Revista de la Facultad de Medicina
ISSN: 2357-3848
ISSN: 0120-0011
Universidad Nacional de Colombia

Barco-Ríos, John; Duque-Parra, Jorge Eduardo; Barco-Cano, Johanna Alexandra
From substance fermentation to action potential in modern science (part two)
Revista de la Facultad de Medicina, vol. 66, no. 4, 2018, October-December, pp. 623-627
Universidad Nacional de Colombia

DOI: 10.15446/revfacmed.v66n4.65552

REFLECTION PAPER

DOI: http://dx.doi.org/10.15446/revfacmed.v66n4.65552

From substance fermentation to action potential in modern science (part two)

De la fermentación de sustancias al potencial de acción en la ciencia moderna (segunda parte)

Received: 08/06/2017. **Accepted**: 12/12/2017.

John Barco-Ríos[1,2] • Jorge Eduardo Duque-Parra[1,2] • Johanna Alexandra Barco-Cano[2,3]

[1] Universidad de Caldas - Faculty of Health Sciences - Department of Basic Sciences - Manizales - Colombia.
[2] Universidad de Caldas - Department of Basic Sciences - Caldas Neuroscience Group - Manizales - Colombia.
[3] Universidad de Caldas - Faculty of Health Sciences - Clinical Department - Manizales - Colombia.

Corresponding author: John Barco-Ríos. Department of Basic Sciences, Faculty of Health Sciences, Universidad de Caldas. Calle 65 No. 26-10, office: M203. Telephone number: +57 6 8781500. Manizales. Colombia. Email: jhon.barco@ucaldas.edu.co.

| Abstract |

Introduction: After conducting a bibliographical review on the works of various researchers at different times to explain the phenomenon of the transmission of nerve impulses, it is observed that since the eighteenth century, when modern science was born, scientific knowledge in the field of physiology had an accelerated development following the creation of new research techniques and the application of the scientific method. Thus, the philosophical theory of "animal spirits" led to the current concept of action potential, understood as a merely electrochemical phenomenon.

Discussion: The establishment of the scientific method and the development of new research techniques led several researchers at different times to unravel the molecular mechanisms involved in the transmission of nerve impulses, which took two and a half centuries to reach the current concept about the origin of action potential.

Conclusion: The notion "animal spirits" was valid for many centuries, while modern science took a little more than two centuries to understand the phenomenon of nerve impulse transmission.

Keywords: History; Physiology; Action Potentials; Science (MeSH).

| Resumen |

Introducción. Después de una revisión bibliográfica sobre los trabajos de diversos investigadores en distintas épocas para explicar el fenómeno de la transmisión nerviosa, se observa que a partir del siglo XVIII, cuando surge la ciencia moderna, el conocimiento científico en el campo de la fisiología tuvo un desarrollo acelerado por la creación de nuevas técnicas de investigación y la aplicación del método científico. Así, de la teoría filosófica de los "espíritus animales" se llegó al concepto actual del potencial de acción, entendiéndose este como un fenómeno meramente electroquímico.

Discusión. Con el establecimiento del método científico y el desarrollo de nuevas técnicas para la investigación, diversos investigadores en distintas épocas fueron desentrañando los mecanismos moleculares implicados en la transmisión de los impulsos nerviosos, por lo que solo bastaron dos siglos y medio para llegar al concepto actual sobre el origen del potencial de acción.

Conclusión. La teoría filosófica de los espíritus animales perduró por muchos siglos, mientras que a la ciencia moderna le tomó poco más de dos siglos para entender el fenómeno de la transmisión nerviosa.

Palabras clave: Historia; Fisiología; Potenciales de acción; Ciencia (DeCS).

Barco-Ríos J, Duque-Parra JE, Barco-Cano JA. From substance fermentation to action potential in modern science (part two). Rev. Fac. Med. 2018;66(4):623-7. English. doi: http://dx.doi.org/10.15446/revfacmed.v66n4.65552.

Barco-Ríos J, Duque-Parra JE, Barco-Cano JA. [De la fermentación de sustancias al potencial de acción en la ciencia moderna (segunda parte)]. Rev. Fac. Med. 2018;66(4):623-7. English. doi: http://dx.doi.org/10.15446/revfacmed.v66n4.65552.

Introduction

Before the birth of modern science, the concepts issued by researchers were accepted almost as dogmas, without being proven by other researchers. Following the scientific revolution, knowledge began to acquire a new dimension, slowly detaching itself from religious influence, leading to dispute and question the concept of animal spirits that controlled our nervous system.

Modern science, an era for reason

The first part of this article, entitled *From animal spirits to scientific revolution in Medicine (first part)*, published in volume 66 issue 2 of this journal, presented a historical review of the way how the functioning of the nervous system based on animal spirits was formerly conceived. This idea prevailed until the seventeenth century, during the scientific revolution, when Giovanni Alfonso Borelli demonstrated that such spirits did not exist. (1)

This literature review of the period between the eighteenth century, when modern science was born, and the first half of the twentieth century, when the phenomenon of nerve impulse transmission was clarified, presents the most outstanding characters and events that prompted knowledge about the way how nerve information is transmitted through neurons and, consequently, the physiology of the nervous system.

Revolution of thought

Although many historians state that the scientific revolution began during the Renaissance, modern science took on its avant-garde role and detached itself from any religious influence only until the eighteenth century. Since then, science developed a way of interpreting the reality that is attempted to be demonstrated by means of the verification of observed facts or data, that is to say, applying the scientific method proposed by Descartes.

Until the eighteenth century, two hypotheses had been proposed to explain that the brain was the place where consciousness, sensation and understanding were located (2), and that many bodily structures were controlled through nerves. The first hypothesis emerged in the sixth century BC and prevailed until the first half of the seventeenth century; it proposed the presence of "animal spirits" that were transported by nerves to make organs work. The second hypothesis, raised in the second half of the seventeenth century, based on the experimental work of Giovanni Alfonso Borelli, an advocate of the scientific method, showed that animal spirits were not transported by the nerves and proposed that muscles contract due to the fermentation of chemical substances. (3)

At the dawn of modern science, a third hypothesis arose to explain the phenomenon of nerve conduction. In 1713, the physicist, mathematician and alchemist Sir Isaac Newton (1642-1727) proposed that the animal spirits promulgated by Galen were actually "ethereal vibrations" that originated in the brain and ran through the nerves to reach the muscles to generate mechanical actions. (4)

In 1752, the Swiss physician and anatomist Albrecht von Haller (1708-1777), considered as the father of modern physiology, based on his experimental work on animals, concluded that only some parts of the body showed sensitivity and that it was a specific property of the nerves, while other parts showed irritability and responded to different stimuli, such as electricity, by contracting; this property is exclusive to the muscles. (5,6) In 1756, the Italian anatomist Leopoldo Caldani (1725-1813), captivated by Haller's work, designed an experiment to verify this theory, for which he used an electric current obtained from a Leyden jar (a device used to store electric charge) for the first time to stimulate muscle tissue in experimental animals, proving that the muscles reacted by contracting. (7) Therefore, the thought that still prevailed over the presence of spirits transported by the nerves began to change to give way to the idea of "electric fluid", whose flow could be controlled by the power of the mind. At this point, the fourth hypothesis arose, which states that nerves transmit electricity.

Animal electricity

Luigi Galvani (1737-1798), Italian doctor and disciple of Caldani, was also interested in the phenomenon of electricity and showed that applying a small electric current on the spinal cord of a dead frog generated abrupt muscle contractions in its limbs. In 1780, Galvani concluded that it was the result of a phenomenon he called "animal electricity" and that the electricity necessary to cause the contractions did not come from the outside but from the inside of the living organism (8,9), apparently from the brain. He also inferred that, after death, the nerves could still retain the ability to drive the electrical impulse and transfer it to the muscle fibers to react to it. (8)

Years later, in 1841, the German physician and physiologist Emil du Bois-Reymond (1818-1896), at the request of his professor Peter Johannes Müller (1801-1858), who was not interested in the field of electricity, confirmed and expanded the findings reported by Matteucci in 1840 on the existence of an electric current that appeared between a damaged segment and another intact part of a muscle. In fact, du Bois-Reymond proved the existence of a current in the injured muscle which he called Muskelnstrom and observed, furthermore, that the amplitude of said current decreased by stimulating the nerve; he called this "negative variation". (10,11) Later, the researcher attached the electrodes of a galvanometer to a nerve and saw the same phenomenon. These works allowed establishing the basic principles of nerve impulse.

In 1850, the German physician and physicist Hermann Ludwig Ferdinand von Helmholtz (1821-1894), who was interested in nerve transmission, which was thought to be impossible to estimate at that time because of the speed at which it was transmitted, designed an experiment to measure said speed. For this purpose, he used the newly dissected sciatic nerve of a frog and the corresponding muscle it innervated, and coupled a clock that started when the nervous stimulus appeared and stopped at the moment of the contraction. After several measurements at different temperatures, Helmholtz was able to calculate that the speed was 27-30 m/s. (8,12) Then, he made some measurements on human subjects and found that the driving speed was much faster, about 60 m/s. (13)

Wilhelm Friedrich Kühne (1837-1900), a German physiologist, used fixation and staining and, in the 1870s, described that nerve endings reached a small formation on the muscle membrane, calling this entire structure "neuromuscular junction". (14) Kühne proposed that the current produced by the nervous impulse excited the muscle fibers in this junction. A few years later, Du Bois-Reymond suggested that the nervous transmission could be of chemical nature, where nerve endings could secrete some chemical agent that excited the muscle causing its contraction. (15)

At that time, the relationship between nerve fibers and nerve cells was not clear, as it was believed that they were two distinct anatomical entities. However, Jan Evangelista Purkinje (1787-1869), a Czech anatomist and physiology professor, and Gabriel Valentin (1810-1883), a German physician and physiologist, believed that these two nervous elements were fundamental in the organization of the central nervous system and that they could be associated, but without an apparent physical connection. (16)

Later, Robert Remak (1815-1865), a Polish embryologist and physiologist, showed that nerve cells were connected to the fibers and that they were extensions of the cell body (17); he further proposed that such cells provide the energy necessary for the transmission of the nervous impulse. But it was Louis Antoine Ranvier (1835-1922), a French histologist, who in 1875 demonstrated the anatomical connection between nerve cells and T-shaped fibers in the dorsal roots of the spinal ganglia. Also, Ranvier explained that one of the two branches of the fiber was directed towards the spinal cord and the other towards the periphery. (18) However, many physiologists of the time still believed that nerve cells were of little importance for the conduction of nerve impulses, including the neurophysiologist Augustus Volney Waller (1816-1870); moreover, such cells were believed to be passive repeating stations.

In 1860, the German neuroanatomist Otto Friedrich Karl Deiters (1834-1863) developed a microdissection technique to isolate nerve cells under the microscope. This researcher was able to obtain clear images of these cells and found that they had two different types of branching processes connected to their body: one, a tree type, with thin and short branches which he called “protoplasmic processes” —later called dendrites by Wilhelm His (1831-1904) in 1889—, and a long fiber with a smaller number of branches that he called “axis cylinder” —later named axon by Rudolph Albert von Kölliker (1817-1905) in in 1891. (19)

A characteristic of nerve physiology that drew the attention of researchers in the late nineteenth century was the relationship between the intensity of stimulation of multiple nerve fibers and the possible responses of the electric potential. In 1871, Henry Bowditch (1840-1911), an American physician and physiologist, demonstrated that a stimulus may or may not cause muscle contraction, and that it depends on the threshold potential of the stimulus applied. This is considered to be the first demonstration of the all-or-none law. (20)

Neural doctrine

By the end of the nineteenth century, it was known that the nerve was made up of multiple fibers. With the establishment of the neuron doctrine, it was understood that the nerve impulse travelled from one nerve cell to another through the axon and that this potential for action occurred quickly and was, perhaps, of the all-or-none type. Meanwhile, since 1888, the Spanish histologist Santiago Felipe Ramón y Cajal (1852-1934) devoted himself to conduct a detailed study of the cellular architecture of a large part of the nervous system, including all its connections, in which he identified the dendritic spines and suggested that they could be involved in learning and memory processes. (21) For this, he used the histological staining technique developed by Camilo Golgi (1842-1926), which consisted in treating the sample with silver solution to impregnate the neurons and visualize them under the microscope; however, only a few cells were stained due to the presence of myelin. Cajal used the same technique to prepare his samples, but he made modifications to the staining method and applied it to cuts of young brains that did not have yet abundant myelin in their structure. The result was surprising, because he managed to see clearly the morphology of nerve cells (22) and then made his famous drawings. The German pathologist Heinrich Wilhelm Gottfried von Waldeyer (1836-1921) coined the term “neurons” in 1891 to name these cells, and also formulated the hypothesis that neurons are the basic structural units of the nervous system, a hypothesis that was demonstrated shortly after by Cajal.

Cajal also developed the neuron doctrine, which stated that neurons are discrete entities that are genetically, morphologically and physiologically independent, and that are also able to communicate with each other, without forming a diffuse reticulum. (23) These postulates went against what many historians of his time thought, among them Camilo Golgi, who defended at all costs the reticular theory, proposed in 1858 by the German anatomist Joseph von Gerlach (1820-1896), who stated that nerve cells were not separate but connected forming a continuous network. (24)

Nevertheless, recent studies have shown that large groups of neurons that establish extensive networks connected by electrical synapses to process neural information can be found in certain parts of the brain, and that such synapses are more common than previously thought. Cajal also observed that dilations were formed in the terminal end of stained axons, which were later called synaptic boutons; in addition, he proposed the law of dynamic polarization to explain that nerve impulse is transmitted unidirectionally from the dendrites to the end of the axon. (25,26) Therefore, Cajal established the basic principles of neuron and nervous tissue functioning, for which he was awarded, along with Camilo Golgi, the Nobel Prize in Medicine in 1906.

Consistent with neuron doctrine, the concept of “histological continuity” with “functional continuity” was established. To name these “contacts” that form between neurons, the English Arthur Woollgar Verral (1851-1912) proposed the name “synapse”, a word of Greek origin meaning “conjunction”. That name was accepted and introduced by Charles Scott Sherrington (1857-1952) in 1897. The concept of synapsis allowed explaining the phenomenon of delay in nerve impulse conduction, which varies in duration between 0.3 and 1 millisecond. Sherrington further observed that the histological substrate for the integrative action of the nervous system was related to multiple synaptic interconnections. (27)

Although electrical theory was used for a long time as an explanation to support the conduction of action potentials by nerves to the muscles, the work done by the German doctor and pharmacologist Otto Loewi (1873-1961) led the idea towards a biochemical explanation. In fact, in 1921, Loewi designed an experiment that occurred to him, in his own words, while sleeping. He dissected two frog hearts, leaving the vagus nerve in one of them, and immersed them separately in saline solution so that they continued beating for a while. Then, he stimulated the vagus nerve in one of the hearts and saw that the heartbeat slowed down. Finally, he took the saline solution in which the heart had been immersed and applied it to the second heart, noting that it also reduced its heart rate. Loewi concluded that the vagus nerve should have released a chemical at the level of the parasympathetic synapse, which he called vagus substance, and which caused the same response in the second heart.

Five years later, Loewi and his collaborator, E. Navratil, proved that the vagal substance was acetylcholine (28), thus becoming the first neurotransmitter identified; they also demonstrated that this substance was rapidly degraded by the enzyme cholinesterase and concluded that the transmission of nerve impulses was of a neurohumoral type. Loewi’s works were widely known and he was awarded the Nobel Prize in Medicine in 1936, which he shared with Henry Hallett Dale (1875-1968), discoverer of acetylcholine in 1913.

Joseph Erlanger (1874-1965), American chemist and physician, and Herbert Spencer Gasser (1888-1963), American physiologist, designed an experiment in which they adapted a cathode ray oscilloscope to amplify the electrical potentials of nerves. These researchers obtained for the first time an exact image of action potentials and discovered that these potentials were formed by waves that moved at different speeds along nerve fibers and that the speed varied in direct proportion to the diameter of the fiber. (29) This way, they established a classification of the fibers as a function of speed: Group A fibers (motor and some sensory), Group B fibers (visceral sensory) and Group C fibers (unmyelinated). They also demonstrated that different nerve fibers can perform different functions. For this work they were awarded the Nobel Prize in Medicine in 1944.

Interested in the electrochemical mechanisms of synaptic transmission, Bernard Katz (1911-2003) and Paul Fatt (1924-2004) proposed the quantal hypothesis of neurotransmitter release of acetylcholine in the motor end plate, which means that acetylcholine is not released continuously by nerve terminals but in small amounts or quanta, where each quantum causes a small signal in the muscle fiber. They also said that acetylcholine binds to specific membrane receptors that act as ionic channels through which ions flow to create an electric current. (30) For his work in nerve physiology and biochemistry, Katz was awarded the 1970 Nobel Prize in Medicine, which he shared with Julius Axelrod (1912-2004) and Ulf von Euler (1905-1983), who also worked on the chemical transmission of nerve endings and the mechanism of storage and inactivation of neurotransmitters.

The invention of the electron microscope, designed by Max Knoll (1897-1969) and Ernst Ruska (1906-1988) in 1931, allowed cell biology to enter a period of intense research activity, which began by interpreting what all those blurred spots that showed the first images obtained meant. With the improvement of the techniques for the preparation of samples that allowed obtaining images of greater quality, the puzzle of that marvelous ultrastructure that shapes the cells was put together.

The American neuroscientist Sanford Louis Palay (1918-2002), using an electron microscope, took on the task of unraveling the ultra-structural details of the synapse in the central nervous system. In 1953, he expanded this knowledge by demonstrating with his images that there is a gap (the synaptic cleft) between the pre- and postsynaptic cells. This definitively validated Cajal's neuron doctrine. (17) In 1954, Palay and his colleague George Emil Palade (1912-2008), a Romanian physician, reported the presence of mitochondria and membranous vesicles in nerve terminals.

In 1947, the Argentine physician Eduardo De Robertis (1913-1988), who was also interested in understanding neuronal ultra-structure by means of electron microscopy, observed for the first time the presence of microtubules inside axons devoid of myelin. But it was until 1954 when he made a momentous discovery: while working in a team with George Bennett, he saw membranous spheres inside the presynaptic terminals, similar to what Palay and Palade saw, and named them synaptic vesicles. De Robertis and Bennett suggested that such vesicles were involved in the storage of neurotransmitters and their transport to the presynaptic membrane. (31)

Ionic flow, the explanation of the problem

As progress was made on the knowledge of the microscopic anatomy of the nervous system, other researchers from different latitudes were striving to unravel the physiological mechanisms involved in the transmission of nerve impulses. In 1952, Alan Lloyd Hodgkin (1914-1998) and Andrew Fielding Huxley (1917-2012), British physiologists and biophysicists, proposed a mathematical model to explain how nerve impulses start and propagate in neurons. This model consists of a series of non-linear differential equations that explain the ionic mechanisms involved in the origin and propagation of action potentials. The researchers used the giant axon of squids as an experimental model, which allowed them to use the voltage clamp technique (32), due to its size, to record the internal ionic currents by means of electrodes.

Thus, according to Hodgkin and Huxley, nerve impulse consists of a rapid and coordinated sodium ion influx and the subsequent potassium ions exit through the membrane of the excitable cells. (33) Thanks to this important work, the British scientists were awarded the Nobel Prize in Medicine in 1963, which they shared with the Australian neurophysiologist John Carew Eccles (1903-1997) for his research on the ionic mechanisms of excitation and inhibition of synapses.

In 1949, Eccles thought that the transmission of nerve impulses in the synapses was strictly electrical. To prove this, he designed an experiment where he took the knee stretching reflex as a model, since only two neurons, one sensitive and the other motor, are involved. In the early 1950s, Eccles realized that his initial assessment was wrong, corroborating what Loewi and Dale had discovered years earlier: when the nerve impulse reaches the end of an axon, a chemical that causes the nerve impulse to pass to the next neuron is released. However, Eccles went further and determined that neurotransmitters opened a channel that causes an influx of sodium ions in the postsynaptic membrane. (15,34)

The conclusions reached by Hodgkin and Huxley led them to hypothesize about the possible existence of ionic channels in the membranes of excitable cells, a fact that was confirmed a few decades later by the physicist and physician Erwin Neher (1944) and physician Bert Sakmann (1942), both of German origin, who developed the patch clamp technique that allows the measurement of the function of ion channels in cell membranes (35); for this work, they were awarded the Nobel Prize in Medicine in 1991.

Discussion

René Descartes (1596-1650), an outstanding French philosopher, mathematician and physicist, defined the rules of the method for "rightly conducting one's reason and of seeking truth in the sciences" (36) in his work Discourse on the Method. Consequently, since the seventeenth century, the implementation of the scientific method as a basic tool and path for the process of research allowed separating scientific knowledge from authority, dogmatic tradition and faith. In addition, the influence of subjectivity on a researcher's work was greatly minimized. Evidently, from then on, researchers in the field of medical sciences assumed an analytical-deductive reasoning in their observations and experiments, as was the case of Giovanni Alfonso Borelli. He was one of the advocates of the scientific method, whose reasoning, detached from any religious and dogmatic influence, led him to conclude that there were no animal spirits transporting themselves through the nerves to control body parts. (3)

After the establishment of modern science in the eighteenth century, scientific knowledge, particularly physiology, underwent an accelerated development driven by the creation of new laboratory tools and techniques, but also by more objective thinking, detached from religious authority and its inquisitorial apparatus. Speculative theories and the subordination of science to religious beliefs had been left behind, and now new knowledge had to be verified and validated by the scientific community. This objective reasoning was evident in the type of theories that have been postulated since then to try to explain the phenomenon of nerve impulse transmission, since they were more coherent with the reality of the moment and easier to measure and verify using the appropriate instrumentation.

Therefore, only two and a half centuries were necessary to unravel the molecular mechanisms involved in the transmission of nerve impulses through the nerves, while the philosophical theory about animal spirits that prevailed until that time was valid for about 23 centuries; it was almost a dogma.

Conclusions

A historical analysis of the evolution of human thought and the events involved in the establishment of a more objective truth makes evident that believing in myths and the fear of questioning paradigms cloud reason and slow down the normal rhythm of knowledge. However, with the development of new research techniques and with the support of the scientific method, researchers in the field of the physiology of the nervous system managed to determine, in a relatively short time, the molecular mechanisms that underlie nerve fibers for transmission of nerve impulses in the form of action potentials.

Conflicts of interest

None stated by the authors.

Funding

None stated by the authors.

Acknowledgements

None stated by the authors.

References

1. **Barco-Ríos J, Duque-Parra JE, Barco-Cano JA.** From animal spirits to scientific revolution in Medicine (first part). *Rev. Fac. Med.* 2018;66(2). http://doi.org/cs4c.
2. **Outes DL, Orlando JC.** Alcmeon de Crotona. El cerebro y las funciones psíquicas. *Rev Arg Clin Neuropsiq.* 2008;15(1):33-49.
3. **Pope MH.** Giovanni Alfonso Borelli-the father of biomechanics. *Spine.* 2005;30(20):2350-5. http://doi.org/b4x6d3.
4. **Henry J.** Isaac Newton: ciencia y religión en la unidad de su pensamiento. *Estud. Filos.* 2008;(38):69-101.
5. **Lain-Entralgo P.** Historia de la medicina moderna y contemporánea. Barcelona: Editorial Científico-Médica; 1954.
6. **Noguera-Paláu JJ.** Albrecht von Haller (Berna, 1708 - Berna, 1777). *Arch Soc Esp Oftalmol.* 2010;85(2):88-90.
7. **Ling GN.** The Origin of Cellular Electrical Potentials. In: Gutmann F, Keyzer H, Editors. Modern Bioelectrochemistry. New York: Plenum Press; 1986. p. 45-68.
8. **Ramón F, Hernández-Falcón J.** El potencial de acción. Historia de su descubrimiento y estudio. México D.F.: División de Posgrado e Investigación y Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México; 2005.
9. **de Micheli A.** En torno a los primeros estudios de electrofisiología. *Arch Cardiol Mex.* 2011;81(4):337-42.
10. **Pearce JMS.** Emil Heinrich Du Bois-Reymond (1818-96). *J Neurol Neurosurg Psychiatry.* 2001;71(5):620. http://doi.org/dr48sf.
11. **Mora-Nvaro OA, Mora-Castillo G.** Historia de la Fisiología: breve revisión con especial referencia a la circulación, respiración, sistema nervioso y glándulas endocrinas. Tenerife: Fundación Canaria Orotava de historia de la Ciencia; 2007.
12. **Sáiz M, coordinator.** Historia de la Psicología. Barcelona: Editorial UOC; 2009.
13. **Latorre R, López-Barneo J, Bezanilla F, Llinás R, editors.** Biofísica y Fisiología Celular. Sevilla: Universidad de Sevilla; 1996.
14. **López-Muñoz F, Álamo C.** Historia de la Psicofarmacología. Tomo I: De los orígenes de la medicina científica: sobre los pilares biológicos del nacimiento de la psicofarmacología. Barcelona: Médica Panamericana; 2005.
15. **Steinberg D.** De cómo el curare fue la clave para el descubrimiento de la transmisión neuromuscular. *Rev Soc Venez Hist Med.* 2010;59(1-2):19-36.
16. **Clarke E, Jacyna LS.** Nineteenth-century origins of neuroscientific concepts. Berkeley: University of California Press; 1987.
17. **López-Muñoz F, Álamo C, García-García P, Boya J.** Relevancia histórica de la teoría neuronal un siglo después del Nobel de Cajal: implicaciones psiquiátricas y psicofarmacológicas. *Psiq Biol.* 2006;13(5):167-82. http://doi.org/bmtwfn.
18. **Barbara JG.** Louis Ranvier (1835-1922): the contribution of microscopy to physiology and the renewal of french general anatomy. *J His Neurosci.* 2007;16:413-31. http://doi.org/dq4bhn.
19. **Finger S.** Origins of Neuroscience: A History of Explorations Into Brain Function. New York: Oxford University Press; 2001.
20. **Riascos-Beernal D, Baltaxe E, Pascual G.** La preparación de Langendorff: corazón de mamífero aislado perfundido. *Universitas Médica.* 2004;45(3):111-7.
21. **Miras-Portugal MT, Avila-de Grado J, Bautista JM.** Homenaje a Ramón y Cajal en el centenario de la concesión del Premio Nobel. *An.* R. *Acad. Nac. Farm.* 2006;72(4):643-59.
22. **Lopera MC.** La teoría neuronal: La tecnología como soporte de un debate científico. Un capítulo en la historia de la ciencia. *Trilogía. Ciencia, Tecnología y Sociedad CTS.* 2011;(4):131-9.
23. **Duque-Parra JE, Barco-Ríos J, Peláez-Cortes FJC.** Santiago Felipe Ramón y Cajal, ¿Padre de la Neurociencia o Pionero de la Ciencia Neural? *Int J Morphol.* 2011;29(4):1202-6. http://doi.org/cqfv.
24. **Finger S.** Minds behind the Brain: A History of the Pioneers and Their Discoveries: A History of the Pioneers and Their Discoveries. New York: Oxford University Press; 2004.
25. **Chuaire L.** Santiago Ramón y Cajal: Cien años de un premio Nobel. *Col Med.* 2006;37(3):247-8.
26. **Belmonte C.** Lección Magistral Andrés Laguna: La exploración del cerebro y la neurobiología española. Aupados a hombros de gigantes. *Educ Med.* 2015;16(2):141-8.
27. **Pearce JM.** Sir Charles Scott Sherrington (1857-1952) and the synapse. *J Neurol Neurosurg Psychiatry.* 2004;75(4):544.
28. **Zimmer HG.** Otto Loewi and the chemical transmission of vagus stimulation in the heart. *Clin Cardiol.* 2006;29(3):135-6. http://doi.org/bj7j34.
29. **McComas AJ.** Galvani´s Spark. The story of the nerve impulse. New York: Oxford University Press; 2011.
30. **Cowan WM, Südhof TC, Stevens CF.** Synapses. Baltimore: Johns Hopkins University Press; 2001.
31. **del Cerro M, Triarhou LC.** Eduardo De Robertis (1913-988). *J Neurol.* 2009;256(1):147-8. http://doi.org/fjsq4w.
32. **Schwiening CJ.** A brief historical perspective: Hodgkin and Huxley. *J Physiol.* 2012;590(11):2571-5. http://doi.org/cqfw.
33. **Hodgkin AL, Huxley AF.** Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. *J Physiology.* 1952;116:449-72. http://doi.org/cqfx.
34. **Schoenfeld RL.** Exploring the Nervous System. Florida: Universal Publishers; 2006.
35. **Neher E, Sakmann B.** Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature.* 1976;260(5554):799-802. http://doi.org/fmr3cb.
36. **Descartes R.** Discurso del método. Buenos Aires: Ediciones Colihue; 2004.