Histol Histopathol (2011) 26: 461-470

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

The primary cilium: A relevant characteristic in interstitial cells of rat duodenum enteric plexus

Concepción Junquera Escribano[1,2], Irene Cantarero Carmona[1,2], Mª José Luesma Bartolomé[1,2], Mario Soriano-Navarro[4], Carmen Martínez-Ciriano[1,2], Tomás Castiella Muruzábal[2,3] and José-Manuel García-Verdugo[4]

[1]Department of Human Anatomy and Histology, Faculty of Medicine, University of Zaragoza, Zaragoza, Spain, [2]Aragon Health Sciences Institute, Zaragoza, Spain, [3]"Lozano Blesa" University Clinical Hospital, Zaragoza, Spain and [4]Prince Felipe Research Center, Valencia, Spain

Summary. Studies *in vitro* have permitted the identification of enteric neural progenitor cells. Now the question arises as to where these progenitor cells are located *in vivo*. The purpose of this paper is to identify possible candidate cells by means of transmission electron microscopy (TEM).

We have located three interstitial cellular types around the rat duodenum myenteric plexus. Type I cells have been identified as Interstitial Cells of Cajal (ICCs). These cells present well defined ultrastructural characteristics, including the triple connexion IC-nervous trunk- blood vessels. Type II cells show characteristics of immature cells, emphasizing the presence of a single cilium with the structure (9+0). To analyse this nanostructure, we have elaborated a reconstruction on ultrathin sections. The two previously described cellular types could be considered to be different functional states of the same cell. Type III cells present ultrastructural characteristics of fibroblast-like cells. This study suggests that Type II cells could be a source of neural progenitor cells.

Key words: Primary cilium, Interstitial cells, Enteric plexus, Progenitor cells, Stem cells, Ultrastructure, Electron microscopy.

Introduction

The enteric nervous system (ENS) is a collection of neurons in the gastrointestinal tract that constitutes the "brain of the gut". This system provides neural control for all functions of the gastrointestinal tract. Subsequent examination of the functional and chemical diversity of enteric neurons revealed that the enteric nervous system closely resembles the central nervous system (CNS). In fact, the ultrastructure of the ENS is more similar to the CNS ultrastructure than to the rest of the peripheral nervous system (PNS) (Gershon et al., 1994; Gershon, 1997).

An increasing body of evidence has accumulated throughout recent years that supports the existence of multipotent neural progenitor cells in the adult mammalian gut, which are capable of differentiating into neurons, glia and other cell types (Kruger et al., 2002; Bondurand et al., 2003).

Rodent enteric neural stem cells have previously been isolated by flow cytometry and cell suspension cultures from smooth muscle layers of the entire gut wall of postnatal and adult animals (Suarez-Rodriguez and Belkind-Gershon, 2004). Recent studies described a different approach to obtain enteric ganglion-derived cells with the properties of neural progenitor cells, using isolated myenteric ganglia from the infant and adult human gut (Rauch et al., 2006; Metzger et al., 2009) and myenteric ganglia from postnatal rat ileum (Silva et al., 2008). These investigations provide evidence that neural crest-derived progenitors are located in the myenteric plexus. These precursors are nestin-immunopositive in vitro, they can be expanded as neurosphere-like bodies under the influence of EGF and FGF-2 and are induced to differentiate into neurons and glial cells (Silva et al., 2008).

Nestin expression is usually associated with dividing or migrating cells (Sahlgren et al., 2001). In addition, nestin is regarded as a marker for progenitor cells in the CNS (Lendahl et al., 1990).

The specific location of enteric neural crest-derived stem cells within the rodent gut wall has not yet been established *in vivo*. It might be expected that they would

Offprint requests to: Prof. C. Junquera, Department of Human Anatomy and Histology, Faculty of Medicine, University of Zaragoza, Domingo Miral s/n, 50009 Zaragoza. e-mail: cjunquer@unizar.es

reside within or, in close association with the enteric ganglia. Interstitial Cajal cells can be found close to enteric plexuses and surrounding the ganglions (Ramón y Cajal, 1893). The ultrastructural characteristics of these cells were established in different locations of rodent gastrointestinal tract (Horiguchi and Komuro, 1998; Komuro, 1998), but not of rat duodenum. Surprisingly, in the small intestine, some ICCs in the plane of the myenteric plexus were found to be nestin-immunopositive as has been demonstrated in humans (Vanderwinden et al., 2002) and some cells were scattered between the two muscle layers and intramuscularly in rat (Cantarero et al., 2010).

Since the nestin-positive stem cells which have generated neurospheres definitely come from the myenteric plexus, our ultrastructural study has focused initially on the identification of the different cell types that appear to related to the enteric plexuses.

Evidently, a critical step towards understanding adult neurogenesis is the identification of the primary precursors that generate new neurons *in vivo*. The identification of a stem cell niche is crucial to understand the factors that regulate these cells.

The purpose of this report is to carry out an ultrastructural study of the enteric plexuses and the cells surrounding the ganglia, where the neural precursor cells must be found.

Material and methods

Animal Use

Four adult Wistar rats, three months old, (Jackson Laboratoires) were used in accordance with institutional guidelines (Ethics Advisory Commission for Animal Experimentation, PI 22/08). Each animal had ad libitum access to food and water and was fed on a complete and balanced standard laboratory diet (Teklad 4% rat diet 7001; Harlan Teklad, Madison, WI). They were housed in temperature controlled rooms (20±1°C) and under natural light.

The rats were anesthetised with pentobarbital sodium and perfused intracardially with 2.5% glutaraldehyde and 2% paraformaldehyde.

Electron Microscopy

The samples were washed in Palade tampon, post-fixed with 2% osmium, rinsed, dehydrated in graded acetones (30%, 50%, 70% with 2% uranyl-acetate, 90%, 100%) cleared in propylene oxide and embedded in araldite (Durcupan, Fluka). Serial semithin sections (1.5 μm) were cut with a diamond knife and stained lightly with 1% toluidine blue. Later, ultrathin (0.08 μm) sections were cut with a diamond knife, collected on Formvar coated single-slot grids, counterstained with 1% uranyl acetate and for 10 minutes with Reynold's lead citrate and examined under a FEI Tecnai G2 Spirit transmission electron microscope. The images were achieved with AMT's CCD imaging system. To determine the cilium ultrastructural characteristics in all its length, we examined 50 serial ultrathin sections.

Results

Myenteric ganglia were located intramuscularly in the connective tissue. In the neurons, the nucleus presented a uniformly distributed chromatin, a thin frame of marginal heterochromatin and a prominent nucleolus (Fig. 1A). Glial cells and thick axonal trunks constituted a part of these ganglion structures (Fig. 1B). We observed three types of non-neural cells in the connective space between the inner circular and the outer longitudinal muscle layers. These cells constitute a cellular net. They are interconnected through their cytoplasmic prolongations that extend from the ganglia periphery up to a capillary (Fig. 2A). Type I cells had an oval or stellate shape presenting a very voluminous nucleus with a finely distributed euchromatin with small condensations and a thin frame of marginal heterochromatin. It was surrounded by a small perinuclear cytoplasm which expanded with radial cytoplasmic processes (Fig. 2B). The cytoplasm of these cells was less electron-dense than the neighbouring smooth muscle cells. The long slender cytoplasmic processes of these cells often overlaid the ganglia (Fig. 1A) and nerve bundles (Fig. 1B) with only a small gap intervening. These cells contained several cisternae of rough endoplasmic reticulum, polysomes, many thin intermediate filaments, abundant mitochondria which extended throughout cytoplasmic processes, and dispersed caveolae along the cell membrane (Fig. 2C). These ultrastructural features corresponded to the currently accepted criteria for interstitial cells of Cajal (ICC) identification. They resembled fibroblasts, but were different on account of the lack of dilated cisternae of endoplasmic reticulum, less dense cytoplasmic matrix and frequent multiple cytoplasmic extensions. IC type I showed intercellular connexions forming a network through the duodenal wall (Fig. 3A,B); these homotypic connexions can be identified with electrodense reinforcement in the membranes of cellular prolongations (Fig. 3A,B). In parallel, more than two cells can be connected (Fig. 3B). These cells also established close contact with neighbouring smooth muscle cells (Fig. 3C); however, fibroblasts do not form close contacts with any other cells.

Type II cells were similar to type I but with certain differences. They had rather larger oval nuclei with simpler cell bodies and fewer cytoplasmic organelles, containing numerous free polyribosomes and limited rough endoplasmic reticulum. Their prolongations established close contacts with nervous trunks (Fig. 4A). These cells exhibited a single cilium with 9+0 pattern, derived from the parental centriole of the diplosomal pair. The single cilium emerging from a typical basal body was projected into the extracellular space. The mother centriole shows basal foot and cap (Fig. 4B). The

daughter centriole was close to the Golgi complex (Fig. 4C). We present three serial electron micrographs selected from a reconstruction to analyse the ultrastructure of this single cilium (Fig. 4).

In the circular smooth muscle layer, a population of interstitial cells of stellate morphology could be observed establishing close association with nerve endings (Fig. 5A,B). Likewise, these cells established a close relationship with blood capillaries (Fig. 6A,B) and a connection with adjacent smooth muscle cells (Fig. 6C). The contact *nervous trunk - interstitial cell - capillary* is a constant interrelation in our observations.

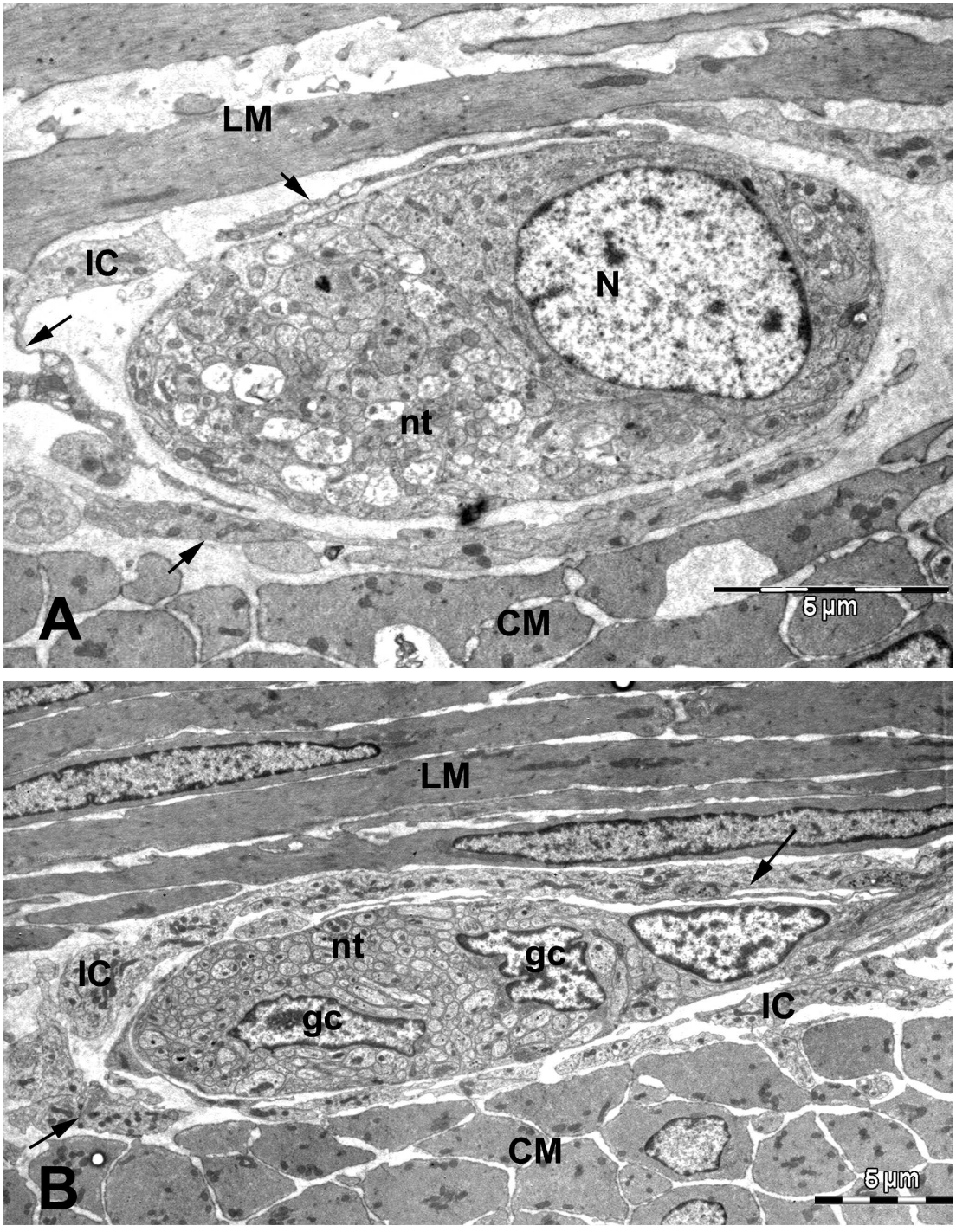

Fig. 1. Ganglion in the myenteric plexus of rat duodenum surrounded by cytoplasmic processes of interstitial cells (arrows). **A.** The nucleus of neurons had finely granular chromatin. **B.** The nucleus of glial cells was smaller and much more irregular, with a compact distribution of heterochromatin and a thin frame of marginal chromatin. N: neuron; gc: glial cell; nt: nervous trunk; IC: interstitial cell; LM: longitudinal muscle layer, CM: circular muscle layer.

The overall appearance of type II cells was suggestive of less-differentiated type I cells, and appeared detached from the IC network, although there was a gradual transition between the type I and type II cells. Type I cells do not present a primary cilium. This cilium emission, like a tiny antenna, is, without doubt, an indicative fact of a specific function of type II cells.

In minor proportion, in the periganglionar intramuscular connective tissue, we found a third cellular type which we identified as fibroblast-like. On an ultrastructural level, the fundamental differences with type I cells were: a fusiform and elongated nucleus, a cytoplasm with a more important electron density, less mitochondrial content and long slender processes which did not present

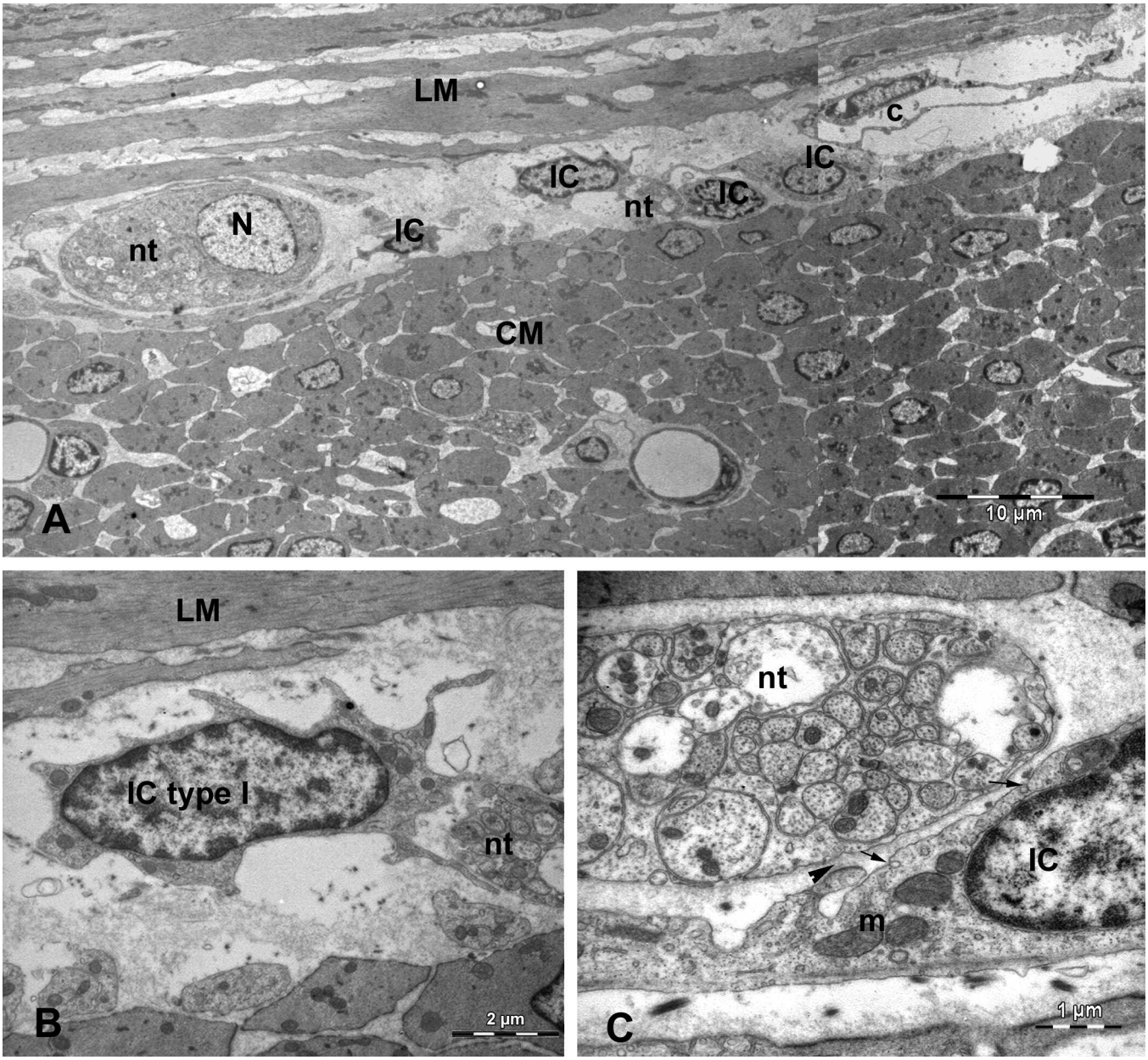

Fig. 2. Interstitial cells located in the connective space between the circular and longitudinal muscle layers. **A.** In the proximity of a ganglion are four interstitial cells between the two muscle coats. These cells show an oval or stellate shape, with long cytoplasmic processes. An interstitial cell with moniliform processes is located between the blood capillary and smooth muscle cells. **B.** Detail of 2A. IC Type I projecting slender cytoplasmic processes in various directions. Their cytoplasm is less electrodense than the smooth muscle cells. **C.** Close association between an IC and a bundle of axons (arrowhead). Some mitochondria and caveolae can be seen (arrows). N: neuron; gc: glial cell; nt: nervous trunk; LM: longitudinal muscle layer; CM: circular muscle layer; c: capillary; m: mitochondria.

caveolae. These fibroblast-like prolongations established contacts with IC type II (Fig. 7).

Discussion

In this transmission electron microscope study, we have identified three cellular types. The ultrastructural characteristics that we described for type I cells coincide with the standards defined as well characterized ICCs. The second type seems to be a less differentiated cell that shows a single cilium as a remarkable characteristic.

A large variety in the structural features of ICCs in different portions of the digestive tract of different species has been recorded by some investigators (Komuro, 1982; Christensen, 1992; Junquera et al., 2001). Komuro refers to the different cytological features of the cells that could represent morphological variations of the same cell type, or a mixture of different cell types, possibly including cells that were not typical ICCs. In the same way, Min and Leabu suggest that the developing gut contains progenitor cells gradually expressing different combinations of proteins. They also suggest that some of these progenitor cells may remain in adult tissues functioning as organ-specific stem cells (Min and Leabu, 2006). Previous results in rabbit duodenum have provided evidence that some populations of ICCs present immunohistochemical and ultrastructural characteristics that are often present in progenitor cells (Junquera et al., 2007). Recent publications have shown evidence of plasticity in these cells (Faussone-Pellegrini, 2005; Faussone-Pellegrini et al., 2006).

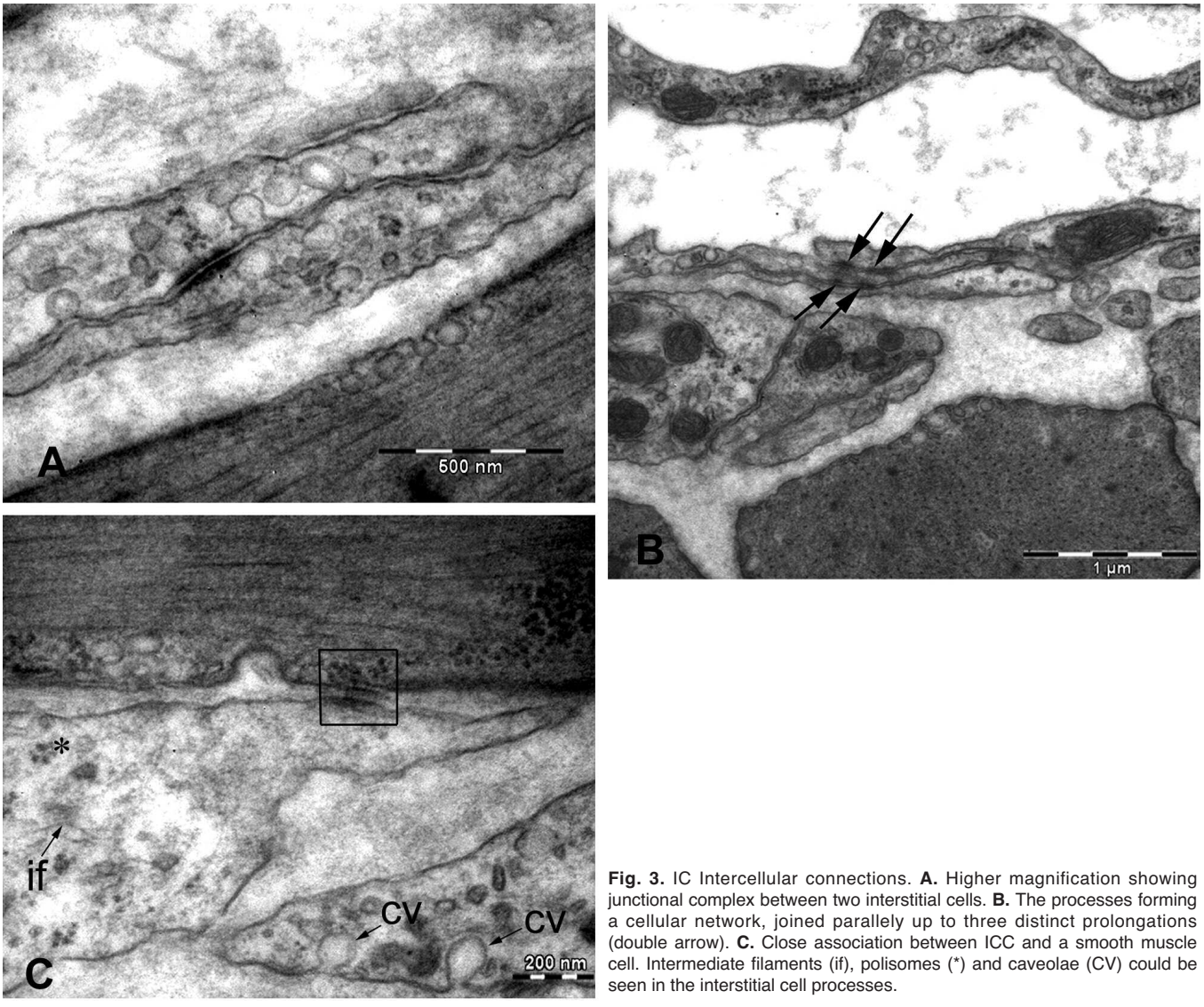

Fig. 3. IC Intercellular connections. **A.** Higher magnification showing junctional complex between two interstitial cells. **B.** The processes forming a cellular network, joined parallely up to three distinct prolongations (double arrow). **C.** Close association between ICC and a smooth muscle cell. Intermediate filaments (if), polisomes (*) and caveolae (CV) could be seen in the interstitial cell processes.

Our view is that the different cytological morphologies found in interstitial cells which surround the ganglia of the myenteric plexus, may represent several degrees of differentiation of a progenitor cell that is located in the connective tissue of the adult intestine. Both cellular types described (type I and type II) could be considered different functional states of the same cell.

The primary cilium, which we found only in type II cells, is a single non-motile cilium derived from the parental centriole of the diplosomal pair; which seems to relocate itself into a position beneath the cell membrane after mitosis (Rieder et al., 1979). It generally differs from a motile cilium by its reduced length, its lack of motility due to the absence of dynein arms and lack of a central pair of microtubules in its axoneme, which are critical for directional controlled beating.

Several authors have reported the presence of a

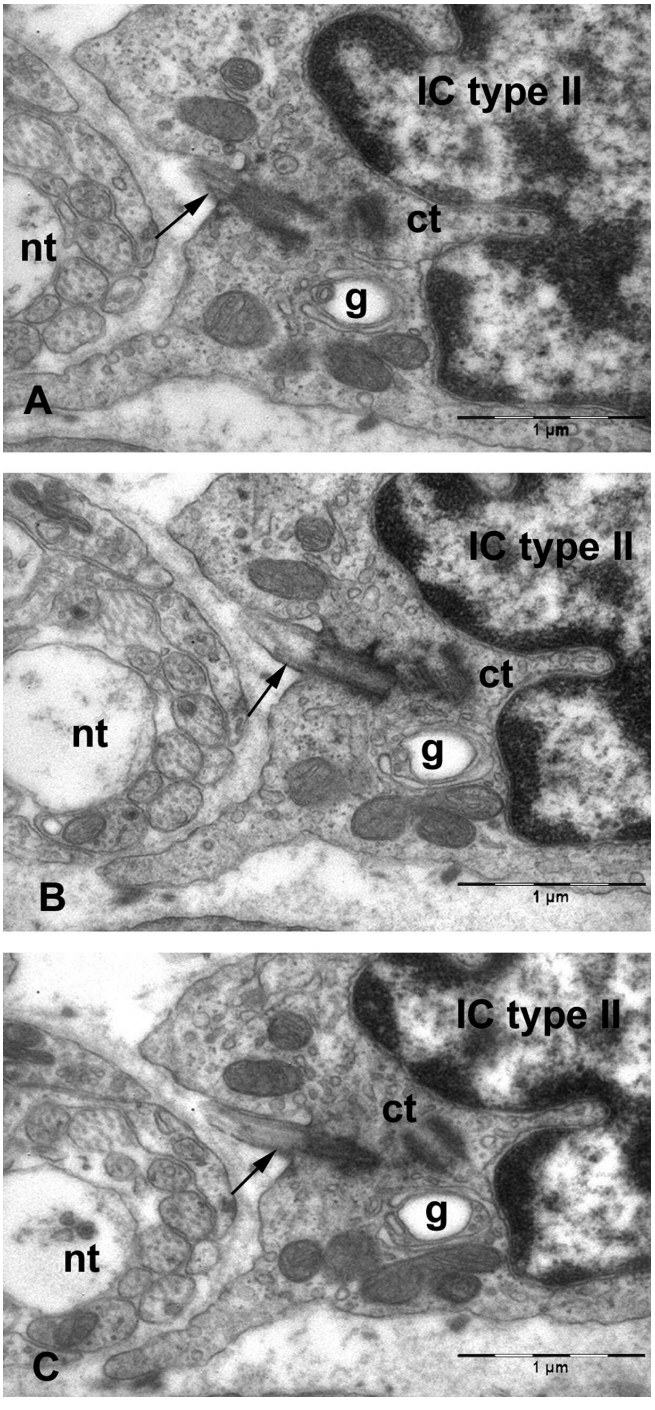

Fig. 4. IC Type II. Three ultrathin serial sections selected from the reconstruction of a primary cilium (arrow). The lack of the central pair of microtubules can be observed. The cilium emerging from a typical basal body that shows basal foot and cap **(B)**. The daughter centriole was close to the Golgi complex; nt: nervous trunk, g: Golgi complex; ct: centriole.

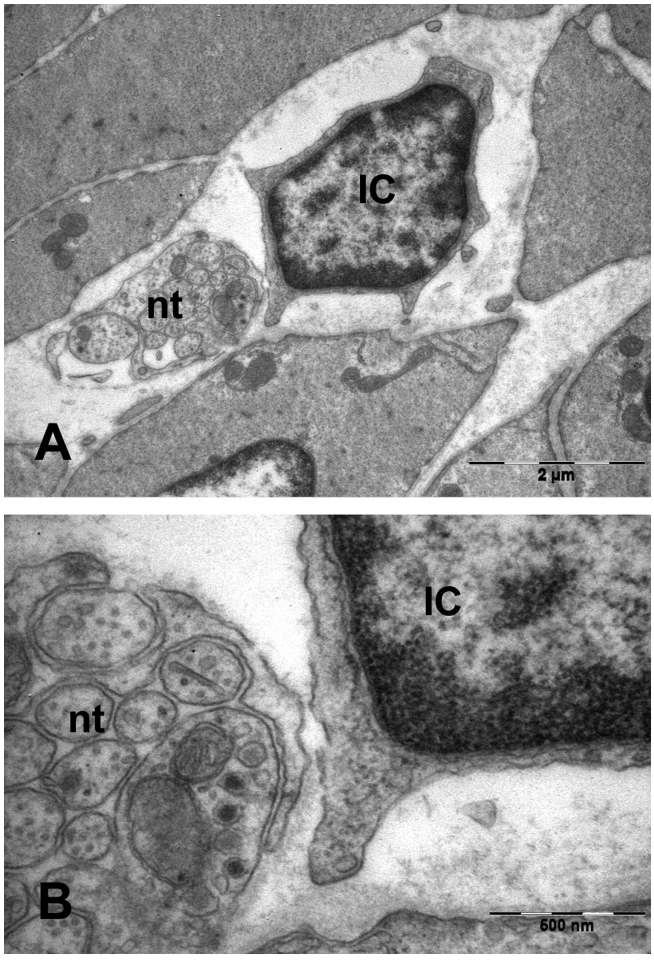

Fig. 5. TEM intramuscular IC. **A.** IC of stellate morphology in the connective tissue that surrounds the circular smooth muscle layer. **B.** Detail of 5A. Close association between IC and a nervous trunk, some varicosity contains peptidergic vesicles with electrondense central core. nt: nervous trunk; IC: interstitial cell.

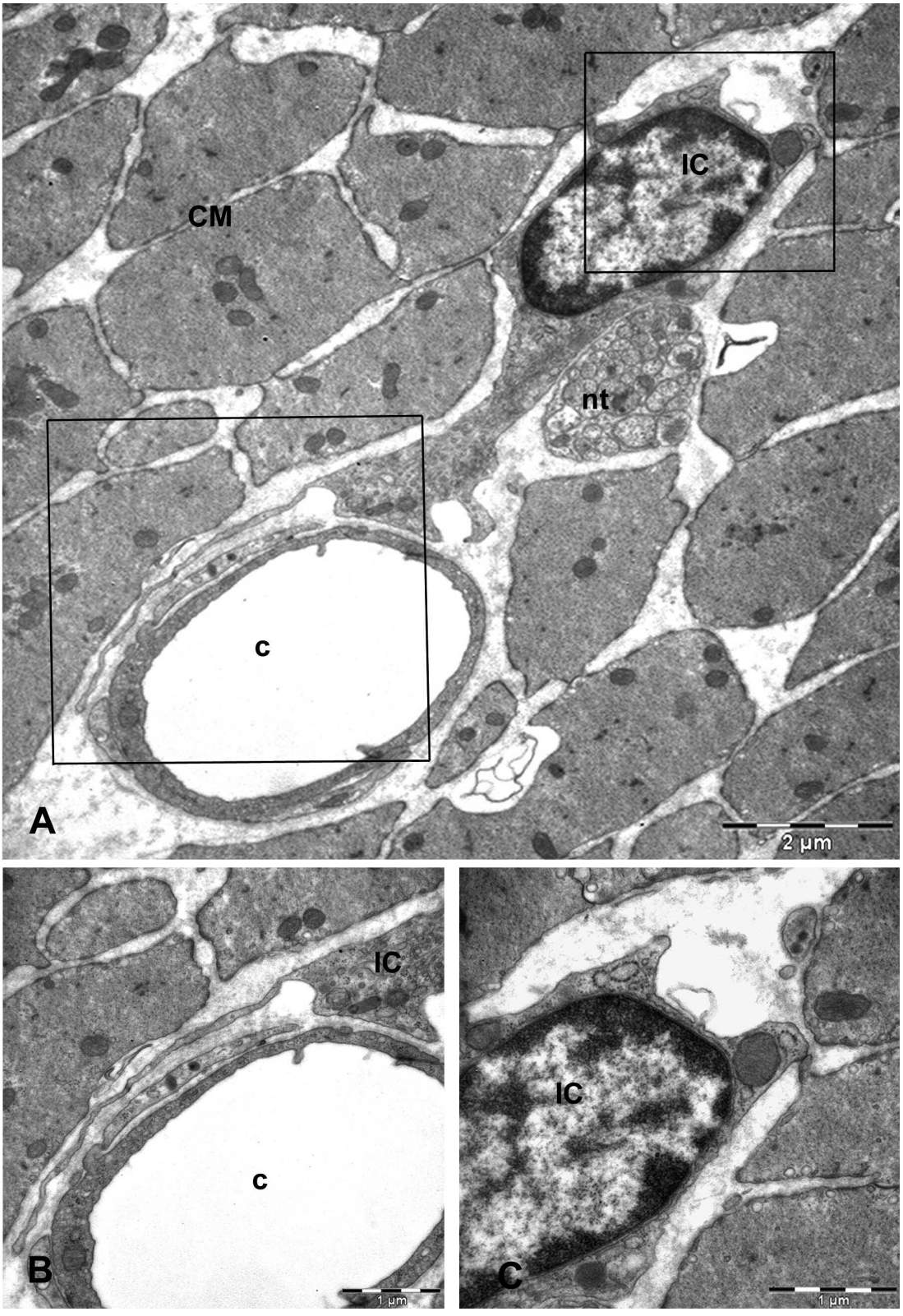

Fig. 6. Triad IC-Nervous trunk-capillary. **A.** Intramuscular IC projecting processes toward a capillary. **B.** Detail of 6A. A filiform process of intramuscular IC surrounding a capillary. **C.** Detail of 6A. The extreme opposite establishes a contact with a muscular cell. nt: nervous trunk; CM: circular muscle layer; c: capillary; IC: interstitial cell.

single cilium emerging from a basal centriole as an ultrastructural characteristic of precursor cells (Seeley and Nachury, 2010). It has also been published that neuronal precursors in the embryonic neuroepithelium extend a single cilium (Cohen and Meininger et al., 1987). The existence of a single cilium characterizes neural progenitor cells in the subventricular zone of the adult mouse (Doetsch et al., 1999; Alvarez-Builla et al., 2001; Alvarez-Builla and Lim, 2004; Doetsch, 2003). Neural stem cells in both embryonic and adult brains have a primary cilium (Han et al., 2008). Primary cilia in the developing dentate gyrus measured 1.5-2μm long and were present in cells containing numerous free ribosomes with little rough endoplasmic reticulum, which is characteristic of dividing progenitor cells. These characteristics are similar to those described in type II interstitial cells.

The dentate gyrus progenitors require primary cilia to mediate Sonic hedgehog (Shh) signalling at a critical stage of their development, when they are transitioning and expanding from embryonic to postnatal neural stem cells (Fu et al., 2004; Spassky et al., 2008). The single cilium is a sensory antenna which concentrates in its surface receptors for Shh signalling. In the same way, Sonic hedgehog regulates the proliferation, differentiation and migration of enteric neural crest cells in the gut (Fu et al., 2004). The presence of a single cilium in interstitial cells type II ("activated" ICCs) could have the same functional meaning as for progenitor cells of the CNS and Shh signalling might act through the primary cilium.

The first objection to this approach is to admit that an ICC's subpopulation, considered to be of mesenchemial origin, could be neural progenitors. However, the existence of a cellular population emerging from the ventral part of the neural tube (VENT cells) and migrating up to the anterior part of the gastrointestinal tract was demonstrated. These cells represent a neurons, glia and ICC source, which suggests the existence of a common progenitor for those three cellular lineages (Sohal et al., 2002).

In the small intestine, the ICCs in the plane of the myenteric plexus and some intramuscular cells were nestin-immunoreactive. Several, but not all, subpopulations of nestin-ir ICCs were Kit-immunoreactive, which indicates some functional subdivisions of the populations of ICCs (Vanderwinden et al., 2002). In rat duodenum, we recently demonstrated nestin-positive cells within myenteric ganglia, but also cells scattered between the two muscle layers and intramuscularly (Cantarero et al., 2010). As a first approach, we might think that at least a subpopulation of interstitial cells, the nestin-ir one, could represent *in vivo* the cells which might give neurospheres in specific culture conditions.

In addition to the two cellular types described in rat duodenum, we have found a third cellular type that we have identified as fibroblast-like, in accordance with Komuro and co-workers studies who identified these cells in rat stomach (Ishikawa et al., 1997) and mouse small intestine (Horiguchi and Komuro, 2000). It has been reported that immature ICCs have fibroblast-like features in Ws/Ws rat colon, where less ICCs were observed.

In vivo, SVZ neural stem cells and their progeny are coupled to blood vessels in a specialized vascular niche; transit-amplifying cells uniquely poised to receive spatial indications and regulatory signals from diverse elements of the vascular system (Tavazoie et al., 2008). SVZ B1 cells in adult mice extend a minute apical ending to directly contact the ventricle and a long basal process ending on blood vessels (Mirzadeh et al., 2008).

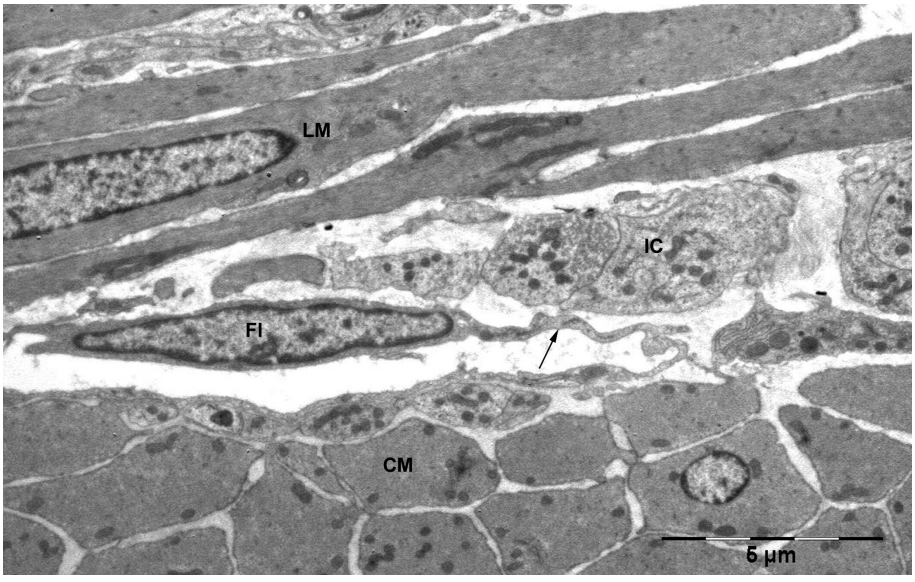

Fig. 7. Fibroblast-like (Fl) in the myenteric region. Its long and filiform process establishes contacts with IC (arrow). No caveolae or close contact with smooth muscle cells was found.

As we have demonstrated in our results, interstitial cells establish, through their processes, contact with blood capillaries of the periganglionar region and with small capillaries located intramuscularly in the thickness of the muscular layer to receive spatial reminders and regulatory signals from diverse elements of the vascular system.

Conclusion

In this paper, we have established the ultrastructural characteristics of the interstitial cells localized around enteric plexuses and intramuscularly in rat duodenum.

The results of this study suggest that at least a subpopulation of *activated* ICCs could be initially the source of neural and ICC progenitor cells and its activation might coincide with the emission of a single cilium (9+0), acquiring a less differentiated morphology. We proposed that the single cilium could represent a characteristic of precursor cells in the enteric nervous system.

Acknowledgements. This research received financial support from Aragon Institute of Health Sciences (I+CS) (PIPAMER 0807) and the European Social Fund (ESF), DGA (B83).

References

Alvarez-Buylla A. and Lim D.A. (2004). For the long run: Maintaining germinal niches in the adult brain. Neuron 41, 683-686.

Alvarez-Buylla A., García-Verdugo J.M. and Tramontin A.D. (2001). A unified hypothesis on the lineage of neural stem cells. Nature Rev. Neurosci. 2, 287-293.

Bondurand N., Natarajan D., Thapar N., Atkins C. and Pachnis V. (2003). Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures. Development 130, 6387-6400.

Cantarero I., Luesma M.J., Lavoie-Gagnon C. and Junquera C. (2010). Distribution of nestin protein: immunohistochemical study in enteric plexus of rat duodenum. Microsc. Res. Tech. Article online in advance of print. DOI 10.1002/jemt.20884.

Christensen J. (1992). A commentary on the morphological identification of interstitial cells of Cajal in the gut. J. Auton. Nerv. Sist. 37, 75-88.

Cohen E. and Meininger V. (1987). Ultrastructural analysis of primary cilium in the embryonic nervous tissue of mouse. Int. J. Dev. Neurosci. 5, 43-51.

Doetsch F. (2003) The glial identity of neural stem cells. Nature Neurosci. 6, 1127-1134.

Doetsch F., García-Verdugo J.M. and Alvarez-Buylla A. (1999). Regeneration of a germinal layer in the adult mammalian brain. Proc. Natl. Acad. Sci. 96, 11619-11624.

Faussone-Pellegrini M.S. (2005). Interstitial cells of Cajal: once negligible players, now blazing protagonists. Ital. J. Anat. Embriol. 110, 11-31.

Faussone-Pellegrini M.S., Vannucchi M., Leder O., Huang T.Y. and Hanani M. (2006). Plasticity of interstitial cells of Cajal: a study of mouse colon. Cell Tissue Res. 325, 211-217.

Fu M., Lui V.C.H., Sham M.H., Pachnis V. and Tam P.K.H. (2004). Sonic hedgehog regulates the proliferation, differentiation, and migration of enteric neural crest cells in gut. J. Cell Biol. 166, 673-684.

Gershon M.D. (1997). Genes and lineages in the formation of the enteric nervous system. Curr. Opin. Neurobiol. 7, 101-109.

Gershon M.D., Kirchgessner A.L. and Wade P.R. (1994). Functional anatomy of the enteric nervous system. In: Physiology of the gastrointestinal tract. 3rd ed. Johnson L.R. (ed). Raven Press. New York pp 381-422.

Han Y.G., Spassky N., Romaguera-Ros M., García-Verdugo J.M., Aguilar A., Schneider-Maunoury S. and Alvarez-Builla A. (2008). Hedgehog signaling and primary cilia are required for the formation of adult neural item cells. Nat. Neurosci. 11, 277-284.

Horiguchi K. and Komuro T. (1998). Ultrastructural characterization of interstitial cells of Cajal in the rat small intestine using control and Ws/Ws mutant rats. Cell Tissue Res. 293, 277-84.

Horiguchi K. and Komuro T. (2000). Ultrastructural observations of fibroblast-like cells forming gap junctions in the W/W(nu) mouse small intestine. J. Auton. Nerv. Syst. 80, 142-147.

Ishikawa K., Komuro T., Hirota S. and Kitamura Y. (1997). Ultrastructural identification of the c-kit expressing interstitial cells in the rat stomach: a comparison of control and Ws/Ws mutant rats. Cell Tissue Res. 289, 137-43.

Junquera C., Martinez-Ciriano C., Castiella T., Serrano P., Aísa J., Calvo E. and Lahoz M. (2001). Enteric plexus and interstitial cells of Cajal: Interrelationship in the stomach of *Podarcis hispanica* (reptilia). Histol. Histopathol. 16, 869-861.

Junquera C., Martínez-Ciriano C., Castiella T., Serrano P., Azanza M.J. and Ramón y Cajal Junquera S. (2007). Immunohistochemical and ultrastructural characteristics of interstitial cells of Cajal in the rabbit duodenum. Presence of a single cilium. J. Cell. Mol. Med. 11, 776-787.

Komuro T. (1982). The interstitial cells in the colon of the rabbit. Cell Tissue Res. 222, 41-52.

Komuro T. (1998). Comparative morphology of interstitial cells of Cajal: ultrastructural characterization. Microsc. Res. Tech. 47, 267-285.

Kruger G.M., Mosher J.T., Bixby S., Joseph N., Iwashita T. and Morrison S.J. (2002). Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. Neuron 35, 657-669.

Lendahl U., Zimmerman L.B. and McKey R.D. (1990). CNS stem cells express a new class of intermediate filament protein. Cell 60, 585-595.

Metzger M., Bareiss P.M., Danker T., Wagner S., Hennenlotter J., Guenther E., Obermayr F., Stenzl A., Koenigsrainer A., Skutella T. and Just L. (2009). Expansion and differentiation of neural progenitors derived from the human adult enteric nervous system. Gastroenterology 137, 2063-2073.

Min K.B. and Leabu M. (2006). Interstitial cells of Cajal (ICC) and gastrointestinal stromal tumor (GIST): facts, speculations and myts. J. Cell Mol. Med.10, 995-1013.

Mirzadeh Z., Merkle F.T., Soriano-Navarro M., García-Verdugo J.M. and Álvarez-Buylla A. (2008). Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. Cell Stem Cell. 3, 265-278.

Ramón y Cajal S. (1893). Los ganglios y plexos nerviosos del intestino de los mamíferos y pequeñas adiciones a nuestros trabajos sobre la médula y gran simpático general. Imprenta y Librería de Nicolás Moya, Madrid.

Rauch U., Hansgen A., Hagl C., Holland-Cunz S. and Schafer K.H. (2006). Isolation and cultivation of neuronal precursor cells from the developing human enteric nervous system as a tool for cell therapy in dysganglionosis. Int. J. Colorectal Dis. 21, 554-559.

Rieder C.L., Jensen C.G. and Jensen L.C. (1979). The resorption of primary cilia during mitosis in a vertebrate (PtK1) cell line. J. Utrastruct. Res. 68, 173-185.

Sahlgren C.M., Mikhailov A., Hellman J., Chou Y.H., Lendahl U., Goldman R.D. and Eriksson J.E. (2001). Mitotic reorganization of the intermediate filament protein nestin involves phosphorylation by cdc2 kinase. J. Biol. Chem. 276, 16456-16463.

Seeley E.S. and Nachury M.V. (2010). The perennial organelle: assembly and disassembly of the primary cilium. J. Cell Sci. 123, 511-518.

Silva A.T., Wardhaugh T., Dolatshad N.F., Jones S. and Saffrey M.J. (2008). Neural progenitors from isolated postnatal rat myenteric ganglia: Expansion as neurospheres and differentiation *in vitro*. Brain Res. 47-53.

Sohal G.S., Ali M.M. and Farooqui F.A. (2002). A second source of precursor cells for the developing enteric nervous system and interstitial cells of Cajal. Int. J. Dev. Neurosci. 20, 619-626.

Spassky N., Han Y.G., Aguilar A., Strehl L., Besse L., Laclef C., Romaguera-Ros M., Garcia-Verdugo J.M. and Alvarez-Builla A. (2008). Primary cilia are required for cerebellar development and Shh-dependant expansion of progenitor pool. Dev. Biol. 317, 246-259.

Suarez-Rodriguez R. and Belkind-Gershon J. (2004). Cultured nestin-positive cells from postnatal mouse small bowel differentiates ex vivo into neurons, glia, and smooth muscle. Stem Cells 22, 1373-1385.

Tavazoie M., Van der Veken L., Silva-Vargas V., Louissaint M., Colonna N., Zaidi B., Garcia-Verdugo J.M. and Doetsch F. (2008). A specialized vascular niche for adult neural stem cells. Cell Stem Cell 3, 279-288.

Vanderwinden J.M., Gillard K., DeLaet M.H., Messam C.A. and Schiffmann S.N. (2002). Distribution of the intermediate filament nestin in the mucularis propria of the human gastrointestinal tract. Cell Tissue Res. 309, 261-268.

Accepted October 29, 2010