

The discoveries of microglia and oligodendroglia: Pío del Río-Hortega and his relationships with Achúcarro and Cajal (1914-1934)

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Presented in part at the Pío del Río-Hortega plenary conference. 64th Annual Meeting of the Spanish Society of Neurology, November 201

ABSTRACT

The discoveries of microglia and oligodendroglia by Pío del Río-Hortega (1882-1945) were undoubtedly among the most important of that researcher's many contributions to neuroscience. In 1912, Río-Hortega moved to Madrid and met Cajal in person; Cajal allowed him to visit his laboratory and observe the projects being carried out there. However, Río-Hortega found the experience disappointing. After this setback, he decided to try again in Achúcarro's laboratory for nervous system histopathology. Here, he gained familiarity with the methods developed by Achúcarro (tannin and ammoniacal silver) and by Cajal (formalin-uranium nitrate). In 1918, using his newly-developed ammoniacal silver carbonate stain, he discovered the cells he was to name microglia. This finding clarified the nature of the compound granular corpuscles which Achúcarro had described. However, it also placed Río-Hortega in a delicate position with respect to Cajal, whose 'third element' theory was not supported by Río-Hortega's findings. Relations with Cajal grew increasingly strained, and the death of Professor Achúcarro left Río-Hortega without valuable support. Tensions arising from misunderstandings with certain members of the Cajal School, and the porter in particular, led Cajal to ban Don Pío from his laboratory in 1921. This event was extremely traumatic for Río-Hortega. He would later set up his own laboratory in Madrid's Residencia de Estudiantes, where he continued to study the origin of mesodermal microglia and their phagocytic functions in different pathological processes. He also published several studies on oligodendroglia. In 1922, Río-Hortega and Cajal met at Café El Prado and the two scientists reconciled.

KEYWORDS

Pío del Río-Hortega, Nicolás Achúcarro Lund, Santiago Ramón y Cajal, microglia, oligodendrocytes

Introduction

Pío del Río-Hortega (Figure 1) was born on 5 May 1882 in the town of Portillo, 24 kilometres from Valladolid; he was the fourth child in a prosperous farming family. He began studying medicine in 1899, and in his third year, he met Leopoldo López García, Chair of Histology and Anatomical Pathology. The following year, the professor chose Don Pío as his honorary department assistant. He was successful in his bid to enter the anatomy programme

as a student intern. He published his first study, a case report, in the student journal *Boletín del Ateneo de Alumnos Internos*. Dr Río-Hortega earned his medical degree in 1905. Between 1908 and 1910 he worked as the district physician of Portillo. In 1909, while still acting as Dr López García's assistant, he presented in Madrid his doctoral thesis on the aetiology and anatomical pathology of brain tumours. In 1911, he was awarded a position as interim assistant lecturer in Dr López García's department. The following year, he had to stand in for his professor on several occasions to present his theoretical



Figure 1. Pío del Río-Hortega (1882-1945)

classes. Río-Hortega began to develop an aversion to the department; he believed that teaching and his own temperament were at odds with his goals as a researcher. He performed better in the peace and quiet of the laboratory and appreciated the practical, observation-based explanations of biological phenomena delivered by experimentation.¹

From that moment on, Río-Hortega began to seek a drastic solution to cure his disenchantment as a university lecturer and satisfy his personal ambition to be fully dedicated to histology research. This decision resulted in several journeys to Madrid to establish contact with Cajal and his school. Dr López García was not pleased by his absences, but he still wrote two letters of recommendation so that Río-Hortega would be able to arrange meetings with Nicolás Achúcarro and with Tello, Cajal's star student.¹

First impressions: Santiago Ramón y Cajal and Nicolás Achúcarro

Don Pío arrived in Madrid in 1912, and the first thing he did was meet Tello in the laboratory at San Carlos Faculty of Medicine. On the following day, he was at last introduced to Cajal (Figure 2), who granted him permission to enter the laboratory every day, accompanied by Tello, to observe the experiments in progress. Don Pío described the short time spent with Tello –less than two months– as fruitless, cold, and disappointing.²

After this setback, he decided to meet with Achúcarro, who presided over the nervous system histopathology laboratory that had been set up in the Natural History Museum by the National Board for Advanced Studies. Although Río-Hortega had a poor preconceived opinion of Achúcarro based on what his professor Don Leopoldo had told him, Achúcarro gave Don Pío a warm welcome and offered him a position in his laboratory. This is almost certainly where Río-Hortega

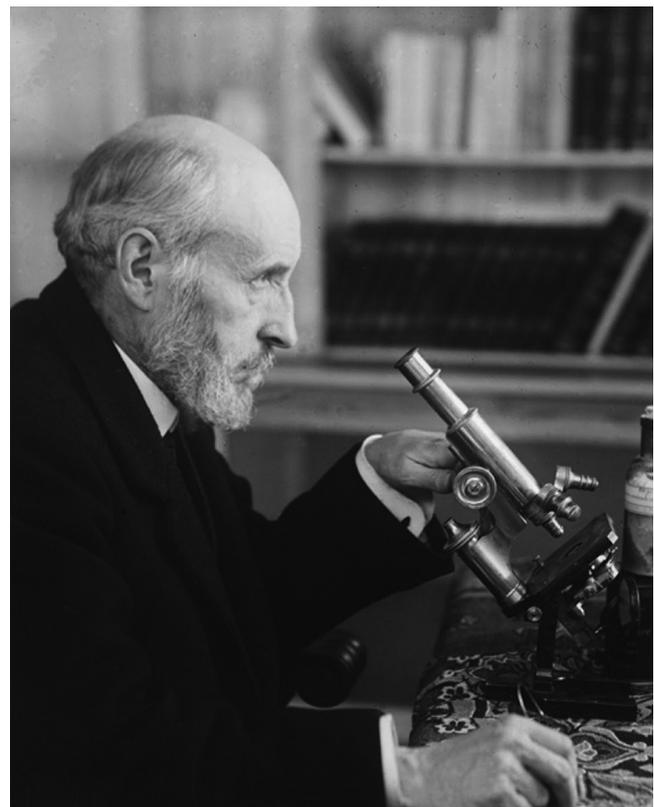


Figure 2. Santiago Ramón y Cajal (1852-1934)

began using the former's tannin and ammoniacal silver oxide method.¹

Tello may have been annoyed that this 'country boy' had decided to try another laboratory after having been granted access to his own; furthermore, the fact that Don Pío sought out Achúcarro, with whom Tello had a strained relationship, may have rankled. Achúcarro's disciples included M. Gayarre, J. M. Sacristán, L. Calandre, Hueto, Father Barbado, and G. Rodríguez Lafora.³ All were passionately devoted to the field of nervous system histopathology.

In addition to the monetary support which postgraduate students received from the Board (Cajal served as its director at the time), another scholarship was posted in January 1913. The prize consisted of working in foreign laboratories for two years in preparation for a six-year minimum stint as department head at the National Institute of Oncology upon the candidate's return. Don Pío was awarded the scholarship.⁴

These two circumstances allowed him to fulfil his goal of full-time dedication to research. This time he was guided by his professor Achúcarro.

Nicolás Achúcarro

Nicolás Achúcarro Lund (Bilbao 1880–Neguri 1918) began his medical programme in Madrid in 1887 (Figure 3), and he was trained by Cajal and Olóriz. Unsatisfied by the Spanish university experience, Achúcarro travelled to Marburg, where he studied different clinical specialties before returning to Spain for his exams in 1900. He also studied under Madinaveitia and Simarro; the latter introduced him to histological research. During this period, he met Giner de los Ríos, and the two would strike up a lifelong friendship.³

Achúcarro had studied in the *Institución Libre de Enseñanza* ('Free Institute of Learning', an elite secular school), which had a marked influence on Río-Hortega's flair for the humanities. After finishing his degree, he went on to frequent some of the most prestigious centres for clinical neurology and anatomical pathology. He worked with Pierre Marie and Babinski in Paris before moving to Florence to study under Lugaro. Following that, he travelled to Munich, where Kraepelin and Alzheimer completed his training in psychiatry and

neurohistology. Alzheimer considered him his best student, and recommended him for the directorship of the Department of Anatomical Pathology in the federal insane asylum in Washington, D.C. He returned to Spain in 1909. The following year, he resumed his activities in Cajal's laboratory and department and was also hired as a doctor at Hospital General.³

In October 1911, he presented the novel staining technique with tannin and ammoniacal silver that still bears his name today.⁵ His scientific activity focused on histopathological studies of microglial rod cells, perivascular cuffing in general paresis, and features of normal and pathological neuroglia.³

According to Río-Hortega, Achúcarro was a world-class thinker whose profound knowledge of neurology and psychiatry extended to its anatomical and physiological

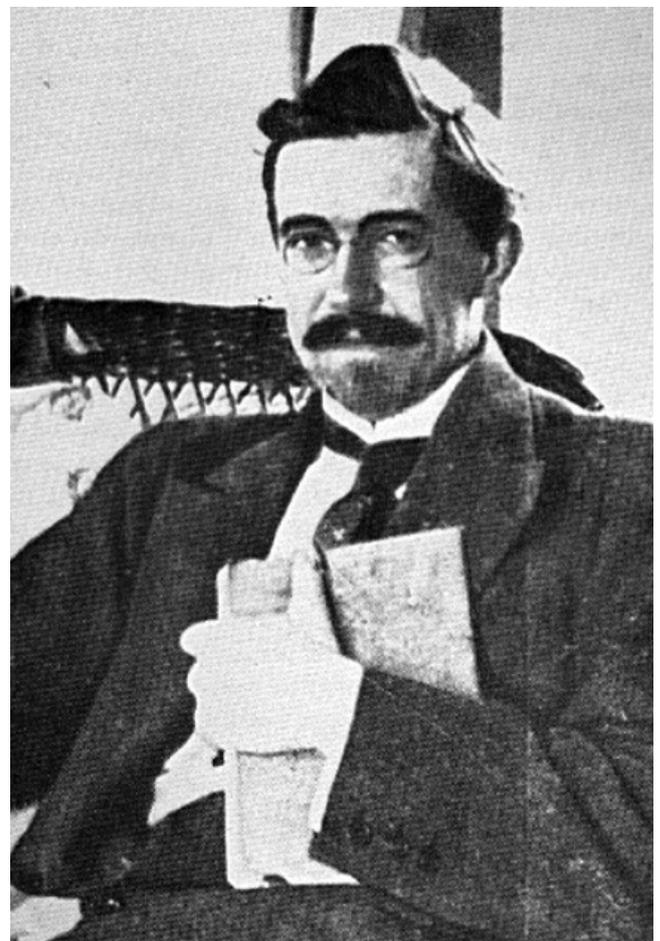


Figure 3. Nicolás Achúcarro Lund (1880-1918)

origins. A tireless worker, he was very enthusiastic about original research. His new professor's qualities as a person and as a scientist encouraged Río-Hortega to begin his tour of Europe.¹

Río-Hortega travelled to Paris in 1913, intending to return to Achúcarro's laboratory at a later date. He had been awarded a scholarship to work with Prenant and Letulle. During his stay, he tried to convince French scientists of the importance of using Cajal's new gold sublimate staining method and Achúcarro's tannin-ammoniacal silver method.¹

In the spring of 1914, Don Pío went to Berlin to study bacteriology and experimental oncology. Adolfo Vila, a former classmate, presented him to his fellows at the Koch Institute. Don Pío describes the experience:

Rather than culturing microbes, which I found uninteresting, I busied myself with transplanting tumours in series after series of mice. Nobody guided me in these experiments, since the head, Joseph Koch, was purely a bacteriologist, but my successes pushed me forward. As one might expect, I did not stop practising Achúcarro's method; in fact, I experienced a small triumph when the technique revealed a syphilis microbe and Loeffler himself came over to see my slides...²

The Great War broke out in summer of that year, and Río-Hortega had no choice but to leave Germany. Upon returning to Madrid, he was surprised to see that Achúcarro's laboratory had been moved to the Museum of Anthropology at Cajal's request. It was now under the same roof as Cajal's Laboratory of Biological Research.

Both laboratories maintained their institutional independence and their 'collective identity', as López Piñero described it.⁶ Don Pío was at first very pleased to find Cajal so close. Years later, however, he recognised that locating both laboratories in the same building was certain to lead to serious clashes.²

Cajal had recently invented a new staining method with gold sublimate which was ideal for staining neuroglia.⁷ This innovation pushed researchers in both laboratories to examine glial architecture. Don Pío had begun to explore Cajal's mysterious and brilliant personality little by little, and as time passed, he was becoming aware of his disappointment: Cajal worked alone, and no one was

allowed to observe while he was working. The door to his office was always closed.²

Don Pío was the only member of either laboratory to dedicate himself exclusively to research, with no other source of work; as a result, Cajal began to notice him. In fact, Cajal recommended that he move to Tello's table where the light was better, since Tello had been out of the laboratory for some time. After their morning duties, Don Pío would sit in on the eloquent and informative conversations between Cajal and Achúcarro, who was still investigating the origin and function of two cell formations found in the glia and known at that time as microglial rod cells and compound granular corpuscles. The discovery of the nature of these cells was to contradict the teachings of Achúcarro's professor Alois Alzheimer, who was a staunch supporter of reticular theory. In 1913, Cajal described the presence of small cells with few or no branches and which could only be stained using the formalin-uranium nitrate method.⁸ He called them adendritic or 'third element' cells of the nervous centres; neurons were the first element, whereas astrocytic, protoplasmic, and fibrous neuroglia constituted the second element. Achúcarro referred to this third element as 'interstitial cells of unknown nervous tissue'.⁶

During the same year, Río-Hortega spent his time using Cajal and Achúcarro's methods while developing his own techniques based on the latter. He studied the origin of neurofibrils, the filamentous structure of the epithelium, and the centrosome of the nerve cells, which he was able to locate and describe perfectly. Don Pío was a keen observer of Achúcarro's method, which was at the time the most appropriate way of staining neuroglia and the building blocks of connective tissue (especially reticulin). In doing so, he became aware that this histological technique was the key to further observations and increasing scientists' knowledge of histology.

Both laboratories remained economically independent, and each one housed a different group of researchers under its own director. However, both groups shared a building, a library, almost the same tools, and mainly the same support staff, including a peculiar and infamous porter named Tomás García de la Torre. Río-Hortega described this situation as follows:

According to Tomás, we were no more than tiresome guests disturbing the peace that would have reigned if only Don Santiago and Domingo Sánchez had been given the run of the laboratory. This is why

he waged his sly campaigns against us; whether he followed his own whims or the perverse urgings of others, I could not say.²

In the meantime, Don Pío continued devoting his time to research. He then received no stipends whatsoever –the length of the Great War prevented the recipients of Cancer Committee grants from enjoying normal disbursements– and he began to ponder how to change his unstable position. In addition, he was not at ease in the Museum of Anthropology. He had good reason to believe that he was still viewed as an intruder in the laboratory, and he was certain that Tello, Cajal's right-hand man, did not think well of him. He also believed the insolent behaviour displayed by Tomás the porter, with whom he had already exchanged heated words, was being encouraged by someone in Cajal's laboratory. Don Pío let Achúcarro know that he had decided to move back to Valladolid and find work as a pathologist, but his professor persuaded him not to by promising him a grant from the Board. Years later, José Miguel Sacristán informed Don Pío that the grant which Achúcarro had arranged actually came from the latter's own monthly stipend (300 *pesetas*).² This leads us to consider what may have been either the professor's 'disinterested' gesture to help his student or a 'calculated' strategy to keep Río-Hortega in Madrid, which was the only place in Spain where histological research was feasible at the time. Don Pío was beginning to show his potential for delivering relevant neurohistological studies, and there can be no doubt that Achúcarro wanted him as his successor.

In October 1916 Achúcarro fell ill. He was diagnosed with Hodgkin disease and was often absent from the laboratory.³ Río-Hortega began to foresee the complications that were about to invade his work and his personal life. Without Achúcarro's protection, things would be much more difficult. Furthermore, Río-Hortega's personality was quite unlike his professor's. He was shy, introverted, silent but restless and nervous, exacting, somewhat suspicious, and he lacked a way with words. It is important to point out that Don Pío had not yet made a name for himself in the scientific sphere. If his professional status had matched Achúcarro's, he might have had more success at resolving his interpersonal problems with some of Cajal's students.

Río-Hortega continued exploring variations on Achúcarro's method and decided to use them to study epithe-

lial fibrils. He made good use of his series of papers on epithelial fibrils to try to build ties with Tello, who had made important contributions in this area some years before. Río-Hortega even credited Tello with the discovery of epithelial fibrils as a gesture of goodwill. His attempts, however, were in vain; he reported that his colleague continued to show signs of scorn and resentment, even going so far as to disparage Don Pío and his work in a meeting of the Spanish Society of Biology. Cajal served as the society's president at the time, and when Tello resigned as secretary, Don Pío was chosen to succeed him.²

Río-Hortega's relationship with Cajal, although still tenuous, would develop over time. Río-Hortega often coincided with him outside of the laboratory. His new position as secretary of the Society of Biology came with new duties. His errands frequently took him to the Moya printing house where Cajal was to be found almost every day "with unbound leaves from his histology and anatomical pathology textbooks, ably reworking the text for new editions being printed, using the originals or the corrected proofs of one of his articles"²

In the winter of 1917, Río-Hortega fell ill with influenza and then contracted epidemic hepatitis. His absence from the laboratory was so long that Cajal was concerned and ordered the porter to visit Don Pío and gather news about his condition. By coincidence, Cajal's photography studio was located in the attic of the building where Don Pío lived (Calle del Prado 10, second floor). Don Pío knew this, but kept it a secret. Cajal himself went to visit his disciple, who was confined to bed. The latter talked at length about his illnesses, including persistent insomnia that he had to treat with Veronal and arteriosclerosis that Achúcarro had diagnosed some time before.²

In late 1917, Río-Hortega's studies on epithelial fibrils and nerve cell centrosomes⁹⁻¹¹ were introduced to American scientists by José Fernández Nonidez¹ and analysed by two leading biologists, Edmund Beecher Wilson and Thomas Hunt Morgan.²

Discovery of microglia and oligodendroglia

In 1918, Río-Hortega's new histological method employing ammoniacal silver carbonate helped him obtain images that would revolutionise the concepts of

nervous system morphology and histology. If Don Pío's increasingly clear findings were confirmed, they would clash with Cajal's theories on the third element of the nervous system. In addition, he began to discover the true function of Achúcarro's microglial rod cells and compound granular corpuscles. Río-Hortega glimpsed the possibility of clarifying the inner workings of the nervous system, but he was also aware that his claims could stir up trouble.²

Once he was certain that his method yielded reliable results, he showed them to Cajal. A few days later, Cajal let him view a few slides that he had prepared according to Don Pío's method, except that he had replaced the silver carbonate with Bielschowsky silver stain, following a variant technique which Río-Hortega had already discovered and shown to him. Cajal's sudden decision to focus on Río-Hortega's field made the latter concerned about the potentially competitive undertones.² Two years later, Cajal published an article on the modified Bielschowsky silver stain version of Río-Hortega's technique.¹² On this subject, Don Pío wrote:

Who could have advised [Cajal] to describe my modified Bielschowsky silver stain technique as if it were his own? My method, with the substitution of a single reagent which he believed he had discovered before me, when I had pointed out its potential use in my article. This very important event took place some time later, when I was absent from the laboratory (...) To my way of thinking, his actions conveyed three things: firstly, Don Santiago was not pleased that I had devised a method that would be advantageous for so many types of experiments; secondly, his studies paralleling my own were not conducted for the reason that I had first believed; and thirdly, he had now published a variant technique that was not new, and of little importance to him, as his glory could grow no greater. The technique would simply come to replace my method, and its primary advantage was that it would bear his name.²

Río-Hortega invariably employed this new staining method in his experiments; he used it on all tissue types, especially nervous tissue in both normal and damaged states. The latter were achieved by using sodium nucleinate to provoke an inflammatory lesion in the specimen (Lafora). He was perfectly certain that his findings were going to clash with Cajal's third element theory, which by that time was widespread and accepted by numerous foreign schools.²

As it turned out, Cajal's third element consisted of cells that differed substantially from those he had described using the formalin-uranium nitrate method. Rather than the apolar corpuscles Cajal had described, Don Pío found fully branched structures with clearly defined protoplasmic arms. In addition, Río-Hortega understood that the so-called third element might in fact include two different cell types. The type later known as microglia, mesodermal in origin, displays different shapes according to its different functional stages (rod-shaped or round granular corpuscles filled with phagocytosed substances). The other type, which Don Pío named oligodendroglia, is ectodermal in origin and pertains to the second element, the neuroglia. These cells exhibit processes that envelop neurons and at the very least are able to generate myelin.²

On 23 April 1918, Dr Nicolás Achúcarro Lund died in his house in Neguri.³ It is no exaggeration to say that as a result, Río-Hortega was left on his own and unsupported. Along with M. Gayarre, Río-Hortega was to direct the part of the histopathology laboratory maintained by the Board. Don Pío was entrusted with two tasks: teaching new students and signing letters to the Board and any bills from providers. As a result, he had daily dealings with Tomás the porter.²

A year after Achúcarro's death, Río-Hortega was awarded the first Achúcarro Prize and named as his master's replacement. He was invited by the Catalan Society of Biology to present a short course on neuroglia at the Barcelona Faculty of Medicine. Don Pío found a welcoming environment there, and he was encouraged by the advice of Ramón Turró, whom he named 'the grand master of biology'.²

Río-Hortega was of two minds about publishing his research. On the one hand, he believed that he should make his discoveries known immediately, as he suspected that a foreign researcher might get there first (especially since his methods had already been published). On the other, he feared how Cajal might react to his revolutionary conclusions.²

Spain's first National Congress on Medicine was held in Madrid in the spring of 1919. Río-Hortega had been asked to give a lecture on the current state of the histopathological problem of neuroglia. It was the perfect time to present his research, but instead, he

hesitated. Some time before, he had shown his results to Juan Negrín in the Board's physiology laboratory (located in Residencia de Estudiantes, the student residence hall); Negrín advised him to wait no longer and publish his work. While Augusto Pi y Suñer and Bellido were in Madrid, he visited Negrín's laboratory once more to show them his discoveries. They also felt that his findings were of great significance and should be published without delay.²

Relationship with Cajal and the Cajal School

Don Pío decided at last to publish his discoveries, but not before showing them to the master himself. Cajal's first reaction, according to Don Pío, was as follows: "I believe that your claims are too daring, and many will disagree with them. Be prepared to argue"² Río-Hortega answered as follows:

I am aware of that, Don Santiago; but I am also certain that if I were to prudently hold my tongue at this time, others would make the same claim in an instant. These observations are too clear to remain unnoticed given that the method for viewing them is now available. In any case, I do not intend to spend much time arguing. I am so certain of my claims that I hope that any debates will take place between convinced and unconvinced factions.²

Ever since Río-Hortega had published his new staining method two years before, his personal relationships with certain members of Cajal's laboratory had grown increasingly strained. Worse still, his recent discoveries had made the tension nearly unbearable. The porter did his best to disturb his work, leaving Río-Hortega exasperated.

At about this time, a medical student from Zaragoza arrived, with the recommendation of Dr Pedro Ramón y Cajal: Lorente de Nó. Tello and Don Pedro's brother Santiago received him with open arms. Don Pío was party to Lorente de Nó's early struggles with histological techniques, and describes the following incident:

I remember one day when the two of us were walking down Atocha and León toward Calle del Prado, where I lived at the time. We were speaking of Achúcarro and his tragic death. I praised his abilities as a teacher and swore that I owed him almost all my knowledge of nervous system pathology. I also added –it was common

LA MICROGLÍA Y SU TRANSFORMACIÓN EN CÉLULAS EN BASTONCITO Y CUERPOS GRÁNULO-ADIPOSOS

por

P. DEL RÍO-HORTEGA

(DEL LABORATORIO DE HISTOLOGÍA NORMAL Y PATOLÓGICA DE LA JUNTA PARA AMPLIACIÓN DE ESTUDIOS)

(Recibido el 1.º de junio de 1920.)

SUMARIO:

- I. Relación entre la microglía y el tercer elemento.
- II. Caracteres normales de la microglía.
 - 1.º Técnica.
 - 2.º Morfología general de la microglía.
 - 3.º Variaciones morfológicas en estado normal.
 - 4.º Distribución y relaciones de la microglía.
- III. Caracteres anormales de la microglía.
 - 1.º Primeros cambios morfológicos.
 - 2.º El problema de las células en bastoncito (*Stäbchenzellen*).
 - 3.º Formación de las células en bastoncito.
 - 4.º Caracteres de las células en bastoncito.
 - 5.º El problema de los cuerpos gránulo-adiposos (*Körnchenzellen*).
 - 6.º Formación de los cuerpos gránulo-adiposos.
- IV. Naturaleza probable de la microglía. Conclusiones.

Hace algún tiempo que, interesados por las investigaciones de Achúcarro—nuestro llorado maestro—, hemos tratado de averiguar la naturaleza verdadera de las células en bastoncito (*Stäbchenzellen*) y de los cuerpos gránulo-adiposos (*Abräumzellen*, *Körnchenzellen*, *Gitterzellen*), que tanto a aquel investigador como a otros muchos preocupara grandemente.

Hace algún tiempo también que constituye tema preferente de nuestras investigaciones, dentro del trabajado campo de la neuroglia, el estudio de las «células adendríticas» o «tercer elemento» de los centros nerviosos, descritas por CAJAL minuciosamente, pero aun mal conocidas, así en lo que respecta a sus caracteres morfológicos y texturales, como a su naturaleza y a su función.

Figure 4. First page of the original study. Río-Hortega P. *La microglía y su transformación en células en bastoncito y cuerpos granuloadiposos*. Trab. Lab. Inv. Biol. 1920;18:37-82.

knowledge— that Don Santiago was so engrossed by his studies that he had little time for his students, at least for those latecomers who were only loosely associated with the Cajal Institute. Very well; I have my reasons for believing that these comments, after being twisted and reshaped, were repeated to Don Santiago with a deliberate purpose.²

The author later adds:

...some undetermined and vicious virus was spreading through the San Carlos histological laboratory, subtly drawing students to Tello's band, inciting them to show their scorn for Achúcarro's group. This dark wave had descended directly

upon me and drenched my colleagues in the process. I believe that the poorly disguised motive behind it all was to widen the absurd breach between our groups, a breach that the porter made plain whenever he insolently hinted that we were not wanted at the Institute.²

At that time, Don Pío was working with Miguel Prados, Luis G. Guilera, Felipe Jiménez de Asúa, Carlos Collado, Manuel López Enríquez, Manuel Sánchez, José María Villaverde, and Augusto Navarro Martín.²

In the summer of 1919, Don Pío edited a special journal similar to those produced by other laboratories run by the Board. Its title was *Trabajos del Laboratorio de Histopatología de la Junta para Ampliación de Estudios* (Projects from the Histopathology Laboratory of the Board for Advanced Studies). This new publication was frowned on by Cajal, who may have thought that Río-Hortega was angling to direct the studies in his division independently.

By the end of the year, Don Pío had focused his efforts on resolving the mysteries of interfascicular glia. He tried out different variations of his method in order to elucidate the morphological and functional properties of what he would later call oligodendroglia (Figure 4). He had very little time to do so, since he also had to teach Cajal's methods to laboratory assistants at the Museum of Anthropology.²

On 4 February 1920, Río-Hortega wrote a letter to Cajal informing him of the porter's behaviour and requesting a key to the laboratory so that he would be able to come and go freely without disturbing anyone. He stressed that his position as a scientist called for this privilege, and he observed that the methods used in histological research could require him to keep irregular hours. Don Pío mentioned that his absence from the laboratory was not only due to a stomach illness, but also to the porter's outrageous behaviour.²

On 8 February, Cajal replied with a series of arguments that illustrate the slander that had reached his ears. The mischief was done, and Cajal had only listened to those parties bent on causing trouble for Don Pío. One paragraph is of particular interest, and it appears below:

While you may of course resume your work if you so choose, I now believe there is but one solution that will prove to be lasting and satisfactory to all: to relocate you and your disciples in your own

laboratory, as was done for Negrín, Cabrera, and Calandre. I am making arrangements to this end. On my visit to the Museum of Natural History and the student residence hall, I located a small area that could be expanded with a little remodelling work. You could set up your laboratory there.²

Don Pío found Cajal's solution to his liking. Both of his wishes would be granted: he would be able to work 'freely' and gain some distance from the hostile environment in the Museum of Anthropology.² Nevertheless, he did return. He was just about to finish a crucial

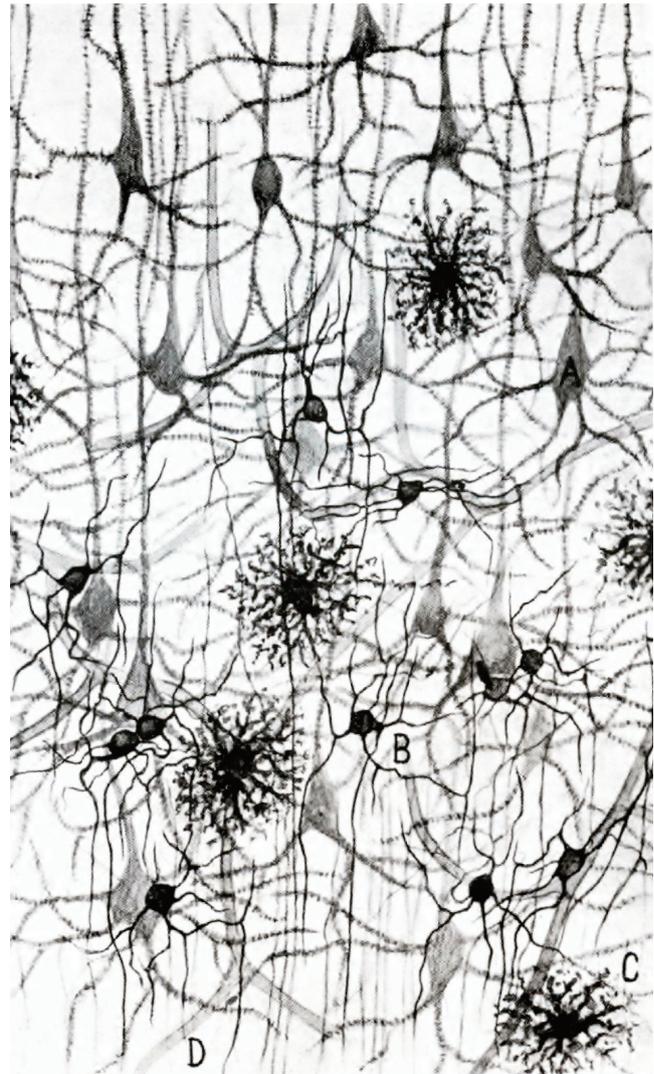


Figure 5. Appearance of the cat cerebral cortex with neurons (A), oligodendroglia (B), protoplasmic glia (C) and vessels (D). Río-Hortega P. *Tercera aportación al conocimiento morfológico e interpretación funcional de la oligodendroglia*. Mem. Soc. Esp. Hist. Nat. 1928; 14: 5-122.

study presenting his conclusions on the morphology and physiological activity of microglia, with particular emphasis on phagocytosis in the nervous system (Figure 5). Río-Hortega's article on the phagocytic activity of microglia¹³ was published in early June; he showed it to Cajal, and describes the master's reaction as follows: "he was not very forthcoming, but assured me that he thought it was good, despite a few venturesome statements; he believed I had progressed considerably toward resolving the problem."²

In July 1920, Río-Hortega attended the International Congress of Physiology in Paris. At the Faculty of Medicine, he gave Eugène Gley a practical demonstration of Cajal's methods for studying neuroglia and the Golgi apparatus, and of his own method for microglia. The histopathologist at King's College in London, Dr Da Fano, was impressed by Río-Hortega's discoveries and soon requested placement at his laboratory to learn his techniques. Da Fano was his first foreign student.¹

Upon his return, he welcomed a new assistant, Vadillo, to the laboratory. Vadillo was struggling to clarify a histological detail that Cajal had been unable to clarify using the same method. Don Pío advised him to give up; if the master himself had not obtained any results using the same technique, no one else was likely to do so. He stressed that if Vadillo did decide to continue, he should try another method instead. Vadillo twisted Don Pío's words considerably when he told his tale, probably to either Lorente de Nó or Villaverde. Cajal obviously heard the story, or at least the mutilated version of it. As Río-Hortega said, "They had finally managed to strike Cajal in a sensitive spot, and the master then gave free rein to his indignation."²

On 9 October 1920, Cajal wrote a fateful letter addressed to Don Pío. Tomás had the pleasure of delivering it personally. Cajal's allegations included the following:

Undeniably trustworthy people have assured me that you have made the statements I enclose. 1) That you have nothing to thank me for, since I have neither supported nor tutored you. 2) That you proclaim yourself Achúcarro's student and his alone, rejecting all complicity with me. 3) That the laboratory journal is being published thanks to you. 4) That you do not allow students to use my working methods, even when the matters being studied call for them expressly. And there are other more bitter and severe affronts that I will not repeat here.²

Cajal's letter ends with the following pronouncement:

In conclusion, to keep our respective laboratories from becoming mired in confusion as we waste our time with bickering and old grudges that may escalate into vexatious personal confrontations, I ask that you refrain from setting foot in my laboratory. You may certainly continue to work in the hospital's laboratory, or Calandre's laboratory in the residence hall. Meanwhile, I will be working with the Board to find you a place where you may freely vent your pride and your temper. With the sincere hope of never seeing you again, to the benefit of your health as well as my own, which has suffered greatly these past days, I send my last regards as your former friend and mentor, S. Ramón Cajal.²

This letter was displayed on the laboratory wall; in addition, someone saw fit to make copies and distribute them throughout the Faculty of Medicine. Río-Hortega fell ill and decided to write to Cajal in an attempt to convince him that he, Don Pío, had been falsely accused. To this end, he named M. Gayarre, G. Rodríguez-Lafora, J. Negrín, J. M. Sacristán, L. Calandre, F. Jiménez-Asúa, and others as witnesses in his defence. One section in the letter reads:

You would do me the greatest of all favours by doing what I ask, because if my letter does not convince you, and I do not believe that it will, my satisfaction will not be complete until there is objective proof that the accusations against me are unfounded. In any case, time will tell. From this day forward, I must deny all responsibility for any statements attributed to me but not signed, or comments which the persons informing you will not repeat in my presence. I hope you will instruct me as to what I must now do. Negrín has since assured me that I may count on his assistance and such guidance as I will need to continue my work, but this can be of little satisfaction if, as your letter states, I have lost your good opinion and friendship forever. In any case, you may be certain that my regard for you remains unchanged, and that despite what I have suffered these past days, I am sorrier still for what you have endured.²

On 20 October 1920, Cajal responded to Río-Hortega's letter.² This letter displays Cajal's attempt at reconciliation, but expresses the typical reservations of a person who flatly rejects the version of the story that paints his 'loyal' disciples as slanderers. In this letter, Cajal states,

One of the reasons I held you in regard was your detachment. You know better than most that the vast majority of the members of the Laboratory are not engaged in science. Rather, they are concerned

with gathering merits to win appointments or comfortable situations that will allow them to attract clients. For the same reason, I confess myself grieved and astounded by the irritating and hurtful expressions which you let fly, perhaps during attacks of inappropriately governed stomach-ache or temper[...]although housed in a separate building, you will remain at the head of the Histopathology Laboratory founded by Achúcarro[...]It is clear that in your present situation in Negrín's laboratory, you will not be comfortable, but you will soon find a larger and better-apportioned space in the student residence hall where you will be able to install the necessary material and resume your work with your team. If the Cajal Institute does indeed materialise, as Castillejo and the Ministry have promised, you will be in charge of a fully independent histopathology division.²

Laboratory of Normal and Pathological Histology

At the end of October, Río-Hortega began a new epoch in his life. He moved into the student residence hall; on the ground floor of the wing named 'El Transatlántico', the Board had provided him with a small area that he would have to outfit as a scientific laboratory. Adjacent to his new quarters, he would find the Microscopic Anatomy Laboratory, directed by L. Calandre from 1912 to 1931; the Physiology Laboratory, which J. Negrín had been leading since 1916; the new Serology and Bacteriology Laboratory, under P. Suárez; and the General Chemistry Laboratory, directed by J. Ranedo since 1913. Another two laboratories had recently closed: the Physiological Chemistry Laboratory (1915-1919), under A. Madinaveitia and J. M. Sacristán, and the Nervous System Anatomy and Physiology Laboratory, which G. Rodríguez-Lafora had directed.¹⁴

Río-Hortega was accompanied by several colleagues: Felipe Jiménez Asúa, Carlos Collado, Manuel Sánchez, and also Vadillo and José María Villaverde; the latter was made his intern and assistant. The assigned workplace was small, cold, and poorly lit, and prospects for renovating it would be limited. Initial funding amounted to only 3000 pesetas per month. However, little by little, Río-Hortega was able to transform his workplace into a world-famous research centre that would welcome a long line of scientists interested in the nervous system. Don Pío was quite content and motivated in his new location, surrounded by his friends. In his own words, "I had escaped from the hornet's nest riddled with stings, but found the antidote to their venom in the residence hall"² The laboratory was first known as

the Nervous System Histopathology Laboratory of the Board for Advanced Studies, but Don Pío soon named it the Laboratory of Normal and Pathological Histology, "to lend it greater scope for action in its investigations"²

Once again, he threw himself into his research with the goal of resolving the mystery of his sparsely branched glial cells. He would later name these cells 'oligodendroglia' and describe their delicate structure and controversial function.

In late 1920, Cajal published two papers that were undoubtedly destined to downplay Don Pío's research. In the first of these articles, as has already been mentioned,¹² Cajal presents himself as the inventor of a variant on the Bielschowsky method in which Bielschowsky stain is used instead of Río-Hortega's silver carbonate. In fact, Don Pío had written about this variant nearly three years before. In the second article,¹⁵ which was more disturbing, Cajal indicated that microglia had first been discovered by the Scottish scientist William Ford Robertson, who in 1900 had published a somewhat confused description of 'mesoglia-cells'. These cells had nothing to do with the microglia described by Río-Hortega, and in fact, Don Pío had shown that most of these cells were really a small type of oligodendroglia. In the words of López Piñero, "it should be stated that Cajal did not consult Robertson's brief report directly, and even cited the author's name incorrectly"⁶

Don Pío worked steadily throughout 1921 in the chilly laboratory in the student residence hall. Within a few months, he had completed his study of interfascicular glia (now known as oligodendroglia) and was close to discovering the origin of microglia.

My first breakthroughs came when I demonstrated the morphological traits of oligodendroglia. This was a perfectly classifiable variety of glial cell, and running counter to Cajal's hypothesis, it did not constitute the true 'third element' of nervous tissue. Without a doubt, that name described the microglia, whose mesodermal origin I had already fully explored by the end of winter, despite my lack of experimental animals. Once I had described the new type of glial cell and classified it within the 'second element' of nervous tissue, I began drawing and writing as if this were my first article. I had to contradict the master's ideas once again, and it was an awkward experience, but there was no sense in trying to turn back time.

The following summer, Río-Hortega travelled to Paris to demonstrate his methods and describe his findings to his

former professor L. Auguste Prenant and to Jean Nageotte, Chair of Histology at Collège de France. Little by little, Río-Hortega became a well-known figure in foreign laboratories and his name became to appear in medical literature from around the world.²

Shortly thereafter, Don Pío published his article: “The third element of nervous centres: histogenesis and normal evolution, exodus and regional distribution of microglia”. It was presented as a communication at the Royal Spanish Society of Natural History on 2 February, 1921.¹⁶ Río-Hortega immediately sent Cajal a copy together with a letter, an excerpt of which appears below:

The fear of displeasing you has proved a heavy burden. Before finally concluding that my utmost priority would be the study of mesodermal glia, I faltered and even considered abandoning the project. I hope you will forgive me for wondering what you might do in such a situation as I was making my decision. If you are still offended by my reasons, please believe me sincere in my wish to spare you any trouble. I hope that you will be satisfied, as I certainly am, to know that by publishing the first study on microglia, I have also shown that this knowledge stems entirely from the Spanish School of Cajal. I only regret being unable to bestow a higher honour.²

On 1 November, Cajal answered his letter and recognised the merit of the article on microglia. Cajal added that he would follow up on the subject by directly observing Robertson’s studies and staining slices with that researcher’s platinum method.²

In mid-January 1922, Don Pío decided to have a talk with Cajal at the former Café del Prado, where the master would come for an hour’s relaxation every afternoon. They spoke of their families, their laboratories, and about microglia and oligodendroglia. Cajal also assured Don Pío that he would be recognised as the discoverer of microglia at the appropriate time. After their meeting, Don Pío wrote, “I rose from the table with the satisfaction that the master’s regard for me had returned, or rather, that it was stronger than ever.”²

Río-Hortega then began work on the pineal gland, a subject that had held his interest for years, especially since Achúcarro and Juan Sacristán had published their studies. He had already presented a brief communication¹⁷ at the Spanish Society of Biology in 1916; now, between 1922 and 1928, he would publish a study of the pineal gland in three parts: parenchymatous cells

(1922)¹⁸; neuroglial substrate (1928); and the secretory activity of parenchymal and neuroglial cells (1928).²⁰ He used silver carbonate impregnation as his histological staining method. The result was the most complete structural study of the pineal gland of its time, and this fact was recognised worldwide.²

Don Pío made frequent visits to Café del Prado to chat with Cajal. They often spoke of how construction of the new Cajal Institute was progressing, but Cajal seemed unenthusiastic about the project. Don Santiago confessed to Río-Hortega that he was conscious of both the lack of fair play in his laboratory and the distrustful attitudes of some of his disciples. Don Pío was well aware that the members of the Laboratory of Biological Research –except for Castro and especially Lafora, with whom he maintained firm friendships– continued to snub him, even in the presence of foreign scientists.²

At this point, I would like to include an interesting observation of Don Pío’s:

It was even more shocking that despite Cajal’s unparalleled fame and the importance of his neurological school, very few foreign students had come here to complete their training. For international scholars, Spain was not yet on the map; not even Cajal, who should have drawn pilgrimages of devoted students from around the world, had achieved that miracle. It is also true that only twenty years earlier, the master had been working in shameful conditions instead of the prestigious institution that Spain should then have offered him. The Cajal School had not expanded; it was tightly closed pocket populated only by Spanish researchers. To quote the popular expression, we formed a small and motley crew. What would explain the phenomenon by which, beginning in 1921, foreign students began to flock to the school?²

When the laboratory was renovated, it was able to hold another eight desks. These were occupied by Don Pío’s eight assistants: F. Jiménez-Asúa, C. Collado, M. López Enríquez, Luna, R. Alberca Lorente, R. Vara López, and A. Gallego. In 1922, two more young scientists arrived: I. Costero and S. Alvarado. At first, they rotated between different desks. Regarding working with his followers, Río-Hortega observed,

I warned them about the lack of space, but how could I prevent them from showing up to observe certain methods? Managing the work of so many was overwhelming at times, but I was happy to do it. However, it did impact my own research, which

was already nearly at a standstill due to the difficulties of conducting experiments. The students themselves were also showed a cooperative spirit. I was able to form a tightly-knit group of friends whose unspoken motto was “all for one and one for all”. Harmony. Mutual assistance. No secrets in the laboratory. Open doors and clear consciences.²

It seems his new environment was totally unlike that in the Laboratory of Biological Research.

In May 1923, Cajal published the third edition of *Recollections of My Life: The Story of My Scientific Work*,^{21,23} taking care to insert a paragraph of fine print to proclaim his current views on the microglia controversy. The master must have felt the moment was right to make his position known. Cajal's statements include the following:

The nerve centre mesoglia or microglia constitute one of the Spanish school's most valuable findings. Nothing was known about these cells until Achúcarro observed them in grey matter; they appeared as fusiform or star-shaped cells with scarce processes that were less ramified. Although Achúcarro only saw them in specific pathological states, he mentioned that their presence in nerve composition might be normal. Some years ago (1913) we too also came across a special element in white matter, which we described as a heterotypical, fusiform neuroglial element with few processes. But we must recognise that the discovery of the nature of this microglial corpuscle, and also the description of the many forms it takes in the brain, are the work of Río-Hortega, who described its phases of development and leucocytic origin. He used his special silver carbonate method to achieve this end. While a foreign author, perhaps Roberston [sic], may have glimpsed these interesting elements in imperfectly prepared slides, he neither drew them nor described them precisely, and it is impossible to identify the entity which he called 'mesoglia'. It was also Río-Hortega who demonstrated that Nissl's *Stäbchenzellen* were in fact a type of microglia.²¹

On another page, he provides the following note:

In recent years, the abundance of devotees and the narrowness of the space available [in the Laboratorio de Investigaciones Biológicas] has necessitated the creation of new laboratories of histology. The most active of these offshoots of the Laboratorio de Investigaciones Biológicas is that directed by Río-Hortega. In it several outstanding pupils

have already distinguished themselves, such as Gimenez Asúa [sic], and Collado.^{21,23}

Don Pío's reflections on what Cajal had said are well worth including here:

Despite the friendly overtures he had extended to me, the injustice remained. Perhaps some impediment to openly recognising the error remained in the depths of his conscience, because this resistance was visible not only in the laboratory journal, but also in the most recent edition of his *Manual de Histología*. Certain particularities can be observed in those pages of his book that had to make mention of microglia. To diminish the importance of this subject, microglia are described in the fine print reserved for only the swottiest students. To avoid naming either Robertson or myself as their discoverer, he slyly began the section with a description of the so-called 'rod cells' which Nissl and Achúcarro had studied long before I did, although neither of those researchers came to understand them. I was the one to demonstrate that these cells developed from microglia. According to this singular explanation, microglia seemed to stem from 'rod cells', like an abnormal state producing a normal one, or a mother born to her daughters. Seen in this light, I would not have discovered the 'third element' of the nervous system, but rather added to the knowledge of 'rod cells'; and since these cells are pathological, there would be no need to describe them in a textbook of normal histology. The surprising part is that later editions maintain the error, and as an outlandish result, any foreign book will offer a better and more detailed description than Cajal's textbook, which was also signed by Tello.²

In early 1924, the influential German researchers H. Spatz and A. Metz²² wrote a ground-breaking article on microglia in which they confirmed the exactness of the events described by Río-Hortega and accept his interpretations. Río-Hortega commented,

Before the paper in question had been published, I had received reports that both researchers were absorbed in their studies of the microglia. They applied themselves to their research with such dedication that they used my method (*Hortegaschemethode*), almost daily; in their laboratory slang, they had formed the verb *hortegieren* ("to do a Hortega"). „*Hortegieren Sie heute?*” they would ask each other, according to Prados, whose unwavering friendship I must praise. If my esteemed friends Sacristán and Sanchís Banús believed that I would bubble over with joy at their news of the Spatz and Metz article, they were somewhat mistaken. Certainly this was good news, but we also know that it was



Figure 6. Felipe Jiménez Asúa, Wilder Penfield, Julio Sánchez Lucas, Rodríguez Valderrama, Pío del Río-Hortega, Antonio Llomba. Residencia de Estudiantes, Madrid, 1924.

became known around the world. His laboratory in the student residence hall received world-class neuroscientists, including Wilder Penfield, Ludwig Aschoff, Percival Bailey, Creutzfeldt, Von Economo, Hussay, Bogaert, Gordon Holmes, and others (Figure 6).¹

With his disciples, he travelled throughout Europe and the Americas to receive the highest honours, including *doctor honoris causa* degrees from the universities of La Plata, Montevideo, Lima, Mexico City, Budapest, Oxford, and Montpellier. He was a visiting professor at the universities of Paris, Florence, Turin, Munich, Freiburg, and Córdoba, Argentina. He was also made a Knight of the Legion of Honour. While he was nominated twice for the Nobel Prize, he never received an award in Spain; in fact, no Spanish academic institutions seconded either of his nominations for the Nobel Prize.¹

His relationship with Cajal was by now completely mended (Figure 7). At the end of his life, Cajal enjoyed his coffee more often with Don Pío than with anyone else.² However, after Cajal's death in 1934, Río-Hortega's enemies came out of the woodwork once more. He was denied Cajal's seat on the Academy of Medicine; more surprisingly, the Academy awarded the seat to Villaverde, one of Don Pío's students.²

Even though Río-Hortega was already in political exile due to the Spanish Civil War, the hospital of Valdecilla and the University of Valladolid both sent telegrams to

late in coming. Our colleagues across the street (in Cajal's laboratory) were not in a celebratory mood, but their disappointment was lessened by the fact that Spatz and Metz were not convinced that microglia were mesodermal in origin.²

Some years later, Río-Hortega would make it his mission to personally convince all of the scientists who still resisted this explanation. Don Pío concluded,

The German authors are familiar with the studies by Robertson and with Cajal's article. However, they staunchly support my views on oligodendroglia and microglia, and after perceiving the differences between Robertson's and my observations, have opted for calling microglia 'Hortega cells'. A fine lesson for the master.²

Beginning in 1925, Río-Hortega's scientific activity increased exponentially, and his teaching abilities

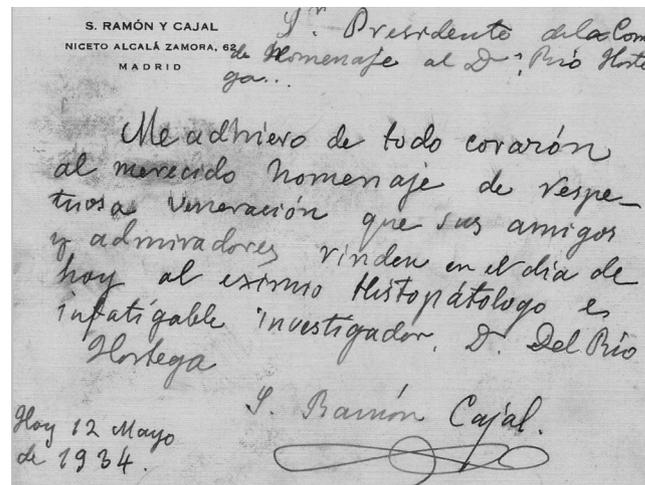


Figure 7. Santiago Ramon y Cajal's letter endorsing the ceremony honouring Pío del Río-Hortega. Madrid, 12 May 1934.



Figure 8. Pío del Río-Hortega, accompanied by Charles Scott Sherrington, in his investiture as Doctor of Sciences (*honoris causa*) by the University of Oxford. Oxford, 25 February 1939.

the University of Oxford in 1939 to protest its decision to grant him the degree of *doctor honoris causa* (Figure 8).¹

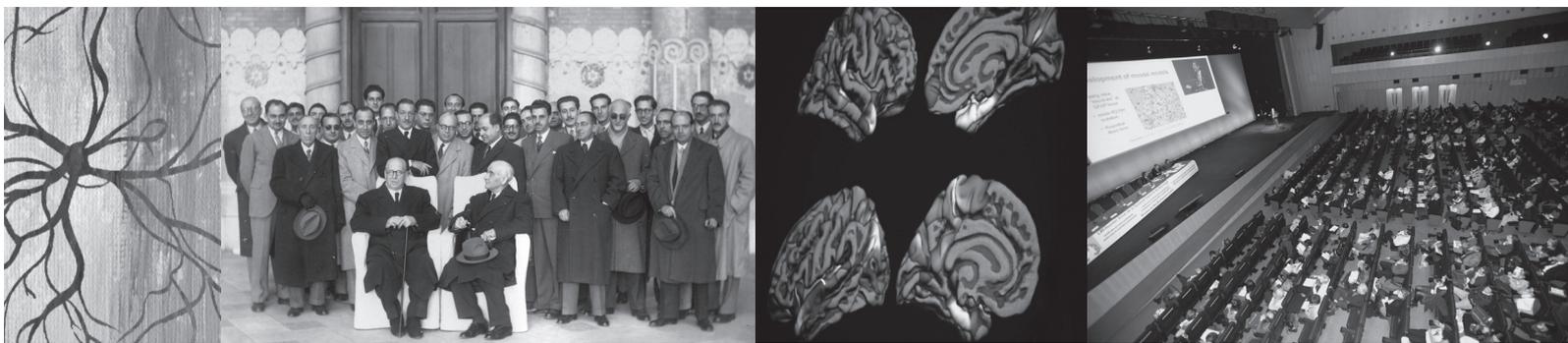
Don Pío's studies in the field of nervous system oncology were outstanding. The current classification system for brain tumours is based on Cushing and Bailey's modified version of his own. His research on the pineal gland remains solid. Furthermore, Río-

Hortega uncovered the defining role of microglia in degenerative processes of the nervous system, and he suspected that oligodendroglia were involved in neurotransmission; researchers of neurotransmitters are now exploring these functions. The scope of Río-Hortega's scientific output covers the entire nervous system. Anyone with an interest in this complex and special branch of biology should be aware of his contributions.

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