

Late events in the migrative behaviour of the retinal ganglion cells. A Golgi study in the chick retina

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Summary. The final displacement of the prospective ganglion neurons toward the ganglion cell layer (GCL) has been analyzed in chicken embryos during days 8 and 9 of incubation with the help of the Golgi method and computer-assisted image processing.

Our findings indicate that some ganglionar soma are still located in the inner nuclear layer (INL) while others pierce the inner plexiform layer (IPL), exhibiting morphological adaptation of their perikaryon.

The changing morphology of these delayed retinal ganglion neuroblasts seems to be due to the late translocation of the cell perikaryon to the GCL. This late migrative displacement of the ganglionar population is discussed in relation to the presence of displaced ganglion cells of Dogiel.

Key words: Retina, Ganglion cell, Displaced ganglion cell, Cell migration

Introduction

With the exception of the displaced ganglion cells of Dogiel, the retinal ganglion neurons are clearly stratified, together with the displaced amacrine cells in the innermost neuronal layer of the adult retina.

This typical stratified arrangement of the adult ganglion neurons takes place during development in the chick, by the production and migration of young ganglion neuroblasts in a pattern of sequential waves (Tello, 1923; Ramón y Cajal, 1960; Sechrist, 1969; Prada et al., 1981, 1991).

Therefore the last population of the prospective ganglion cells may still be located in the intermediate strata of the embryonic retina intermingled with other retinal neuroblasts, before their perikaryon acquire their final situation (Genis-Galvez, 1977; Rager, 1980; Prada et al., 1981).

This sequential process is clearly expressed, not only by differences in the topographical localization of the cells, but also in the pattern of dendrite morphology which is very simple in the last differentiating ganglionar population when compared with the first.

Most attention has been paid to the differentiating morphological pattern of the first ganglion population (Nishimura, 1980; Rager, 1980; Prada et al., 1981). However, the migrative behaviour of the last differentiated ganglion cells is poorly known.

This report shows the morphological changes that the last chick ganglion cells undergo, during days 8 and 9 of incubation when they pierce the IPL before reaching their definitive position in the GCL.

Materials and methods

White Leghorn chick embryos were incubated at 37.8 °C and staged, according to Hamburger and Hamilton (1951). Whole embryos were fixed by immersion in Stensaas' solution (Stensaas, 1967) for 24 hours. The embryos were then rinsed in tap water and in a small volume of 0.75% silver nitrate solution, before silvering in darkness in a fresh, larger volume of the same solution for 48 hours. This was followed by dehydration in ethanol, embedding in low viscosity nitrocellulose and clearing of the blocks in cedarwood oil. Serial sections were obtained at a thickness of 60 µm. Twenty satisfactorily impregnated embryos at days 8 and 9 of incubation were used in our observation. To avoid taking into consideration broken cells at the cut surfaces of the sections, we accepted only those cells which were shown by over- and underfocusing to be fully contained within the section.

Differential localization of Golgi-stained young ganglion neurons was visualized using an image-processing system based on a Commodore Amiga 2000 computer equipped with an Image Analyzer. Patterns of retinal neurons from a single vertical sections were captured with a SONY TV camera and digitalized as two separated images. Each cell image was processed assigning a specific color to enhance differences

between them. Colors were also assigned to the IPL image and superimposed for a better analysis.

Results

At days 8 and 9 of incubation (Stages 34-35) the structural organization of the central retina showed a well-defined, but incomplete, IPL. This layer was mainly formed by the processes of some amacrine and ganglion neurons whose dendritic fields extended into the IPL in a limited but differentiated pattern.

In Golgi preparations young, less differentiated, ganglionar neurons were observed which can be classified into three varieties according to their topographical location in relation to the incipient IPL. All were identified by impregnation of the axon, which could then be followed entering the Optic fibre layer (ONFL) of the retina.

The first group located their perikaryons at different levels in the immature INL. Those cells with the most external perikaryon had a long vitreal prolongation which became thinner when it crossed the plexiform and ganglionar layer (Fig. 1A). The thick sector of this prolongation had a few short coarse processes sprouting at various levels of its length (Fig. 1A, arrows). A retracting scleral process could frequently be seen in these elongated ganglion neurons (Fig. 1A, curved arrow). Other cells also have their perikaryon at the INL but close to the IPL (Fig. 1B-E, Fig. 2A). Their thick cytoplasmic sector could be seen piercing the IPL where it enlarged considerably in a sprouting mass with several spinelike processes (Fig. 1B-E, arrows). The sprouting of this enlarged cytoplasmic sector always began while the perikaryon was still within the INL. The perikaryon was separated from the sprouting portion by an attenuated neck. From the sprouting mass, the inner prolongation pierced the GCL and immediately adopted a short tangential deviation and then continued by the most internal strata of the incipient ONFL (Fig. 1B and Fig. 2A, arrowheads).

A second group of immature ganglion cells showed their perikaryon in an external position within the IPL (Fig. 1F, I-K, Fig. 2B,C). The sprouting sector of the vitreal prolongation of these cells was generally not clearly separated from the perikaryon as in the first group. In many impregnated neurons the sprouting sector seemed to have a greater volume than that of the corresponding perikaryon (Fig. 2B).

Young ganglion neurons of the third group had their perikaryon located in the external row of the GCL, but they still showed a limited portion of cytoplasm within the IPL (Fig. 1G, H, I-P, Fig. 2D). This portion of the

cell appeared as a spheroidal thickening (Fig. 1G, H, L, M, curved arrows) which may represent a retracting or trailing remnant of the cell.

Discussion

According to previous observations, the synaptic inner plexiform layer is clearly outlined during stages 34 and 35 (Coulombre, 1955; Genis-Galvez et al., 1977; Nishimura, 1980; Rager, 1980; Spira et al., 1987).

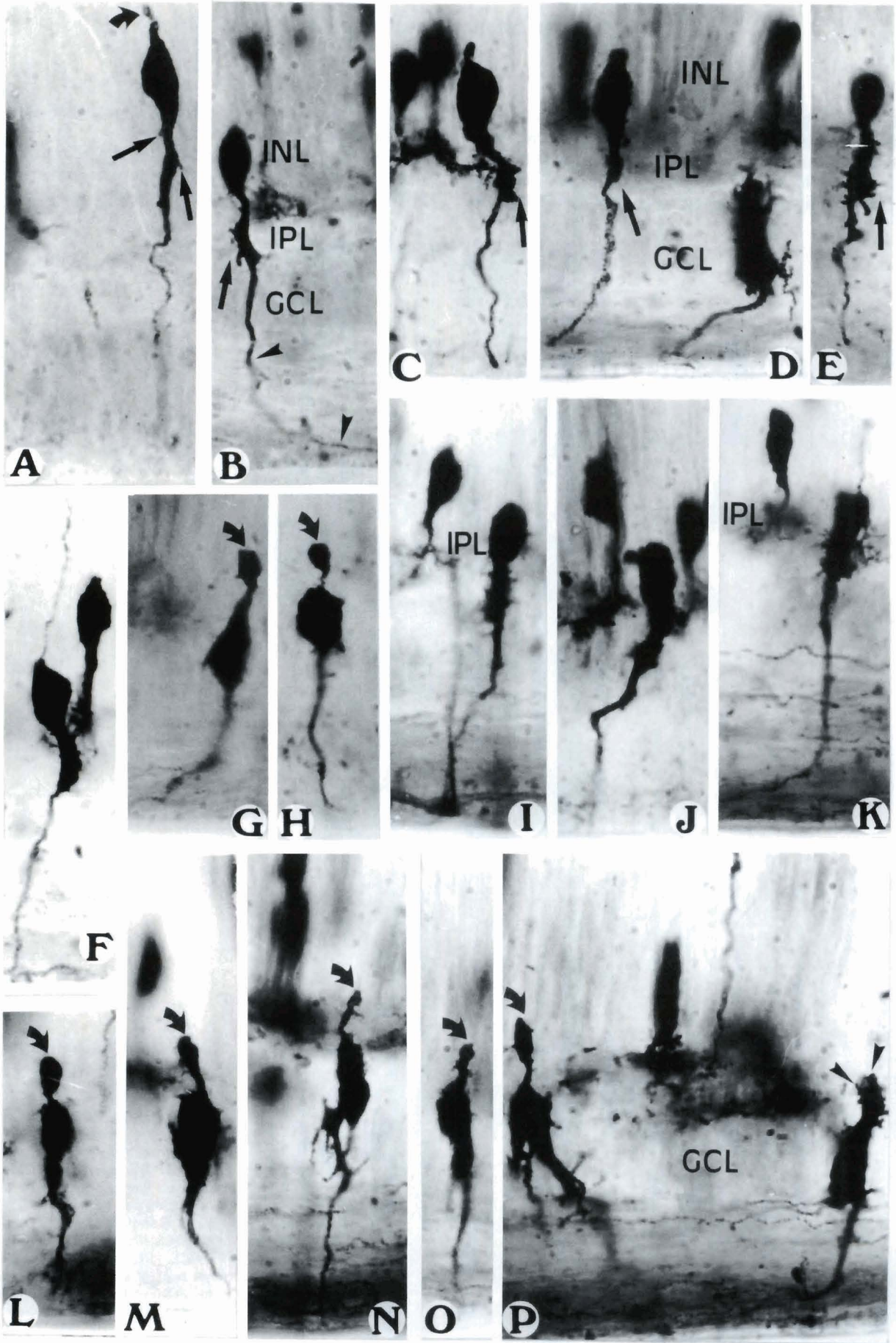
Although it was thought, from the studies of Kolliker (1882), Ramón y Cajal (1892, 1960) and Morest (1970), that the IPL appears only after the formation of the GCL, our observation in the chick supports the conclusion that during the 8th and 9th day of incubation there is a significant number of young ganglionar neurons in the central retina whose perikaryon are still located within both the INL and IPL.

The results obtained in the present study also support the possibility that a significant ganglion cell population, whose axonal processes were precociously itinerant through the ONFL, displaces its perikaryon through the IPL to be finally located in the GCL together with early morphologically differentiated ganglion cells. While piercing the IPL, these cells show evident morphogenetic and adaptative changes which may be seen as trailing pediculated thickenings.

In a previous contribution (Prada et al., 1981) we reported our observations on retinal gangliogenesis in embryos ranging from day 5 to 6 of incubation when the anlage of the IPL is still not found. We observed, at stage 28, that some presumptive ganglion cell perikaryon were located in the middle retinal strata, and had a long leading process bending into the ONFL. We may consider these as younger stages of the ganglion cells described in the present study, before the IPL is formed. There is also the possibility, as was observed in the rat by Morest (1970), and also in our studies in the chick (Prada et al., 1981) and by Watanabe et al. (1991) that although the vitreal attachment feet of anaxonic cells soon acquire the growth cone and axonal characteristics, perikaryon translocation to GCL can be delayed until after formation of the IPL.

As is well known, a population of adult ganglion cell perikaryon remains displaced permanently along the inner border of the IPL, predominantly in the peripheral fields (Prada et al., 1989). Our present analysis of the central retinal fields does not invalidate the possibility that a fraction of the young ganglionar neurons of the first group here described remains, with their perikaryons always scleral to the IPL and therefore transformed into adult displaced ganglion cells (Prada et

Fig. 1. Photographs of Golgi-impregnated presumptive ganglion cells of 8 and 9 days of incubation. Vertical sections. x 1,000. **A to E.** Young ganglion neurons whose perikaryon are clearly located at different levels of the amacrine cell layer. Subperikaryal cytoplasmic enlargements show sprouting of delicate phylopodia. Compare with the definitely located ganglion cell to the right (D). **F, I, J, K.** Young ganglion cells with perikaryon located within the IPL. **G, H, L to P.** Young ganglion cells with trailing pediculated remnants. The arrows show short processes in the inner prolongation. The curved arrows show retracting processes or trailing remnants of the cell. The arrowheads show the axon of the cell in photograph «B» and the birth of dendrites in «P». (INL) inner nuclear layer. (IPL) inner plexiform layer. (GCL) ganglion cell layer.



Migrative behaviour of ganglion cells

al., 1992). Previous studies on the formation of ganglionar dendrogenesis in the chick (Nishimura, 1980; Rager, 1980) have not paid attention to the ganglion cells found in this study. Immunocytochemical analysis recognizing antigen associated to ganglion cell neurons

does not seem to detect cells, as reported in this study (McLoon and Barnes, 1989).

No Golgi images of pediculated thickenings have been described previously. These thickenings do not show any necrotic features either, suggesting a

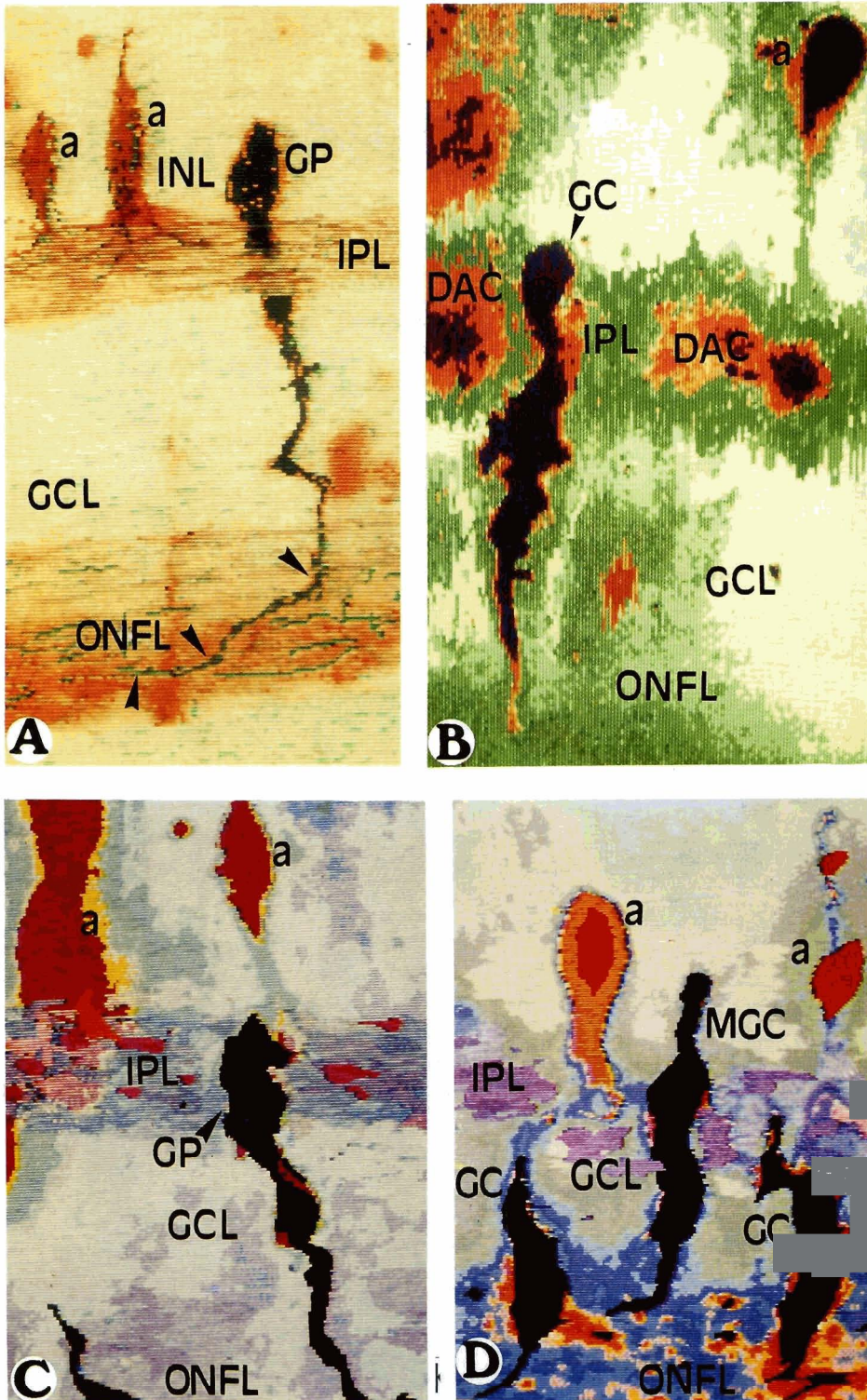


Fig. 2. Colour digitalized computer images of Golgi-impregnated retinal neurons at day 8 of incubation. Vertical sections. **A.** Young ganglion neuron percing the IPL with the perikaryon (GP) located at the INL. The arrowheads show the axon of this cell reaching the optic nerve fibre layer. **B.** Ganglion cell (GC) with a lobulated soma located in the IPL. **C.** Ganglion cell perikaryon (GP) into the IPL. **D.** Ganglion cell migrating (MGC) through the IPL and ganglion cells (GC) in the GCL. (IPL) inner plexiform layer. (INL) inner nuclear layer. (GCL) Ganglion cell layer. (ONFL) Optic nerve fibre layer. (a) Amacrine cells. (DAC) Displaced amacrine cells.

regressive change resulting in neuronal death. It is possible that these retracting cytoplasmic remnants may be used, after the accomplishment of perikaryon translocation, as the sprouting sector from where the definitive dendrites of these delayed ganglion cells arise and extend outward to the IPL (Fig. 1P, arrowheads).

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