The Discovery of Sensory Nature of the Carotid Bodies – *Invited Article*

F. De Castro

Abstract Although the carotid body (or *glomus caroticum*) was a structure familiar to anatomists in the XVIIIth century, it was not until the beginning of the XXth century that its role was revealed. It was then that the German physiologist Heinrich Hering described the respiratory reflex and he began to study the anatomical basis of this reflex focusing on the carotid region, and the carotid sinus in particular. At this time, the physiologists and pharmacologists associated with Jean-François Heymans and his son (Corneille) in Ghent (Belgium) adopted a different approach to resolve this issue, and they centred their efforts on the cardio-aortic reflexogenic region. However, at the *Laboratorio de Investigaciones Biológicas* (Madrid, Spain), one of the youngest and more brilliant disciples of Santiago Ramón y Cajal, Fernando De Castro, took advantage of certain technical advances to study the fine structure of the carotid body (De Castro, 1925). In successive papers (1926, 1928, 1929), De Castro unravelled most of the histological secrets of this small structure and described the exact localisation of the "chemoreceptors" within the *glomus*. Indeed, his was the first description of cells specifically devoted to detect changes in the chemical composition of blood. Heymans was deeply interested in the work of De Castro, and he extended two invitations to the Spanish neurologist to visit Ghent (1929 and 1932) so that they could share their experiences. From 1932–1933, Corneille Heymans focused his attention on the carotid body and his physiological demonstration of De Castro's hypothesis regarding chemoreceptors led to him obtaining the *Nobel Prize in Physiology or Medicine* in 1938, while Spain was immersed in its catastrophic Civil War.

Keywords Carotid body · Glomus caroticum · De Castro · Heymans · Sensory organ · Intracarotid gland · Microganglia

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1 Introduction

The carotid body (also known as the carotid corpuscule, carotid ganglion, carotid gland and *glomus caroticum*[1]) is an anatomical structure situated at the bifurcation of the internal and external carotid from the primitive carotid artery. This structure became known in the XVIIIth century, although its function remained elusive to scientists for centuries. In the mid XIXth century, the experiments performed by the Weber brothers and Henle to modulate cardiac frequency by electrical stimulation of the vagus nerve (even stopping the heart) can be considered to have set the first stone in a new line of research in the cardio-aortic-carotid region. The studies that were carried out in the following decades explored the cardio-respiratory reflexes and the physiology of this system in general. The work published by Marey in 1859 was also particularly relevant, demonstrating the direct and opposing relationship between arterial pressure and cardiac frequency: if one increases, the other falls, and vice versa. Although Cyon and Ludwig (1866) demonstrated the implication of the vagal nerves in the origin of bradycardia and hypotension, the studies of François-Franck (1876–1878) situated the origin of this reflex in the brainstem (that is, in the central nervous system -CNS-).

However, Pagano and Siciliano proposed in 1900 that the cardio-respiratory reactions were reflexes originated in the carotid region independent of the CNS. Indeed, for more than fifty years, this contradiction with François-Franck's postulates was ignored by the scientists studying this problem. However, finally it was Heinrich Hering and his collaborators who elegantly demonstrated that either the electrical or mechanical stimulus of the carotid sinus (a dilatation of the bifurcation of both carotid arteries from the primitive one) triggers a reflex mechanism that results in bradycardia and arterial hypotension, baptised as the "sinus reflex". Indeed, this physiologist from Köln (Germany) also discovered that this region was innervated by a branch of the glossopharyngeal nerve, named the "sinus nerve" or "Hering's nerve" (1923–1927).

In parallel, Jean-François Heymans and his son Corneille, and their disciples,[2] applied the famous technique of two living dogs in parabiosis to reach the conclusion that hypertensive bradycardia is a reflex mechanism, independent of the central structures (Heymans and Ladon, 1925). They stated that the "main regulation of the respiration depends on the cardio-aortic region, and it is conditioned by the pressure and composition of circulating blood"[3] (Heymans and Heymans, 1927). Once this had been established, the study of cardio-respiratory reflexes and physiology began to move its centre of gravity towards Madrid, the capital of Spain. There, one of the youngest and the last direct disciple of the reputed Santiago Ramón y

[1] Throughout this text, we will preferentially use two terms: carotid body and *glomus caroticum*, which represent the more modern concepts of this anatomical structure.
[2] All the physio-pharmacologists who worked with J.-F. and C. Heymans are collectively known as the "Ghent school", since the Institute for Pharmacology was situated in this Belgian city.
[3] Translated into English by the author of this article.

Cajal,[4] Fernando de Castro, began to publish his anatomo-histological observations that were to transform this research field.

2 De Castro, 1926

In the middle of the 1920's, the rich blood supply and sympathetic innervation of the *glomus caroticum* had become evident, but the fine details behind the organization of this anatomical structure, as well as its physiological relevance, remained completely unknown. After his remarkable studies on the structure and organization of the sensory and sympathetic ganglia (the main articles related to this research are: De Castro, 1921; De Castro 1922), Fernando de Castro began to study the aorto-carotid region. He found that the entire heads of animals could be fixed by adding nitric acid (3–4%) to the classic fixatives (urethane, chloral hydrate, formol or especially somnifene), perfectly preserving the nervous structures within their skeletal casing, as well as the peripheral innervations to which Cajal's famous reduced silver impregnation method could be applied (De Castro, 1925). The technique De Castro developed is especially effective in small animals, such as rodents, and it reduces the staining of the connective tissue, thereby increasing the contrast of peripheral nerve structures like the *glomus caroticum*. In his own words, this technique was crucial to be able to study the detailed innervation of the carotid sinus and the carotid body (De Castro, 1926). In fact, the first microphotographs of the rich vascular supply and the innervation of the *glomus caroticum* were published by the De Castro in his methodological paper (De Castro, 1925).

The first detailed study of the carotid region was published by De Castro in 1926, using tissue from both adults and embryos of different mammalian species (small rodents, cats, dogs, rabbits and humans). With the aid of microphotography (a technique introduced into the Cajal Institute after 1922), he could perform an exhaustive and intensive study of this area enabling him to make the first crucial clarification regarding this structure. Accordingly, De Castro showed that the nerve fibres do not form a closed plexus around the carotid body but rather, they project into this structure from the superior cervical ganglion (sympathetic system) and to a lesser extent, from the glossopharyngeal nerve (the intercarotid branch of the glossopharyngeal nerve that exclusively innervates the carotid body). Years previously, Hering had identified this intercarotid branch as the "sinus nerve", which innervates the carotid sinus and is responsible for the "sinus reflex" (see above). However, it was De Castro who clearly stated that the sinus nerve is a branch of the vagus nerve, which while sometimes lying close to the intercarotid branch of the glossopharyngeal nerve, is not the same at all (De Castro, 1926).

In this paper, De Castro described the complicated structure of the *glomus caroticum* in detail: a real tangle of small blood vessels, sympathetic axons and

[4] Santiago Ramón y Cajal (1852–1934) and Camillo Golgi (1843–1926) shared the Nobel Prize in Physiology or Medicine in 1906.

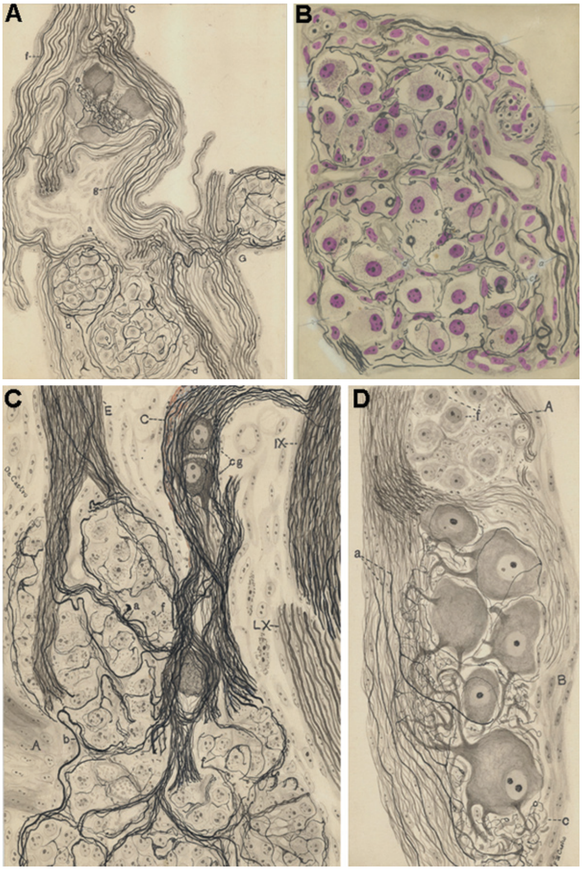

Fig. 1 De Castro describes the detailed fine structure of the carotid body (1926). Original drawings from Fernando De Castro, all of them included in his first publication on the innervation of the carotid region (De Castro, 1926). (**A**) Arrival of the inter-carotid nerve (a branch of the glossopharyngeal nerve; *c*) to the carotid body (*d*) of a young human. In the carotid body, different glomeruli with fine innervation can be identified. A sympathetic microganlgion is also illustrated (*e*). (**B**) Illustration of the *glomus caroticum* of a young human. Glomic cells are illustrated with coloured nuclei. The sensitive innervation surrounds these cells, but they do not comprise a closed plexus around the carotid body. (**C**) Illustration of the carotid body, forming different glomeruli close to the carotid artery (*A*). The sympathetic nerve (E) arrives from the superior cervical ganglion, but its contribution to the innervation of the carotid body is not the most relevant. The same can be said of the vagus nerve (*LX*) in the vicinity of the carotid body. By contrast, the most relevant contribution comes from the intercarotid nerve (*C*), a branch of the glossopharyngeal nerve (*IX*). Within this nerve, a sympathetic microganglion can be seen (*cg*). (**D**) Detailed illustration of one of the sympathetic microganglia that can be observed within the intercarotid nerve (see **A**, **C**)

glandular cells, which may form small glomeruli within the carotid body as well as a minuscule and complicated plexuses of glossopharyngeal fibres surrounding these glomeruli (Fig. 1A, C–D). The complexity of these nervous plexuses within the carotid body is even greater in humans than in the other mammals studied, although by contrast, there is less complexity around the carotid body (Fig. 1A, B).

With this paper (De Castro, 1926), Fernando de Castro began a detailed study of the innervation of the *glomus caroticum* and its physiological implications, which he continued in his second paper on this subject (De Castro, 1928) and intermittently, in successive papers until the end of his life. At that time, the controversy among the physiologists interested in the function of the carotid body and the anatomical basis of the Hering's reflex was being strongly pursued. In 1924, Hering attributed the profuse innervation of the carotid sinus to the fact that the mechanical or electrical stimulation of this region results in bradychardia and a decrease in arterial pressure (Hering, 1924). One year later, through Drüner, merely due to his intuition and without the support of any experimental data, Drüner launched the hypothesis after which that the intercarotid gland (the carotid body) was responsible for Hering's reflex (Drüner, 1925). In the same year, Hering responded that the mechanical pressure of the intercarotid territory, as well as that of the carotid body, does not fire the reflex, confirming that the "Sinus reflex" must be due to the excitation of the arterial wall in the sinus (Hering, 1925).

3 De Castro, 1928

Although his first paper directly affected the controversy between Hering and Drüner, De Castro decisively resolved this scientific joust with his second article on the subject, published in 1928. Using material from humans, monkeys, cows, cats, rabbits and rodents, De Castro fell back on the methylene blue reaction of Erlich-Dogiel and Bielschowsky's method (the protocol modified by Boecke) to complement the observations obtained with Cajal's technique. This paper can be considered in two main blocks: the confirmation of the physiological existence of the carotid sinus and the study of its innervation (baro-receptors); the detailed study of the innervation of the carotid body and the description of a different kind of receptors (chemoreceptors). Although the present work focuses on the chemoreceptors, we must briefly review the results obtained by Fernando de Castro on the innervation of the carotid sinus.

In agreement with the prior description by Hering (1927), De Castro confirmed the existence of the carotid sinus in all the species and at all ages studied, except in the human foetus in which the sinus is not macroscopically evident. Similarly, in the cow where there is no internal carotid artery, the carotid sinus is present in the bifurcation of the primitive carotid and the occipital arteries. This study ruled out other hypotheses about the nature of the carotid sinus, such as that of a pathological malformation (Binswanger and other anatomists in the last part of the XIXth century), or the existence of enlargements in each vascular bifurcation, as suggested by other prestigious scientists (Henle, Luschka, etc.).

In this second paper, Fernando de Castro described the sensory innervation of the carotid region in detail. He showed that the fibres concentrate in the part of the carotid sinus immediately prior to the origin of the internal carotid artery. This is where the vessel's wall is thinner and it is bordered by two concentric bands, one with little sensory innervation and the second with almost no such terminals,

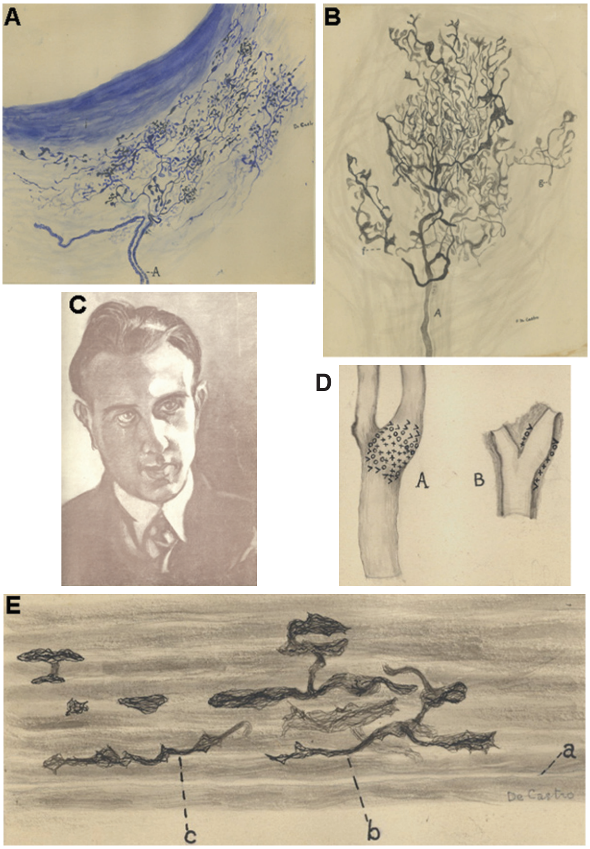

Fig. 2 De Castro describes the baroreceptors in the carotid sinus in detail (1928). Fernando De Castro's original drawings from his second publication on the innervation of the carotid region (De Castro, 1928). (**A)** Illustration of a type I (diffuse) baroreceptor close to the adventitia of the artery from a young human, stained with methylene *blue*. (**B)** Detailed illustration of a type II (circumscribed) baroreceptor of the human carotid sinus stained by silver impregnation. The myelin trunk is marked by *A*. (**C)** Portrait of Fernando De Castro by the Hungarian painter Férenc Miskolzy, brother of the famous neurologist Deszö Miskolzy, the founder of modern Hungarian Neurology. The portrait reflects the aspect of the Spanish neurohistologist at the time of his studies on the innervation of the carotid body and sinus. (**D)** Schematic distribution of the baro-receptors in the human carotid sinus. The symbols identify dense terminals from (*o*) to (>), passing through (+). In the section of the artery (*B*) the situation of the terminals in the thinner part of the arterial wall is illustrated. (**E)** Fine terminals of baroreceptors (*b*, *c*) intermingled with the collagen fibres (in diffuse *grey*, *a*), a distribution that allows the changes in volume of the blood vessel to be better detected. In some cases, the nerve terminals form a meniscus

a kind of "sensory penumbra" (Fig. 2D). This region shows virtually the densest innervation of the entire arterial tree and the fact that this distribution exists in all the species studied, including the cow, coupled with the specific location of the carotid sinus (see above), confirms that it is not merely anecdotic. Rather, in all animals the sensory fibres are specifically situated at the origin of the artery that irrigates the brain. Depending on their morphology and distribution, De Castro identified the different types of sensory receptors (basically either "disperse" or "circumscribed" receptors; Fig. 2A, B). However, it is even more interesting that he described that the fibres are terminals that extend and ramify through all the different planes of the adventitial layer of the artery, even the deepest one. Moreover, he described them as nude terminals, devoid of any kind of cover, so that they can sense the changes in the volume of the vessel derived from the changes in arterial pressure (Fig. 2E). Unfortunately, the silver method used by De Castro for this study does not stain the elastic fibres of the arterial vessels, so he could not study the intimate relationship between the sensory receptors and the elastic fibres that determine the movement of the artery. In none of the thousands of sensory terminals studied by De Castro did he observe any kind of cellular anastomosis. Indeed, the author highlights that this observation was not due to the *esprit d'ecole*, in clear reference to Cajal's "neuronal theory", even though he and the Master himself were the most ardent defenders of this theory against the continued attacks of the supporters of the "reticularist theory". However, these observations confirmed those published earlier, in 1924 and 1926, where De Castro showed that the arborization innervating the carotid sinus is more profuse in the big mammals and simpler in the small rodents (De Castro, 1928).

De Castro ingeniously studied the nature of carotid sinus innervation. To disprove the hypothesis of Boecke about the origin of these nerves in a sympathetic ganglion (approx., 1918), he ablated all the ganglia of the cervical chain and he failed to detect any sign of degeneration in the terminals innervating the carotid sinus. The same results were obtained when the glossopharyngeal, vagus and spinal nerves were sectioned. From the results of such trophic deprivation, De Castro concluded that the fibres innervating the carotid sinus must be sensory fibres, neurons projecting towards the central nervous system, which provides them with sufficient trophic support to survive. At that point, he proposed that these observations reflect the morphological basis of the physiological responses described by Hering (1927), the "sinus reflex". The hypothesis launched by Drüner was definitively defeated by the combination of the physiological studies of Heinrich Hering (1927) and the anatomo-histological ones of Fernando De Castro (1926 and mainly, 1928).

Having reviewed the studies of the carotid sinus and the baroreceptors, I will now focus on the second big block of results published in 1928, regarding the *glomus caroticum* and the chemoreceptors. Just when his first observations on this subject were published in 1926, De Castro postulated that the cells of the carotid body were probably cells with a paracrine or autocrine function, in accordance with the suggestion of many scientists at that time that the *glomus caroticum* was a real gland (the intercarotid gland). However, when he performed the elegant ablation experiments described above, De Castro observed that a few motile sympathetic fibres within the *glomus* did not degenerate once the sympathetic ganglia of the cervical chain were extirpated, indicating that they originated from the sympathetic neurons of the microganglia present within the *glomus* (see above: De Castro, 1926). By contrast, sectioning of the glossopharyngeal nerve where it exits the skull resulted in the fast (a mere 5–6 days after lesion) and almost total degeneration of the fibres forming the nervous plexus in the carotid body and the terminals connecting with glomic cells. This degeneration affected the cell cytoplasm and mitochondria, but the feochrome reaction of Henle and Vulpian was not observed, ruling out the possibility that the *glomus* might be a paraganglion, as suggested by Kohn (1900–1903). Moreover, De Castro thought that a organ as exquisitely innervated as the carotid body would not be a residual or involutive organ.

Accordingly, he decided to paint the surface of the carotid artery of adult cats with phenol to kill the terminals innervating the carotid sinus, and to study the possibility that the *glomus caroticum* might be involved in regulating arterial pressure. However, he detected only minimal changes in the latter, which suggested that the carotid body contributed little to the sinus reflex. This minimal contribution to the control of blood pressure might be due to motile fibres from the sympathetic microganglia within the carotid body (or even to a minimal branch coming from the superior cervical ganglion). However, this would not seem to be the main function of the carotid body because electrical stimulation of the intercarotid nerve (which remains intact when the glossopharyngeal nerve is sectioned) did not result in any relevant vasoconstriction of the artery. Also in 1928, a group of Rumanian scientists insisted that the *glomus caroticum* participated in the regulation of arterial pressure (Jacoborici et al., 1928), as suggested by Drüner three years ago, although De Castro's experiments completely ruled out this possibility.

De Castro performed a second series of experiments to clarify the function of the *glomus* in which he sectioned the glossopharyngeal and vagus nerves in cats and dogs, and studied the effects of this procedure on the carotid body. The degeneration of the fibres was fast and almost total, from which he deduced that the terminals innervating the *glomus* belong to sensory neurons from the nuclei of both nerves (glossopharyngeal and vagus).[5] Given that this sensory nature is different from that which determines the arterial pressure through Hering's "sinus reflex", De Castro hypothesised that these nerve receptors within the *glomus* would detect changes in the chemical composition of the blood. Indeed, blood pressure needs more urgent control exerted by the baroreceptors in the carotid sinus, while qualitative changes in the composition of the blood should be detected by a second system, not far from the first, such as the chemoreceptors of the *glomus caroticum*. De Castro even declared in his article that the changes in composition would not be detected directly by the nerve terminals, because they were not in direct contact with the blood. Rather, he stated, the glomic epithelial cells should perform this task via an "active protoplasmic process" extending from these cells, centripetally transmitted to the nerve terminals.

[5] The sensitive ganglia of the IXth craneal nerve are the Ehrenritter's and Andersch's ganglia, those from the Xth nerve are the jugular, the nodose and the petrose ganglia.

4 Heymans in the 1930's and the Nobel Prize in Physiology or Medicine

As stated previously, in the decade from 1920 to 1930 the two most important figures in cardiac and respiratory physiology research were Heinrich Hering (Köln University, Germany), and Jean-François Heymans along with his son Corneille (both working at the University of Ghent, Belgium). Fernando de Castro communicated his studies in different meetings of the *Association d'Anatomistes* held at Liege in 1926 (Belgium), at London in 1927 (Great Britain), and at Bourdeaux in 1929 (France), and together with the aforementioned publications, the work from Spain raised the attention of the entire scientific community interested in the field, including some physiologists. As a result, Professor Goormaghtigh, the chair of Pathology at the University of Ghent, transmitted an invitation from Corneille Heymans to Fernando de Castro to visit Ghent and to discuss his work with him. Just after the meeting, De Castro visited his sister who lived in La Roche-Chalais, in the French Périgord (not far from Bourdeaux), where he received a letter from Heymans again inviting him to visit Ghent (Fig. 3A). De Castro accepted and his own words are better than any other description:

> I went to Ghent and I was with him [*Heymans*] for two or three days, during which we performed some experiments on the carotid sinus of dogs. At that time, he was not interested in the carotid body. Rather, he had studied the vasoconstriction phenomena on the cardio-aortic region and he had just started to study the carotid region. His work on the carotid body came afterwards, and he said to me. *I'm deeply interested in your idea about this. It would be great if I could demonstrate it!*[6] (Gómez-Santos, 1968).

This was Fernando de Castro's first visit to the laboratory of Corneille Hyemans in Ghent (April, 1929) and there was to be a second one. However, the Belgian physiologists did not directly address the study of the physiological role of the carotid body and they took years to abandon their hypothesis that the respiratory reflex originated in the carotid sinus. For example, although in his first work about the physiological reflexes originated in the carotid sinus (Heymans, 1929), Heymans recognised that the anatomy and the histology of the sinus region had been described "recently, in a precise and detailed manner by De Castro",[7] he did not pay any attention to the *glomus caroticum*, and even less so to De Castro's hypothesis on the function of this small structure in chemoreception (Heymans, 1929). In their detailed study the following year, Heymans and his collaborators described the primacy of the carotid sinus (even ahead than the respiratory centre in the brainstem) to detect changes in arterial pressure and in the concentration of H^+, CO_2 and O_2 in the blood. However, although they cited the work of De Castro (1928) in the

[6] Translated into English by the author of this article.
[7] Translated into English by the author of this article.

Fig. 3 Corneille Heymans and Fernando De Castro, a long friendship full of mutual admiration. **(A)** Letter from Heymans, dated the 28th March 1929, inviting Fernando De Castro to his lab at Ghent University. As can be seen on the envelope (upper right), the letter was sent to "Chez Lagoubie, La Roche-Chalais, Dordogne", the family name of De Castro's French brother-in-law where the Spanish histologist was staying for a few days after the meeting of the Association d'Anatomistes in Bourdeaux (France). (**B)** De Castro's drawing showing two dogs in parabiosis (the famous physiological technique in which the Heymans -father and son- were consumed masters). The notes in Spanish complete the information for the experiments. (**C)** Letter from Heymans, dated 29th December 1939, in answer to a previous letter from Fernando De Castro (dated 15th December 1939) where he congratulated the Belgian physio-pharmacologist for the Nobel Prize award in Physiology or Medicine. (**D)** Picture of the portrait of Corneille Heymans, dedicated to De Castro (see hand-writing in blue at the lower-right corner of the image) and dated 1939 by the painter. Fernando De Castro kept this dedicated picture on his bureau until he died in 1967. (**E)** De Castro prepared to perform one of his famous and complicated nerve anastomosis in a cat, in the precarious conditions of his laboratory in Madrid (circa 1941)

bibliography, they did not discuss his studies in the text (Heymans and Bouckaert, 1930; Heymans et al., 1930). This is likely to be the origin, at least in part, of the persistent error in failing to differentiate between the site at which blood pressure and it composition are detected (Gallego, 1967).

It was not until 1931, when these researchers published a study (focused on the study of bradycardia induced by different drugs) in which they explicitly accepted the hypothesis of De Castro:

> "…our experiments indicate that the starting point of the reflexes triggered by the injection of chemical substances may be localised in the region of the carotid bifurcation and, more exactly, in the glomus caroticum, as proposed by De Castro" (Heymans et al., 1931).

And even more explicit, perhaps the reference published in a second paper about the same subject one year later:

> …the substances injected in the common carotid abandon this slowly through the small arterial branches which have not been sutured, and then they can act for a relatively long period of time, on the region of the carotid ganglion which is, as De Castro and we have demonstrated, the starting point of the reflexes triggered by the chemical excitants[8] (Heymans et al., 1932).

Coinciding with the second visit of Fernando de Castro to the *Pharmacological Institute* at the University of Ghent (where he repeated experiments on dogs together with Heymans; Fig. 3B), the Belgian physio-pharmacologists once and for all adopted the hypothesis of Fernando de Castro. At that time, they began to study more closely the region of the *glomus caroticum* and the physiological importance of the chemoreceptors:

> The reflexogenic hypothesis of vascular sensitivity in the regions of the carotid sinus was already postulated by De Castro in 1928 based on experimental morphological observations[9] (Heymans et al., 1932).

In 1933, Heymans grouped all the discoveries published by his group up to that date in a book (Heymans et al., 1933). In this text, the different types of reflexes are profiled and it is reflected that changes in arterial pressure (detected by the baroreceptors from the carotid sinus) or in the chemical composition of blood (detected by the chemoreceptors of the *glomus caroticum* or carotid body) control respiratory frequency, the heart rate and the arterial pressure. From this year on, Heymans and his collaborators will cite this book and less frequently their initial publications on the subject (1929–1932).

5 The Scientific Path of Fernando De Castro Between 1929 and 1936

During this period, Fernando de Castro was following difficult and complex approaches in Madrid using experimental surgery to demonstrate that the neurons of the *glomus* respond to changes in the chemical composition of blood. For this purpose, he developed different anastomosis between the glossopharyngeal, vagus and hypoglossal nerves to create artificial reflex arches. This approach required the

[8] Translated into English by the author of this article.
[9] Translated into English by the author of this article.

prior and detailed study of the regeneration of the sectioned preganglionic branches. These studies (De Castro, 1930, 1933, 1934, 1937), necessary to the ulterior demonstration of the physiological role of arterial chemoreceptors, distracted Fernando de Castro from his original goal by raising other scientific questions, which led him to work with the world famous and reputed biologist Giusseppe Levi at Turin (Italy), both in 1932 and particularly in 1934. One of Fernando de Castro's last collaborators, Prof. Jaime Merchán, could not understand why de Castro "did not try to put two living animals in parabiosis, a classical physiological technique and certainly easy enough for someone with the surgical skills of your grand-father [*Fernando de Castro*]".[10] It is possible that these parabiosis experiments, after all similar to those performed by the Heymans, and by Fernando de Castro and Heymans, in Ghent (see above), were those he referred to at the end of his work published in 1928 (see above).

In these years, a scientist close to the octogenarian Santiago Ramón y Cajal was worried about the poor international repercussion of the studies carried out by many members of his school (mostly published in Spanish; Penfield, 1977) and in particular, of the works of De Castro on the carotid sinus and carotid body (see for example the letters from Cajal to Fernando de Castro, dated February 18th, 1932 and March 15th, 1933 -both conserved in the Fernando de Castro Archive-). This led Cajal to take two significant steps: (i) from 1923 onwards, the journal published by the Cajal Institute ("Trabajos del Laboratorio de Investigaciones Biológicas") was published in French as "Travaux du Laboratoire de Recherches Biologiques"; (ii) together with Fernando de Castro, Cajal decided to compile all the different Histological protocols and experimental procedures that made up the technical corpus of the Spanish School of Neurologists (or Cajal's School), and to publish them in a book (Cajal and De Castro, 1933).

However, it was Rafael Lorente de Nó, another young disciple of Cajal and a close friend of Fernando de Castro, who tried to draw the attention of their Maestro and of his colleague and friend to the error in their ways. In his opinion, they should not forsake the study of the carotid region and of its physiological implications in order to start working on the regeneration of the nervous system in vitro, which seems clear after the letter written by Cajal on May 19th 1934, to Fernando de Castro, ill in Turin (Italy) at that time:

> I received a letter from Lorente regretting, that after many years of work in a difficult histological speciality, with an excellent orientation and dominion of scientific bibliography, you have changed your bearing to work in a field that, if not exhausted, does not initially offer to the researcher unexpected fruits. When I answer him, I will inform him that both paths converge and not only will no harm be done, but it is favourable to air one's intelligence in other scientific domains.

This episode suggests that the change of course in Fernando de Castro's scientific career was adopted with Cajal's agreement. Although there are additional events that may help understand the stance adopted by Cajal, as indicated in the letter above,

[10] Professor Jaime Merchán (UMH, Spain), personal communication (March, 2008).

including events associated with the research into arterial chemoreceptors, it seems clear that Lorente de Nó was not entirely wrong.

In Febraury1934, De Castro went to Turin to work with Levi, and there his life ran a serious risk due to a rare gastric hemorragia. Santiago Ramón y Cajal died in October 1934 (with the ensuing organizational changes at the Cajal Institute), and the political and social events in Spain (and Europe) became complicated, leading up to the Spanish Civil War in July 1936.

6 Corneille Heymans, Nobel Prize in Physiology or Medicine in 1938

In 1938, the Nobel Committee evaluated the proposal of Cornelius Heymans for the Nobel Prize in Physiology or Medicine. It was a year in which the number of scientists nominated was quite numerous.[11] Professor Liljestrand, member of the committee, together with Ulf von Euler[12] (both collaborators of Cornelius Heymans) evaluated the candidature of the Belgian physio-pharmacologist,[13] and they left this testimony in the records of the Nobel Foundation regarding the prize for Physiology or Medicine:

> Hering's work was twice submitted to special investigation. In 1932, the reviewer exposed some doubts about his qualification for a prize like this, taken into account the obvious analogy with previous research performed on the aortic nerves and those from their predecessors mentioned above [*Pagano, Siciliano, Sollmann y Brown*]; afterwards, it was considered the division of the prize between Hering and Heymans, but the Committee was still sceptic about the merits of the first one. Pagano was also nominated by his compatriots, but only in 1943 (Liljestrand, 1962).

In fact, when consulting the database of the Nobel Foundation, it can be seen that the German physiologist Heinrich Hering was proposed for the prize in 1932, 1933, and 1937, as well as sharing a nomination with the Louis Lapique and Corneille Heymans in 1934. But in 1938, no one nominated the physiologist from Köln for the Nobel Prize.

The case of Heymans merits certain attention because he was nominated for the Nobel Prize in 1934, 1936, 1938 (when it was awarded to him) and also in 1939. Curiously, only in the proposals from 1938 and 1939 is there any explicit mention of his studies on the baro- and chemoreceptors of the blood, while on the previous occasions he was nominated for his studies on blood circulation in general. It also seems interesting that while in 1938 he was nominated by the Hungarian professor Mansfeld, on all the other occasions he was supported by Belgian

[11] Consulting the database of the Nobel Foundation, there were a total of 96 scientists nominated for the Nobel Prize in Physiology or Medicine in 1938. Among them, Hess, Houssay, Stanley, Sasaki, Lapique, Erlanger, Gasser, Cushing . . . to cite only some of the most relevant names.

[12] Future Nobel Prize in Physiology or Medicine in 1970.

[13] Database of the Nobel Foundation: http://nobelprize.org/nomination/medicine/nomination.php?action=simplesearch&string=Heymans&start=11

professors and medical doctors, including the deluge of proposals supporting his candidature in 1939 (either alone or in conjunction with Louis Lapique and Walter Hess).

So what about Fernando De Castro? A legend circulates that during the deliberation of the Nobel Committee over lunch, someone argued in favour of Fernando De Castro's nomination and that another committee member, remembering the war that was desolating Spain at that time, asked: "But, does someone know if De Castro is still alive?".[14] Once again, the database of the *Nobel Foundation* is clear (which opens all its documents to the public 50 years after the concession of each Prize): nobody either in Spain or in any other country nominated Fernando De Castro for the Nobel Prize in those years.[15] By contrast, the Spaniard Pío del Río-Hortega was nominated in 1929 by a professor at the *University of Valladolid* and in 1937 (in the middle of the Spanish Civil War) by two professors from the *University of Valencia*.[16] After carefully consulting the database of the Nobel Foundation, it can be seen that Santiago Ramón y Cajal supported only one nomination during his life (together with another eight Spanish scientists and medical doctors, and he did not lead the proposal): that of the French immunologist Richet in 1912.[17] It remains a mystery that during the 28 years after he was awarded the Nobel prize and as active as they were until close to his death in 1934, this Spanish genius remained at the margin of these scientific jousts.

The draft written by Fernando De Castro to felicitate Heymans for the award of the prize, dated December 15th 1939, is conserved in his archives, as is the hand-written answer from the Nobel Prize dated December 29th of the same year. Following on from the studies of the Ghent group, Comroe discovered the chemoreceptors in the so called *glomus aorticum* (innervated by the depressor nerves) had a minor role in the respiratory reflexes given that they merely respond to extreme cases of hypoxemia (Comroe, 1939). And Fernando De Castro continued working in the fine structure and physiology of the carotid body just from the end of the Spanish Civil War (April, 1939) in penury of technical support and in difficult personal and economical circumstances (Fig. 3E), something that did not really change until the decade of 1950.[18]

In the presentation of the Nobel Prize in Physiology or Medicine to professor Corneille Hyemans, at Ghent on January 1940,[19] the Swedish professor

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[14] Professor Gunnar Grant's personal communication (meeting of the *Cajal Club*; Stockholm, May 2006).

[15] This information was corroborated by a letter dated April 3rd, 2007 written by the Administrator of the Nobel Committee in answer to the author of the present paper.

[16] While his 1929 nomination was evaluated, that in 1937 was not.

[17] Charles Richet was awarded the Nobel Prize in Physiology or Medicine in 1913 "in recognition of his work on anaphylaxis".

[18] This was his main research labour till he died in April, 1967.

[19] Although the IInd World War commenced in September 1939, it was not until May 10th 1940 that the German troops invaded Belgium, The Netherlands and Luxembourg in their race towards France. Thus, life in these countries would have been relatively normal in January 1940, which

G. Liljestrand recognised the work of Fernando De Castro as fundamental in the path that Heymans followed towards his final success:

> Since the end of the 18th century we know of the existence of a curious structure in the region of the sinus, the glomus caroticum or carotid body which, in man, extends over only a few millimetres. The glomus consists of a small mass of very fine intertwining vessels arising from the internal carotid and enclosing various different types of cells. It has been considered by some as being a sort of endocrine gland similar to the medulla of the suprarenal glands. De Castro, however, in 1927 demonstrated that the anatomy of the glomus could in no way be compared to that of the suprarenal medulla. De Castro suggested rather that the glomus was an organ whose function was to react to variations in the composition of the blood, in other words an internal gustatory organ with special ≪chemo-receptors≫. In 1931, Bouckaert, Dautrebande, and Heymans undertook to find out whether these supposed chemo-receptors were responsible for the respiratory reflexes produced by modifications in the composition of the blood. By localized destruction in the sinus area they had been able to stop reflexes initiated by pressure changes, but respiratory reflexes could still continue to occur in answer to changes in the composition of the blood. Other experiments showed that Heymans's concepts on the important role played by the glomus in the reflex control of respiration by the chemical composition of the blood were undoubtedly correct".[20]

Due to the IInd World War, Heymans did not give his Nobel lecture until December 12th, 1945 (when he received the prize, *de facto*). It was then surprising that he scarcely cited the earlier work of Fernando De Castro:

> Histological research carried out by de Castro, Meyling and Gosses, and our own experimental findings, obtained with J. J. Bouckaert and L. Dautrebande in particular, has led to the locating of the carotid sinus chemo-receptors in the glomus caroticum and of the presso-receptors in the walls of the large arteries arising from the carotid artery.[21]

When asked both indirectly as well as directly by the interviewer to explain why Cornelius Heymans did not share the Nobel Prize he received in 1938 with Fernando De Castro, he explained:

> He performed the physiological demonstration, not the anatomical one. Obviously, there was a tremendous loss of time from 1936 to 1938 due to the war and because at that time I was performing the experiments on nervous anastomosis. This was to automatically register the phenomenon of the carotid reflexes on the eye, with the nervous anastomosis detecting chemical changes in the blood. Years after, I presented this work in a symposium held at Stockholm, in which the opening conference was entrusted to Heymans and the second to me. There, I presented the work that I couldn't finish during the war. I had no more cats during that difficult time, they died of hunger or I was forced to sacrifice them. For this reason, I could not complete my experiments at that time (Gómez-Santos, 1968).

In this respect, the comments on this chapter of the life of Fernando De Castro published by the Chilean scientist Juan de Dios Vial, who worked with De Castro in the fifties, are very interesting:

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allowed this ceremony to be celebrated. These circumstances delayed holding the Nobel ceremony in Stockholm for six years.

[20] Prof. G. Liljestrand (Karolinska Institutet); presentation of the 1938 Nobel Prize in Physiology or Medicine to Prof. C. Hyemans (Ghent, Belgium; January 16th, 1940).

[21] Nobel Lecture, Prof. C. Heymans (Stockholm, Sweden; December 12th, 1945).

The period of intense activity around 1930, which had ended by the proposal of the idea that the glomus was a chemoreceptor, was followed by a lull. This may be due to the fact that his contribution was widely ignored as coming from Spain. He opened the way to Heymans' discoveries but did not receive due credit, even at the moment when the latter was awarded the Nobel Prize. 1 never heard De Castro himself refer to that circumstance, but his disciples and friends often did, and were somewhat bitter about it. These were also the years of Cajal's death, and of organizational changes in his Institute (Vial, 1996).

7 Heymans and De Castro: A History of Mutual Admiration

In Fernando De Castro's archives, dozens of letters remitted by Corneille Heymans and other close collaborators are conserved, as well as several drafts of the correspondence maintained by Fernando De Castro with all the members of the Ghent School. Although most of the correspondence conserved corresponds to the period between 1930 and 1960, the first letters sent by Heymans date from the early twenties. Some of these documents are especially significant, like the draft of the felicitation for the concession of the Nobel Prize sent by De Castro as mentioned above, the dedicated photograph of Heymans' portrait that was all the time in De Castro's office (Fig. 3D), two invitations sent in 1952 by Bouckaert and Heymans himself inviting Fernando De Castro to take part in the symposium held in the honour of the Belgian Nobel prize winner and even a letter from Heymans' wife (written on February 17th, 1953),[22] in which she personally welcomes Fernando De Castro to the cited hommage or inviting him to the wedding of Heymans' daughter in 1957. All these are examples of the friendship between Corneille Heymans and Fernando de Castro, as published by the son of the latter: "full of mutual admiration, their friendship continued over the years until April 15th 1967, the date of my father's death".[23]

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References

Comroe, J.H. (1939) The location and function of the chemoreceptors of the aorta. *Am. J. Physiol.* 127, 176–191.

Cyon, E., and Ludwig, C. (1866) Die Reflexe eines der sensiblen Nerven des Herzens auf die Motorischen der Blutgefässe. *Ber. Sächs. Ges. Wiss. Leipzig*. 18, 307–329.

[22] Fernando De Castro invited Heymans to the symposium in honour of Santiago Ramón y Cajal, celebrated to commemorate the centenary of his birth, and held in Madrid in 1952.

[23] F.-G. de Castro in letter to the Director of the journal ABC (Madrid) published on November 20th, 1974.

De Castro, F. (1921) Estudio sobre los ganglios sensitivos del hombre adulto en estado normal y patológico. *Trab. Lab. Invest. Biol.* 19, 241–340.

De Castro, F. (1922) Evolución de los ganglios simpáticos vertebrales y prevertebrales. Conexiones y citoarquitectonia en algunos grupos de ganglio en el niño y en el hombre adulto. *Trab. Lab. Invest. Biol.* 20, 113–208.

De Castro, F. (1925) Technique pour la coloration du système nerveux quand il est pourvu de ses étuis osseux. *Trav. Lab. Rech. Biol.* 23, 427–446.

De Castro, F. (1926) Sur la structure et l'innervation de la glande intercarotidienne (glomus caroticum) de l'homme et des mammifères, et sur un nouveau système d'innervation autonome du nerf glosopharyngien. *Trav. Lab. Rech. Biol.* 24, 365–432.

De Castro, F. (1928) Sur la structure et l'innervation du sinus carotidien de l'homme et des mammifères. Nouveaux faits sur l'innervation et la fonction du glomus caroticum. *Trav. Lab. Rech. Biol.* 25, 331–380.

De Castro, F. (1929) Ueber die Struktur und innervation des glomus caroticum beim Menschen aund bei den Säugetieren. Anatomisch-experimentelle Untersuchungen. *Zeist. Anat. Entwicklungs.* 89, 250–265.

De Castro, F. (1930) Recherches sur la dégénération et la régénération du système nerveux sympathique. Quelques observations sur la constitution des synapses dans les ganglions. *Trav. Lab. Rech. Biol.* 26, 357–456.

De Castro, F. (1933) Quelques recherches sur la transplantation de ganglions nerveux (cérebrospinaux et sympathiques) chez les mammifères. Etudes comparatives sur la capacité réactionnelle et la résistance vitale des neurones survivants dans les greffes. *Trav. Lab. Rech. Biol.* 27, 237–302.

De Castro, F. (1934) Note sur la régénération fonctionnelle hétérogène dans les anastomoses des nerfs pneumogastrique et hypoglose avec le sympathique cervical. *Trav. Lab. Rech. Biol.* 29, 307–316.

De Castro, F. (1937) Sur la régénération fonctionnelle dans le sympathique (anastomoses croisées avec des nefs de type iso et hétéromorphe). Une référence spéciale sur la constitution des synapses. *Trav. Lab. Rech. Biol.* 31, 271–345.

Drüner, L. (1925) Ueber die anatomischen Unterlagen der Sinusreflexe Herings. *Deutsche Mediz. Wochenschr.* 51, 559.

François-Franck, C.E. (1876) Physiologie experimentale. *Trav. Lab. Marey* 2, 221–288.

François-Franck, C.E. (1877) *Trav. Lab. Marey* 3, 273–288.

François-Franck, C.E. (1878) Sur les effets cardiaques et respiratoires des irritations de certains nerfs sensibles du coeur, et sur les effets cardiaques produits par l'irritation des nerfs sensibles de l'appareil respiratoire. *Trav. Lab. Marey* 6, 73.

Gallego, A. (1967) La contribución de Fernando De Castro al descubrimiento y estudio de los preso-receptores vasculares y quimio-receptores sanguíneos. *Arch. Fac. Med. Madrid* 11, 406–439.

Gómez-Santos, M. (1968) *Cinco grandes de la Ciencia española*. Ed. Biblioteca Nueva, Madrid, Spain.

Hering, H.E. (1923) Der Karotidsdruckversuch. Münch. *Med. Wschr.* 70, 1287–1290.

Hering, H.E. (1924) Der Sinus caroticus an der Ursprungsstelle der Carotis interna als Ausgangsort eines hemmenden Herzreflexes und depressorischen Gefässreflexes. *Münch. Med. Wschr.* 71, 701–705.

Hering, H.E. (1927) *Die Karotissinusreflexe auf Herz und Gefässe*. Ed. T. Steinkopff, Dresden-Leipzig, Germany.

Heymans, C. (1929) *Arch. Int. Pharmacodyn.* 35, 269–313.

Heymans, C., and Bouckaert, J.J. (1930) Sinus Caroticus and Respiratory Reflexes. *J. Physiol.* 69, 254–266.

Heymans, C., Bouckaert, J.J., and Dautrebande, L. (1930) Sinus carotidien et réflexes respiratoires. II. Influences respiratoires réflexes de l'acidôse de l'alcalose, de l'anhydride carbonique, de l'ion hydrogéne et de l'anoxémie: Sinus carotidiens et échanges respiratoires dans le poumons et au delá des poumons. *Arch. Int. Pharmacodyn.* 39, 400–448.

Heymans, C., Bouckaert, J.J., and Dautrebande, L. (1931) Au sujet du m6canisme de la bradycardie provoquée par la nicotine, la lobéline, le cyanure, le sulfure de sodium, les nitrites et la morphine, et de la bradycardie asphyxique. *Arch. Int. Pharmacodyn.* 41, 261–289.

Heymans, C., Bouckaert, J.J., Von Euler, U.S., and Dautrebande, L. (1932) Sinus carotidiens et reflexes vasomoteurs. *Arch. Int. Pharmacodyn.* 43, 86–110.

Heymans, C., Bouckaert, J.J. and Regniers, P. (1933) *Le Sinus Carotidien.* Ed. G. Doin, Paris.

Heymans, J.F., and Heymans, C. (1927) Sur les modifications directes et sur la régulation réflexe de l'activité du centre respiratoire de la tête isolée du chien. *Arch. Int. Pharmacodyn.* 33, 273–370.

Heymans, C., and Ladon, A. (1925) Recherches physiologiques et pharmacologiques sur la tête isolée et le centre vague du chien. I: Anémie, asphyxie, hypertension, adrénaline, tonus pneumogastrique. *Arch. Int. Pharmacodyn.* 30, 145.

Jacoborici, J., Mitzescu, I.I., and Pop, A. (1928) Sur la fonction de la glande (paraganglion) carotidienne. La glande et le réflexe du sinus carotidien. *Compt. Rend. Soc. Biol.* 98(9).

Kohn, A. (1900) Ueber den bau und die entwicklung der sogen carotidsdrüse. *Arch. F. Mikroscop. Anat. Bd.* 56, 81–148.

Kohn, A. (1903) Die Paraganglien. *Arch. F. Mikroscop. Anat. Bd.* 62, 263–365.

Liljestrand, G. (1962) *Nobel the Man and His Prices.* Ed. Elsevier, Amsterdam, The Netherlands.

Marey, E.-J. (1859) Des causes d'erreur dans l'emploi des instruments pour mesurer la pression sanguine, et des moyens de les éviter. *Compt. Rend. Soc. Biol.* 1, 55–58.

Pagano, G. (1900) Sur la sensibilité du Coeur et des vaisseaux sanguins. *Arch. Ital. Biol.* 33.

Penfield, W. (1977) *No Man Alone: A Neurosurgeon'S Life.* Ed. Little Brown & Company, Boston-Toronto, USA-Canada.

Ramón y Cajal, S. and De Castro, F. (1933) *Técnica micrográfica del sistema nervioso.* Ed. Tipografía Artística, Madrid, Spain.

Siciliano (1900) Les effets de la compression des carotides sur la pression, sur le coeur et sur la respiration. *Arch. Ital. Biol.* 33, 338–344.

Vial, J.D. (1996) A tribute to Fernando De Castro on the centennial of his birth. In: *Arterial Chemoreception*. Eds. Zapata et al., Plenum Press, New York, USA.