The Pattern of Neurogenesis in the Retina of the Rat

D. Kent Morest*
Department of Anatomy, Harvard Medical School
and Eaton-Peabody Laboratory of Auditory Physiology,
Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, U.S.A.

Received February 9, 1970

Summary. The morphological features of the retina of neonatal rats have been analyzed with the rapid Golgi method in an attempt to provide some embryological observations crucial to the study of neuronal specificity in the visual connections. The retinal neurons and photoreceptors form from primitive epithelial cells that assume the characteristics of neuroblasts. Initially they extend from the internal to the external limiting layers. There is no evidence of free cellular migration or of cells resembling the neuroblast of His. While their perikarya are situated deep within the matrix zone, the first signs of differentiation appear at the external and internal limiting membranes, where the receptor inner segments and ganglion cell axons begin to form. Subsequently the perikarya move through the primitive epithelial processes to the prospective outer nuclear or ganglion cell layers. In the receptor cells, this is accompanied by the differentiation of the rods and cones and of the receptor fibers. In the ganglion cells, the perikaryal translocation is followed by the differentiation of the dendrites and the internal plexiform layer. The amacrine and bipolar cells follow a similar sequence of changes. The receptor outer segments form in conjunction with the processes of pigmented epithelial cells; the differentiation of the ganglion cell dendrites occurs in association with the formation of the amacrine and inner bipolar processes. The amacrine and ganglion cells begin to differentiate first, followed closely by the receptor cells and the bipolar cells. Müller's cells and astrocytes differentiate last. Horizontal cells were not studied. There is a gradient of differentiation, such that the axons and dendrites of the ganglion cells near the optic nerve head differentiate earlier than those located more peripherally. The implications of the findings for the analysis of the mechanisms controlling growth, differentiation, and neuronal specificity in the visual system are discussed.

Key-Words: Vision -- Embryology -- Neurogenesis.

This study provides an unexpected insight into the neurogenesis of the retina. Although it is only an episode in a series of neurogenetic studies (Morest, 1968a, c; 1969a, b; 1970), it seems worth reporting at this time, for the early and promising analyses with the Golgi method by Ramón y Cajal (1960), who regarded the retina as his first love, and the subsequent efforts with less demonstrative techniques do not account for the morphogenesis of the synaptic architecture of the retina. The present observations pertain to the movements of the neuroblasts and the differentiation of their axons and dendrites in the retina of the newborn rat.

Materials and Methods

The observations are drawn from rapid Golgi impregnations of the retinas from two littermate series of six albino rats, newborn, one-day, and three-day old. The tissue was fixed in situ by immersion in the rapid Golgi fixative according to Morest and Morest (1966) after a dorsal exposure of the living brains and the optic nerves and bulbs. After impregna-

*Supported by U.S. Public Health Service Research Grant NS 06115 and GRS Grant 5 SOI FR 05381-08 to Harvard University.
tion the entire heads were embedded in celloidin and sectioned serially at 80—160 µ in the horizontal plane. In one littermate series, one eye was processed for parasagittal sections. Other details of the methods and their artifacts have been recorded previously (Morest, 1968b, c; 1969b).

Note on the Drawings. It is traditional to portray the cells impregnated by the Golgi techniques as solid black figures on a white background, as if the virtue of the method were to display the cells as bare silhouettes. But this practice belies the microscopist's experience and could frustrate an accurate interpretation of the microscopic images. For the real value of the impregnations derives from their three-dimensional properties. This consideration applies not only to the spatial relationships and minute details of the cells and their processes but equally to the plastic aspects of their cytological form. Solid black silhouettes cannot convey such information. Photomicrographs are even less demonstrative. The present illustrations are unconventional. They purport to be accurate portraits of the shapes, surfaces, textures, and dimensions of the cellular details and the intercellular relations, insofar as pen and ink permit. Beyond that the reader may gain some impression of what the impregnated cells actually look like to the microscopist. However, these are not black and gray shadows. The cellular surfaces shine with colorful reflections. The cytoplasm is alive with granules and fibrils, embedded in a bubbly, frothy matrix.

Results

In the retina of the newborn rat the ganglion cell and inner plexiform layers have appeared. The inner nuclear layer is barely detectable; the outer nuclear layer is absent. These layers and the external plexiform layer continue to form within the course of the first three to seven days after birth (Weidman and Kuwabara, 1968). In the rapid Golgi preparations of the neonatal rat the cell layers are as fully represented and as readily identified as in aniline-stained preparations, since the perikarya are stained orange-gray by the chrome-osmium mixture of the fixative (Figs. 1, 2). In the rat by the time of birth the ganglion cell layer appears in all regions of the retina. However, the more fully differentiated ganglion cells that are impregnated in the newborn retina occur near the optic nerve head. The observations reflect the gradient of differentiation in the retina, arranged concentrically with respect to the optic nerve head, or possibly the area centralis. It is possible to confirm the sequence of changes deduced from the observations by comparing the cells impregnated in more remote portions of the retina with those surrounding the nerve head and by comparing the cells from corresponding sectors in older retinas. Astroblasts or the cells of Müller do not appear in the newborn preparations. The retina appears to be avascular in the present series, although many vessels are impregnated in the vitreous body adjoining the internal limiting membrane (Figs. 1, 3 Right, 9A). The internal and external limiting membranes are clearly indicated in the Golgi preparations (Fig. 9 A).

The undifferentiated state of the retinal neuroepithelium may be illustrated by the cells appearing more commonly in a peripheral field or in the margin of the retina (Fig. 3 Right). These cells resemble the primitive epithelial cells illustrated in Golgi preparations of the embryonic mouse retina (Ramón y Cajal, 1911: Fig. 226; 1960: Chap. 18) and with more detail in the fetal cerebral cortex (Morest, 1970: Fig. 2) and optic tectum (Morest, 1968a). Some of these cells are cuboidal or cylindrical cells confined to the matrix zone. Many are elongated cells, the perikarya of which appear at various depths within the