1938a). The scope of the two cortical papers is surely not linked to that but to Cajal and Sherrington tradition. Interestingly, Lorente organized his 1933 paper as an updating of Cajal publications, putting his own findings in that perspective:

“As in my description I would have to repeat all the details published by Cajal, I think it best to translate Cajal’s words and reproduce some of his figures, adding afterwards the new facts that appear in my preparations.”

Both reports have a descriptive section quite similar to that in his initial paper. There we see the same scrupulous description of neurons, fibers, and dendritic and axonal plexuses we have seen in his 1922 publication (Fig. 3). In the Ammon's horn paper (Lorente de Nó, 1934), a novelty is the subdivision of the hippocampus proper in fields, a subdivision based on connectivity patterns: it is the division into hippocampal CA1–CA3 fields that has resisted the passage of time.

In the most speculative parts of these papers, we see Lorente making a big step toward finding possible principles of functional organization of the cerebral cortex, by defining the elementary functional unit and the basic neuronal circuits of the cortex. His ideas on these matters are rooted in what he knew about reflex arcs from the literature and from his own work in Upsala. This is not new in itself, for Cajal had already followed Sherrington principles to interpret the cortex functionally as an ensemble of reflex arcs (Cajal, 1904, but see Berlucchi, 1999). The real advance here is that Lorente ventured further by incorporating short axon cells in diagrams of neuronal chains. Lorente seemed to avoid giving an enormous importance to this, for he said here (and again in his final cortical essay, Lorente de Nó, 1938b) that the same fundamental neuron chains were present in all the brain regions he had studied, other than the cerebral cortex. Cajal considered that short axon cells were more prominent in the cerebral cortex or the cerebellum than elsewhere in the CNS (Cajal, 1901), but Lorente was in profound discrepancy at that time. Referring to his previous work on the VIII nerve, he argued that the brainstem nuclei had plenty of such neurons. At this moment, however, it is relevant to recall that the then widely used term ”internuncial neurons” was loosely related with short axon cells. In Lorente writings, it is clear that short axon cells were internuncial neurons, but that also local connections of principal neurons made them internuncial as far as their local axon projections were concerned (Fairén, 1993).

From his first publication, not in vain he had studied the barrelenfield of the somatosensory cortex, he intuited the functional unit had to be inside a column; it will never be in a cortical layer. On that assumption, he elaborated on his conception of cortical circuit as a reflex arc, in which he implied “cortical neurons” (including short axon cells, but also the intrinsic axon collaterals of projections neurons) as “regulators” of the activity of the neuronal chains; we would say now of neuronal networks. We will see immediately what he meant by regulators:

“The afferent impulses carried by the afferent fibres leave again the cortex through the axons of the star and pyramid...
Fig. 2 – On the top stands Fig. 13 in Lorente de Nó (1922). From my point of view, the axon arborizations are exact reproductions made with a camera lucida. A–D are different examples of short axon cells. A and D resemble interneurons with axonal arcades that are frequently found in Golgi preparations of rodent cortex. A and C are similar to the cell labeled with an asterisk in Fig. 1. The axonal arbors are in layer IV, likely related to barrels. On its turn, cell body B is in upper layer IV and the axon arbor looks more dispersed than in A and C. Cell F does not show any detail to comment. Photomicrographs in 1 and 2 (second row) display examples of cells tagged with alkaline phosphatase; in 1, the section was Nissl stained. These are interneurons that presumably originated at the medial ganglionic eminence at E12.5. Cell bodies are at the bottom of layer IV and dendritic and axonal branches relate to barrels in layer IV. Preparations from Hynek Wichterle (Wichterle et al., 2001). Photomicrograph 3 displays several GFP* interneurons of mouse line X94; photomicrograph by Ariel Agmon (Ma et al., 2006). We see again (a, b) the forms of Lorente’s cells A–C. These cells co-express glutamate decarboxylase and somatostatin, and belong to a novel subtype of interneurons with peculiar firing properties and characterized by targeting layer IV (Ma et al., 2006). The lower row of images is from the paper by Ma et al. (2006). These are images of dendritic (green) and axonal arbors (red) reconstructed in 3D with a modern camera lucida (Neurolucida). Arrows indicate the points where axonal branches turn to reach layer IV again. Scale bar: 50 μm. Lower row: Copyright 2006 by the Society of Neuroscience.
cells. The role of the cortical cells cannot be any other than to regulate the discharge of the efferent ones. Therefore, the “units” in the cortex are constituted by each afferent fibre, the efferent cells that will carry the impulses further, and the internuncial neurons that have to regulate the discharge of the efferent ones. As in the area entorhinalis each afferent fibre generates a ramification having more or less the form of a vertical cylinder with the fibre itself as axis, we may consider each cylinder with all the cells it contains and the neurons underneath as a physiological unit.”

The emphasis is Lorente’s. This kind of cylindrical unit could not the only one that may be thought of. For Lorente, the elementary unit depended on time: “Almost any cortical cell represents the axis of a similar cylindrical unit ..., might it be a pyramidal cell, or a ‘cell with short axis cylinder’ [...] But no matter what kind of cylinders we choose,—all are equally well justified and every one will have a real physiological existence at determined moments of cortical function.”

The idea of modules operating at definite times is original and attractive, and preludes more modern formulations such as Szentágothai’s (1975). We witness here an episode of the infancy of a functional cortical neuroscience.

4. A hypothesis on how synapses and neurons operate

Lorente adopted as a first premise that dendrites receive the impulses and the axons carry them further, according to Cajal and Van Gehuchten. He recognized the contribution by Sherrington (1897) that synapses are unidirectional (he gave additional experimental support for it in Lorente de Nó, 1935b). However, how was this in physiology terms? He adopted an additional premise, the existence of graded levels of excitation in the cell somata and dendrites (Sherrington, 1925).

To explain neuronal excitation and inhibition in spinal reflexes, Sherrington (1925) proposed that synaptic inputs a neuron receive would cause central excitatory states (c.e.s.) that modify the functional state of the neurons. These states are able of temporal and spatial summation. When the neuron c.e.s. reaches a threshold value, the neuron discharges. Independently, other sets of synapses produce what Sherrington called a central inhibitory state (c.i.s.), which would lower the possibility of the neuron to reach threshold. Thus, synapses would give effect to two separate types of actions, excitatory and inhibitory (see also, Eccles, 1935). C.e.s. and c.i.s. were later assimilated into the postsynaptic potentials revealed by intracellular recordings (Brock et al., 1952; Eccles, 1990). Lorente liked the concept as a theoretical reference because he thought it was the less committal for defining the state of the neurons. The proposal did not intend to define the nature of these states, excitatory or inhibitory. However, at the time it was not even thought that the synapses were from different neurons (Eccles, 1990).

The neuron for which Sherrington made his proposal was the motor neuron, and integration was considered only at the level of the cell bodies. Lorente speculated with the neural chains of the cortex, which is a most complicate anatomical framework. The most important consequence is that dendrites have to be taken into account and, as he noted, “nothing is known about the conduction in dendrites.” He considered that, since dendrites receive a large quantity of synapses (he adventured: “several thousand” on a pyramidal cell), “we must face the possibility of a dendrite receiving many thousands of impulses each second,” thence “summation of impulses...
arriving at different points of a dendrite [was] very difficult to understand." Let us follow Lorente in his own words:

"...the impulses arriving to the neuron [h]rough one of its numerous synapses; each impulse raises a certain amount of c.e.s. and as soon as the c.e.s. reaches threshold value the neuron discharges. After each impulse [of the neuron] the neuron enters in a refractory state" “a single impulse is surely not able to set up a discharge [...]“ we could have [...] that there are synapses in a nerve cell that in spite of temporal summation never are able to raise threshold c.e.s., thus setting up a discharge."

He thought how to define a circuit, considering types of neurons that could or could not be excited by the afferent fibers. The former would be cells that received direct contacts; the latter “may be brought to discharge only as a result of intracortical activity.”

A curious note on inhibition follows in the writing. Although Lorente conceived the existence of inhibition, and wrote that internuncial neurons might be the siege of it, then he disdained this concept absolutely, and did not mention it further. That is, he ignored in this paper the central inhibitory states he had just introduced after Sherrington. Lorente maintained his ideas on this point: an interesting later discussion (Lorente de Nó, 1938a) is out of the scope of this paper. The existence of inhibitory interneurons had to wait, unfortunately, neurophysiology and biophysics did not advance fast enough along these years so as to incorporate that complicated anatomy into experiments.

In Fig. 35 in Lorente de Nó (1934) (Fig. 5 here), Lorente represented two CA3b pyramidal cells (Py. 1, Py. 2) receiving perforant path contacts (afferent fibers, A.f.) on the distal dendritic tuft in stratum moleculare (str.m.c.), and mossy fiber (m.f.) contacts on the proximal part of the apical dendrites. Perforant path axons also contacted granule cells (G.c.) of the dentate gyrus and certain short axon cells located, for instance, in the stratum lacunosum (Str. l.), stratum radiatum (Str. r.c.) and stratum pyramidale (a pyramidal basket cell, B.c.). An additional short axon cell in the stratum oriens (Str. o.c.) could not receive contact from the afferent fibers. Lorente explained in a footnote certain additional details: the figures

"[...] are diagrammatic and not intended to show the structure of the synapses. The contacts of the fibres with the dendrites belong to the class of "collateral" synapses [...]"

Note that Lorente’s collateral synapses are synapses “en passant.” Lorente noted that, in cortical pyramidal cells, “the synapses of different kinds are not mixed, but rather grouped in special regions of the cell” as if it were a “functional segregation” of types of cortical interneurons. This is quite an advanced idea for that time.

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5. The elementary circuits

Lorente utilized circuit diagrams to test how the premises he had summarized serve to formulate hypotheses of the function of cortical neuron chains. This is neuronal modeling in the 1930s (Swanson, 1993). In previous papers, Lorente had elaborated schemes of elementary circuits conceived as neuron chains (Lorente de Nó, 1933a) describing two basic types (Fig. 4). The first class is the open circuit with multiplicity of connections that is in accord with Cajal’s idea of avalanche conduction (Cajal, 1899). The other class of circuit is closed, with reciprocal connections, first introduced by Lorente (see Graybiel, 1979). This is what we know now as feed-back loops.

The circuit diagrams in the two papers of 1933 and 1934 were anatomically minded and embedded his model circuits, open and closed. In all diagrams, arrows are as in Cajal, but Lorente talked about volleys and activation of synapses. Unfortunately, neurophysiology and biophysics did not advance fast enough along these years so as to incorporate that complicated anatomy into experiments.

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Let us listen to Lorente’s arguments:

“If a single volley of afferent impulses arrives to the Ammonshorn through the afferent fibers (A.f.), the pyramids will receive several volleys of impulses. A first volley (that of the afferent impulses themselves) will arrive at the pyramids through the synapses in the dendritic bushel in the Str. moleculare. A second volley through the synapses in the big thorns at the origin of the shaft; the impulses of this volley will have traversed through the granules of the Fascia dentata. A third volley will arrive through the synapses on the body after having passed through the pyramidal basket cells. A fourth volley will arrive through the cells with short axis cylinder of the Stratum moleculare, etc.

Here, Lorente proved by contradiction: imagine that dendrites conducted in the same way axons do, thus the chain simply could not be operative:

“...only the first arriving impulse is effective, and that if one of the other impulses delayed in a long chain of regulator cells [italics mine], arrives when cell Py [1 or 2] has recovered, the following afferent impulse will be ineffective.”

Notice that Cajal had proposed beforehand the concept of avalanche conduction (Cajal, 1899; Herrick, 1918; see Llinas, 2003). Avalanche conduction concept was one on which Lorente has apparently ruminated for a long time. Lorente (Lorente de Nó, 1934) was worried that avalanche conduction concept led to the idea that the number of neurons engaged in the transmission of a peripheral stimulus should increase each time that a new central station is passed,” which was not in accord with his experiments (Lorente de Nó, 1933a). However, this does not occur, says Lorente,

“[...] undoubtedly because due to the threshold of the neurons only a number of the anatomically possible transmission paths are accessible to the afferent impulses. The role of the central stations will consist in concentrating the stimuli in a few conductors rather than increasing the number of active conductors.”

Lorente found a hypothetical explanation to allow a neuron to process all the impulses it receives:

“The only possibility [...] is that each synapse sets only a subliminal [...] change able of summation and second, that the conduction through the synapses is not followed by a refractory period. The subliminal changes are summated first in the dendrites and then in the surrounding of the axon. When the charge reaches threshold value, an explosive discharge through the axon takes place.”

Logically, modern readers will find some difficulty. Is this sufficient to explain how closed circuits may work if inhibition is not considered?

Fig. 6 (Fig. 19 in Lorente de Nó, 1933b) illustrates the possible position of a short axon cell (cell 4) in a neuronal chain. Lorente represented there three layer III pyramidal cells (cells 1, 2, 3) of the entorhinal cortex. The short axon cell in question had its dendrites and axonal ramifications within the limits of one anatomical layer.

“The fibre a.f. is an afferent fibre carrying impulses to the cortex. The synapses established by this fibre are indicated
with s, and those of the endfibrils of the short axon with s.a. The impulse or impulses arriving though the fibre a.f. raise threshold c.e.s. in cells 1 and 4 because the number of s synapses is high, but cells 2 and 3 remain in sub-threshold excitatory state and it is only when the impulse of cell 4 reach them through the s.a. synapses that they are able to set up a discharge. Cell 4 makes the discharge of the cells of the layer more regular and on the other hand, when its impulses arrive on neurons that already have discharged, produce a facilitation of the following impulses.

Thus, Lorente indicated that the short axon cell would be in a position to reinforce the nervous flow “in a similar way as a relay may reinforce a weak electric current.” Cajal (1901) had earlier expressed this same view. This is a formulation of a hypothesis about the functional role of the short axon cells, in which it is implicit that their discharge will produce central excitatory states in the pyramids. Besides reinforcing activity, short axon cells could regularize the discharge of efferent neurons.

Along this line, then he proposed that local connections through short axon cells would permit “autogenous activity” (after-discharges) due to their participation in closed loops. In other words, activity was maintained by internuncial bombardment. Fig. 7 (Fig. 21 in Lorente’s report, 1933b) shows the circuit under discussion. Lorente emphasized cell 8, a short axon cell. Lorente explains in the figure legend:

“...deep pyramids (4, 5) discharge cell 8 reaches threshold c.e.s. The 8 impulses excite again cells 1, 2, 3, that already were facilitated by the recurrent collaterals [...] and the cyclic process starts again. The discharge of such closed chains will constitute the autogenous activity of the cortex; the frequency of the impulses being dependent on the number of active links within the chain.”

In this case, the short axon cell contributed to a self-re-excitation chain. Remember that, in the hippocampus (Fig. 5), the basket cell B.c. is in a closed loop: it receives contacts from Py. 1 and sends axons back to the same pyramid Py. 1.

“It is impossible to ascertain which neurons constitute each time the self-re-excitation chain, but there can be but little doubt that only a mechanism of this kind is able to set up a prolonged after discharge of a center obeying the all-or-nothing law.”

Here Lorente continued with the problem he had formulated in 1922: How is it thus possible that the excitatory state did not extend to the whole mass of cortical cells and, how will the self-re-exciting chains come again to resting state? The activity of the cells with short axon cells might account for both, he suggested: first, that some short axon cells might have high threshold and, second, because of refractory periods.
Short axon cells could participate in a different manner depending on the span of their axonal ramification, be it limited, it would be a concentration mechanism (Fig 3, cell 2), and if long (Fig 3, cell 1), it would cause association between adjacent cortical circuits. Finally, how the reverberant circuits would come back to a resting state could be explained by high threshold. Timing, refractory periods, and conduction times could in his view contribute to stop after-discharges.

We have thus seen how Lorente envisaged how the cortical ensembles of neurons could operate. Unfortunately, experiments to put these premises to test could not easily follow at that time. It is important to recall that there are two main themes in this exposition: one, the explanation of circuit activity in terms of central excitatory states and summation and two, on the role of the neuron chains in their self-maintained activity.

6. Lorente writes to Cajal, 1934

In the celebrated arrow diagrams of Cajal (see for instance Fig 6 in Sotelo, 2003 and Fig 2B in Llinas, 2003), interneuronal connections are always feed-forward. Cajal has never he had considered feedback. Just with one exception, when he describes the connections between parallel fibers to Purkinje cells and to Golgi cells. As it is well known, Golgi cell axons contact the dendritic digits of granule cells at the periphery of the glomerule. This closed a reverberant circuit, although Cajal did not consider such a possibility in his discussion (Cajal, 1901). Nevertheless, the existence of such circuits seemed to have posed a conceptual point of disagreement between Cajal and Lorente. This is reflected in the following passage, which I take from Freeman (1984):

“Three years ago in a workshop at Irvine I presented some data on the properties of mitral cells in the olfactory bulb, from which I inferred that they formed a mutually excitatory neural population (i.e., one having positive feedback). I cited this as confirmation of Ramón y Cajal’s (1909) hypothesis of “avalanche conduction,” in which a weak olfactory stimulus might undergo “amplification” (as we would say now). Rafael Lorente de Nó, in the audience, stated that I was in error; Cajal had in mind feed-forward recruitment of mitral cells and disavowed the notion of feedback. Lorente later recalled (personal communication) that in the mid-1920s he prepared a manuscript on the cytoarchitecture of the cerebral cortex, in which he concluded that feedback relations were a prominent feature. After reading the manuscript, Cajal strongly urged Lorente not to publish it because it would be unacceptable to the scientific community and might blight his career. Out of respect for his mentor, Lorente elected not to publish the material while Cajal lived, when he did publish (Lorente de Nó, 1934), the work established itself as one of the enduring classics in neuroanatomy. Its influence on neuropsychology (through Donald Hebb), on neural modeling (through Warren McCulloch), and on computer design (through John von Neumann by way of McCulloch) has been incalculably great.”

Lorente was surely certain that Cajal had not suspected the existence of recurrent circuits. However, it is not that evident how exactly Cajal gave Lorente the advice of delaying the publication of the next Lorente papers on the cerebral cortex (Lorente de Nó, 1933b, 1934). According to the recently published correspondence (Rodríguez Quiroga, 2002), Lorente had sent Cajal in 1934 a reprint of his 1933 paper. The letter is dated March 6, 1934:

“I enclose several reprints, among those one about the cerebral cortex that doubtless will interest you.1 Another three papers are in press, one on the Ammon’s horn and afferent pathways, another on the cortex of the human visual area cortex and [finally] another on the cortex of the remainder of the occipital lobe, also in human.”

The next letter in Rodríguez Quiroga (2002) is dated 10 April 1934. Because of its interest to understand the relationships between Cajal and Lorente, I translate it almost entirely:

“My dear master: Many thanks for your kind letter of 26 March.

The figures you refer to in your letter (figures 19, 20 and 21 of my paper on the entorhinal cortex) are schematic, in those it was not intended to give an idea of the forms of the contacts but simply of their existence; those figures are diagrams destined to show how the nervous currents circulate through the cerebral cortex so that the synapses have been exaggerated, giving them the form of small plates, to appeal the readers’ attention and allowing them to observe their occurrence. The true form of the synapses was indicated in figures 10 and 11, which are in agreement with figures 52 and 48 of your paper in Archivos de Neurobiología [Cajal, 1933]. On the cell bodies one can find terminal boutons or similar things, which very likely are, at least in part, termination of short axons. On the dendrites, one can find only what I named “collateral synapses” because you did not assign any appropriate name to them; the fibers en passant enter into contact with the dendrite. The only difference between your observations and mine is that I observed with extraordinary frequency – and you have mentioned them only exceptionally – are dilatations of the fibers or even small collateral balls. Perhaps such a difference was due to [the fact that] I used more aged animals, almost useless for the Golgi staining, where synapses were more developed [than in your material]. I thus do not believe that my observations require that you examine the issue once more. At least, not in the brain. Collateral spines are doubtless points of contact. Frequently, I have observed very satisfactory images such as those reproduced in figure 10 of my paper on the entorhinal area. In such a figure taken, from a mouse almost one month old, the fibers already presented the enlargements and small balls, which are also observed in adult animals. Certainly, perhaps doubtful images are also found, which make one to believe that the spines are portions of the fiber and not of the dendrite. This happens especially with the short axon cells— which in general lack spines. In those cells the contacts look different than in the pyramids. This I had already mentioned

1 Lorente de Nó (1933a,b).
2 These two manuscripts were never published.
in my paper of 1922. In the next paper that I ever write I will insist again on this subject to eliminate any doubt. […]

I enclose a copy of the printer proofs of a second work on the cerebral cortex (it is not urgent, or even necessary that you send them back to me, unless you wish [me to make] some change in the text). I hope you will like the form in which the subject matter subject is treated. As you will see the work brings the confirmation of many of the things that you described in 1901. The situation of bankruptcy in America [i.e., the United States] and Germany has made it impossible to reproduce your figures, as had been my wishes; but at every step I refer the readers to your book, especially to chapters IV and VIII [Cajal, 1911] […]

The transcription of a letter from Cajal, dated 15 October 1934, and presumably his last letter, was made public recently (Vázquez Tapiles, 2005). The letter is cheerful and polite and in it, Cajal made a remark on the last paper by Lorente: “the forms of dendritic spines (“note that they are not pointed excrencencies but gemmules, spines ending in a ball. The pedicle is sometimes too pale”). This is obviously a comment on Fig. 35 of the Lorente de Nó (1934; reproduced here as Fig. 5) whose galley proofs Lorente has sent Cajal in April that year. It is there where I see such pointed spines. The other comment by Cajal is perhaps more severe: Cajal has not seen many new anatomical data in that paper and notes that the mouse hippocampus is not the best suitable for the Golgi method.

7. From the neuron modeled as an axon to the chains of internuncial neurons

Just as he had mentioned to Cajal in a letter in 1934 (Rodríguez Quiroga, 2002), Lorente was conducting in Saint Louis experiments of neurophysiology using the cathode-ray oscillograph. Looking for a model system, he chose the motoneurons of the III nerve. In a rapid succession of papers (Lorente de Nó, 1935a, b,c) he build up an interpretation that he seemed to suggest was of general application. Lorente devised a preparation in which “[…] no internuncial neurones are present between the excited fibres and the motoneurones and in which errors due to unknown speeds of conduction can not occur” (Lorente de Nó, 1935a). He measured of synaptic delay and refractory periods using orthodrome or antidromic stimulation of the motoneurons. What he found was that the synaptic delay in these neurons was quite similar to that of the axons:

“[…] the refractory period of the motoneurones (dendrites and body including the synapses) “[…] can not be longer than 0.6 s [i.e., ms] which is the absolutely refractory period of the stimulated fibres themselves.”

“It does not seem necessary to accept for the motoneurones (synapses, dendrites and body) anything fundamentally different from the process happening in the neuromuscular junction.” […] “After all the body and the dendrites of the neurone have to be considered only as a specialized part of the axon, adapted to the function of receiving the excitatory impulses.”

“The neurone is […] a nerve fibre having two specialized endings, the receptor and the effector ones. The trophic centre with the nucleus may be located within the fibre itself and acquire the shape of cell body with dendrites.”

In the context of such an interpretation, it is the intercellular connections that are functionally important, that is, the activity of the chains of internuncial neurons, rather than the integrative functions of somata and dendrites derived from Sherrington’s (1925) proposal. Although the latter had dominated successful scientific progress at the time, neuroscience had also evolved to incorporate network concepts, in which Lorente was a pioneer. Lorente’s choice of focusing on neuron chains is overwhelming in his final synopsis of cortical organization (Lorente de Nó, 1938a,b, 1949).

His subsequent analysis of neuronal chains and of the role played by internuncial neurons in the chains (Lorente de Nó, 1938a) smoothed his early interpretations somewhat. Once more, Lorente put in a first place the irrefutable fact of the intricate anatomical organization of the CNS, which demanded his attention as a neuroanatomist:

“[…] few exceptions, at least one pathway can be found connecting any two central neurons in a manner so that an impulse may be conducted from one to the other neuron […]” “Obviously many of these complicated paths are physiologically impassable, because the impulses sooner or later fail to reach the threshold of an intervening neuron, but others actually do play an important role in the physiology of the central nervous system.”

Lorente then proposed to reduce all complexity to elementary circuits (Fig. 4):

“The chains may be longer or shorter, they may contain a larger or smaller number of parallel branches, but in every case they remain essentially the same.”

“[…] the cerebellum as an organ is, from the point of view of elementary physiology, a giant chain of internuncial neurons, superimposed upon the reflex arcs in the spinal cord and the medulla.” […]

“The interest of the analysis is that it is possible to reduce the actual anatomical complexity of the nerve centers to simple diagrams suitable for theoretical arguments.”

On these premises, he set out to analyze responses of motoneuron pools in the brainstem after stimulation of neuron pools of various natures. It is interesting that the experiments led him discuss the respective effects of internuncial bombardment and of facilitation in the neurons themselves:

“Internuncial bombardment […] has all the properties of c.e.s. […], and since a motoneuron despite any possible lowering of threshold due to previous, intrinsic or extrinsic activity does not fire unless impulses are delivered to its synapses […], there is no doubt that the “central excitatory state” leading to motor discharge is due to internuncial activity and bombardment”
This is, as shown by Barbara (2006) in an illuminating review, an attempt at reconciling his ideas on circuits with the integrative properties of neurons of Sherrington (1925). Lorente relativized his differences:

“[c.e.s.] were assumed to develop and accumulate within the individual neurons, while internuncial bombardment places the excitatory and facilitatory mechanisms outside the cell […] in fact, the result obtained is essentially the same, whether the one or the other concept is used […] nor it is necessary to alter the discussion of the transmission of impulses across the cerebral cortex made elsewhere [he refers the reader to Lorente de Nó, 1933b] on the basis of Sherrington’s c.e.s.”

This was fortunate, for it made it possible the synthesis of cortical neurobiology of 1938.

8. The cortical chapter

Lorente summarized his concepts of the cerebral cortex in a chapter of John F. Fulton’s Physiology of the Nervous System (Lorente de Nó, 1938b). I used the third edition (Lorente de Nó, 1949). As one could expect from the preceding, the account started with one condition:

“[…] after important simplification the cortex is considered as a chain of neurons built on the plan of reflex arcs. How this can be done is shown in figure 74 [Fig. 8 here]”

When a given cortical area is studied in different mammals,

“it is found that some of the structural details remain constant despite the variations in cell number, cell form and size, and the disappearance in the lower mammals of many types of cells found in the brain of the monkey and above all of man. What remains constant is the arrangement of the plexuses of dendritic and axonal branches, i.e., of the synaptic articulations through which nerve impulses are transmitted”

Emphasis is Lorente’s. Note this means emphasis on the arrangement of neurons in synaptic chains and this is the issue in Fig. 8. First note the simplified diagram on the right, where the theme is feedback connectivity: The afferent fiber af. contacts a large pyramid that is at the origin of an efferent fiber ef., but that also activates a series of internuncial neurons (i1–i3) that in turn activate the pyramidal cell. In the large diagram, the passage of impulses is signaled at synapses (s) with numbers (s1–s5) that indicate the number of synapses the impulse had traversed to arrive to the synapse in question. Arrows have a meaning slightly different from that in Cajal’s drawings: “it is assumed that synapses marked by an arrow are traversed by impulses.” And,

“[…] the fact must be borne in mind that since synaptic stimulation demands simultaneous activation of several

Fig. 8 – Reproduction of Fig. 74 of Lorente de Nó (1939b). The small diagram “exemplifies the broad plan upon the central nervous system is organized”: this is a reflex arc with an afferent fiber (af) and an efferent fiber (ef) on which chains of “internuncial neurons” are superimposed; there are both feed-forward (f1, f2) and feed-back connections (f3, plus the same fiber on f1, or on f2). The large diagram is only moderately realistic. a, a’ are afferent axons, and axons of cortical neurons are marked a, e are recurrent collaterals of pyramidal cells. s are synapses and are numbered 1–5 to indicate the number of previous synapses in the path.
synapses, the transmission of impulses though the cortex requires the activation of many more cells that can be included in a diagram”

Of interest is to note that Lorente distinguished two different types of chains: the one was formed by neurons in a unique layer (e.g., 25–8’ in layer V, or 24–5’ in layer IV), the other included cells of different layers (e.g., 18–2, 15–4, 8′–2’). Lorente called them short and long chains, respectively. He went on to say that the long links varied little among mammals, but short links increased progressively in number from mouse to man. Thus, Lorente admitted that the cortex of the mouse contains a lower number of short axon cells. “Lower species” would contain, therefore, an elementary pattern of cortical organization, essentially constant during mammalian evolution.

At the end of his studies on the human cortex, Cajal had concluded that the complexity of the cerebral cortex reaches a peak in humans. As Lorente put it, “Cajal assumed that the large number of cells with short axons was the anatomical expression of the delicacy of function of the brain of man” (Lorente de Nó, 1933B). It is interesting to note that Cajal indeed described such a progression in his studies of the human cortex (Cajal, 1904) but where he put great emphasis was only in his autobiography (Cajal, 1917). Cajal had also thought that pyramidal cells had to do with phylogeny: he had baptized the pyramids as “psychic cells” (Cajal, 1893; see Goldman-Rakic, 2002), “las mariposas del alma,” the butterflies of the soul.

“Descending in the [phylogenetic] scale of the vertebrates, the form of the psychic cell is simplified, corresponding decreasing in length and volume.”

Since Golgi staining is stochastic (Strausfeld, 1980), Cajal’s conclusion of a richer complement of short axon cells in the human brain with respect to other mammals was technically untenable. However, if one of the investigators of the classical times had claimed the opposite, e.g., the rodent cortex had a rich complement of interneurons, the latter assert had to be taken with respect. Examples are in the first early cortical paper by Lorente, and in his studies of the entorhinal cortex (Lorente de Nó, 1922, 1933B). As we see it today, this is because the Golgi staining of neurons serves in most cases to bring forth hypotheses, but not conclusive evidence. In a modern perspective, we should reckon today, this is because the Golgi staining of neurons serves in most cases to bring forth hypotheses, but not conclusive evidence.


