Julio Perales García, a disciple of Luis Simarro from Valencia

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ABSTRACT

Julio Perales García was a disciple of Luis Simarro Lacabra, who supervised his doctoral thesis in 1890. Besides his thesis, which was cited by Cajal in 1896, Julio Perales was probably the least well-known of Simarro's disciples, and few biographical details are available.

The present study provides a global review of Simarro's main disciples, followed by an analysis of the life of Julio Perales, based on documentary sources identified in Madrid and Valencia. We also analyse the content of his original thesis, evaluating his micrography work and its impact on the scientific oeuvre of Santiago Ramón y Cajal.

Julio Perales was born in Valencia in 1866; he studied in the city for the first five years of his medical degree, moving to Madrid in 1887 to complete his medical studies and to prepare his doctoral thesis with Simarro.

Cajal wrote in 1896 that Perales' micrography work had been totally overlooked by other authors. This was due to the fact that the thesis was never published; neither was the hypothetical book mentioned in the thesis, which was to be published imminently by Perales' master, Simarro. Cajal emphasised the functional significance of the results reported in the thesis, and had the integrity to acknowledge that Perales had been the first to make these discoveries, despite what was reported in other European publications.

Julio Perales ceased his micrography research in 1905, not without first attempting to obtain a stable university position. Unlike other disciples of Simarro, Perales had no links later in his life with the Junta para la Ampliación de Estudios, and was not involved in the histological school that materialised around Ramón y Cajal in the first third of the 20th century.

KEYWORDS

Julio Perales, Luis Simarro, neurohistology, Ramón y Cajal

Introduction

Over the last two decades, the available information on the life and interests of Luis Simarro Lacabra (1851-1921) has grown considerably with the appearance of various historiographical works, some of which were published to mark the centenary of his death.

These publications shed light on new aspects of the figure of Simarro, although much remains unknown about his complex scientific career, largely due to the limited amount of written work that he produced.

Simarro was in essence a Socratic teacher, who wrote little and whose instruction fundamentally took place in the private environment of the micrography laboratory he established at his own home.[1] However, Simarro enjoyed considerable scientific prestige at the time,[2] and was known to have influenced the magnificent work of

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Santiago Ramón y Cajal and the origin and development of the so-called Spanish Histological School,[3] which included several of Simarro's disciples.

Simarro's scientific interests over his lifetime were heterogeneous, and included the micrography work he performed at his own laboratory; research in neuropsychiatry, which he practised at a private clinic; and scientific psychology, which began to gain institutional status when Simarro occupied the first chair of experimental psychology at a Spanish university, in 1902.

In the field of scientific psychology, while Simarro never created a true school, he did have a large group of students and collaborators (Francisco Santamaría Esquerdo [1866-1925], Martín Navarro Flores [1871-1950], Juan Vicente Viqueira López [1886-1924], José Verdes Montenegro [1865-1940], Alejandro Díez Blanco [1894-1967], Cipriano Rodrigo Lavín [1882-1972]).[4-8] From 1908, several of these disciples received grants from the Junta para la Ampliación de Estudios (Board for Study Extensions; JAE) and later (with many becoming professors) disseminated ideas about the new scientific psychology that they had received from Simarro, an effective importer of knowledge.

It was at Luis Simarro's laboratory that Nicolas Achúcarro Lund (1880-1918), Gonzalo Rodríguez Lafora (1886-1971), and Miguel Gayarre Espinal (1866-1936) began their training in histology. Like Simarro, these three men would later combine their micrography research with neuropsychiatric practice, eventually joining the Laboratorio de Investigaciones Biológicas and the Spanish Histological School, led by Cajal. In addition to these names, we should mention those of certain unexpected, or less well known, physicians, such as José María Villaverde Larraz (1888-1936),[9] with whom Simarro published a single work in 1913,[10] and Julio Perales García, mentioned in recent publications by Campos Bueno[11-13]; Perales is probably the least well known of Simarro's disciples.

The objective of this study is to describe the figure of Julio Perales García, analysing the micrography work he performed under Simarro's supervision, to which he dedicated his doctoral thesis. We will evaluate and discuss the impact of this work on the publications of Cajal, and provide some details of his subsequent medical practice in Valencia from 1905. From that date, Perales ceased his micrography research, and never became a member of the Spanish Histological School that formed around Cajal in the first third of the 20th century.

Material and methods

The primary documentary sources used for this study were Julio Perales García's doctoral thesis, which he defended at the Universidad Central de Madrid in 1890 and is currently available digitally[14]; and his academic record from the same university,[15] which was transferred from the Universitat de València.[16]

Other documentary sources from the city of Valencia also provide information on the biography of Julio Perales. The latter sources were obtained at the historical archives of the Universitat de València,[17] the historical archives of the city of Valencia,[18] and from the Valencian Medical Institute,[19] of which Perales was a member. Searches were also conducted in the *Gaceta Médica de Madrid* and in the archives of the JAE, both of which are available digitally.

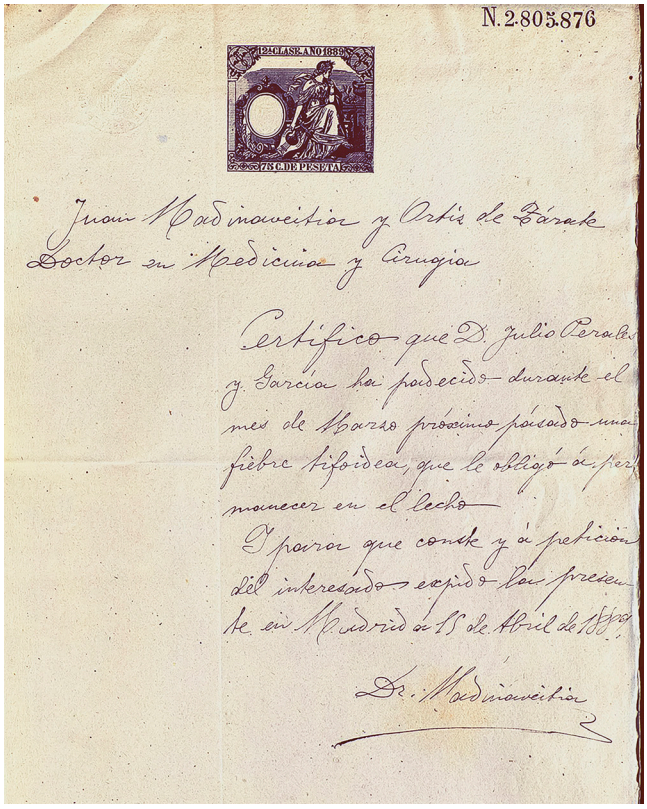
Development

Documentary data on the biography of Julio Perales García

Julio Perales García (1866-1939) was born in Valencia on 4 February 1866, the second of three children born to the lecturer Baltasar Perales Boluda and his wife María Amelia García Salabert.[15,16]

At the age of 12 years, in 1878, Julio Perales entered the Instituto Provincial de Segunda Enseñanza[16] (formerly, Colegio Real de Nobles de San Pablo de Valencia), the city's only secondary education institution at which pupils could obtain the title of *bachiller* (high school diploma); in 1882, he received the diploma with a classification of outstanding. Perales' teachers at the school included Vicente Boix Ricarte and Jaime Banús Castelví. A decade earlier, both men had been mentors and protectors of Simarro,[19,20] who had been a boarding student at Colegio de Nobles.[21]

Before obtaining his diploma, Julio Perales also took classes at Escuela Normal de Valencia to become a teacher.[16] This school's facilities belonged to the Colegio de Nobles,[22] and Perales' father was a lecturer there. His father was a noteworthy figure who undeniably influenced Julio Perales' academic education.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12ª CLASE AÑO 1888

75 CTS DE PESETA

Juan Madinaveitia y Ortiz de Zárate
Doctor en Medicina y Cirugia

Certifico que D. Julio Perales y García ha padecido durante el mes de Marzo próximo pasado una fiebre tifoidea, que le obligó á permanecer en el lecho

Y para que conste y á petición del interesado expido la presente en Madrid á 15 de Abril de 1889.

Dr. Madinaveitia

Figure 1. A doctor's note from Dr Juan de Madinaveitia y Ortíz de Zárate, a close friend of Luis Simarro, certifying that Julio Perales had fallen ill with typhoid fever in March 1889. The document is included in Perales' academic record at the Universidad Central, and was presented by Perales to justify his absences from the university while he was studying for his doctorate in 1889. Source: academic record of Julio Perales, Universidad Central – Archivo Histórico Nacional (leg. 1620, exp. 29).

Baltasar Perales Boluda (1836-1906), born in Mogente (Valencia), had worked as a teacher at rural schools, and later as a lecturer at the Escuela Normal; he was considered an early member of the revolution in teaching in Valencia. He also taught at schools for adults, which were promoted by the Valencian Royal Economic Society of Friends of the Country. His students included Joaquín Sorolla Bastida (1863-1923), who was practically the same age as his son Julio Perales, and who also attended the practical school attached to the Escuela Normal. The advice and the influence of Baltasar Perales appear to have played a decisive role in Sorolla's decision to enrol in night-time drawing classes at the School of Artisans and Apprentices of Valencia,[22] an institutionalist centre created in 1868 by the revolutionary Junta of Valencia,[23] and with which Simarro maintained links throughout his life.[24]

In 1882, after obtaining his *bachiller*, Julio Perales enrolled at the faculty of medicine in Valencia, where he completed the first five years of his medical degree, until the 1886-1887 academic year.[16] In his fifth year, he decided to move to Madrid to complete his medical studies there and very probably to start conducting micrography research alongside Luis Simarro. Thus, by 30 September 1887, Julio Perales was residing at number 17, Calle de la Salud, Madrid, and had enrolled at the Universidad Central for the classes he needed to complete his medical degree.[15]

It is unclear who introduced Julio Perales to Simarro, who was also living in Madrid at the time, following a long spell in Paris. One possibility, though unconfirmed by documentary evidence, is that Epifanio Lozano's widow Beatriz Tortosa Perales introduced the two. Beatriz Tortosa Perales, probably a relative of Julio Perales, was a close friend and benefactor of Simarro; the two always maintained close contact, as shown by their respective last wills.[24]

In Madrid, Simarro was enjoying growing fame as a neuropsychiatrist, and his dedication to micrography work was well known. Cajal, in turn, was still the chair of anatomy in Valencia. It was also during this period that the famous meeting between Cajal and Simarro took place. In March 1887, Simarro had recently married in Valencia,[21,24] but it was at his laboratory at number 41, Calle del Arco de Santa María (now, Calle de Augusto Figueroa), in Madrid, where he met Cajal. There, Simarro showed Cajal histological preparations stained with the Golgi technique, which played a decisive role in Cajal's decision to study the histology of the nervous system.[3,13]

During the 1887-1888 academic year, at Universidad Central de Madrid, Julio Perales took the four outstanding classes he needed to complete his medical degree.[15] At the end of the academic year, he requested the permission of the rector of the Universidad Central to complete his final examination at the Universidad de Valencia, in his hometown. He was examined in Valencia on 28 June 1888, by a board comprising Peregrín Casanova (anatomy), Julio Magraner (clinical medicine), and Francisco Moliner (specialist medical pathology). He passed the examination with a grade of outstanding, after conduct-

ing a practical examination in which he diagnosed a patient with "muscular rheumatism."[16]

However, this examination in Valencia was merely a brief intermission in Perales' time in Madrid. This is demonstrated by two details in his academic record: firstly, he re-enrolled in Madrid within three months to study four doctorate courses; and secondly, he requested that the Universidad de Valencia "forward [my] medical degree to the civil government of Madrid, as [I am] residing there to complete [my] doctorate study and works."[16] This information suggests that by June 1888, Perales had already decided to develop his thesis with Simarro, and had already started conducting micrography work at the latter's laboratory.

According to the dates included in his academic record,[15] Julio Perales' time in Madrid spanned the period from September 1887 to June 1890 (the exact date is unclear), when he defended his doctoral thesis.[14]

The exact date that Perales subsequently left Madrid is unknown. However, his academic record includes two additional documents that on the one hand demonstrate once more his relationship with Simarro, and on the other hint at the dates when he may have left Madrid.

The first document is a doctor's note dated March 1889, certifying that Perales, now a licensed physician, had fallen ill with "typhoid fevers requiring bed rest," preventing him from sitting classes for his doctorate (Figure 1). The note is signed by Juan de Madinaveitia y Ortiz de Zárate (1861-1938), who was such a close friend of Luis Simarro that, years later, the two set up a shared laboratory in adjacent rooms in their respective homes, which were attached to one another in a single building,[23] where they analysed necropsy samples that Madinaveitia supplied to Simarro.

The second document indirectly refers to Perales' departure from Madrid. It a letter written in 1905 in Soneja (Castellón), Perales requests that the Universidad Central de Madrid exempt him from attending a doctoral investiture ceremony, because he was working as a physician in Soneja; furthermore, he indicates his civil status as married (Figure 2).[15]

Evidence from the *Gaceta de Madrid* confirms that, after defending his thesis in 1890, Julio Perales attempted unsuccessfully to obtain a university teaching position, participating in 1892 in the competitive examinations for the chair of histology in Cádiz[25] and in 1902 for the

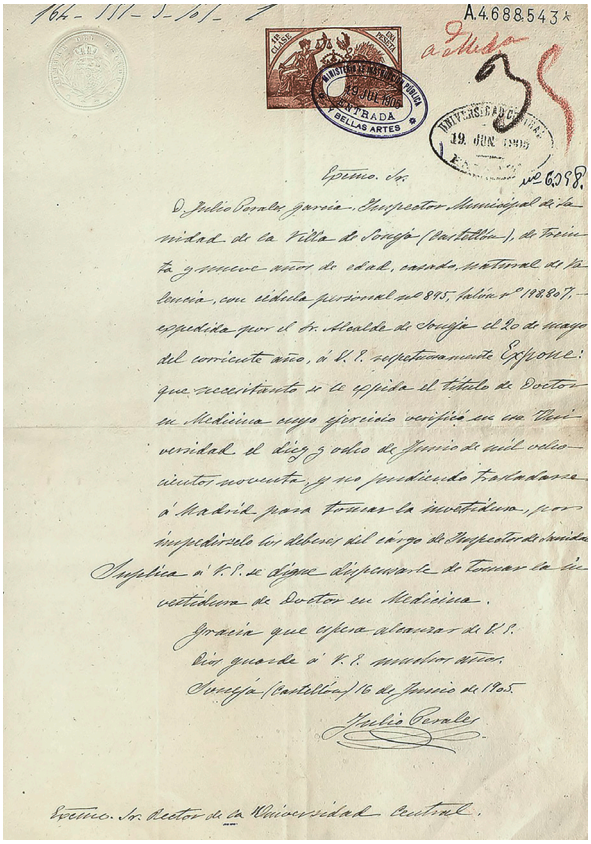


Figure 2. A letter from Julio Perales to the rector of the Universidad Central, drafted in Soneja (Castellón), requesting that he be exempted from attending a doctoral investiture ceremony. In the letter, Perales argues that at the present time he is working as a municipal physician in Soneja, and indicates his civil status as married. Source: academic record of Julio Perales, Universidad Central – Archivo Histórico Nacional (leg. 1620 – exp. 29).

chair of legal medicine in Salamanca,[26] probably seeking stable employment that would be compatible with his micrography work. Perales did not win either of these competitions, and the municipal register of inhabitants of Valencia confirmed that he had returned to the city by 1904; he is registered as an unmarried man of 38 years of age, living once more at his parents' home at number 5, Calle Ruzafa, Valencia.[17] The JAE archives, which cover the period 1907-1939, do not contain any grant application from Julio Perales.

It is unclear whether Julio Perales continued frequenting Simarro's laboratory in the period between the presentation of his doctoral thesis (June 1890)[14] and the competi-

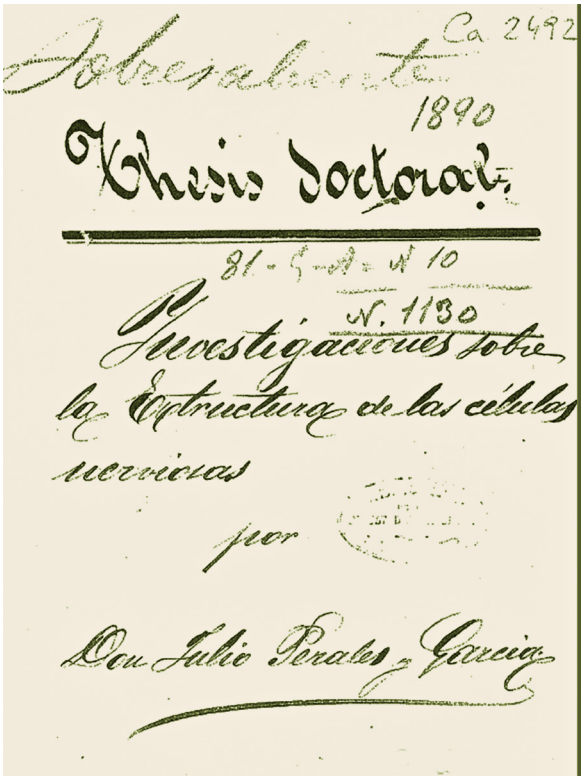


Figure 3. Front page of Julio Perales García's handwritten doctoral thesis "Investigaciones sobre la estructura de las células nerviosas" (Research on the structure of nerve cells), which he defended on 18 June 1890 at the faculty of medicine of Universidad Central. Source: Biblioteca Histórica Marqués de Valdecilla de la Universidad Complutense de Madrid, Fondo Antiguo Biblioteca de Medicina, Ca2492(1130).

tive examinations he took (April 1892 and June 1902).[25,26] If he did, it is very likely that Perales also would have collaborated in the experimental work that led Simarro to develop his photographic staining method. This hypothesis is based on the fact that Simarro was occupied with this experimental work for nearly two years, beginning in February 1899, which falls between the two competitive examinations in which Perales participated. Simarro only published the results of his photographic staining method applied to the study of the nervous system in Cajal's journal late in 1900,[27] but he had previously communicated his findings in Federico Rubio y Galí's journal *Revista Íbero-Americana de Ciencias Médicas*,[28] noting that since February 1899 he had performed various studies to perfect the new method,[28(p333)] whose initial formulation involved in vivo poisoning of rabbits using hypodermic injections of bromide or sodium/potassium iodide.

The exact reasons for Perales' return to Valencia are unclear, but it is reasonable to suspect that his failure to obtain stable employment, his mature age of 38 years, and the desire to marry would have influenced his decision to abandon his micrography research and dedicate himself to clinical work, which would have provided a stable income. Therefore, after his departure from Madrid to live at his parents' home in Valencia, he married María Luisa Díez, from Mallorca, at some time in the period 1904-1905, coinciding with the start of his new employment as a municipal physician in the village of Soneja.

Two years later (1907), Perales and his wife returned to Valencia. The couple lived in Plaza del Ángel,[17] and Perales was listed as a clinical physician in the city (register no. 834 in the official college of physicians). Meanwhile in Madrid, the JAE was established in January 1907, with Simarro as a board member. However, unlike other disciples of Simarro, Perales did not apply for any kind of grant to study abroad or within Spain.

From that time, Perales was a distinguished member of the Valencian Medical Institute (IMV, for its Spanish initials)[18] and of the Valencian Royal Economic Society of Friends of the Country. In 1914 he was general secretary of the IMV; in this role, he promoted the creation of a German language school for members of the Institute, among other projects,[18] although there is no evidence of academic activities related to the Universidad de Valencia. Clearly, upon his return to Valencia, Perales definitively abandoned the micrography work he had conducted in Madrid with Luis Simarro. In the political sphere, Perales was a member of the city council of Valencia from 1925 to 1928, during the Primo de Rivera dictatorship; the mayor was Luis Oliag, who represented the Royal Economic Society of Friends of the Country, of which Perales was also a member.[17] A decade later, in November 1939, Julio Perales died at the age of 73 years, having been hospitalised for asthmatic bronchitis at Hospital Provincial de Valencia.

Micrography work in the doctoral thesis of Julio Perales

Julio Perales' doctoral thesis, entitled "Investigaciones sobre la estructura de la célula nerviosa" (Research on the structure of nerve cells; Figure 3),[14] was the only scientific work by the author identified in the literature

search. The results of his thesis did not appear in a subsequent publication; therefore, the original manuscript of the thesis is the only documentary source enabling us to review Perales' micrography work.

He defended his thesis in Madrid on 18 June 1890, receiving a grade of outstanding. The president of the examining board was Juan Magaz (physiology), and the remaining members were José Grinda Forner, Andrés del Busto López, Adolfo Moreno Pozo, and Alejandro San Martín (Figure 4), all of whom were professors at the faculty of medicine and were involved in the micrography work conducted at the San Carlos faculty and at the Histological Society of Madrid.

The hand-written thesis is held at the Historical Library of the Universidad Complutense de Madrid, and is currently available digitally.[14] The work comprises 113 unnumbered pages, with two pages of figures (Figure 5) at the end of the document; after a short preamble, the work is developed over three chapters.

Chapter I is a literature review, with 22 works cited in footnotes, beginning with the first observations on the texture of the nervous system by Anton van Leeuwenhoek in 1722 and ending with the reports made by Golgi in his 1883 work. Where he cites Golgi's work, Perales notes that the treatise published by Cajal in Valencia in 1889 and the content of the *Revista Trimestral de Histología Normal y Patológica*, founded by Cajal in Valencia in 1888,[29] demonstrate how Golgi's technique was, in Cajal's hands, the most fruitful method for studying the nervous system. Thus, with reference to these two works by Cajal, Julio Perales asserts that: "[…] the noteworthy research by this Spanish professor, though it does not specifically address the structure of cells, merits attention as it led to the ultimate consequences of the application of Golgi's method, which in the hands of this most distinguished professor from Barcelona has become a novel and fruitful method of study."[14]

Chapter II of the thesis is also a literature review, this time addressing the methods used to process the nervous system for microtomy. It contains 24 references, which in this instance are inserted into the body of the text. The entire bibliography of the thesis is clearly influenced by the rich library of Simarro, with over 20% of the works cited by Perales corresponding exactly with books and publications in his master's collection. After a number of vicissitudes, a total of 4333 titles from Simarro's well-stocked library are available today in the Simarro collection,[30] and were cross-checked against Perales' references for the present study.

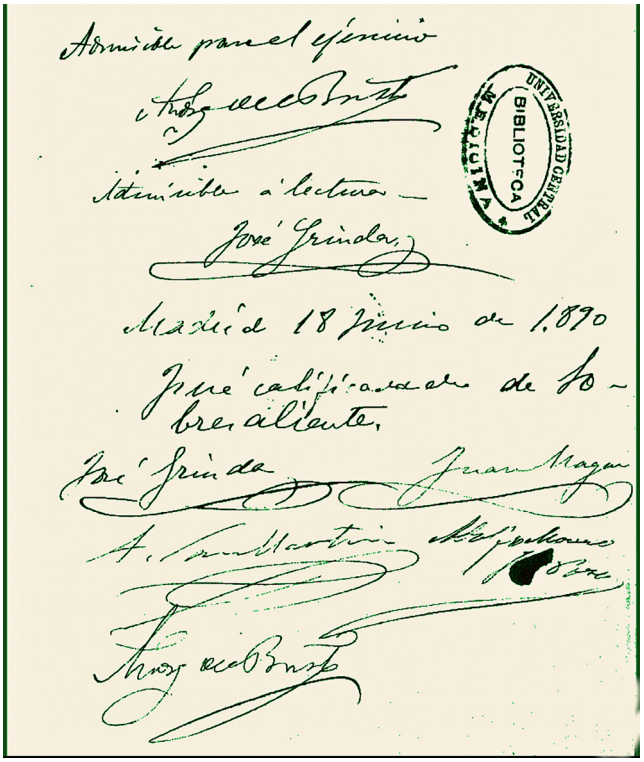


Figure 4. Report from the examination board, classifying Julio Perales' thesis as outstanding, signed by the president, Juan Magaz (physiology) and the other four members, José Grinda (histology), Andrés del Busto (obstetrics), Adolfo Moreno (surgical pathology), and Alejandro San Martín (surgery). Source: Biblioteca Histórica Marqués de Valdecilla de la Universidad Complutense, Fondo Antiguo Biblioteca de Medicina, Ca2492(1130).

In Chapter II, Perales reviews the methods used for hardening of nervous tissues, preferring the use of ether, applied with an atomiser. He also reviews the available methods for the inclusion and staining of nerve cells and fibres, addressing the advantages and disadvantages of each; this demonstrates that he tested each method in Simarro's laboratory. He always insists on the superiority of the stain used by his master, noting that "Dr Simarro's method of colouration, using methyl blue, enables us to discover a new facet of the structure of nerve cells" (Figure 6).[14] One striking detail of the thesis is that it does not mention the stain developed by Franz Nissl in 1884, which the latter mentioned in his own doctoral

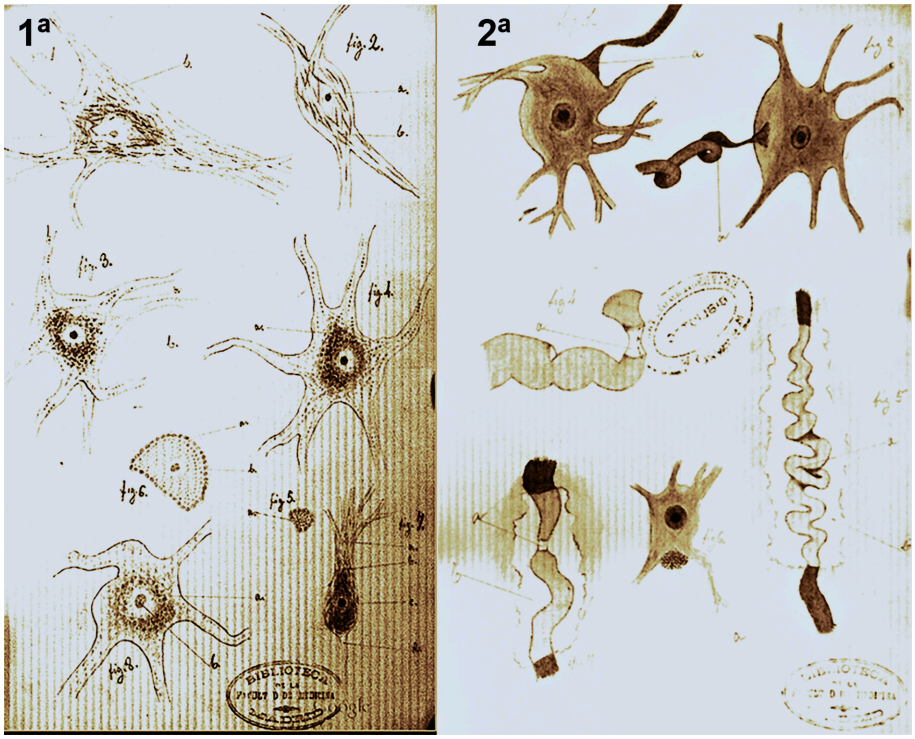


Figure 5. Sheets 1 and 2 of figures from Julio Perales' thesis. Sheet 1 illustrates the fusiform bodies stained with methylene blue in nerve cells, and the post mortem progression of "chromatic spindles" (fragmentation, granular transformation) after the four time intervals analysed. Sheet 2 shows nerve cells dissociated with lithium carbonate, with labels indicating the nodes of Ranvier, and corkscrew-shaped axis cylinders. The latter phenomenon (corkscrew-shaped axis cylinders) was mentioned and discussed in Cajal's 1899 work *Textura del sistema nervioso del hombre y de los vertebrados*.

thesis, using the same dye as Simarro. Nissl's thesis, presented in 1884, was followed by several works published between 1884 and 1894, resulting in widespread dissemination of his method. However, Perales' thesis does not mention any of these studies; this stands in contrast to subsequent works by Cajal, which extensively cite Nissl.

Chapter III of the thesis presents the results of Perales' micrography work using the methyl blue technique, which he once more refers to as "Dr Simarro's method." Regarding the tissues analysed in the thesis, Perales notes that he fundamentally used fresh samples taken from the anterior horn of the lumbar spinal cord.

He described how the methyl blue stain revealed fibrils within the cytoplasm of nervous cells, which thickened to form spindles; these structures were present in dendritic protoplasmic processes, but never in axis cylinders. He also reported having verified the same observations in Purkinje cells to ensure the consistency of the data obtained. Furthermore, he reports that he checked the accuracy of his findings with a series of studies using double staining and modification of the fixatives used.

Perales also reports that he studied changes to this fibrillary structure in the protoplasm with the post mortem progression of nerve cells, analysing it in autopsy samples after 24 hours (observing segmentation of the spindles), beyond which time fibrils began to present granular transformation, and eventually disappeared. This post mortem progression of the fibrils (described by Perales in necropsy studies performed after four different periods post mortem) was studied both in the spinal cord and in the cerebral cortex and cerebellum, using necropsy samples obtained after different periods of time

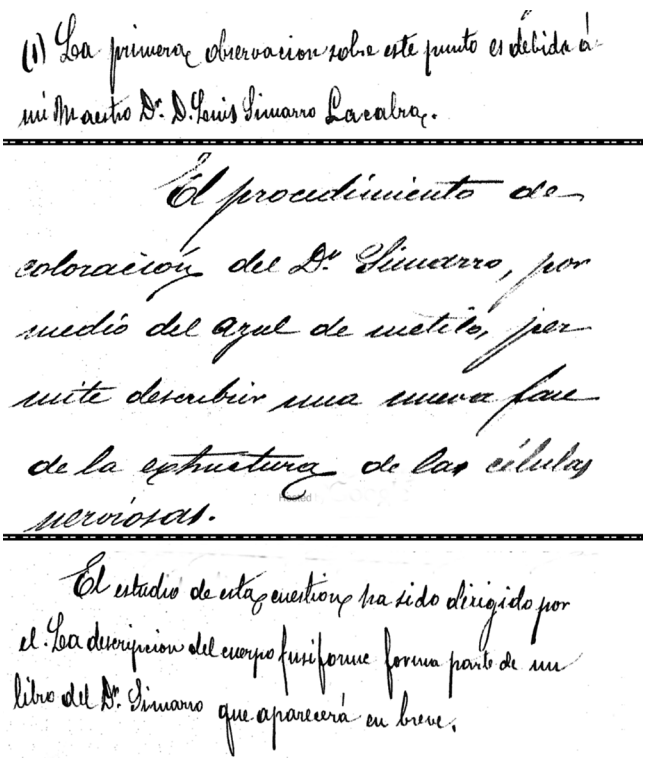
post mortem. In addition to this, Perales also reproduced the study in the spinal cord and vagal lobe of the common carp (*Cyprinus carpio*), suggesting that this protoplasmic structure may be present in all nervous cells in vertebrates.

The latter point demonstrates that Perales applied the same methodology in the micrographic study of the nervous system in fish, a practice that persisted over time at Simarro's laboratory, as shown by the fact that a decade later, Achúcarro[31] and subsequently Gonzalo Rodríguez Lafora[32] also conducted studies in fish. We should also mention the possible source of the fresh human tissue samples studied. According to Perales, these samples came from necropsy studies. In all likelihood, the autopsies would have been performed at Hospital General de Madrid, where Madinaveitia was head of ward and personally responsible for conducting autopsy studies of deceased patients, with samples being analysed at the laboratory of his close friend Luis Simarro.[33]

In the final section of Chapter III, Perales also comments that the post mortem progression of the fibrillary structure, analysed with methyl blue, may in future produce useful results for forensic medicine. He concludes by arguing that the spindles or fusiform bodies observed in nerve cells and their cytoplasmic expansions, but not in axis cylinders, must play an important and distinct role in nerve cells, stating his intention to continue this research in the future, studying fresh spinal cord samples to develop a comprehensive idea of the structure.

The final pages in Chapter III are the two sheets of illustrations (Figure 5). Sheet 1 shows the fusiform bodies and spindles, and the changes observed post mortem in this fibrillary structure. Sheet 2 shows nerve cell elements dissociated with lithium carbonate, presenting corkscrew-shaped axis cylinders, and intraradicular fibres with nodes of Ranvier. As we shall see, the presence of these corkscrew-shaped axis cylinders was discussed and interpreted by Cajal in 1899.

Throughout the three chapters of his thesis, Perales frequently cites Simarro, acknowledging his mastery and the excellence of his histological technique. Furthermore, one reference mentions a forthcoming book written in collaboration with Dr Simarro, in which they will describe these fusiform bodies (Figure 6). The book was never published. Similarly, Perales' thesis never appeared as a separate publication.



(1) La primera observacion sobre este punto es debida á mi Maestro D. D. Luis Simarro Lacabra.

El procedimiento de coloracion del Dr. Simarro, por medio del azul de metileno, permite descubrir una nueva fase de la estructura de las células nerviosas.

El estudio de esta cuestion ha sido dirigido por el. La descripcion del cuerpo fusiforme forma parte de un libro del Dr. Simarro que aparecerá en breve.

Figure 6. Mentions of Simarro, whom Perales refers to as "my master," in the text of the thesis. In one reference, Perales mentions a forthcoming book written in collaboration with Dr Simarro in which they will describe the fusiform bodies. Source: Biblioteca Histórica Marqués de Valdecilla de la Universidad Complutense, Fondo Antiguo Biblioteca de Medicina, Ca2492(1130).

Discussion

The analysis of Julio Perales' life and of the micrography work in his thesis raises several questions to be clarified with documentary data, regarding Perales' biography and the way in which his thesis is cited by Cajal.

Firstly, this study seeks to document the career of a little-known Valencian disciple of Luis Simarro; after working at the latter's laboratory for at least three years, he moved on from micrography research, dedicating himself to clinical work in his hometown.

Evidently, Julio Perales definitively ceased all aspects of micrography work from 1896. In all likelihood, the decision to return to Valencia and work in the clinical setting was influenced by his inability to obtain a stable university position. These circumstances were not exclusive to

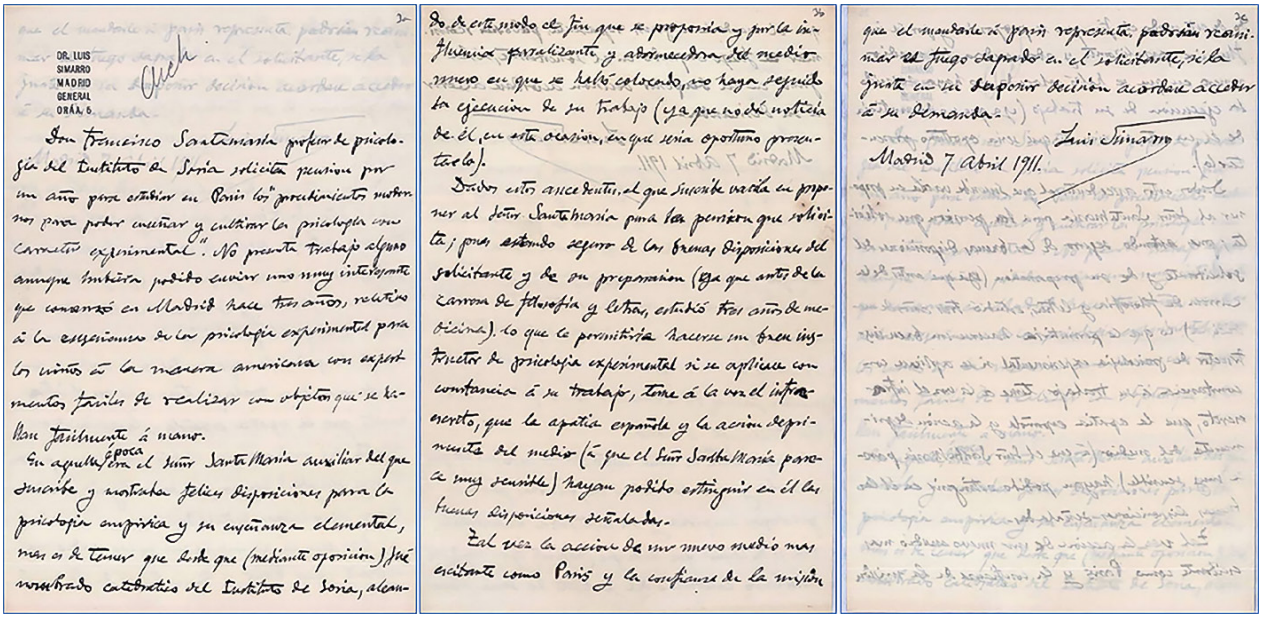


Figure 7. A letter written by Simarro in April 1911, endorsing an application for a JAE grant to study in Paris, submitted by his disciple Francisco Santamaría Esquerdo, whose doctoral thesis Simarro had supervised. Santamaría's application included no publications, and Simarro's letter of endorsement provides a partial explanation of this lack of published work, although he states that, given his familiarity with the criteria of the JAE, he had hesitated to propose Santamaría as a candidate for the grant. Source: JAE file 135/310, on Francisco Santamaría Esquerdo.

Julio Perales: very few opportunities were available for micrography work, and those that did exist were almost completely restricted to the university setting.

Unlike Achúcarro, Rodríguez Lafora, and Gayarre, Perales did not seek to join Cajal's laboratory after defending his thesis. Simarro, who described himself as an "occasional histologist, because histology is not for me an end in itself, but rather a means of studying neurology-,"[34(p296)] advised several of his students to seek to continue their micrography work at Cajal's laboratory. However, we were unable to establish that Perales followed this advice. To explain this, we may consider two relevant details partially related to the chronology of Perales' lifetime: firstly, the distance that formed between Simarro and Cajal at the time of the competitive examination for the Madrid chair; and secondly, the fact that Cajal did not live in Madrid until April 1892, and his laboratory (called the Instituto de Investigaciones Biológicas at the time) only began operating in 1902.

However, we did establish that Cajal was a member or president of the examining boards for the university positions to which Perales unsuccessfully applied in 1892 (histology in Cádiz)[25] and in 1902 (legal medicine in Salamanca).[26] The fact that Perales had never published any scientific work beyond his thesis very probably would have worked against him in these competitive examinations.

We also found no documents establishing the individuals or circumstances that facilitated Perales' contact with Simarro in 1887, or whether, after 1906, Perales maintained any correspondence or other contact with Simarro. The latter point may explain why, from 1907, Perales did not explore other opportunities to continue his work with Simarro, or to expand his studies outside of Spain with the help of the recently created JAE.

The JAE was established in January 1907, on the initiative of the government minister Amalio Gimeno, another disciple of Simarro and a colleague of Cajal, initially in

Valencia and later in Madrid. Cajal was appointed president of the JAE, and the members of the board included two individuals who could have supported an application from Perales: Luis Simarro and Joaquín Sorolla.[35] The former, as we have discussed, was Perales' master; the latter must have been well acquainted with Perales, as they were similar ages and frequented the same places during their time at school in Valencia.

The JAE issued the first call for grant applications in 1907, although the first grants were not awarded until 1908. However, the JAE archives[36] include no grant applications from Perales, either to pursue training outside of Spain or to work at Simarro's laboratory. The contrary was the case for Verdes Montenegro,[36,37] a psychologist who studied under Simarro; a most distinguished secondary school teacher, he received a grant from the JAE to study memory in schoolchildren with Simarro.

Various other disciples of Simarro's also applied for grants from the JAE,[36] with examples including Achúcarro in 1907, Rodríguez Lafora in 1907 and 1908,[38] Viqueira López in 1912, Rodrigo Lavín in 1918, and Santamaría Esquerdo in 1911.[36,39] Some of these applications were ultimately unsuccessful, and the specific case of Santamaría bears some resemblance to that of Perales. Both men were from Valencia, and both wrote their doctoral theses, which they never published, during their time working with Simarro; Santamaría only ever published a single, short, pamphlet. Santamaría applied for a JAE grant to travel to France in 1911, but the application was ultimately rejected,[39] despite the support of Simarro, who wrote a letter endorsing his candidacy and explaining why he had not made any publications (Figure 7).

A second point to be considered in this discussion is the fact that Cajal mentions the content of Perales' thesis in various of his publications. Julio Perales defended his doctoral thesis on 18 June 1890. This date has a certain significance, as Simarro wrote to Cajal seven days earlier (on 11 June)[34(p296-7)] to inform him that he planned to enter the competitive examination for the chair in Madrid, as long as Cajal did not also enter. This letter marked the beginning of a rift that formed between the two men, which persisted until the examination concluded in 1892. The call for candidates to the Madrid chair was issued a month later (21 July 1890) and, despite the poor relations between the two researchers, the exchange of scientific information between them was never completely interrupted[11,40]; Cajal (who was living in Barcelona) was aware of the work being conducted at Simarro's laboratory, and specifically of the content of Perales' thesis.

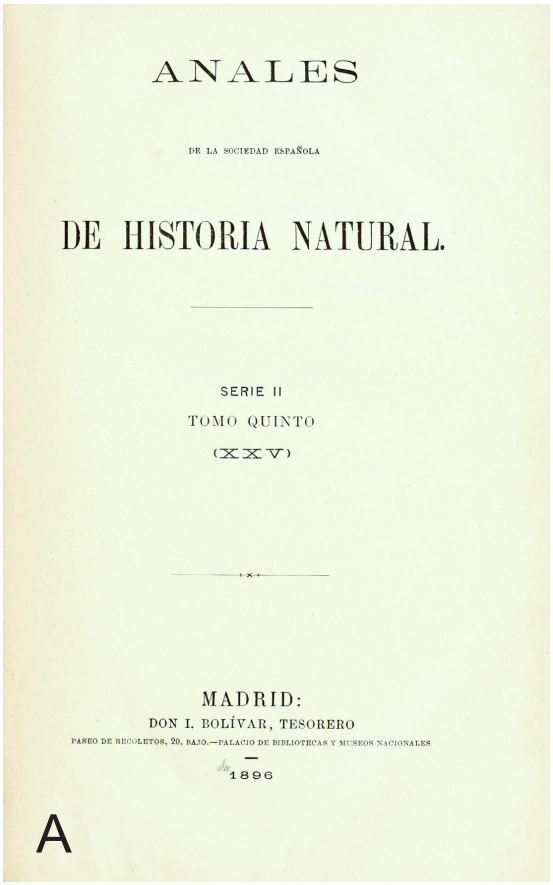
Cajal demonstrated this familiarity with Perales' thesis in an article published in 1896 in *Anales de la Sociedad Española de Historia Natural*,[41] under the title "Estructura del protoplasma nervioso" (Structure of the nervous protoplasm; Figure 8). In the study, Cajal includes a footnote graciously acknowledging that Simarro and Perales were the first to describe the distinct localisation of "chromatic spindles" and their absence in axis cylinders. This acknowledgement contradicted the internationally disseminated idea that it was Karl Schaffer, in Budapest, who had first described this finding. In fact, Schaffer had reported similar results to those of Perales and Simarro in 1893,[42] three years after Perales defended his thesis.

Unlike Simarro, Cajal was highly aware of the importance of disseminating his results. For that reason, the same work ("Estructura del protoplasma nervioso"), with no modifications to the title or text, was also published in volume 1 of *Revista Trimestral Micrográfica*, which Cajal began editing in Madrid in 1896 (Figure 9A).[43]

In this study, Cajal explored in great detail the possibilities of Ehrlich's in vivo staining method with methylene blue. The content of this work by Cajal is both more extensive and more significant than Perales' thesis. His study on the structure of the nervous protoplasm was developed in the context of an exhaustive and systematic general work plan, the basis of what came to be known years later as neuron theory. This study demonstrates how, initially in Barcelona and later in Madrid, Cajal had studied in depth these protoplasmic fibrils and their possible functioning through the analysis of different regions of the nervous system, different animals, and different developmental stages; adopting an ontogenetic method, he followed the same general work plan that he had previously used with Golgi's method.

In addition to his 1896 study citing Perales' thesis, Cajal also published in the first issue of *Revista Trimestral Micrográfica* of 1896 a comprehensive study of the Ehrlich reaction and methylene blue staining,[44] indicating that he had dedicated six months of work to the task, enabling him to corroborate his previous findings with the Golgi stain, both in adult and in embryonic tissues from lower vertebrates, birds, and mammals.

According to Durán Muñoz and Alonso Burón,[45] Cajal was probably following the advice of Kölliker when he


ANALES

DE LA SOCIEDAD ESPAÑOLA

DE HISTORIA NATURAL.

SERIE II

TOMO QUINTO

(XXV)

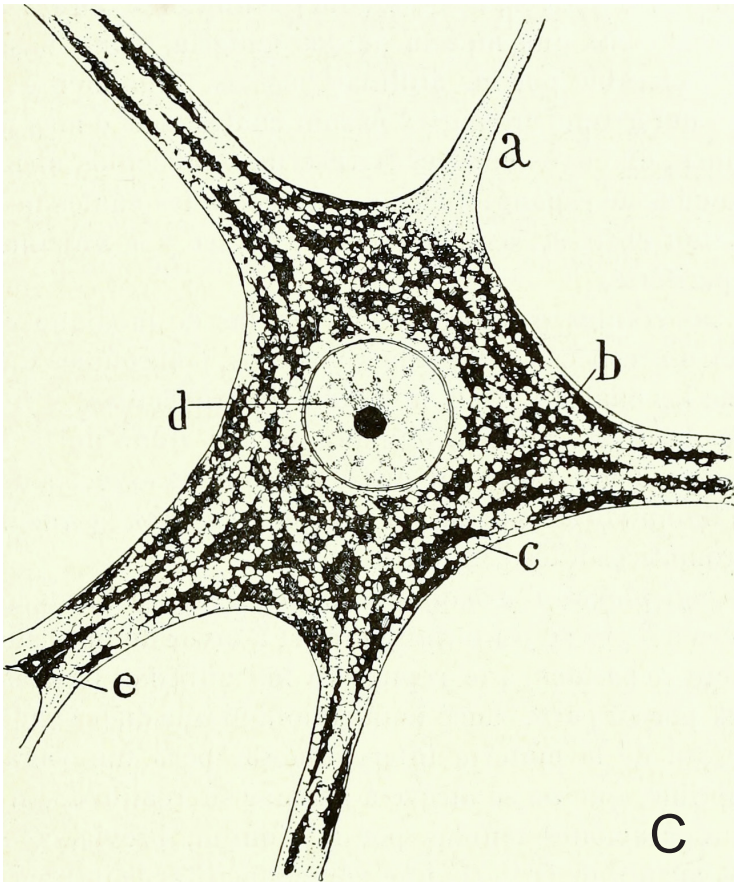
MADRID:

DON I. BOLÍVAR, TESORERO

PASEO DE RECOLETOS, 20, BAJO.—PALACIO DE BIBLIOTECAS Y MUSEOS NACIONALES

1896

A



C

(1) Las opiniones del Dr. Simarro se exponen en la tesis del doctorado de J. Perales, titulada *Investigaciones sobre la estructura de las células nerviosas*. Mayo de 1890. En este trabajo, totalmente desconocido de los sabios, se consigna con toda claridad el hecho, generalmente atribuído á Schaffer, de que los husos cromáticos faltan por completo en el arranque y trayecto del cilindro-eje, hallándose constantemente en el cuerpo celular y porción inicial de las prolongaciones protoplásmicas. Estas investigaciones de Simarro son tanto más interesantes, cuanto que han sido hechas en estado fresco á favor de un modo especial de aplicación del azul de metileno (disociación de las células vivas en porta-objeto, imbibición por el azul de metileno, aplastamiento del preparado con el cubre-objetos, fijado del color bajo la influencia de la temperatura, decoloración en alcohol y montaje en bálsamo), método que, dicho sea de paso, excluye la idea de que los husos cromáticos se deban á la acción coagulante del alcohol ó bicloruro de mercurio.

(2) SCHAFFER: *Kurze Anmerkung über die morphologische Differenz des Achsencylinders im Verhätnisse zu den protoplasmatischen Fortsätzen beim Nissl's Färburg. (Neurol. Centralbl.*, pág. 849, 1893.)

B

Figure 8. Cajal's article "Estructura del protoplasma nervioso" (Structure of the nervous protoplasm), published in 1896 in *Anales de la Sociedad Española de Historia Natural* (A), in which Cajal cites works by Simarro and the thesis of Julio Perales, noting that they had described this finding prior to the publication by Karl Schaffer, three years later in 1893 (C). Cajal includes a figure (B) depicting the presence of "chromatic spindles" in the protoplasm and in dendritic expansions, and their absence in axons (labelled "a").

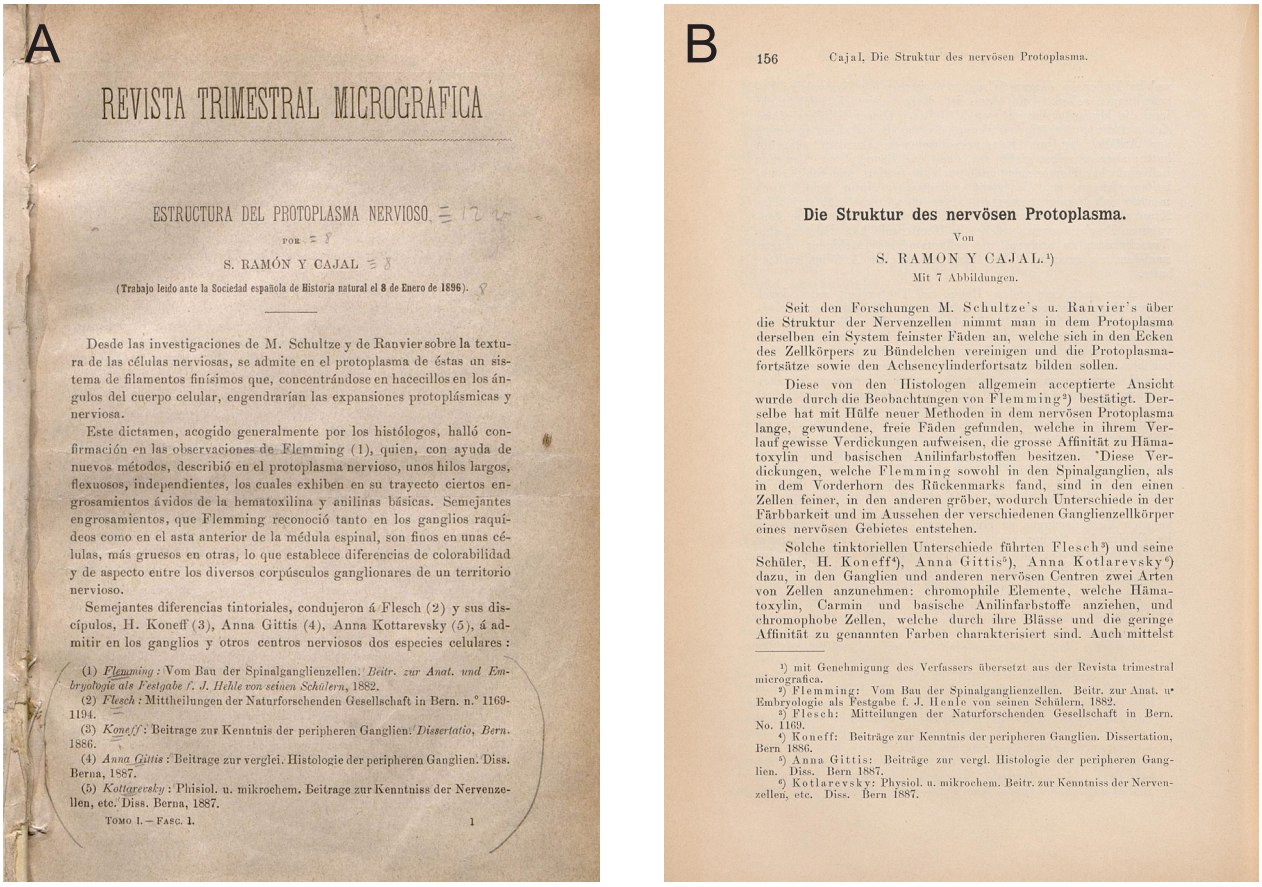
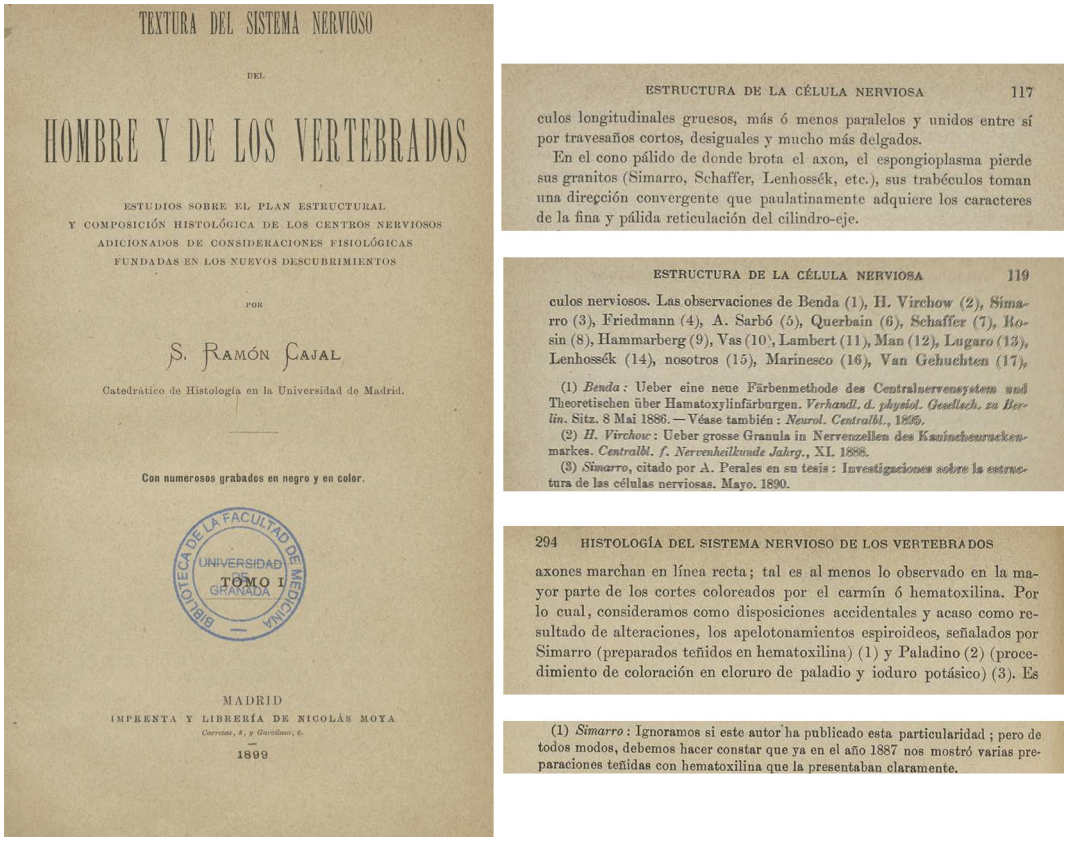


Figure 9. To increase the dissemination of his results, Cajal included the article "Estructura del protoplasma nervioso" (Structure of the nervous protoplasm) in the first issue of *Revista Trimestral Micrográfica*, which he began editing in Madrid in 1986 (A). Similarly, on the invitation of Ziehen and Wernicke, he sent the text to Germany, and a German translation of the article appeared a year later in *Monatsschrift für Psychiatrie und Neurologie* (B).

started using methylene blue. Thus, they assert that "in letters that we possess," the distinguished German anatomist wrote to Cajal in December 1891 telling him that methylene blue was a very good technique for studying nerve cells.[45(p250)] Curiously, this letter from Kölliker does not appear in the collection of Cajal's correspondence published by Fernández Santarén, which includes only three letters from Kölliker, all written in 1893, demonstrating once more how Cajal's correspondence has not been conserved or kept together, and many letters have probably disappeared.[34] Regardless of the content of Kölliker's letter, by the time in question (1891) Cajal clearly already had reliable information from Perales' thesis and Simarro's work with methylene blue.

Furthermore, the aforementioned collection of Cajal's correspondence includes a letter dated 22 September 1896 from the German psychiatrist Theodor Ziehen,[34(p564)] informing Cajal that he and prof Carl Wernicke would begin editing a new monthly journal of psychiatry and neurology (*Monatsschrift für Psychiatrie und Neurologie*), in which they hoped that the anatomy of the nervous system would play a key role. In his letter, Ziehen asks Cajal for an article for the new journal, indicating that he and Wernicke would handle the translation of the article into German, and that Cajal would be paid 40 marks for a 16-page article.

Cajal saw this as an excellent opportunity to disseminate his results, and decided to send Ziehen his ar-



TEXTURA DEL SISTEMA NERVIOSO

DEL

HOMBRE Y DE LOS VERTEBRADOS

ESTUDIOS SOBRE EL PLAN ESTRUCTURAL
Y COMPOSICIÓN HISTOLÓGICA DE LOS CENTROS NERVIOSOS
ADICIONADOS DE CONSIDERACIONES FISIOLÓGICAS
FUNDADAS EN LOS NUEVOS DESCUBRIMIENTOS

POR

S. Ramón Cajal

Catedrático de Histología en la Universidad de Madrid.

Con numerosos grabados en negro y en color.

BIBLIOTECA DE LA FACULTAD DE MEDICINA
UNIVERSIDAD DE GRANADA

TOMO I

MADRID
IMPRENTA Y LIBRERÍA DE NICOLÁS MOYA
Carretas, 8, y Garcilaso, 6.
1899

ESTRUCTURA DE LA CÉLULA NERVIOSA 117

culos longitudinales gruesos, más ó menos paralelos y unidos entre sí por travesaños cortos, desiguales y mucho más delgados.

En el cono pálido de donde brota el axon, el espongioplasma pierde sus granitos (Simarro, Schaffer, Lenhossék, etc.), sus trabéculos toman una dirección convergente que paulatinamente adquiere los caracteres de la fina y pálida reticulación del cilindro-eje.

ESTRUCTURA DE LA CÉLULA NERVIOSA 119

culos nerviosos. Las observaciones de Benda (1), H. Virchow (2), Simarro (3), Friedmann (4), A. Sarbó (5), Querbain (6), Schaffer (7), Rosin (8), Hammarberg (9), Vas (10), Lambert (11), Man (12), Lugaro (13), Lenhossék (14), nosotros (15), Marinesco (16), Van Gehuchten (17),

(1) *Benda*: Ueber eine neue Färbenmethode des Centralnervensystem und Theoretischen über Hamatoxylinfärburgen. *Verhandl. d. physiol. Gesellsch. zu Berlin*. Sitz. 8 Mai 1886. — Véase también: *Neurol. Centralbl.*, 1895.

(2) *H. Virchow*: Ueber grosse Granula in Nervenzellen des Kaninchenruckenmarkes. *Centralbl. f. Nervenheilkunde Jahrg.*, XI. 1888.

(3) *Simarro*, citado por A. Perales en su tesis: Investigaciones sobre la estructura de las células nerviosas. Mayo. 1890.

294 HISTOLOGÍA DEL SISTEMA NERVIOSO DE LOS VERTEBRADOS

axones marchan en línea recta; tal es al menos lo observado en la mayor parte de los cortes coloreados por el carmín ó hematoxilina. Por lo cual, consideramos como disposiciones accidentales y acaso como resultado de alteraciones, los apelotonamientos espiroideos, señalados por Simarro (preparados teñidos en hematoxilina) (1) y Paladino (2) (procedimiento de coloración en cloruro de paladio y ioduro potásico) (3). Es

(1) *Simarro*: Ignoramos si este autor ha publicado esta particularidad; pero de todos modos, debemos hacer constar que ya en el año 1887 nos mostró varias preparaciones teñidas con hematoxilina que la presentaban claramente.

Figure 10. *Textura del sistema nervioso del hombre y de los vertebrados*. (Texture of the nervous system of man and the vertebrates). Cajal began publishing his magnum opus, in three volumes, in 1899. Volume I includes several references to Simarro and Perales, with a footnote summarising the images of the corkscrew-like appearance of axons (p294) shown on sheet 2 of the illustrations to Perales' thesis. Cajal admits that he does not know if Simarro ever published this finding, whose preparations he showed Cajal in 1897.

ticle on the structure of the nervous protoplasm, once more acknowledging the work of Perales and Simarro, mentioned previously. As a result, the same article was published a year later in Wernicke's journal, in two submissions (in issues 2 and 3 of the first volume of 1897), under the title "Die Struktur des nervösen Protoplasma" (Figure 9B).[46] Thus, Cajal's study was published in three different editions, with two appearing in Spanish, almost simultaneously in 1896, and another in German, in 1897. All three explicitly acknowledged the work of Simarro and the unpublished thesis of Perales.

It was through these publications by Cajal[41,43,46] that we are aware today of Perales' thesis, as Cajal, no doubt moved by a sense of scientific honour and "laborious love of Spain,"[47] stressed the primacy and originality of his compatriots' findings, which had not previously been published and had been completely overlooked in the international context.

The fact that Perales had not published the results of his thesis also invites comparison of the figures of Simarro and Cajal, a subject previously addressed by Campos Bueno.[11-13] Cajal was tireless in his laboratory work. His micrography activity from 1887, after his meeting with Simarro, was almost frenetic: he systematically explored the Golgi technique; successfully (with great perseverance) overcame the "inconstancies of chrome-silver impregnation" that had led Simarro to "[abandon] his efforts in discouragement"[48(p309)]; and in 1888 (using his original chrome-silver impregnation technique) produced a structural analysis of the nervous system, beginning with the study of birds and mammals, until he was able to demonstrate the individuality of the nerve cell,

first in the cerebellum and subsequently in the retina and other locations.[40]

In addition to his great capacity for micrography work, Cajal was also a great publicist, and was able to capture the attention of international figures including the German researcher Albert von Kölliker, in 1893, and the Swedish scientist Gustaf Retzius, in 1896.[40] For this reason, Cajal knew the importance of publishing and disseminating his results, in the great languages of science. This is the context in which we should interpret the publication of his results from 1896, in which he mentions Perales and Simarro: he published the work almost simultaneously in two separate journals in Spanish,[41,43] and a German translation the following year, in 1897.[46]

In December 1897, Cajal began to publish his work *Textura del sistema nervioso del hombre y de los vertebrados*[49] (Texture of the nervous system of man and the vertebrates; Figure 10) in instalments with Nicolás Moya's publishing house. Cajal considered the work his magnum opus, and eventually published it in two volumes in 1904. In volume I, Cajal cites works by Simarro and the thesis of Perales on a further five occasions, once more recalling the primacy of their works before that of Schaffer. Cajal robustly argues that Simarro's "chromatic spindles" and the so-called Nissl's granules are one and the same thing[49(p118)]; later, he explicitly refers to Simarro's discovery about the corkscrew-shaped axons, also illustrated on sheet 2 of Perales' thesis, which Cajal interprets as random, adding in a footnote that: "I am not aware whether Simarro has published this peculiar finding, but I must acknowledge that he showed them to us in 1887 in preparations stained with haematoxylin, in which they were clearly present."[49(p294)]

At this time, when Cajal began submitting the first instalments of his magnum opus, understanding of the internal structure of nerve cells was seen as a crucial subject, as some reformulations were beginning to be made to the reticular theory, in which the neurofibrils present in nerve cells were thought to form a continuous, interneuronal network responsible for nerve impulses. The solution to these reformulations of reticular theory, proposed by István Apáthy, Albrecht Bethe, and Hans Held in the early years of the 20th century, reached Cajal in 1900 thanks to Simarro's photographic staining method,[21,27,28] mentioned previously, which Simarro had begun using in 1889, probably with the assistance of Perales in the early days. Three years later, Cajal modified Simarro's procedure, eventually establishing his famous reduced silver nitrate method.[50] This method was once more applied systematically by Cajal and his school over the course of a decade, and was crucial in the total victory of the neuron doctrine over the criticisms from the new defenders of reticular theory.[40]

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