SKATE (RAJA OSCELLATA) RETINA HORIZONTAL CELLS: PRIMITIVE NEURONS WITH HIGH LEVELS OF THE ENZYME CARBONIC ANHYDRASE

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The vertebrate neural retina possesses three spatially and functionally distinct neuronal cell strata: The photoreceptor layer; the layer of interneurons; the ganglion cell layer. Light reception and transduction follows a path of stimulation of the photoreceptor cells, transmission and signal integration in the interneuronal layer and then finally transmission to the brain via the ganglion cell layer. Several types of interneurons exist which are involved in the complex process of signal modification and integration. Understanding of the phenomena which occur at this level of light signal processing is poor and relatively simple models can be helpful in developing such an understanding.

The retina of the skate (Raja oscellata) is arguably among the most primitive of vertebrate retinas in that it contains only rod type photoreceptor cells. Another remarkable feature of the retina of the skate is that a class of interneuron in this retina the horizontal cell (HC) seems to possess characteristics of both neuronal and nonneuronal cells. As a consequence, the skate HCs have been viewed as a model for very primitive retina neurons. Among the primitive characteristics of these cells are the fact that these cells communicate laterally (i.e. with other HCs) by electrical junctions rather than by chemical synapses. Also, unlike most other neuronal cells which have been described, skate HCs contain extremely high concentrations of immunoreactivity for the enzyme carbonic anhydrase II (CA-II). The long range goals of my research with the skate retina system include determining whether or not the high levels of CA-II in horizontal cells influences the electrical communication between these cells by virtue of the influence this enzyme has on intracellular pH. The immediate goals of my research for the 1988 season at the MDIBL were to examine the potential heterogeneity of skate HCs and to establish methods for long term tissue culture of isolated HCs which would permit electrophysiological studies of the role of CA-II in these cells.

Female skates were used in most experiments. In a few cases male skates were used with results identical to those obtained with females. To assess the potential heterogeneity of HCs, retinas were isolated and fixed as described previously (Linser et al J. Comp. Neurol. 237:264-272, 1985). Tissues were either prepared for paraffin histological sectioning or for clearing and immunostaining as a whole mount (Linser and Irvin Develop. Biol. 121:499-509, 1987). Monoclonal and polyclonal antibodies to CA-II were used for immunostaining as before.

Isolated HCs were prepared by papain digestion of retina tissue followed by repeated trituration (Lasater et al J. Neurosci. 4:1966-1975, 1984). Suspensions of single cells were plated in a variety of tissue culture media developed for elasmobranch cells. The cells were plated onto glass cover slips by low-speed centrifugation. The coverslips had an etched grid on the surface which permitted daily observation of a series of specific locations and hence individual cells. Prior to cell plating, coverslips were made adhesive by

successive exposure to 1% protamine sulfate followed by 0.2mg/ml Concanavalin-A. Immunohistochemical staining of the cultured cells was as previously described (Linser and Moscona Develop. Biol. 96:529-534, 1983).

Immunohistochemical analyses of sectioned and whole-mount retina preparations showed that both monoclonal and polyclonal antibodies to chicken CA-II labelled skate HCs. Muller glial cells also labelled but to a lesser extent than the HCs. Distribution of HCs through the retina was nonuniform. An apparent "visual streak" was defined by HC density. In the area of the retina associated with the reflective tapetum, HCs were small and very closely packed relative to one another. In other regions of the retina the HCs became larger and less densely distributed in an inverse relationship with proximity to the region of the reflective tapetum. Whether or not the skate possesses a "visual streak" type region of high visual acuity is not known. Our results suggest that HC density may be useful in an analysis of visual acuity in this model retina. These results also suggest that HCs are morphologically heterogeneous in the skate retina as a function of position (figure 1) explaining earlier results with sectioned retinas.

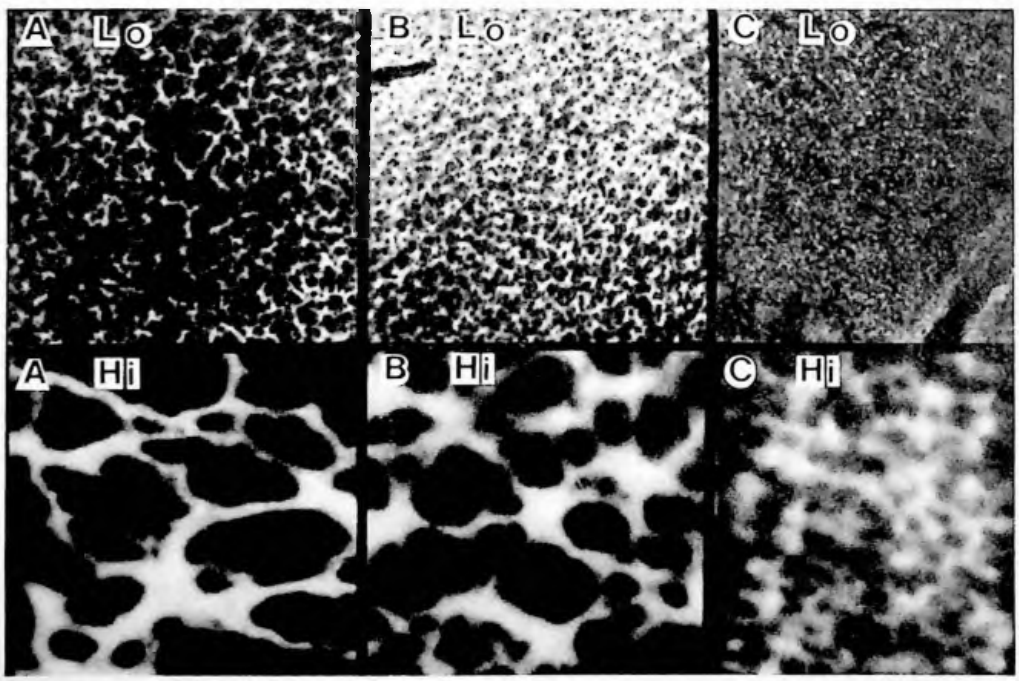

Figure 1. This figure shows the distribution of HCs in a whole mount immunostained with the monoclonal antibody 7C6-1 against CA-II. Low (A,B,C Lo) and high (A,B,C Hi) magnifications of regions of sparse HC concentration (left) to the dense region (right) of the visual streak are shown.

Comparison analyses of the retina from dog fish (Squalus acanthias) which possesses both rod and cone photoreceptors showed a complicated overlapping multilayered arrangement of HCs which could not be clearly visualized in whole mount preparations. Our results indicated that although there was positional heterogeneity in HC size and distribution in skate retina, there was apparently only one lamina of these cells in contrast to other elasmobranch

retinas which possess both rod and several types of cone photoreceptors. Our results supported the idea that skate retina possesses only one type of HC in parallel to the single type of photoreceptor.

Isolated HCs placed in monolayer tissue culture also showed some morphological heterogeneity which seemed to parallel the size and shape differences seen in whole mount preparations. By counting several hundred individual HCs per culture and assessing their survival from day to day, tissue culture medium as described by Valentich and Forrest (MDIBL Bull. 26: 91-94, 1986) was found to promote the highest percentage of HC survival of four different media tested. Temperature was also evaluated and 15 degrees C gave best survival with 4 and 21 degrees C only slightly less effective. Cell shape underwent stereotypical changes with time in culture with initial loss of branched morphology (after 1 day) to gradual reemergence of neuronal processes (5 days to 2 weeks). Skate HCs in culture continued to exhibit the capacity to immunostain for CA-II for as long as 2 weeks in culture (figure 2). These results therefore demonstrated the potential utility of skate HCs in future studies of the role of CA-II in primitive neuronal cell physiology.

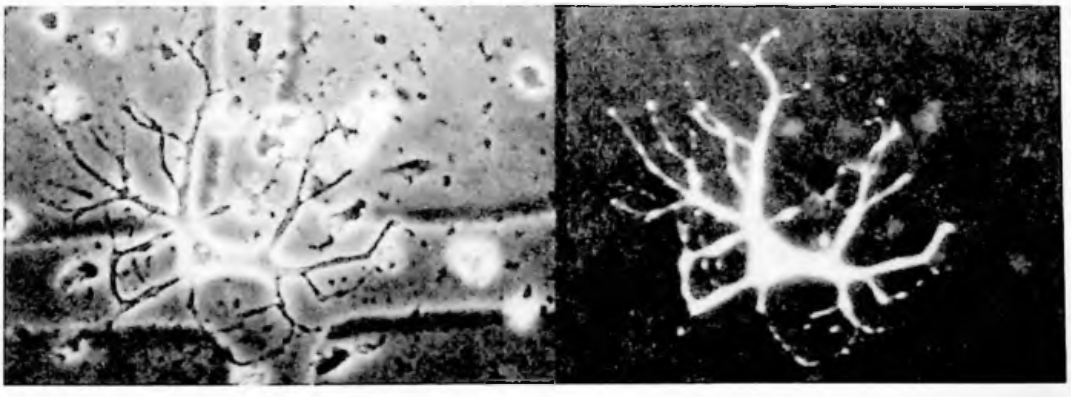

Figure 2. This figure shows an individual skate retina HC after two weeks in tissue culture. Phase optics (left) and immunofluorescence of CA-II localization (right) micrographs are shown.

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