

Persistence of Cajal-Retzius cells in the adult human cerebral cortex. An immunohistochemical study

R. Martín¹, A. Gutiérrez¹, A. Peñafiel¹, M. Marín-Padilla² and A. de la Calle¹

¹Department of Cell Biology, Faculty of Sciences, University of Málaga, Málaga, Spain and

²Department of Pathology, Dartmouth Medical School, Hanover, New Hampshire, USA

Summary. The presence of Cajal-Retzius cells in the adult human prefrontal and visual cortices has been demonstrated with calcium binding protein immunocytochemistry and NADPH-diaphorase histochemistry. These cells expressed parvalbumin, calbindin and calretinin calcium binding proteins and displayed NADPH-diaphorase enzyme activity. The three basic morphological profiles-horizontal, pyriform and multipolar-were observed. The morphologies of labelled cells resembled those of neurons observed in Golgi studies of the human cerebral cortex. The presence of calcium binding proteins and NADPH-diaphorase in these cells suggests a possible inhibitory role as GABAergic neurons. The persistence of Cajal-Retzius cells in the adult cerebral cortex supports the idea that they undergo developmental dilution rather than postnatal degeneration.

Key words: Cajal-Retzius cell, cerebral cortex, calcium binding proteins, NADPH-diaphorase, human.

Introduction

Cajal-Retzius (C-R) cells are the first cortical cells to differentiate in the neocortex. Their morphological characteristics have been thoroughly studied by Golgi methods in different species including humans (Marín-Padilla, 1988, 1990, 1998). C-R cells are believed to play a crucial role in corticogenesis (Marín-Padilla, 1988, 1990, 1998; Ogawa et al., 1995), however, their postnatal fate and function in the adult mammalian brain is a matter of controversy. Some authors have suggested that these cells: i) degenerate during late corticogenesis (Duckett and Pearse, 1968; Derer and Derer, 1990), ii) are diluted by the developmental growth of the neocortex but some persist in the adult brain (Marín-

Padilla, 1988, 1990; Mrzljak et al., 1990), or iii) are transformed into non-pyramidal cells (Parnavelas and Edmunds, 1983).

In human brain, several studies have reported the existence of C-R cells expressing calretinin calcium binding protein throughout all prenatal developing stages to adulthood (Glezer et al., 1992; Belichenko et al., 1995; Fonseca and Soriano, 1995; Verney and Derer, 1995; Gabbott et al., 1997). Parvalbumin and calbindin are also expressed by human fetal C-R cells (Verney and Derer, 1995; Cao et al., 1996). However, the expression of these two calcium binding proteins by C-R cells in the adult human brain has not been reported. Furthermore, NADPH-diaphorase (NADPH-d) activity in human C-R cells has only been demonstrated during cortical development (Duckett and Pearse, 1968; Meyer and González-Hernández, 1993).

The present study shows the persistence of parvalbumin and calbindin immunoreactive C-R cells in human cerebral cortex. These cells also depict NADPH-d activity.

Materials and methods

The post-mortem brains from eight patients (48-89 years old), who died without history of neurological or psychiatric disorders, were studied. Time interval between death and the post-mortem removal of the brains ranged between 8 and 10 hours. Brains were perfused *ex situ* via the internal carotids and basilar arteries with 0.1M phosphate buffer, pH 7.3 (PB) followed by 2 litres of 4% paraformaldehyde, 0.5% glutaraldehyde in PB. The brains were then suspended by the basilar artery in the same fixative for 12 hours. Several 1 cm thick tissue blocks were dissected from the prefrontal and visual areas and postfixed for an additional 24 hours. Tissue blocks were cut at 50 μ m in a vibratome and sections collected in cold PB. Free-floating sections were processed for immunocytochemistry using the peroxidase-anti-peroxidase (PAP) method or for the histochemical detection of NADPH-d activity. Autopsies and experimental procedures were

Offprint requests to: Dra. Adelaida de la Calle, Departamento de Biología Celular, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos, 29071 Málaga, Spain. Fax: +34-5-2132000. e-mail: delacalle@uma.es

conducted in conformance with the policies and guidelines of human protection and ethics.

Polyclonal antibodies against parvalbumin (SWant Antibodies, Berlinzona, Switzerland) diluted 1:1000, calretinin (SWant Antibodies, Berlinzona, Switzerland) diluted 1:2000, and calbindin-D28K (donated by Dr. Emson, Cambridge, UK) (Ichimiya et al., 1988) diluted 1:1000 were used. Bound antibodies were revealed with 0.05% 3,3'-diaminobenzidine tetrahydrochloride, 0.05% nickel ammonium sulphate and 0.03% H₂O₂. Immunocytochemical controls, in which the primary antibodies were omitted or substituted by normal rabbit serum, did not show tissue immunostaining.

For NADPH-d histochemistry, sections were preincubated in PB containing 0.25% Triton X-100 for 10 min followed by incubation in PB containing 0.5 mg/ml β -NADPH, 0.2mg/ml nitro-blue tetrazolium (NBT) and 0.25% Triton X-100 for 2-18 hours at 37 °C. In control sections, incubated without β -NADPH or NBT, NADPH-d reactivity was absent.

Results

The presence of occasional C-R cells was demonstrated within layer I of adult human prefrontal and visual cortices. These cells were immunolabelled using antibodies to three different calcium binding proteins (parvalbumin, calbindin and calretinin), and labelled by NADPH-d histochemistry. Immunolabelled or NADPH-d reactive C-R cells displayed staining in the whole somata and primary thick dendrites. Labelled C-R cells were distributed throughout layer I in the cortical areas studied, prefrontal and visual. Some C-R cells were located just beneath the pial surface (Fig. 1A-C, E), whereas others were seen at the middle portion (Fig. 1D) or even lower, within layer I. All of them were non-pyramidal cells showing different morphologies: 1) large horizontal bipolar cells with two prominent horizontal dendritic trunks extending from their opposite poles (Figs. 1A-C) and a descending axon (Fig. 1B), 2) pyriform cells with a thick main dendrite branching horizontally (Fig. 1E), and 3) irregular or multipolar cells with various dendritic processes (Fig. 1D). In both prefrontal and visual cortices, calretinin-immunoreactive C-R cells were located throughout layer I, but they were more frequent in the upper half of this layer (Fig. 1A, B). We also saw C-R cells expressing calbindin (Fig. 1C) or parvalbumin (Fig. 1D) located in the prefrontal and visual cortices of adult human brain. Although these cells were more frequent under pial surface, we also observed some parvalbumin or calbindin cells throughout this layer. NADPH-d-positive C-R cells in the

prefrontal and visual cortices were mainly located in the upper portion of layer I (Fig. 1E).

Discussion

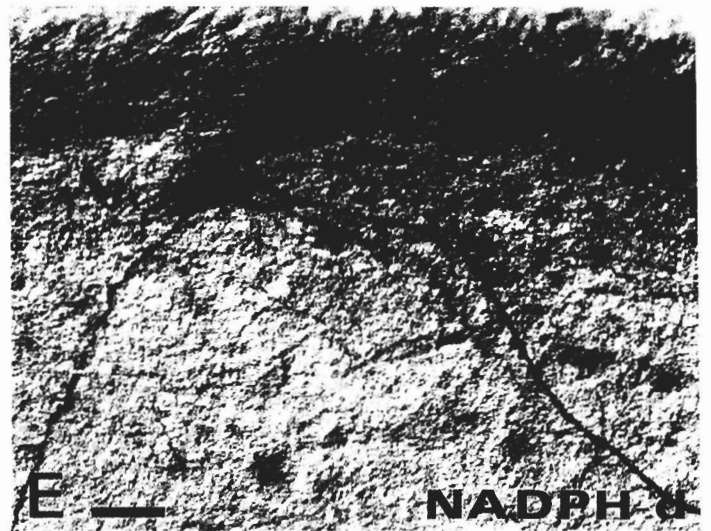
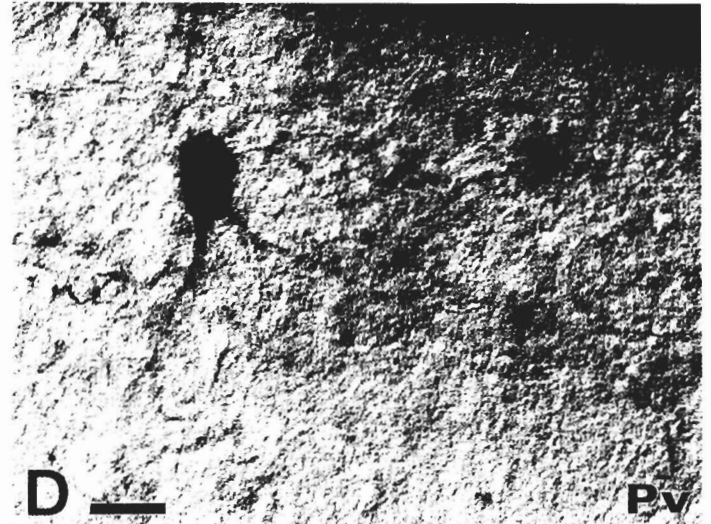
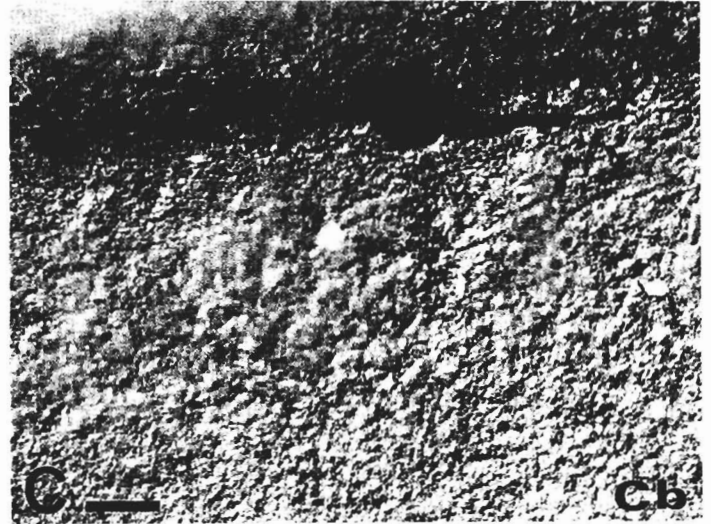
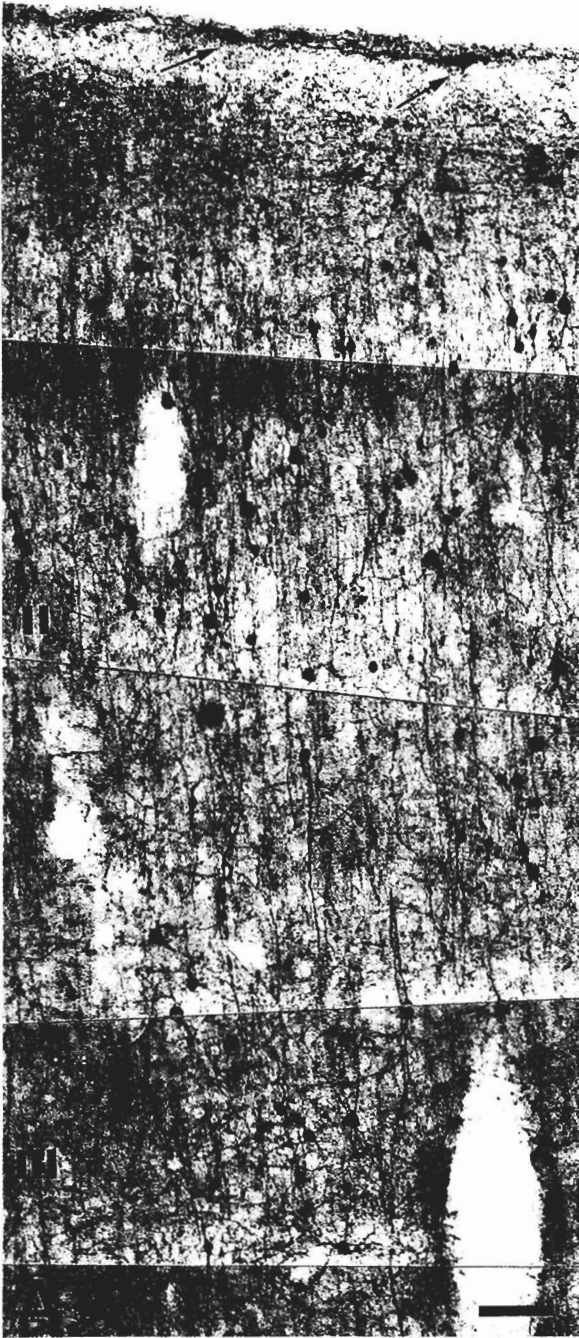
In the present report, we have demonstrated the existence of immunolabelled cells with anti-calretinin, anti-parvalbumin or anti-calbindin, in layer I of prefrontal and visual cortices from adult human brain. These cells also showed NADPH-d activity as revealed by histochemistry. We have observed three morphological varieties, horizontal, pyriform and multipolar, of these nonpyramidal cells. The morphology and position in layer I of these labelled cells coincided with C-R cells seen in prenatal (Marín-Padilla, 1988, 1990, 1998) and adult human brain (Belichenko et al., 1995; Fonseca and Soriano, 1995). In the adult brain C-R cells are few and difficult to find. This is probably due to their developmental dilution as a consequence of the postnatal cortical growth (Marín-Padilla, 1988, 1990, 1998).

The presence of calretinin in C-R cells has been demonstrated by other authors in adult human brain (Glezer et al., 1992; Belichenko et al., 1995; Fonseca and Soriano, 1995; Leuba and Saini, 1996). In the present report we corroborate these findings; however this is the first study reporting the presence of C-R cells containing parvalbumin and calbindin calcium binding proteins in the adult human cerebral cortex. Human C-R cells showing NADPH-d activity have only been observed during brain development (Duckett and Pearse, 1968; Meyer and González-Hernández, 1993); however, we have revealed that C-R cells of adult human prefrontal and visual cortices also express the enzyme NADPH-d. To our knowledge, these findings provides the first evidence of C-R cells containing calbindin or parvalbumin proteins or expressing NADPH-d activity in the adult human cerebral cortex.

On the basis of their early origin and strategic localisation, C-R cells have been considered to have an important role in cortical development (Marín-Padilla, 1988, 1990, 1998; Ogawa et al., 1995). However, the functional significance of these cells in the adult brain is still unknown. It has been suggested that they continue transmitting a common input to the apical dendrites of all pyramidal cells throughout the cerebral cortex, regardless of their specific, motor, sensory, visual or acoustic function (Marín-Padilla, 1998). Also, their chemical content suggests that they might have an important functional role in the neural networks of the adult human cerebral cortex. The presence of GABA in these cells in adult human brain has not been reported.

Fig. 1. Calretinin immunoreactivity in human visual cortex (layers I-III) from an 89-year-old patient (A). Note the presence of two horizontal C-R cells (arrows) under the pial surface in layer I. One of these cells at higher magnification (B) shows the initial portion of its descending axon (arrow). Calbindin D-28K immunoreactive C-R cell located just under the pial surface (C), parvalbumin immunoreactive C-R cell with irregular morphology located at the middle part of layer I (D) and NADPH-d positive C-R cell with a pyriform cell body and a thick dendritic process which profusely branches into the lower part of layer I (E), in human prefrontal cortex of a 72-year-old patient. Scale bars: A, 16 μ m; B, 5.4 μ m; C-E, 4.4 μ m.

Cajal-Retzius cells in adult human brain



We have studied GABA immunocytochemistry in human sections without success, probably due to the rapid diffusion of GABA molecules in post-mortem tissue. However, characterisation of GABAergic neuronal subpopulations by calcium binding protein immunocytochemistry is a well established criteria (Celio, 1990; Baimbridge et al., 1992; Gabbott and Bacon, 1996). Moreover, NADPH-d activity has been used as a marker of GABAergic interneurons (Valtschanoff et al., 1993; Gabbott and Bacon, 1996). Thus, the labelled C-R cells reported in the present study might correspond to different GABAergic inhibitory interneuronal subpopulations.

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