Temporary contacts formed between developing optic fibers in the chick

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SUMMARY

Temporary junctions were observed between developing optic fibers of the chick embryo and were distributed along the entire length of the axons from the cell body to the tip of the growth cone. These junctions were present in all material studied between days E-3 and E-18, the latter being the start of myelin formation of the optic tract. Junctions between adjacent axons were focal in nature and showed a decrease in the size of the intercellular space caused by a close apposition of the plasma membrane. With the experimental techniques used final identification of these junctions could not be made but are thought to be of two types—the gap and occludens junctions. Temporary fasciae adherentes junctions were observed at the end of the axon, between the growth cone and adjacent neural tissue. Speculation was made on the functional role of these temporary junctions.

INTRODUCTION

While considerable research attention has been given to a physiological description of retinotectal specificity (Gaze, 1970), few morphological studies have been made on the normal formation of such specific projection patterns. Recently, Crossland, Cowan & Rogers (1975) used auto-radiographic techniques to study the distribution of the optic nerve on the tectum of the chick at various stages of development. In a series of developmental experiments currently being conducted in our laboratory we are using electron microscopy to study the formation and growth of the optic nerve in the chick. This paper will describe temporary connexions observed between fibers in the developing optic nerve.

MATERIALS AND METHODS

The material studied was from chick embryos (E) 3, 5, 8, 10, 15, 18 and 20 days. Heads of animals (E) 3, 5 and 8 days were fixed by total immersion while those 10 days and older were fixed by cardiovascular perfusion. The optimum fixative for outlining the unit membrane was a mixture of 3 % glutaraldehyde, 1 % paraformaldehyde in 0.1 M sodium cacodylate buffer. After initial fixation

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for 1 h, the tissue was post-fixed for $1\frac{1}{2}$ h in 2% osmium tetroxide, washed in 0.1 M maleate buffer, en bloc stained in uranyl acetate, dehydrated in graded ethanol, embedded in epon, sectioned, and examined on a transmission electron microscope.

**RESULTS**

Optic fibers were examined at several sites along their entire expanse from the eye to the tectum. A feature common to all material studied was the appearance of junctions between adjacent axons and between growth cones and other neural elements. In order to rule out the possibility that these junctions were due to fixation artifacts various percentages of the initial fixative of glutaraldehyde and paraformaldehyde were used with either a phosphate or sodium cacodylate buffer, all at several osmolalities. While the procedure discussed above yielded the best results for outlining the unit membrane, the junctions were present in all fixation combinations studied. These junctions were periodically spaced between 5 and 8 μm apart along the entire length of the optic pathway starting soon after axons emerged from the retinal ganglion cells and extended to the tip of the growth cones. The connections were present in all material studied from day E-3 and remained until the start of myelin formation on day E-18 at which time the junctions disappeared.

Connexions between adjacent axons except at the growth cones were of the macula variety and extended between 0.05 and 0.2 μm in length (Figs. 1, 2). At these junction sites one characteristic feature was a decrease in the size of the intercellular space caused by the two trilaminar membranes of adjacent axons coming into closer apposition than at nonjunctional sites. Most junctions showed an intercellular zone between 2 and 4 nm in width (Figs. 1, 2) while at other, but fewer sites, no electron-lucent space was evident between the two trilaminar membranes (Fig. 3). These morphological characteristics are the usual descriptions given to two distinct junction types - the gap or nexus junction and the occludens junction respectively (McNutt & Weinstein, 1973). However, the limitations of using only thin sectioning and transmission electron microscopy to identify these junctions accurately have been well reviewed by several investigators (Revel & Karnovsky, 1967; McNutt & Weinstein, 1973; Goodenough & Gilula, 1974). Since correct identification of these junctions can be made only with the use of techniques other than those used in this study, e.g. freeze-cleave and freeze-etch replications (Revel & Karnovsky, 1967; McNutt & Weinstein, 1973), labeling of these junctions has been purposely avoided.

The type of junction that occurs between the growth cone of optic fibers on the surface of the tectum and an adjacent plasma membrane differs from that observed between adjacent axon shafts. At the growth cone the junctions appear to have the morphological characteristics of a fasciae adherentes (Farquhar & Palade, 1963). These junctions appeared to be formed by two parallel membranes having an intercellular space between 20 and 25 nm (Fig. 4). In the interspace
Fig. 1. Longitudinal section through two adjacent axons of optic nerve between optic fissure and chiasm of 8-day chick embryo showing 2 nm space between adjacent membranes (× 162000).

Fig. 2. Two adjacent axons of optic nerve between optic chiasm and tectum in 15-day embryo. A 2 nm gap appears between membranes (× 183000).

was contained an amorphous-appearing material which according to our method of tissue preparation was of low electron density. These junctions were observed between a growth cone and an adjacent soma, axon, glial process or other growth cone (Fig. 4). Junctions at the growth cones were never of the gap or occludens variety. The junctions are present soon after the axons emerge from the retinal ganglion cell and remain until the start of myelin formation on about
Fig. 3. Longitudinally sectioned axons from 10-day chick embryo. No intercellular space is apparent at the junction site (× 158000).

day E-18. While it is assumed that the formation of myelin around the plasma membrane of an axon leads to the separation of these junctions the mechanisms involved in their disappearance are not known.

**DISCUSSION**

Junctions similar to the gap and occludens variety have been observed between neuroblasts in the developing spinal ganglia of the chick on days E-5 and E-6 (Pannese, 1968). When satellite cells developed and extended between the neuroblasts within the ganglia, these junctions disappeared. In our material it is assumed that the formation of myelin around the plasma membrane of an axon leads to the separation of these junctions. However, the mechanisms involved in their disappearance are not known.

In addition, similar gap junctions have been identified in the developing visual system of *Daphnia* (Lopresti, Macagno & Levinthal, 1974) and *Xenopus* (Dixon & Cronly-Dillon, 1974). In *Daphnia*, gap junctions were shown to form for a short period of time between the growing lead optic fiber and the neuroblast that was wrapping around it. In the retina of *Xenopus*, gap junctions were present to stages 30/31 of development between two pigment epithelial cells and between pigment epithelial and retinal cells.

At present, one can only speculate on the functional role that these junctions serve in a developing system of axons. In the establishment of synaptic connexions Weiss (1941) has proposed that subsequent fibers grow out along the line laid down by pioneering axons. Factors involved in directing the earliest
Fig. 4. Growth cone (1) from 5-day chick embryo showing fasciae adherentes connexions with neural processes (arrows) on the surface of the tectum (× 62000).
formed (pioneering) axons to the tectum are not fully understood, as yet. However, subsequently formed axons could form junctions with each other that stabilize and guide the directions of growth along the path of the pioneering axons to the correct destination. In addition to serving in an adhesive capacity, junctions between adjacent axons could represent sites of informational exchange of a chemical or electrical nature that also might be necessary for maintaining a proper direction of growth (Lowenstein, 1972).

Similar speculation can be made on the functional role of the fascia adherens junction observed at the growth cone. As observed in vivo (Speidel, 1941), the filopodia which are found at the end of each growth cone are constantly expanding and retracting. This phenomenon continues throughout the entire growth process of each axon, giving the appearance that the growth cone is in constant search of its terminal site. Thus whatever information is necessary for identifying the terminal site of an axon also might be transmitted at junction sites identified as fasciae adherentes.

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REFERENCES


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