An electron microscopic study of synapse formation, receptor outer segment development, and other aspects of developing mouse retina

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Synapse formation and several other developmental features of potential functional significance were followed by electron microscopy in mouse retina through the postnatal developmental period. Tight junctions between pigment epithelial cells, which are postulated to play a possible role in blood retina barriers, were well established in the newborn animal. Observations pertaining to receptor outer segment development indicated that saccule formation began between the fifth and sixth postnatal days and appeared to occur by a process of plasma membrane invagination. The formation of various types of synaptic complexes in both plexiform layers was studied in an attempt to ascertain the sequence in which these complexes mature in developing retina. Although layering phenomena observed with the light microscope suggest that retinal maturation proceeds from inner to outer levels of the retina, with ganglion cells maturing first and receptors last, this did not appear to be the sequence with which synapses matured. Synaptic developments began early and proceeded rapidly in receptor terminals in contrast to inner retinal components which showed a relative lag in achieving synaptic maturity. This finding is discussed in light of certain electroretinographic data on developing retina which suggest that at least some aspects of functional maturation proceed centrifugally from outer to inner levels of the retina.

Much of the information available concerning retinal neurogenesis derives from the early classical histologic studies of Cajal and others. It was observed by Cajal that ganglion and amacrine cells appeared to differentiate and organize into layers quite early, whereas constituents destined to mature as horizontal, bipolar, and photoreceptor cells remained intermixed without order in outer retinal regions until relatively late in the developmental period. Cajal identified the primitive horizontal cell in newborn mouse retina but observed that its processes wandered extensively and did not become oriented horizontally at an outer plexiform level until approximately the twelfth postnatal day. He was unable to positively identify the bipolar cell early in the several species he studied but conjectured that it probably extended an axonal process into the inner plexiform layer prior to the development of the outer plexiform layer.
which, in mouse retina, would be prior to the fourth postnatal day. Cajal believed receptors underwent an initial unipolar phase of development in which they possessed only a sclerally oriented process. The development of a vitreally oriented process was not described in detail but he considered the maturation of outer plexiform receptor terminals to be a late occurring phenomenon (from the twelfth to eighteenth day in mouse retina). On the other hand, Cajal suggested that the receptor cell, in combination with the action of light, might constitute a neurotropic factor promoting the definitive organization and orientation of inner neural elements. Such a neurotropic effect of receptors on the maturation of inner retinal elements would seem to suggest the possibility that receptors mature earlier in some essential features than the cells influenced by them, though Cajal did not emphasize this implication of his neurotropic hypothesis.

The organization of retinal cell types into layers and the sequence in which these layers are formed in the developmental period has been described in several routine2-4 as well as classical histologic studies. It has become a generally accepted interpretation5-6 of such studies that morphological maturation of retinal cells proceeds in a centrifugal sequence from inner to outer levels of the retina, with ganglion cells being the first and receptors the last to mature. This very general thesis, however, is highly inferential with respect to developmental events at cellular and subcellular levels of organization beyond the range of sensitivity of the light microscope. In fact, data from a number of electrophysiologic studies, which will be compared below with the electron microscopic findings of the present study, suggest the possibility that certain aspects of functional maturation proceed centripetally from outer to inner levels of the retina.

Electron microscopy of developing retina has been limited to the region of photoreceptor outer segments and the pigment epithelium,7-8 with the exception of a brief description by Cohen9 of the appearance of inner and outer limiting membranes in the mouse embryo and neonate. The retina proper, including the plexiform layers which contain the entire synaptic apparatus of the retina, has not been examined electron microscopically in the developmental period. In the present study of developing mouse retina an attempt has been made to follow the morphogenesis of synaptic structures in each of the neural cell types in the retina and, to the extent possible, to ascertain the sequence in which retinal cells become synaptically involved with one another in the developmental period. Receptor outer segment formation was also followed and will be described briefly in this report in that data available on this subject pertain to species other than the mouse.

Materials and methods

Twelve litters of Swiss albino mice (48 surviving animals) and three litters of C57-BL6 black mice (12 surviving animals) ranging in age from 1 to 15 days were studied. Retinas from more mature animals (18, 20, 30, and 60 days) were also examined to establish the appearance of more mature synaptic complexes for comparison with those in developing retinas. To assure accurate orientation, retinas were fixed in situ. Several fixation regimens were explored initially, including osmium tetroxide alone or following prefixation with glutaraldehyde. The most satisfactory preservation was obtained with 2 per cent osmium tetroxide alone in a buffered medium consisting of 35 c.c. of Earles balanced salt solution, 15 c.c. of distilled water, and 0.5 c.c. of 0.1M cacodylate buffer. The relative hypotonicity of this mixture (290 mOsm. per liter) was found to be more suitable for neonatal retina than media of higher tonicity. Light microscopic sections approximately 1.5 μ thick, stained by the method of Richardson and colleagues,10 were reviewed from each specimen prior to cutting ultrathin sections on an MT-2 Porter Blum ultratome. Sections were mounted on formvar-coated slot grids and stained with both lead and uranium salts before viewing in an Elmiskop 1A electron microscope. Since retinal maturation is known to proceed most rapidly at the posterior pole, observations on this region were used as reference in establishing the order of first appearance of synaptic structures. Other regions were routinely
examined also because the small size of the neonatal mouse eye permits inclusion of all regions from the ora serrata to the posterior pole within the same ultrathin section. Multiple specimens (at least 10) from each of several animals (at least 3) representing each day of neonatal development from days 1 to 15, were studied. As no differences were found in the maturation schedules of the two mouse strains studied, no distinctions will be made between them in the results reported below.

Results

Light microscopy. Light microscopic observations were essentially in agreement with those contained in prior reports. In the newborn animal, the ganglion cell, inner plexiform, and amacrine cell layers were established as relatively distinct layers, whereas the remainder of the retina consisted of a mantle of cells appearing poorly differentiated with oblong nuclei and scant cytoplasm (Fig. 1, A). The mantle was comprised of approximately fourteen tiers of nuclei representing cells destined to mature as Muller, horizontal, bipolar, and photoreceptor cells. There was no gap in the mantle suggesting the beginning of an outer plexiform layer. During the first 4 to 5 neonatal days mitotic figures were frequently seen at the primitive ependymal surface. Sizable fiber bundles were noted in the fiber tract region on the first postnatal day, suggesting significant centripetal development of ganglion cell axons.

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Fig. 1. Light micrograph showing different stages in the formation of retinal layers in mouse retina. A, Newborn. Inner plexiform, amacrine, and ganglion cell layers are recognizable and the fiber tract is present, but there is no gap in the outer mantle of cells. B, Fifth postnatal day. A gap is appearing in the outer portion of the mantle representing the formation of an outer plexiform layer. C, Tenth postnatal day. Separation of nuclear layers is complete and the outer plexiform layer is well established. The inner plexiform layer shows only minimal change from the fifth day. Outer segments are becoming recognizable. D, Eighteenth day. There is a striking increase in the length of outer segments and an apparent decrease in the concentration of cell bodies in nuclear layers, but the volume of the inner plexiform layer is approximately double that of the tenth day. (x360.)
A gap between photoreceptor nuclei and those of inner neurons began to develop between days 4 and 5 (Fig. 1, B). However, separation of nuclei into outer and inner nuclear layers was not completed until approximately the tenth day (Fig. 1, C). With the light microscope, it was not possible to perform a critical examination of the make-up of the plexiform layers in which synaptic connections were presumably developing and it was difficult to distinguish receptor outer segments from inner segments before approximately the ninth postnatal day.

Between the tenth and twentieth days the inner plexiform layer became considerably thicker, implying a significant increase either in the number or size of processes making up that layer (bipolar, amacrine, Muller, and ganglion cell processes) (Fig. 1, D). In contrast, the concentration of cellular components in nuclear layers appeared to decrease. A severalfold increase in the size of receptor inner and outer segments was a conspicuous developmental feature during this late period. By the twentieth day the retina appeared essentially mature in all layers.

**Electron microscopy.**

**Outer retinal surface.** On the first postnatal day the inner surface of the pigment epithelium and outer retinal surface were closely apposed to one another (Fig. 2). Villous processes of the pigment epithelium entered interstices between components of the outer retinal surface but formed no specialized contact with them except in the region of the ora serrata, where these surfaces were joined by tight junctions (zonulae occludens). Junctional complexes were conspicuously present between processes at the outer retinal surface and between cells of the pigment epithelium. Complexes between pigment epithelial cells included tight junctions (Figs. 2 and 3) whereas those at the outer retinal surface appeared to be limited to the desmosomal variety (maculae or zonulae adherens). Cohen, who described the same finding in mature retina, suggested that tight junctions at the inner margins of epithelial cells might contribute to blood-retina barriers. If these junctions are indeed related to certain blood-retina barrier phenomena, it is noteworthy that they were well established in the newborn retina.

**Photoreceptor cells.** A significant degree of photoreceptor differentiation had occurred by the first postnatal day in that some of the processes abutting at the outer retinal surface (Fig. 2) contained ultrastructural features characteristic of those which have been described in receptor inner segments of adult mice. Initial formation and longitudinal growth of the ciliary stalk (Fig. 3) were the only developmental features related to receptor outer segment formation observed until approximately the third postnatal day, after which a process of ballooning of the outer one half of the stalk occurred. Between the fifth and sixth postnatal days saccule formation in the ballooned portion of the stalk could be detected in a few receptors. Once begun, saccule formation proceeded at a remarkable rate, at least in some receptors, in that examples could be found by day 9 of outer segments containing over 100 saccules. Consistent with Nilsson's recent findings in developing tadpole retina, several examples were found in which saccules appeared to be forming by a process of plasma membrane invagination at the inner aspect of the ballooned ciliary stalk (Fig. 4).

In the adult guinea pig retina, Mountford, using the criteria of Sjostrand and others, has described receptor synaptic terminals as falling into one of three morphological categories; alpha, beta, or paranuclear. Cohen has described receptor terminals of adult mouse retina as spherules (including both alpha and paranuclear configurations) or pedicles (beta configuration). In developing mouse retina, each type of terminal could be distinguished relatively early in its developmental course. Beta terminals usually contained from 5 to 10 (and sometimes more) synaptic lamellae (ribbons or bars) and
characteristically had a thick stalk coning out into a wide basilar expansion, whereas the alpha terminal typically contained only 1 or 2 lamellae and had a more slender stalk terminating in only a minimally expanded sac. The paranuclear terminal had neither a stalk nor terminal expansion and merely consisted of 1 or 2 lamellae confined to a narrow cytoplasmic space between the plasmalemma and nuclear membrane.

In both alpha and beta type receptors, an occasional synaptic lamella could be detected as early as the second postnatal day and by the fourth day they were present in many receptors. In the earliest detected stages of their formation these organelles appeared in paranuclear regions of the receptor cytoplasm in the form of small, amorphous densities already encircled by a halo of clear synaptic vesicles. Characteristically, in addition to the vesicle accompaniment, there were numerous free ribosomal particles in the nearby cytoplasm (Fig. 5). In receptors in which multiple lamellae were developing, these structures appeared to migrate in cluster formation down the axonal stalk to arrive at the base of the terminal by approximately the sixth postnatal day. Thereafter, a partially dispersed cluster was commonly found in the base of the terminal, and individual lamellae, which had presumably migrated away from the central cluster, were found either at or near developing synaptic sites (Fig. 6). In this stage of their development, lamellae were considerably larger than those detected earlier in more proximal regions of the receptor cytoplasm. The specific mechanism by which this growth took place was not readily apparent. By the seventh postnatal day the majority of receptor terminals at the posterior pole were oriented in a row at the outer margin of the developing outer plexiform layer. Each terminal was diffusely filled with synaptic vesicles and contained a variable number of synaptic lamellae, some of which had already located at synaptic stations where contact with second order neurons was evident (Fig. 7). Between the seventh and twelfth days morphogenesis of synaptic terminals proceeded essentially to completion in the majority of receptors, although a partially dispersed cluster of lamellae could still be seen in an occasional terminal as late as the twelfth day. Contact with inner neurons, involving invaginated processes presumed to be of both bipolar and horizontal cell origin, appeared to be well established at most terminals by the tenth postnatal day (Fig. 8) but it was not ascertained in what order such contacts had become established.

In adult guinea pig receptor terminals, Mountford described a cross-striated filamentous structure, not known to occur in receptors of other species, which she designated the "synaptic spindle."
Fig. 5. Fourth postnatal day. A presumptive beta receptor cell. From six to eight small densities surrounded by synaptic vesicles are aggregated in the paranuclear region of the receptor cytoplasm prior to migration to the synaptic terminal. Numerous free ribosomal particles are present in the nearby cytoplasm. (The line = 0.5 μ.)

Fig. 6. Eighth postnatal day. Base of a beta receptor terminal illustrating a central cluster of synaptic lamellae and one or two others on the left assuming positions at synaptic sites opposite invaginated processes of inner neurons. Serial sectioning of this terminal revealed that its full complement of lamellae was seventeen. (The line = 0.5 μ.)
Fig. 7. Seventh postnatal day. Beta terminal containing a partially dispersed cluster of lamellae in the base of the terminal. Degree of development in this terminal by seventh postnatal day is suggested by the two individual lamellae which have assumed positions at synaptic sites opposite processes of presumptive bipolar or horizontal cell origin. (The line = 0.5 μ.)

Fig. 8. Tenth postnatal day. Two alpha receptor terminals appearing essentially mature in their synaptic configuration and content (right and left). A synaptic spindle (S) is seen in longitudinal section in the receptor terminal at center. (The line = 0.5 μ.)
Figs. 9-12. For legends see opposite page.
structure could be detected by the fourth postnatal day in a few receptor terminals of mouse retina, and by the tenth day it was observed as a relatively conspicuous component in many terminals (Fig. 8). Contrary to Mountford's findings in the guinea pig, only one type of spindle was found in mouse retina and it was seen in all three types of synaptic terminals. Similar structures were also identified in receptor terminals of adult mouse but in a preliminary study of developing rabbit retina none were detected.16

Horizontal cell. The ultrastructure of the horizontal cell has been studied in fish, turtle, and human retina by Yamada,17 and in rabbit and cat by Dowling,18 but has not been described in the mouse. In the present study, certain features similar to those described by Yamada in horizontal cells of fish and turtles were recognized in large horizontally oriented processes in the outer plexiform layer of adult mouse retina. A particularly striking feature of these processes was their dense filamentous content (Fig. 9). They also were noted to contain dense-core vesicles of approximately 1,000 Å diameter (Fig. 10) similar to those which have been described in processes of the inner plexiform layer of rabbit24 and rat25 retina and in neural components in other regions of the central nervous system. These presumptive horizontal cell processes were found in a few instances in synaptic contact with bipolar components. This synaptic complex, not noted by Yamada, but described in rabbit and cat retina by Dowling,19 was of conventional configuration, involved the horizontal cell presynaptically, and was always found in the innermost aspect of the outer plexiform layer (Figs. 9 and 11). Similar synaptic complexes were first detected in developing mouse retina on the tenth postnatal day (Fig. 12), but the few examples found in this period involved presynaptic processes which usually could only be equivocally identified with the horizontal cell. The difficulty in identifying the horizontal component in these synapses with confidence probably related to the immaturity of the horizontal cell at this stage of development. Specifically, the presynaptic processes were not as rich in filaments as were those in adult retina. The possibility that other types of synaptic complexes may exist in this region, for example interhorizontal cell synapses, remains under investigation.

Bipolar cell. Investigators of retinal synapses have described the bipolar axon as a relatively conspicuous component of the inner plexiform layer because of its rich vesicle content and of the unique configuration of dyad synaptic complexes on its
Figs. 13-16. For legends see opposite page.
terminal expansion.\textsuperscript{14, 19, 20} An additional identifying feature of the inner plexiform bipolar process is its relatively frequent postsynaptic involvement with what are believed to be amacrine presynaptic contacts. The only presynaptic involvement which has been described for the bipolar cell is at the dyad synapse where it contacts a pair of processes, at least one of which contains vesicles and both of which may show postsynaptic membrane densification. The presynaptic complex in the mature bipolar terminal resembles that seen in receptor terminals in that it consists of a synaptic lamella accompanied by a halo of vesicles and sometimes an underlying arciform density (Fig. 13). To date, no inner plexiform processes other than those with dyad-containing terminals have been attributed to the bipolar cell.

At the outset of this study it was assumed that the dyad synapse, being of conspicuous morphology and the only configuration of its kind in the inner plexiform layer, would be easily detected early in the developmental period. However, neither synaptic lamellae nor any configuration of structures conforming to the above description of the dyad synapse could be found in the inner plexiform layer prior to the tenth postnatal day. Between the tenth and twelfth days a limited number of sites of membrane specialization characteristic of the dyad synapse were seen, some accompanied by small synaptic lamellae and others without such accompaniment (Fig. 14). No evidence was found to suggest that bipolar synaptic lamellae developed in regions of the cell remote from the synapse or that lamellae migrated down the axon to terminal synaptic sites, as appeared to occur in developing receptor terminals. On the tenth and eleventh days, the few dyad synapses found were primarily in the outer half of the inner plexiform layer, but between days 11 and 14 there was both an increase in the number of identifiable dyads and a shift in their distribution to include the inner as well as the outer aspect of the inner plexiform layer. Bipolar terminals appeared to continue increasing both in number and size and to assume new synaptic relations both pre- and postsynaptic until approximately the eighteenth postnatal day, by which time the majority of these terminals had become essentially indistinguishable from those in adult retina.

Processes seen in the inner plexiform layer prior to the tenth postnatal day with a significant vesicle content but lacking synaptic involvement could not be identified with confidence as bipolar axons since amacrine neurites are also known to

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**Fig. 13.** Inner plexiform layer of adult mouse retina showing bipolar terminals containing dyad synaptic sites (★) at upper left and lower right. Synaptic lamellae, synaptic vesicles, arciform densities, and paired postsynaptic processes with membrane densifications are evident at these sites, constituting a conspicuous configuration which bears some resemblance to that of outer plexiform receptor terminals but does not resemble any other configuration in the inner plexiform layer. (The line = 0.5 μ.)

**Fig. 14.** The inner plexiform layer on the tenth postnatal day. A site of membrane specialization, with a configuration resembling that seen at dyad synaptic sites (★) but lacking a synaptic lamella, is seen in lower center. A similar site is present at upper right (★) which contains a small synaptic lamella. (The line = 0.5 μ.)

**Fig. 15.** The inner plexiform layer on the third postnatal day showing one of the very few synaptic complexes present in this early period. The presynaptic process contains a few diffusely distributed vesicles and other vesicles are clustered at the presynaptic membrane. As was usually the case in the early development of this type of synapse, there is very little difference in density between the pre- and postsynaptic membranes. (The line = 0.5 μ.)

**Fig. 16.** A ganglion cell soma (G) and Muller process (M) with a subsurface cistern underlying the ganglion cell plasmalemma. Tenth postnatal day. (The line = 0.5 μ.)
contain a variable content of synaptic vesicles. Furthermore, some vesicle-containing processes found prior to the tenth day also contained presynaptic complexes of conventional configuration (Fig. 15), a feature which has been attributed to amacrine cells but not bipolar cells by investigators of this retinal level. Vesicle-containing processes involved postsynaptically at synapses of conventional configuration, though very uncommon, were also seen prior to the tenth postnatal day. While these could conceivably have been bipolar processes which had not yet developed dyad synapses, interamacrine synapses or perhaps synaptic contacts between centrifugal fibers and amacrine neurites would fit the same description. Thus, although the bipolar process may have been represented in the inner plexiform neuropil prior to its development of dyad profiles on the tenth postnatal day, evidence for such representation was not established. The Golgi method in combination with electron microscopy as described in several recent studies could be employed to clarify this issue further.

Amacrine and ganglion cells. In keeping with light microscopic appearances, both amacrine and ganglion cells appeared to have achieved a significant degree of differentiation by the first postnatal day in that their cell bodies were organized into relatively distinct layers separated by an appreciable inner plexiform region comprised largely, if not entirely, of the processes of these two cell types.

Evidence of synapse formation in the inner plexiform layer on the first day was equivocal in that the occasional sites of membrane specialization detected between cell processes appeared to be desmosomal type contacts. Between the second and third postnatal days, however, a few complexes containing features more characteristic of conventional synapses were detected (Fig. 15). During the first 8 to 10 days the number of these conventional synapses increased, but only at a slow rate, and the majority of those seen in this period were of a configuration consistent with the presumptive appearance of the amacrine-ganglion cell synapse in adult retina. These complexes were characterized by a variable content of clear vesicles in the presynaptic process, a cluster of such vesicles at the presynaptic membrane with variable densification of the postsynaptic membrane, and an absence of vesicles in the postsynaptic process. The amacrine neurite is the only retinal cell believed to contribute presynaptic complexes of conventional configuration to the inner plexiform layer. It presumably establishes such conventional contacts with bipolar terminals and other amacrine processes as well as with ganglion cell dendrites. Probably a few of the conventional synapses observed prior to the tenth postnatal day were inter-amacrine, though none was traced as such. The remote possibility that bipolar terminals lacking dyad synaptic sites were involved postsynaptically in these early synapses has been considered earlier. The possibility must also be considered that centrifugal fibers originating in the central nervous system were responsible for some of the synaptic complexes of conventional morphology developing in this early period. Unfortunately, centrifugal fibers have not been studied in mouse retina nor has the issue of whether any mammalian retina is supplied with such fibers been clarified from studies on other species. Between the eighth and tenth days the number of conventional synapses in the inner plexiform layer began to increase at a more rapid rate than was apparent earlier, and from approximately the tenth to eighteenth postnatal days, while many bipolar dyad synapses appeared to be arising de novo in the inner plexiform layer, an accelerated rate of increase in the number of conventional synapses also occurred.

Subsurface cisternae were first detected at the somal surfaces of ganglion and amacrine cells on approximately the third postnatal day. In this early period these membranous configurations were quite small and only infrequently observed. By the tenth day they were larger, more con-
Fig. 17. A process from an unidentified cell type in adult mouse retina containing vesicles of approximately 1,000 Å in diameter with dense cores. This process is in synaptic contact with an amacrine soma (A) but the dense-core vesicles are positioned in no particular relation to the synaptic site. (The line = 0.5 μ.)

Fig. 18. A portion of an amacrine cell soma from adult mouse retina including Golgi zone and numerous dense-core vesicles of several sizes. The mitochondria of the cell (m) have a different appearance from those in the macrophage in Fig. 19. (The line = 0.5 μ.)

Fig. 19. A macrophage containing numerous dense-core structures of variable size in an eight day mouse retina. This cell is positioned between two amacrine cells (A) but can be distinguished from them by the appearance of its mitochondria. (The line = 0.5 μ.)

spicuous, and more frequently seen (Fig. 16). Cisternae at the ganglion cell surface were usually, though not invariably, at a site of contact between the ganglion cell plasma membrane and that of a Muller process, as is clearly the case in Fig. 16. These cisternal configurations were not confined to the soma in that both amacrine neurites and ganglion cell dendrites in the middle of the inner plexiform layer were occasionally found to contain them.

Considerable speculation has arisen recently concerning granulated or dense-core vesicles which are found in many parts of the nervous system, often in the proximity of synapses, and are thought to contain monoamines. Such dense-core vesicles have been reported in the retina of rabbit and rat where they were restricted to certain processes in the inner plexiform layer and to cell bodies of certain cells in the amacrine and ganglion cell layers. It has been postulated that retinal dense-core vesicles contain dopamine since fluorescence studies have traced the presence of monoamines to the
same general areas where such vesicles are found, and in biochemical studies of rabbit retina, significant concentrations of dopamine, but only trace amounts of other monoamines, were found.

In the mature mouse retina a dense-core vesicle approximately 1,000 A in diameter was typically seen in scattered processes of the inner plexiform layer (Fig. 17). This type of vesicle and other larger vesicles of the same morphological appearance were also found in cell bodies of the amacrine and ganglion cell layers (Fig. 18). Although vesicles in inner plexiform processes were sometimes seen in the general region of a synapse, they were rarely located close to the synaptic cleft and it was common to see them in processes which did not appear to be involved in a synapse at all. A similar dense-core vesicle showing no intimate relation to synapses was also seen occasionally in horizontal cell processes in the outer plexiform layer (Fig. 10).

On the second postnatal day processes in the inner plexiform layer of the mouse were found to contain dense-core vesicles which were approximately 1,000 A in diameter. However, an attempt to identify the vesicles found in such processes with those believed to contain monoamine stores in the adult became complicated by the appearance, in the inner plexiform layer within the first few neonatal days, of a presumptive macrophage which contained numerous dense-core vesicles of variable size from 500 to 10,000 A in diameter in its soma and of approximately 1,000 A diameter in its processes. This cell type appeared to accompany the vasculature in that it made its appearance at various levels of the retina at the same time and in close proximity with capillary ingrowths. A phagocytic function was assumed for this cell in that nuclei and cytoplasmic components of degenerating cells were seen engulfed in its cytoplasm on several occasions. Vesicles contained in this cell were presumably lysosomes but many of them were morphologically indistinguishable from vesicles considered as possible monoamine stores in the retina and some other parts of the nervous system (Fig. 19). Because processes of the presumptive macrophage intermingled early with other inner plexiform processes it was difficult to be certain that vesicles appearing early in inner plexiform processes were those putatively related to monoamines rather than those of macrophagic origin and presumably of lysosomal make-up. Histochemical and autoradiographic techniques in combination with electron microscopy would be useful for clarifying the nature of each type of vesicle.

Discussion

A graphic recapitulation of the data presented above concerning the order in which synaptic complexes were established in the developing mouse retina is presented in Fig. 20. The postnatal course of retinal development in the mouse spans approximately an 18 to 20 day period which, for purposes of discussion, can be conveniently subdivided into an early period comprised of the first 10 days and a late period from the tenth to twentieth days.

Ultrastructural evidence for synaptic connections in the inner plexiform layer was equivocal in the first few days and only a relatively small number of synapses had appeared in this region by the end of the early 10 day period. The bipolar synaptic complex (dyad) was not evident in the inner plexiform layer in the early period. Therefore, with respect to the establishment of functional connections, the developmental picture in the inner plexiform layer during this period was one of relatively slow change, even though amacrine and ganglion cells by light microscopic appearances were already well differentiated and organized from the very beginning of this period. In contrast, the receptor cell, generally considered from light microscopy to be the last component in developing retina to mature, showed unequivocal ultrastructural evidence of early synapse morphogenesis which, in the majority of terminals, reached essential com-
Fig. 20. Each line depicts the schedule of morphological maturation observed for the synaptic complex designated by initials on the line. Receptor outer segment (ROS) maturation is also included. R, receptor; H, horizontal cell; BP, bipolar cell; A, amacrine cell; G, ganglion cell; C, centrifugal fibers. Heavy lines indicate a period of accelerated development. Broken lines refer to periods of relative quiescence or very slow development. The possible contribution of centrifugal processes to inner plexiform synapses in either the early or late developmental period is an open question represented by a dotted line. As is indicated, it is unclear whether a ganglion cell dendrite is represented postsynaptically at each dyad synapse or whether, in some instances, both postsynaptic members are amacrine neurites.

Completion by the end of the early period. Horizontal cell synaptic contacts with bipolar components were not detected in the early period.

In the late period, from days 10 to 20, the reverse picture was seen for each of these components. Although outer and inner segments continued to increase in length, receptors otherwise appeared relatively quiescent, having already established their full component of terminals, each complete in its synapse morphology and probably in its contacts with inner neurons. But horizontal cells were just beginning to make presynaptic contacts with bipolars, bipolars were just establishing presynaptic contacts at dyad sites with amacrine, and ganglion cell processes and presumptive amacrine processes appeared to be entering an acceleration phase in establishing new presynaptic relations throughout the inner plexiform layer. These data suggest that synaptic complexes are not established in developing retina in a centrifugal sequence as might be assumed from light microscopic layering appearances. Cajal apparently anticipated such a possibility when he postulated, in connection with his neurotropic theory, that some degree of receptor maturation must occur as a precondition to definitive maturation of inner retinal components. Ultrastructural data
are consistent with such a view, in that all presynaptic complexes detected in bipolar and horizontal cells and the bulk of those attributable to amacrine were established only after receptor synaptic developments were relatively well advanced.

A comparison of developmental data from physiologic studies with those from morphologic studies is of potential value for identifying the structural correlates of functional activity. To be most meaningful, such comparisons should be made between morphologic and electrophysiologic observations made by techniques of comparable sensitivities. For example, because electron microscopy visualizes developmental changes at the single cell or subcellular level, it should ideally be compared with single unit recordings reflecting functional changes in each cell type in the various stages of its development. Unfortunately, single unit recordings taken from various retinal levels at appropriate time intervals during the developmental period, as would be required to establish the sequence in which cell types mature functionally, are not available. However, electrophysiography, which records mass responses of retinal cells to light stimuli, has been employed to study functional maturation in developing retina of various species. It has been reported, not only in the mouse, but in rat, rabbit, dog, chick, and frog, that the a-wave, which is believed to arise at an outer retinal level, could be elicited several days earlier than the b-wave, which is believed to arise at an inner retinal level. A centripetal sequence of at least certain aspects of functional maturation, from outer to inner levels of the retina, is suggested by these electrophysiographic data. The ultrastructural findings presented here are more consistent with this functional interpretation than with the light microscopic assumption of a purely centrifugal progression of maturational events from inner to outer levels of the retina.

Brown and colleagues have presented considerable evidence from studies of adult retina that the a-wave arises from the photoreceptor cell and probably involves its synaptic terminal. If the a-wave does indeed arise from the receptor cell, its appearance early in the course of retinal development implies early functional maturity of the receptor cell. The electron microscopic finding of early maturation of synaptic components in receptor terminals lends direct morphologic support to this interpretation. An alternative view was recently postulated by Dowling that the horizontal cell, rather than the receptor, may give rise to the a-wave. Since horizontal cells are presumably activated by receptors, receptor maturity is required for either hypothesis. Based on Golgi studies, horizontal cells are classically regarded as linking one class of receptors with another. It has only recently been shown that some horizontal processes terminate on bipolar dendrites and it remains to be clarified whether such processes arise from a subclass of horizontal cells, represent a subclass of processes from all horizontal cells, or whether the classic view may be wrong. In any event, horizontal processes terminating on bipolars were just beginning to appear on the tenth postnatal day, which is also the first day Noell reported eliciting an a-wave from developing mouse retina. Unfortunately, such a precise temporal correlation between ultrastructural and electrophysiographic observations is difficult to interpret without knowing the concentration and numbers of functionally mature cells required to generate activity detectable by a given electrophysiographic technique. Theoretically at least, it should be possible by electron microscopy to detect early examples of a given synapse prior to the time it is either mature enough or present in sufficient numbers to generate electrophysiographically detectable wave activity.

Because the b-wave arises in the inner retina and the ganglion cell can apparently be eliminated as a candidate for the source of this wave, the remaining candidates
are the amacrine and bipolar neurons. Since bipolars provide the only vertical stimulus to amacrines, neither the bipolar nor amacrine could give rise to light-evoked activity (b-wave) until the bipolar has achieved functional maturity. In the present study, maturation both of bipolar terminals and of amacrine contacts with these terminals occurred relatively late by comparison to maturation of receptor terminals. A rough correlation, therefore, might be considered between such late morphological developments and the late occurrence of the b-wave, just as the early maturation of receptor terminals could roughly be correlated with the early occurrence of the a-wave. For reasons stated above, however, it would be of dubious value to attempt a more precise correlation.

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