Nicolás Achúcarro and the histopathology of rabies: a historical invitation from Nissl and Alzheimer

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ABSTRACT

Introduction. Nicolás Achúcarro (1880-1918), a brilliant disciple of Cajal, was invited by Nissl and Alzheimer to write on the subject of experimental rabies. The chapter, published in 1909 in German, has never previously been translated into Spanish.

Material and methods. The study "On the understanding of the central nervous system histological pathology in rabies" was obtained from the University of Bonn, Germany, and translated into Spanish by one of the authors (FM). We researched the context of the study; its relevance to the epidemiology, diagnosis, and histopathology of rabies encephalitis; and its influence on Achúcarro's scientific career.

Results. The study was conducted in rabbits, a dog, two hens, and a brain specimen from a man who died due to rabies. It was presented as a doctoral thesis in Madrid in December 1906. The German-language publication, from 1909, comprises 51 dense pages of text with 13 illustrations; a summary in Spanish was published in 1914. Achúcarro rejected the idea that Negri bodies were parasites, confirming Cajal's observations on Alzheimer neurofibrillary degeneration and argyrophilic fibres in rabies. He underlines the transformation of glial cells in the cornu ammonis into elongated elements (rod-like cells or *Stäbchenzellen*) to adapt to the parallel arrangement of pyramidal cells in the stratum radiatum. Achúcarro suggests that these cells are also involved in the capture and elimination of fat and degenerative residues (granulo-adipose bodies) and possibly also in the phenomenon of neuronophagia.

Conclusions. Achúcarro's study was the first to describe the morphology and functions of microglia. Del Río Hortega identified Cajal's "third element" as two separate cells (microglia and oligodendroglia), demonstrating the mesodermic origin of microglia. The study on experimental rabies was decisive in Achúcarro's career as a neuropathologist.

KEYWORDS

Achúcarro, rabies, rod-like cells, granulo-adipose bodies, microglia, Negri bodies, neurofibrillary tangles.

Introduction

Despite dying at a young age, Nicolás Achúcarro Lund (1880-1918) may have been the most brilliant and most productive member of the Spanish Neurohistological School (Figure 1). Initially studying under Simarro, and with Cajal indisputably being his master, Achúcarro chose Madrid as his home, with Germany as his model: combining neurological and psychiatric experience with high-level histopathological study in clinical practice, together with cutting-edge histological research.[1-4]

Due to his early death, before the age of 38 years, Achúcarro was the great "missed opportunity" for the implementation of neurology as an independent specialty in Spain, which was unjustifiably delayed by many years.[5] "Neurology is the victim of history," as Gonzalo Moya[6] used to say. Achúcarro's obituaries highlight his extraordinary qualities. "One of the ten or

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twelve Spaniards of greatest intellectual merit," wrote Ortega y Gasset (*El Sol*, 26 April 1918). "An exceptional Spaniard, barely known in Spain, who excelled for his great originality," added Gregorio Marañón (*El Liberal*, 25 April 1918). His bourgeois upbringing in Bilbao, the path his life followed, and the qualities adorning his tremendous personality have been analysed by various authors (Figure 2).[7-10]

After earning a degree in medicine from the Universidad Central in the autumn of 1904, Achúcarro undertook a long journey, staying in different European cities including Paris, Florence, and Munich, in order to receive training in neurology, psychiatry, and neuropathology. As noted by Gonzalo R. Lafora,[7] it was in Munich where he consolidated his calling for diseases of the nervous system: "inspired by the neurologist Lewandowsky, with whom he had become friends in Paris, he travelled to Munich […] and in the laboratory of Dr Alzheimer he published the most detailed histological study to date of nervous lesions in rabies." In fact, at the invitation of Nissl and Alzheimer, Achúcarro wrote a chapter for the third volume of their magnum opus, the six-volume *Histologic and histopathologic studies of the cerebral cortex*, conducting experiments with a rabbit, a dog, two hens, and the nervous system of a 26-year-old man who had died due to rabies. The study was entitled "On the understanding of the central nervous system histological pathology in rabies."[11]

As noted by Marañón in his obituary of Achúcarro (*El Liberal*, 25 April 1918), it was unusual for figures of the stature of Franz Nissl (1860-1919) and Alois Alzheimer (1864-1915) to have the deference to invite a young Spanish researcher to contribute to a work of such significance. This was one of their most distinguished works, wrote a disciple of Nissl.[12] Overall, few Spanish authors have cited the article,[9,10] and it is omitted from extensive studies.[13] In 1977, Manuel Vitoria Ortiz[8] translated only the conclusions in his excellent biography. To our knowledge, the original German-language version of this study of the histopathology of rabies has never been translated.

Nicolás Achúcarro's contribution to the encyclopaedic work by Nissl and Alzheimer was published in 1909. He further advanced his research between 1908 and 1911, with three additional studies on rod-like cells, hypertrophy of the endoplasmic reticulum, and granulo-adipose bodies.[14-17] Finally, he eventually published a monograph in Spanish in 1914 as a doctoral thesis.[18]

Figure 1. Nicolás Achúcarro Lund (Bilbao, 14 June 1880 - Neguri, 23 April 1918), circa 1910 (image taken from Vitoria[8]).

This study aims to raise awareness of the 1909 article, which has not previously been published in Spanish, and Achúcarro's subsequent contributions on rabies encephalitis, as well as Alzheimer's laboratory in Munich and its influence on Achúcarro's work as a neuropathologist.

Material and methods

Achúcarro's original German-language article, published in 1909 in *Histologische und histopatologische Arbeiten*

Figure 2. Family photo, circa 1912. Aniceto Achúcarro (1) and Juana Lund (2) and their three children, Nicolás (3), Severino (4), and María (5) (image taken from Vitoria[8]).

(vol. III, part 1),[19] was obtained from the University of Bonn and translated into Spanish by one of the authors (FM). The monograph published as a doctoral thesis in 1914 is available online (Catálogo Cisne, Biblioteca Complutense, tesis doctorales). His studies on the subject between 1909 and 1914, published in the journals *Laboratorio de Investigaciones Biológicas* and *Boletín del Instituto de Sueroterapia*, were consulted at the National Library of Spain and at the Cajal Institute (Spanish National Research Council), in Madrid. In addition to the important references on Achúcarro's biography, we conducted a search for relevant aspects of rabies, such as its history, neuropathology, pathophysiology, and prophylaxis.

Results

1906: defence of his doctoral thesis

Contemplating a future university career, Achúcarro took advantage of his research into experimental rabies at Alzheimer's laboratory and escaped to Spain to present it as a doctoral thesis.[10,20] This was a purely administrative exercise, and he took the chance to spend Christmas with his family. He defended his thesis, entitled *Contribution to the study of the anatomical pathology of rabies*, at the Faculty of Medicine in Madrid on 10 December 1906. It is unclear how the session unfolded, but having received his undergraduate degree two years previously, Achúcarro would have been known to the members of the examination panel. The panel was presided by Alejandro San Martín Satrústegui (1847-1908), from Navarre, of whom Cajal wrote that "I was bound by close ties of affection and sincere intimacy"[13(p487)]; and included José Gómez Ocaña (1860-1919), from Málaga, with whom Achúcarro had worked at his laboratory of experimental physiology, and Manuel Márquez Rodríguez (1872-1962), from Toledo, a famous ophthalmologist who had collaborated with Cajal. The final members of the panel were J. Ribera y Sanz (1852-1912), a prestigious Catalan paediatric surgeon, and J. Trigueros, both of whom served as spokesmen. Overall, Madinaveitia, Gómez Ocaña, and Simarro were Achúcarro's greatest influences in his time as a student in Madrid (Figure 3).

1909: invitation from Nissl and Alzheimer

In early January 1907, shortly after collecting his doctorate certificate, which qualified him as "outstanding," Achúcarro returned to Munich to continue his research on rabies. Nissl and Alzheimer, who were familiar with his work, invited him to write on the subject for the third volume of their magnum opus, which resulted in the chapter "Zur Kenntnis der pathologischen Histologie des Zentralnervensystem bei Tollwut" ("On the understanding of the central nervous system pathology in rabies") (Figure 4, left). Achúcarro's article, drafted over a century ago, contains archaisms and concepts and terminology yet to be defined, which makes translating it an arduous task. The work clearly shows the influence of his master Alois Alzheimer, whom he repeatedly thanks for his guidance.[11] The complete Spanish translation, the original German-language text, and the original figures may be consulted in the supplementary material.

1908-1911: further research on rod-like cells (*Stäbchenzellen*), granulo-adipose bodies, and neurofibrillary tangles

Aware of the originality of his work, Achúcarro conducted further research, publishing in French and Spanish, between 1908 and 1911. Rather than the rabies virus, he

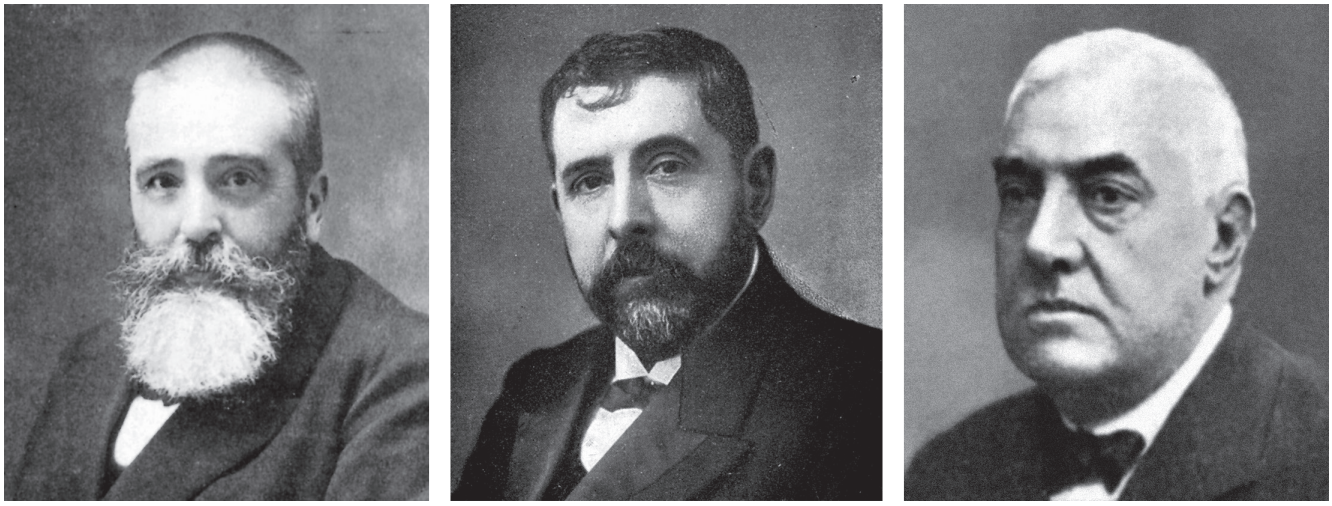


Figure 3. Three figures who influenced Achúcarro's training in Spain. Left: Juan Madinaveitia y Ortíz de Zárate (1861-1938), head lecturer of medicine at the Hospital Provincial de Madrid. He became a personal friend of Achúcarro. A plaque, which has since disappeared, used to read "The great Madinaveitia taught medicine here." Centre: Don José Gómez Ocaña (1860-1919), in whose laboratory at the San Carlos medical school Achúcarro first studied experimental medicine. Right: Don Luis Simarro Lacabra (1851-1921). Achúcarro began studying histology at his laboratory on Calle General Oráa. Source: RANM.

used granulomatous lesions secondary to inoculation with fungi (*Sporothrix schenckii*) or the application of different caustic substances to several areas of the cornu ammonis.[14-16]

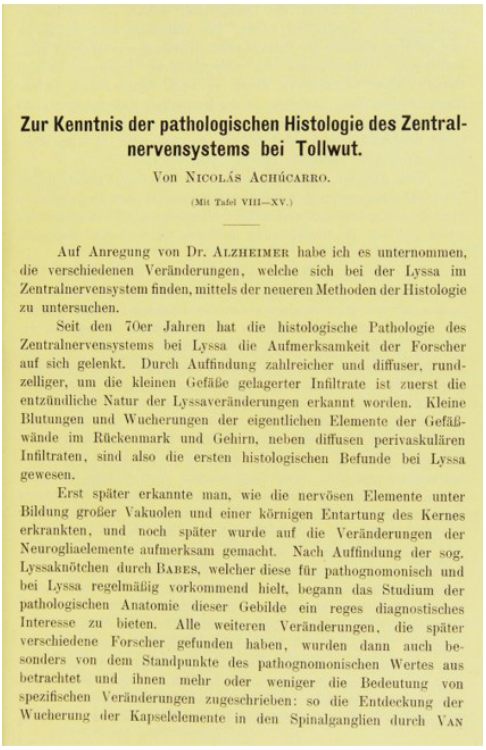
1914: monograph on the neuropathology of rabies

The monograph *Contribution to the study of the anatomical pathology of rabies*[18] comprises 37 pages without images or a bibliography (Figure 4, right). A footnote clarifies that "a more extensive version of the article, including figures and bibliographical data, has been published," referring to the study conducted at Alzheimer's laboratory. It should be noted that the two publications are far from being the same, no doubt because the author wished to include more recent developments. He summarises that "rabies virus causes exudative inflammation throughout the nervous system, with hyaloid degeneration involving ganglion cells, glia, and the vascular system." Achúcarro dedicates considerable space to the normal morphology of the cornu ammonis and to the numerous staining methods available and their specific affinities for viewing different histological structures.

With the header *Doctoral thesis*, presented and graded eight years earlier, it was published at a time of Achúcarro's life when he had achieved his most cherished goals. He had returned from his "American adventure" at the St. Elizabeths Hospital in Washington, D.C., which began in September 1908 with a voyage from the port of Le Havre to New York,[10] and had joined the Biological Research Laboratory, under the chair of Santiago Ramón y Cajal. On 1 January 1912, he had passed a competitive public examination for a position on the medical staff of the Hospital Provincial de Madrid, earning a monthly salary of 1760 pesetas, which alleviated previous hardships that he and his wife had endured. Furthermore, on Cajal's recommendation, the Board for Study Extensions afforded him a laboratory at the Residencia de Estudiantes, where he soon gathered a host of brilliant interns: Gonzalo R. Lafora, Pío del Río Hortega, Miguel Sacristán, Felipe Jiménez de Asúa, Luis Calandre, and Miguel Gayarre. Achúcarro had created his own school of followers.[20]

Discussion

Rabies is a form of encephalitis caused by *Lyssavirus*, family Rhabdoviridae, a neurotropic RNA virus transmitted through the saliva of infected animals. It has been a scourge on humanity since time immemorial,



Zur Kenntnis der pathologischen Histologie des Zentralnervensystems bei Tollwut.

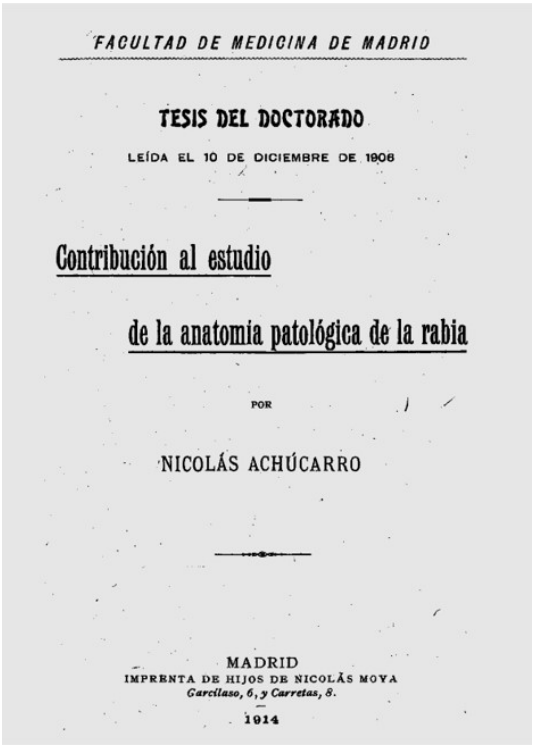
Von Nicolás Achúcarro.

(Mit Tafel VIII—XV.)

Auf Anregung von Dr. Alzheimer habe ich es unternommen, die verschiedenen Veränderungen, welche sich bei der Lyssa im Zentralnervensystem finden, mittels der neueren Methoden der Histologie zu untersuchen.

Seit den 70er Jahren hat die histologische Pathologie des Zentralnervensystems bei Lyssa die Aufmerksamkeit der Forscher auf sich gelenkt. Durch Auffindung zahlreicher und diffuser, rundzelliger, um die kleinen Gefäße gelagerter Infiltrate ist zuerst die entzündliche Natur der Lyssaveränderungen erkannt worden. Kleine Blutungen und Wucherungen der eigentlichen Elemente der Gefäßwände im Rückenmark und Gehirn, neben diffusen perivaskulären Infiltraten, sind also die ersten histologischen Befunde bei Lyssa gewesen.

Erst später erkannte man, wie die nervösen Elemente unter Bildung großer Vakuolen und einer körnigen Entartung des Kernes erkrankten, und noch später wurde auf die Veränderungen der Neurogliaelemente aufmerksam gemacht. Nach Auffindung der sog. Lyssaknötchen durch Babes, welcher diese für pathognomonisch und bei Lyssa regelmäßig vorkommend hielt, begann das Studium der pathologischen Anatomie dieser Gebilde ein reges diagnostisches Interesse zu bieten. Alle weiteren Veränderungen, die später verschiedene Forscher gefunden haben, wurden dann auch besonders von dem Standpunkte des pathognomonischen Wertes aus betrachtet und ihnen mehr oder weniger die Bedeutung von spezifischen Veränderungen zugeschrieben: so die Entdeckung der Wucherung der Kapselelemente in den Spinalganglien durch Van



FACULTAD DE MEDICINA DE MADRID

TESIS DEL DOCTORADO

LEÍDA EL 10 DE DICIEMBRE DE 1906

Contribución al estudio

de la anatomia patológica de la rabia

POR

NICOLÁS ACHÚCARRO

MADRID

IMPRENTA DE HIJOS DE NICOLÁS MOYA

Carcilaso, 6, y Carretas, 8.

1914

Figure 4. Left: title and first page of the German-language study published in 1909, on the same subject as his doctoral thesis (1906). Right: eight years after defending his thesis, he published a somewhat less extensive monograph in Spanish.

and continues to cause approximately 60 000 deaths annually in African and Asian countries.[21,22] With some exceptions,[23] patients with the furious form die after a course of shocking symptoms, with violent, painful spasms typically triggered by the sight or intake of water, excessive sweating and salivation, and mydriasis; awareness is preserved until the terminal phase.

Compared to other forms of viral encephalitis, rabies is unusual due to the low incidence of inflammatory manifestations (perivascular cuffs, microglial nodules, and neuronophagia) and its unique pathogenesis. With tropism particularly affecting the salivary glands and acetylcholine nicotinic receptor, the virus penetrates the neuromuscular junction and subsequently spreads to brain areas with abundant cholinergic innervation, such as the cortex and limbic system, in the hydrophobic form.[21] The virus remains sequestered in Negri bodies, in the soma of some large neurons, enabling it to evade the immune response, with the bodies and dendrites of neurons remaining intact; it may also cross the blood-brain barrier for highly variable incubation periods, which range from several weeks to four years.[24]

Rabies in Spain

In 1907, the Valencian writer Vicente Blasco Ibáñez wrote a short story entitled *La rabia* ("Rabies"), in which he described with extreme realism the inevitable death of the son of the protagonist Caldera. He had been bitten by stray dogs that roamed in the sugar plantations of the Albufera estuary near Valencia. Unable to contemplate his moribund son, and with no other recourse, Caldera made a fatal decision: "so he won't suffer," he repeated to himself as he fired his shotgun upon his marriageable son.[25] This cannot have been an unusual occurrence: in France, a law passed in 1810 expressly prohibited the murder of patients with invariably fatal diseases.[26]

In Galicia, the towns of Chantada, Tuy, and Monforte de Lemos were famously attacked by packs of rabid wolves

between 1880 and 1900.[27] The last outbreak in Spain was in Málaga in 1975, and caused the deaths of three people, one of whom was attended by the late neurologist Mariano Pastor (personal correspondence with SGR). The disease was eradicated in Spain in 1978, and the few cases reported since have been related to Spain's southern neighbour, Morocco.[28] For example, in a health alert in Madrid on 25 June 2013, 29 children received prophylactic treatment at La Paz children's hospital; another individual died in Barakaldo after being bitten by a cat (*Diario Vasco*, 16 December 2019). A new threat is the European bat lyssavirus type 1 (EBLV-1) variant: two individuals from Huelva and Valladolid have been bitten by bats carrying the variant; both survived. In Germany, a 46-year-old woman was bitten by a fox suspected of carrying rabies; foxes are an infrequent reservoir for the virus (*Frankfurter Allgemeine*, 24 April 2017).

Experimental rabies in rabbits: background

The selection of rabbits for the experimental model was not solely based on their being easier to handle than dogs. Louis Pasteur (1822-1895) had created an attenuated rabies virus by passing the virus numerous times from one animal to another. In a historic development, the desiccated spinal cord from rabbits infected with the attenuated virus was used to manufacture the first post-exposure vaccine for humans.[29] In any case, transcranial inoculation of the "fixed" virus shortens the incubation period to seven days, causing progressive paralysis and intense neuropathological alterations, predominantly affecting the cornu ammonis region of the hippocampus (Figure 5).

In the rabbit model, Cajal had described "Alzheimer lesions" (neurofibrillary degeneration or neurofibrillary tangles) in the reticular apparatus of the cerebral cortex and hippocampus, using ammoniacal silver oxide and Bielchowsky staining techniques.[30,31] Eighteen preparations from this research are preserved at the Cajal museum in Madrid.[32] On 4 November 1906, Alois Alzheimer had delivered a lecture in Tübingen (Germany) entitled "Über eine eigenartige Erkrankung der Hirnrinde" ("A characteristic disease of the cerebral cortex").[33] The most interesting finding from his study of the brain of the 55-year-old Auguste Deter was a marked alteration of intraneuronal neurofibrils, which presented increased thickness and strong affinity for silver stains (neurofibrillary tangles).[34,35] In turn, Nissl had described *Stäbchenzellen* or rod-like cells in general paresis.[36]

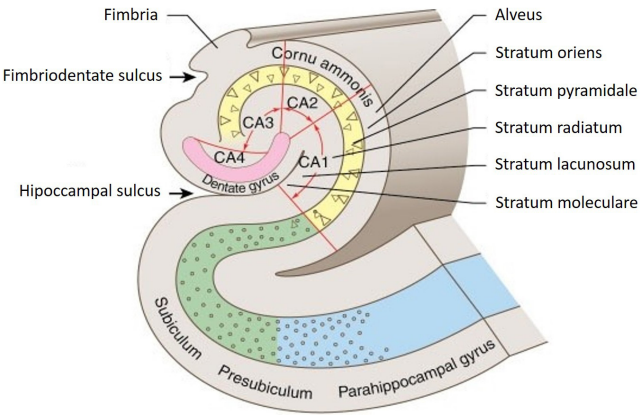


Figure 5. Schematic drawing of the complex anatomy of the hippocampus. The labels show the locations of the cornu ammonis and stratum pyramidale, where Achúcarro observed rod-like cells (*Stäbchenzellen*) in his experimental study of rabbits inoculated with the rabies virus. Figure found online, original source unknown.

Alzheimer's laboratory in Munich

Between 1870 and 1914, Germany and Austria became the global hub of neuroscience in general and neuropathology in particular. Proof of this was the creation in 1917 of three histopathology units at the Deutsche Forschungsanstalt für Psychiatrie (later called the Max Planck Institute), under the directorship of Franz Nissl, Korbinian Brodmann (1869-1918), and Walther Spielmeyer (1879-1935).[37] Lewandowsky made no mistake in recommending that Achúcarro visit Emil Kraepelin's (1856-1926) research institute in Munich, where he learned an innovative approach to the study of mental disorders. The revolutionary concept of "organic dementia" arose from the histopathological study of general paresis, the prototype for the application of clinical anatomical methods in psychiatry.[38] The new nosological entities described by Kraepelin, such as *dementia praecox* (schizophrenia) and bipolar disorder, were yet to be researched. His objective was "to help psychiatry through the microscope."[39,40] To that end, it was essential to have a well-equipped histopathology laboratory, and there was nobody better than Alzheimer to lead such a centre: at the time, he already had extensive experience in conducting pathological studies of the cerebral cortex.[40] In the autumn of 1903, Alzheimer

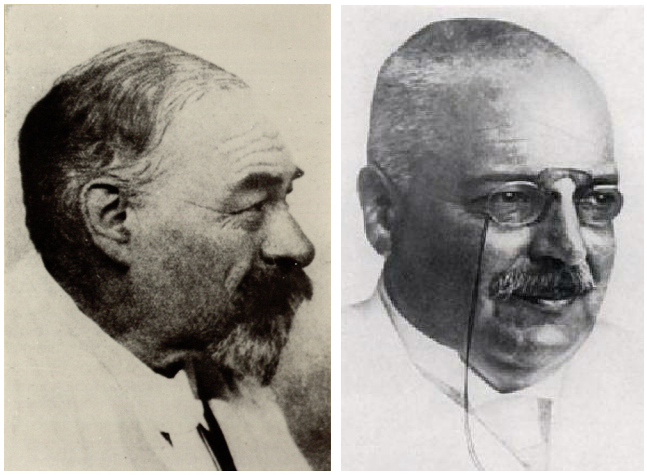


Figure 6. The two figures who invited the young Achúcarro to participate in their magnum opus *Histologic and histopathologic studies of the cerebral cortex*. Left: Franz Nissl (1860-1919); right: Alois Alzheimer (1864-1915).

left the Frankfurt Municipal Mental Asylum, where he had worked for 15 years, and travelled to work with Kraepelin in Munich, where he became the director of the neuropathology laboratory at the Nervenklinik, the brand new institute.[41]

Nissl and Alzheimer first met in December 1888, when Alzheimer was working as Nissl's assistant at the Stätischen Heilanstalt für Irre und Epileptische (Municipal Asylum for the Insane and Epileptics) in Frankfurt am Main, where they worked together for seven years. The two became good friends but had to part ways in 1895 when Nissl decided to move to Heidelberg to work with Kraepelin, leaving Alzheimer in charge of the Asylum (Figure 6).[42,43] Nissl had trained Alzheimer in the pathological study of the brain and the application of different histopathological techniques, using an eosin and methylene blue staining method to observe Negri bodies and rod-like cells in rabies, as well as assisting Brodmann in his important work on the cytoarchitecture of the brain.[44]

Despite his talent as a writer and his indisputable intelligence, Kraepelin was tyrannical and conceited, and had little empathy with his patients. The atmosphere at the institute was far from relaxed: "[assistants were] more or less invisible […] almost totally subordinates, and although they supported and carried out the research of their chiefs […], they received little or no credit for their efforts. […] Almost never did their names appear on the publications, nor did they participate in the presentations. The director was usually the sole visible entity."[39,45] Alzheimer could not have been more different. Those who knew him emphasised his creative, affable, optimistic, caring, and fun-loving nature,[42] as compared to the cold, distant Pierre Marie, whom Achúcarro had met in Paris. These circumstances meant that Alzheimer's laboratory received scholars from around the world (Figure 7), regardless of their origin, colour, or creed. Solomon Carter Fuller (1872-1953), born in Monrovia and a pioneer of neuroscience in the United States, is a representative case.[45] He performed a literature review on neurofibrillary tangles in 1912, using images that may have been provided by Alzheimer.[42,46]

1906-1914: Achúcarro's histopathological contributions on rabies

The eosinophilic bodies observed in rabies encephalitis were described in 1903 by the Italian researcher Adelchi Negri (1875-1912), a disciple of Camillo Golgi.[47] These formations, which particularly affect the cornu ammonis of the hippocampus and are considered pathognomonic, are round in shape, and occur inside large neurons which are otherwise surprisingly well preserved (Figures 8 and 9). Today, they are studied using specific fluorescent antibodies.[48] Negri believed them to be protozoa, showing different stages of differentiation and even spores; this theory was defended for a long time.[49] Unlike such species as dogs, rabbits do not present abundant Negri bodies,[50] although Achúcarro had access to brain specimens from various animal species and from a young man who died due to rabies. It was in the human specimen that he described abundant Negri bodies in Purkinje cells, ruling out their supposedly parasitic nature. The structure of Negri bodies is related to the synthesis and aggregation of viral, genomic, and antigenomic RNA, a "virus factory" inside neurons allowing replication of the pathogen without triggering an immune response.[51,52] Hypertrophy of neurofibrils is associated with increased expression of two proteins of the cytoskeleton: MAP2 and high molecular weight neurofilament.

Achúcarro ruled out that infiltrates from proinflammatory perivascular cells may play a role in viral entry, the theory

Figure 7. Alois Alzheimer with his group of collaborators in 1909-1910. Achúcarro, beside him, is easily recognisable.

accepted at the time. After observing that an inoculated hen survived 42 days with massive perivascular infiltrates, he deduced that inflammatory infiltrates may be related with the duration of the process. "Babes nodules," considered by the Romanian physician and biologist Victor Babeș (1854-1926) to be pathognomonic in rabies,[53] are now interpreted as microglial nodules. Achúcarro erroneously deduced that they were clusters of nucleoli from degenerated cells, possibly due to the small size and rounded shape of normal microglia.

Cajal had described "Alzheimer lesions," thickening of the reticular apparatus with marked argyrophilia, in experimental rabies in rabbits.[30,31] Achúcarro demonstrated these neurofibrillary changes in early stages of the process, with sinuous thickening of the endoplasmic reticulum, particularly in the apical part of pyramidal cells in the cornu ammonis. He rejected the hypothesis that they appeared exclusively in rabies encephalitis, as Francisco Tello had demonstrated in hibernating reptiles.[54]

The stratum oriens, the area showing the most intense glial proliferation, is the layer of the cornu ammonis displaying the most pronounced disintegration of pyramidal cells. Some glial cells, unlike ganglion cells, become laden with fat as they move toward the adventitia of blood vessels. Achúcarro proposed a novel hypothesis, according to which the function of certain glial formations was to move toward and phagocytise the products of tissue disintegration, thereby transforming into granulo-adipose cells.

The role of glia in the phenomenon of neuronophagia had previously been suggested by Marinesco, in 1905, and Cerletti, in 1907. Achúcarro hypothesised that glial cells are occasionally observed moving toward the soma of ganglion cells, adjacent to their membrane, and even inside them. Always a prudent thinker, he did not dare to confirm their apparent role in neuronophagia.

Regarding *Stäbchenzellen*, or rod-like cells, he refuted the theory of Nissl and Alzheimer about their genesis

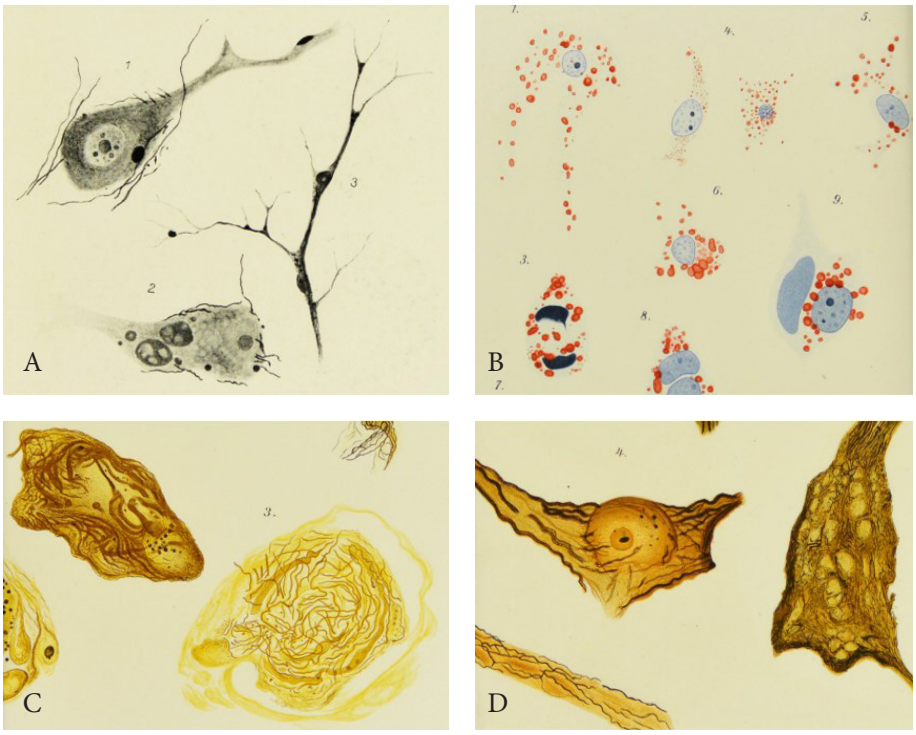


Figure 8. A) Morphological varieties of Negri bodies (sheet XIII, fig. 8). B) Fat-laden glial cells in different stages (sheet IX, fig. 1-20). C) Cajal's silver staining method. Ganglion cells in the Gasserian ganglion, with intense impregnation of neurofibrillary changes (sheet X, fig. 1 and 2). D) Cajal's silver staining method. Neurofibrillary changes of differing intensity in a rabbit that died due to rabies (sheet XI).

in connective cells of the vascular adventitia,[19,36] initially favouring Cerletti's glial theory. Rod-like cells are elongated, sometimes fusiform elements that infiltrate the interstitial space between degenerated pyramidal cells in the stratum radiatum (Figure 10). In this region, both cellular elements maintain a perpendicular position with respect to the cortex of the cornu ammonis. This is not the case in the stratum moleculare, the location of the dendritic tree of pyramidal cells, despite the intense proliferation of neuroglia, as Achúcarro demonstrated in the case of the patient with rabies. Achúcarro considered rabies to attack first the nuclei of ganglion cells (neurons), and particularly the pyramidal cells of the cornu ammonis. Overall, the precise location of the cortical lesion affects the morphology of the glia proliferating at its borders. Thus, in the stratum radiatum, where approximately ten pyramidal cells running in parallel form a dense mesh, glial cells become elongated to occupy the spaces left by degenerated cells. On the contrary, in the stratum ambiens, which lacks this characteristic mesh, the same cells form star or globular shapes (Figure 11).[15-17]

Cajal's "third element"

Achúcarro soon recognised the need for a staining method capable of distinguishing between the different types of glia. This was the path that led him to discover the tannin and ammoniacal silver staining method.[55] The method enabled him to distinguish hypertrophic astrocytes containing modified fibrils (gliofibrils) from the large "sucker feet" adhered to the vascular adventitia. These were somewhat different than the rod-like cells (microglial elements of mesodermic origin), which were able to maintain their shape in the stratum pyramidale, lacked gliofibrils, and had no connection with blood vessels.

In Achúcarro's time, astrocytes were known simply as "neuroglia." Years later, Cajal referred to an undefined

"third element," apolar cells, which were distinct from neurons and astrocytic glia, and could only be stained with his formalin-uranium nitrate method,[56,57] one of his alleged "errors."[58] This question was not clarified until Pío del Río Hortega developed his silver carbonate method. He denied the existence of "apolar cells" because the "third element," oligodendroglia, showed subtle protoplasmic arms.[59] Del Río Hortega had been appointed to the histopathology department of Hospital Provincial de Madrid, and after the death of Achúcarro became director of the Laboratory of Normal and Pathological Histology at the Residencia de Estudiantes.[60] The tranquillity of the laboratory at Dr Velasco's anthropological museum was disrupted when del Río Hortega showed him his conclusions. Cajal was displeased. They exchanged strongly-worded letters and their friendship was broken.[57,61]

In a regrettable episode for the Spanish Neurohistological School, del Río Hortega was forced to leave the laboratory.[57] In reality, the "third element" was not one but rather two distinct classes of cells: oligodendroglia in the white matter, and microglia in the cerebral cortex.

The microglia is currently considered to consist of mononuclear phagocytes intrinsic to the nervous system, involved in the first line of defence, as well as having a role in immune regulation. These cells are responsible for neuronophagia, a feature used to diagnose neurodegenerative processes, and their morphology adapts according to local anatomical conditions. The Spanish Neurohistological School, including Achúcarro and del Río Hortega, made essential contributions to the understanding of the microglia.[62]

Achúcarro, clinician and neuropathologist

Too little emphasis may have been placed on Achúcarro's calling to work as a clinician, both in neurology and psychiatry, and at the same time as a neuropathologist. Even before Achúcarro completed his studies, the chair Juan Madinaveitia entrusted him to tutor his peers, leading seminars on neurological examination.[63] Javier Martín Artajo recalled how, as a child, "his uncle Nicolás would auscultate him at a neurology and psychiatry consultation he had installed with great enthusiasm on the sunny side of calle Lista" (today, calle Ortega y Gasset).[64,65] He achieved a degree of fame as a clinician; for instance, when Cajal became desperate due to intractable insomnia and unbearable headaches, he decided to consult "the friendly and wise Dr Achúcarro," already a prestigious neurologist.[66]

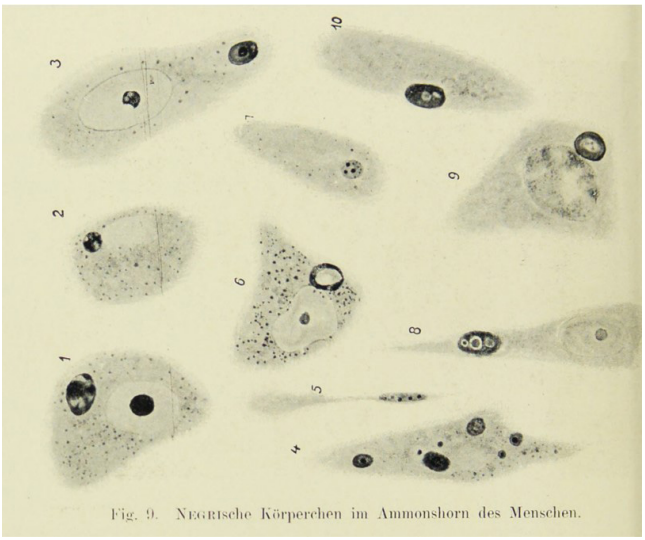


Figure 9. Negri bodies in the human cornu ammonis (sheet XIV, fig. 9).

Having surpassed the notorious public service entry examination, he was appointed in 1912 to a ward at the Hospital Provincial de Madrid as a *médico de número*; the title did not denote specialism in any particular area of medicine, although some flexibility was allowed in this regard. Alzheimer had examined the famous Auguste Deter in 1901, and in 1906 he received her brain from the hospital where he had been working.[35] Achúcarro may have thought that he could follow in his master's footsteps, combining clinical neuropsychiatry with neuropathology. But Spain was not Germany, and Achúcarro received a severe official warning from the director of the Hospital Provincial, which has not previously been published[a]:

> Mr Serra requested that inspectors visiting the Hospital send him details of the number of patients attended at Dr Achúcarro's ward and the types of diseases they presented, in order to determine

[a] Boletín Oficial de la Provincia de Madrid, Thursday 1 January 1916. Session of the 14 May 1915 (p. 2).

[b] Boletín Oficial de la Provincia de Madrid, Thursday 23 February 1916. Session of the 19 June 1915.

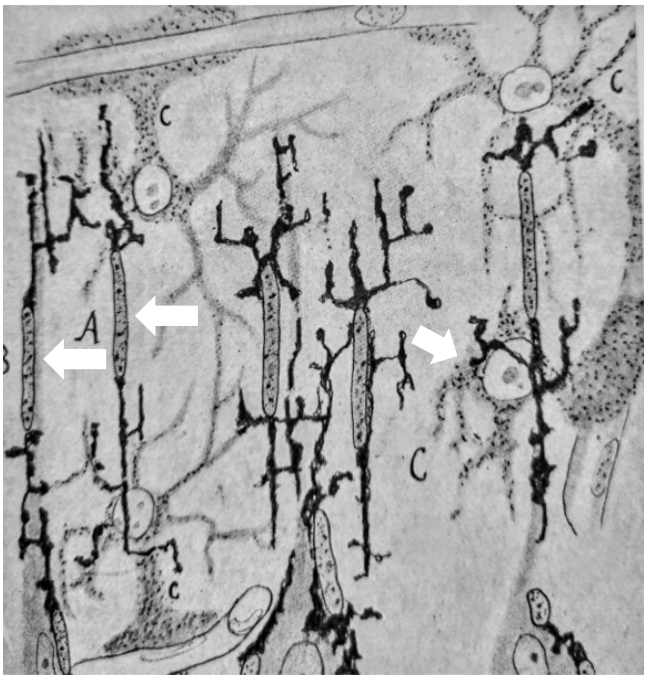


Figure 10. Semi-schematic drawing by Achúcarro showing, to the left, rod-like cells (long arrows), of elongated morphology, running parallel to the mesh of pyramidal cells of the cornu ammonis. To the right, a large astrocyte (short arrow) projects a "sucker foot" around the adventitial layer of a vessel (Achúcarro and Gayarre, 1914).[76]

> whether the ward, which was intended only to receive acute patients, was in fact receiving patients with diseases of the nervous system, some of whom were demented and had caused disturbances on the ward, sometimes even leading to fears that some misfortune may occur.

Essentially, the problem today would be mean duration of hospitalisation and the admission of neurological patients instead of general medical emergencies.

The response took some time, probably due to the checks that had to be made by hospital inspectors.[b]

> According to Mr Prida, after a detailed inspection, "the majority were admitted this year, but three were admitted in 1913." While he agreed that "stays should not have been so long," he asserted by way of explanation that "the disease prevents their discharge and they do not have families." Serra responded with a stern warning, reminding the neuropsychiatrist that "the ward is simply another medical ward for acute patients, not for nervous diseases, whereas some patients have been diagnosed with dementia."

Achúcarro is known to have "established a neurology clinic in his small service,"[8] which Dionisio Nieto recalls being on the ground floor of the Hospital Provincial de Madrid.[67] It was not meant to be: the obstinate, disciplinarian administration was unable to recognise the merits with which Achúcarro, now an expert neuropsychiatrist who was also able to apply the histopathological techniques of Cajal, Nissl, and Alzheimer, had triumphed in Germany and the United States. Ramón y Cajal[68] listed some of Achúcarro's neuropathological research in his eulogy to his disciple: rabies, Alzheimer disease, general paresis, brain softening, alcoholism, chorea, early-onset and senile dementia, glioma and neuroglioma, and lathyrism, among other conditions.

Achúcarro's first experience of neuropathology was at the neurology clinic of Pierre Marie, at the Hôpital Bicêtre in Paris. It took some time before he had the honour of being received by the director, but the interview was far from satisfactory. Pierre Marie was not an easy man to be around[69,70]: "Somewhat formal and cold […], strong, authoritarian, and independent." He warned the Spaniard, who was frustrated by a lack of resources, "that his work should be serious and continuous." However, he was not completely cold: in July 1905, he facilitated a communication by Achúcarro to the Société Française de Neurologie, reporting an anatomical-clinical case of supposedly post-traumatic syringomyelia.[71,72] Achúcarro made good friends in Paris, for example with the Italian physician Catola, with whom he began a study into amyloid bodies at the Florence Psychiatric Clinic, and the German Lewandowsky, who did not hesitate to recommend him at the Munich Psychiatric Clinic.[73]

However, it was Alois Alzheimer himself who most supported Achúcarro as a neuropathologist, recommending him to the director of the St. Elizabeths Hospital in Washington, D.C., to work as director of the new histopathology laboratory of the former asylum.[74] In a significant commitment, he gave a lecture to the centre's 34 psychiatrists, many of whom were self-declared psychoanalysts, such as superintendent Williams A. White and the centre's official pathologist, Dr Blackburn. The Spanish pathologist warned that they should not expect histopathology to resolve the essence of mental illness, but promised that it was a science with practical applications, such as differentiating general paresis from other syphilitic processes, or senile psychosis from cerebral arteriosclerosis.

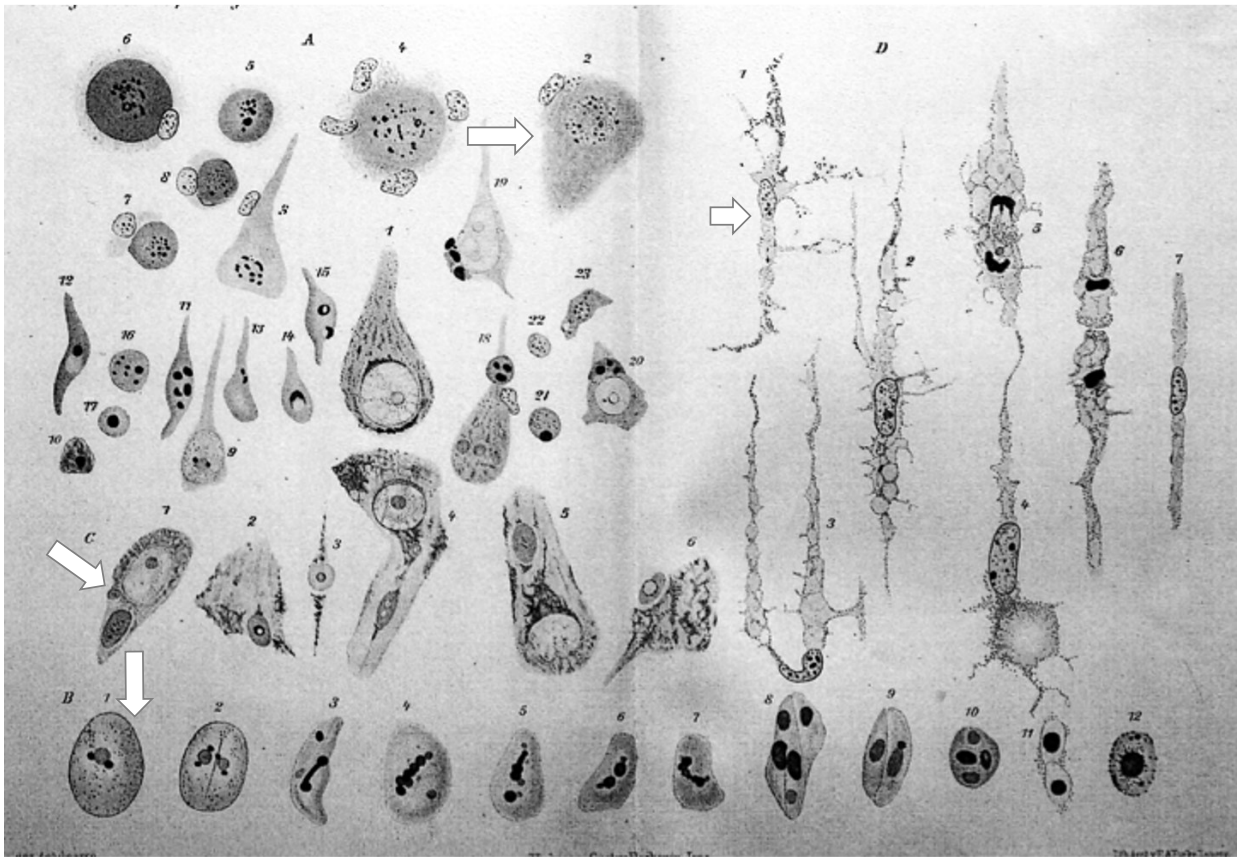


Figure 11. Sheet VIII, panoramic view. A, 2 (horizontal arrow). Rabbit with rabies. Degenerative changes in a ganglion cell: loss of Nissl substance, fragmentation of the nucleus, partially visible cell membrane. B, 1-12 (vertical arrow). Rabbit with rabies. Different stages of degeneration of glial cells in the cornu ammonis. C (diagonal arrow). Rabid dog. Cornu ammonis. Negri bodies in a ganglion cell. D (short horizontal arrow). Rabbit with rabies. Rod-like cells; to the right (3), a pyramidal cell. Colour versions of the images and other details may be consulted in the supplementary material.

Final remarks

We may ask a final, perhaps unanswerable, question: what motivated Achúcarro to publish the monograph as a thesis in 1914, eight years after he completed his doctorate? Achúcarro died four years later and it was known "that he had been ill for some time" (Gregorio Marañón, *El Liberal*, 25 April 1918). A year before, in March 1913, for unknown reasons, he declined an invitation from the renowned psychiatrist Adolf Meyer to participate in the inauguration of the Henry Phipps Psychiatric Clinic in Baltimore, which would have been his third trip to the United States.[75] He complained of "abdominal discomfort and tension,"[55] which he reiterated in the letters he sent from the United States to his mother, Juana Lund Ugarte ("my guts," he joked).[5] The answer to the question is unclear, but it is possible that, concerned by the course of his disease, he may have decided in 1914 that his legacy should be an update of what he considered to be his most important contribution to histopathology: a compendium of the work he started in Munich alongside his good-natured master Alzheimer.

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Supplementary material

1. Spanish translation of the chapter by Nicolás Achúcarro: "Zur Kenntnis der pathologischen Histologie des Zentralnervensystem bei Tollwut" ["On the understanding of the central nervous system histological pathology in rabies"]. In: Histologische und histopathologische Arbeiten über die Grosshirnrinde. F. Nissl and A. Alzheimer (eds). Jena: Gustav Fischer; 1909. Vol. 3. p. 143-194.

2. Front page, contents, and original German-language text by Nicolás Achúcarro from an edition from 1910: Histologische und histopathologische Arbeiten über die Grosshirnrinde mit besonderer Berücksichtigung der pathologischen Anatomie der Geisteskrankheiten. Herausgegeben von Franz Nissl und Alois Alzheimer. Jena: Verlag von Gustav Fischer; 1910. Electronic copy available from: https://archive.org.

3. Original prints from the 1910 edition. The prints and figures are explained at the end of Supplementary Material 1, after the bibliography, following the distribution of text and imagery in the original work.

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