REGENERATION OF VERTEBRATE SENSORY RECEPTOR CELLS
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Symposium on Regeneration of Vertebrate Sensory Receptor Cells, held at the Ciba Foundation, London 4–6 December 1990

The topic of the symposium was proposed by Professor Edwin W. Rubel

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Introduction

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This symposium originated from the notion that it would be fun to bring together a group of people who study the regeneration of different cell populations but who do not usually have the chance to discuss common themes and problems. Subsequently, Greg Bock visited Seattle, the idea was discussed, and a group of participants was evolved. After that, the Ciba Foundation took over. The result is the present symposium.

Sensory receptor cells are our link with the environment: they connect us to the environment and allow us to act upon changes in the environment as well as perceive the consequences of these actions. Sensory receptors vary markedly between species and between receptor cell populations. In mammals, particularly in humans, many receptor cell populations are permanent; they are not replaced when they are damaged. Although the condition is not life threatening like heart disease or cancer, it represents a major alteration in the quality of life that can be a permanent change for as long as a person is alive. These changes have an impact on our society in major ways. About one in every 700–800 babies is born with significant hearing loss that severely compromises the development of communication and the number of people with serious hearing loss increases to 50% by the age of 65. A rough calculation suggests that something like 600 million people in the world suffer significant impairment of their hearing. More than 100 million people around the world are thought to have significant taste and smell disorders. Similar numbers can be found with respect to permanent vision disorders. Most receptor cell populations—although this isn’t necessarily true of the olfactory and taste cells—aren’t replaced after injury resulting from disease or other kinds of damage. Many investigators are attempting to understand why sensory receptors are replaced in some other groups of vertebrates, and why they cannot be replaced similarly in humans. Part of the impetus behind this symposium was to see if we can learn from these species and apply this knowledge to the replacement of receptors in our own species.

The organization of the meeting was an attempt to bring together people who work on the regeneration of receptor cell populations of various kinds, to determine the state of knowledge in various systems, and what we can learn from each other. In addition, by bringing in cell biologists who work on the
control of mitosis, on the differentiation of cells, and on the factors regulating these processes, we hoped to be able to discover new approaches and new experiments that will provide new directions for our research. For three days we shared this information among ourselves and in this volume we hope to communicate what we’ve learned with our colleagues at large. My hope as chairman was that we would have very free-wheeling discussion periods, in which people working on sensory systems and receptor systems other than the one being immediately discussed would not hesitate to participate. This end was achieved, and I think the discussions that follow each chapter are at least as valuable as the chapters themselves. They provide a framework of the relative state of understanding in each sensory system. What is more, I think they are fun to read.

What I think we achieved from this meeting, and what I hope the reader will achieve, is increased information about sensory systems other than the one each of us works on, as well as an understanding of the present state of knowledge on regeneration in other systems. Even more important, from the meeting and, I hope, from the volume, we shall be able to return to our laboratories and do different, better, experiments. The major purpose is to be able to take what we learn here, go back, and then modify our research programmes in order to achieve a better understanding of the ways of Nature.
The general architecture of sensory neuroepithelia

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Abstract. All neuroepithelia are sheets of cells lining an internal or external surface of the body and resting on a basement membrane. They consist of at least two kinds of cell, receptor cells and sustentacular (supporting) cells. Some contain undifferentiated precursor cells and senescent or degenerating cells. The potential for plasticity and regeneration in different sensory neuroepithelia varies widely according to their origins and structure in any individual animal and according to the species in which they occur. Four sensory neuroepithelia are described as examples of the range of construction, complexity, and life history.


Although neuroepithelia differ in their embryological histories, in their specific structure, and in their capacity for regeneration, they display certain common architectural features. First of all, they are epithelial; that is, they are sheets of more or less similar cells resting upon a basal lamina and mesodermal connective tissue and fronting upon an external or internal open space. Second, they consist of at least two types of cell, specific sensorireceptor cells and supporting cells. Many of them contain a third cell type, precursors that give rise to the replacements of the other two cells. Third, the free or apical surfaces of the receptor cells, and in some cases also of the supporting cells, are decorated with elaborate motile or non-motile appendages—cilia or microvilli. Often these appendages protrude into or are attached to a dense extracellular secretory material, which may have an important role in the function of the ensemble as a sensory receptor organ. Fourth, the sensory elements are connected to the central nervous system by way of specific nerves, which either originate from the sensorireceptor cells themselves or synapse with them. In this way the neuroepithelia differ from a variety of other peripheral sense organs in which the sensory element is itself a terminal of a nerve fibre originating from a dorsal root ganglion cell. These general features accommodate a wide variety of
different receptor organs responsive to photic, mechanical, kinetic, electrical, or chemical stimuli.

In order to cover this enormous variety of structure and function, I would have to go into much more detail than the space allotted to this communication allows. Therefore I have chosen to describe only four kinds of neuroepithelia, which illustrate both their common features and a range of problems involved in understanding their architecture. In addition I shall include some remarks about their development.

**Taste buds**

I begin with the taste bud because it appears to be a simple cluster of cells (Fig. 1). Actually its structure has been the centre of considerable controversy, and it exemplifies many of the problems associated with understanding the organization and life histories of neuroepithelia. A taste bud consists of a rounded cluster of epithelial cells mostly oriented in a vertical direction with their bases lying on the basement membrane and their apices extending microvilli into a narrow channel on the free surface, the taste pore. The cells are of at least two types, gustatory receptor cells and sustentacular (supporting) cells, but the categories of cells vary in different species and in different kinds of taste buds on different parts of the tongue (or different parts of the body in those animals that have taste buds on their exposed surfaces). The species variations are at least partly responsible for the vigorous contention that has arisen over the question of cell types in the gustatory organs during the past century and is still going on. Not only are there different numbers of cell types, but also the cell types are differently described in different species of animals and kinds of taste organs.

In taste buds on the rabbit's tongue, four cell types have been described (Murray 1986, Kinnamon 1987, Royer & Kinnamon 1991). Type I cells are fusiform cells extending from the basal lamina to the taste pore. The apical cytoplasm of these cells contains characteristic large dense granules and extends into fine short microvilli. Type I cells enclose their neighbouring cells and intragemmal nerve fibres in extended lamellar processes similar to those of Schwann cells around unmyelinated nerve fibres. Type II cells are also fusiform but lack the lamellar processes and the dense vesicles of the Type I cells. In addition, they are fairly large plump cells with vacuolated cytoplasm. Type III cells are generally thin, dark, and fusiform. They contain characteristic dense core vesicles, 80–140 nm in diameter, which crowd the basal and perinuclear cytoplasm, but decrease in concentration in the apical cytoplasm, where they are replaced by clear vesicles. The tips of Type III cells are crowned by elongated extensions that surpass the microvilli of the other cell types. Type IV cells lie at the base of the taste bud and do not possess apical processes. They are usually dark, triangular cells. In addition to these four cell types, other cells may be distinguished that have dark cytoplasm or that contain dense lamellar inclusions.
The appearance of these cells suggests that they are undergoing degeneration and that they may be end stages in the life cycle of Type III cells.

Which of these cell types should be regarded as sustentacular cells and which as receptor cells? In the rabbit, only Type III cells are in synaptic contact with nerve terminals. The presynaptic zones are usually round patches on the basal or perinuclear regions of the cell and are marked by prominent dense fibrillar material and dense projections attached to the cytoplasmic surface of the cell. Small clear vesicles aggregate in small or large clumps in this presynaptic cytoplasm. The tips of the afferent nerve fibres contain vesicles of various sizes, but no obviously specialized modification of the postsynaptic membrane. In the taste buds of mice, in contrast, all types of cells—dark, intermediate and light—engage in synapses with nerve fibres (Kinnamon et al 1985, Royer & Kinnamon 1988). Thus, if the presence of innervation is a minimal criterion for identification of the sensorireceptor cell in taste buds (Royer & Kinnamon 1991), different cell types can be sensorireceptor cells in different species, and indeed in different taste buds in any individual animal.

This inherent ambiguity has an important bearing on our understanding of the development of the cells in the taste bud. It has long been known that the cellular components of taste buds continually undergo renewal. The life cycle of any one cell occupies about fourteen to twenty-one days. Occasional mitotic figures seen among the basal cells suggests that these Type IV cells are stem cells that give rise to the other cell types. But there is considerable debate about whether the different types represent stages in a continuous cell line in which Type III is the final mature stage, or whether each cell type represents the product
of a different cell line. Experiments in which taste buds are allowed to degenerate and regenerate after transection of the glossopharyngeal nerve have produced conflicting results. It is entirely possible that there are species differences in the generation of cells in taste buds corresponding to the differences in the distribution of innervation within the taste bud.

Both the development and persistence of taste buds require the presence of gustatory nerve fibres. When these specific nerve fibres are interrupted the taste buds atrophy and when the nerve fibres regenerate the taste buds reappear. As the gustatory cells are continually undergoing desquamation and replacement, the terminals of the nerve fibres must continually form fresh synaptic connections on the new cells. The discovery of how this is accomplished should be a fascinating study in neuroectodermal plasticity.

The olfactory epithelium

Taste buds are not the only neuroepithelial structures that undergo continuous renewal. The olfactory epithelium sustains a similar turnover of its component cells. Like other neuroepithelia, the olfactory epithelium is stratified and consists of three cell types—sustentacular cells, receptor cells, and basal cells (Fig. 2). The receptor cells, however, are unique in being true nerve cells, with dendritic processes extending to the surface and axonal processes extending to the brain, where they end in complicated synaptic formations known as the olfactory glomeruli. These are the only nerve cells in vertebrates that reside on the surface of the body, exposed to the outside world. They are also the only nerve cells in mammals that continue to be generated throughout the life of the animal (Graziadei & Monti-Graziadei 1979, Costanzo & Graziadei 1987). The receptor cells have a life cycle of 4–8 weeks. When degenerating cells die, their axons also disintegrate and are replaced by the growing axons of new receptor cells in the epithelium. These axons grow along the surviving axons in the olfactory nerves and must establish new connections in appropriate glomeruli in the olfactory bulb. The mechanisms that regulate and guide this natural and normal regenerative process might provide insights for ameliorating and enhancing plastic repair under pathological conditions in the central nervous system.

The olfactory epithelium originates from two embryological sources, the superficial ectoderm and the olfactory placode, a broad thickening in the ectoderm that forms very early in embryonic life almost simultaneously with the neural plate (Costanzo & Graziadei 1987). As development of the head end of the embryo proceeds, the cells of the placode and the superficial ectoderm intermingle, the latter providing the precursors of the sustentacular cells and the placode cells giving rise to the neuronal, sensory receptor cells of the olfactory epithelium. Undifferentiated cells, presumably from both cell lines, persist in the depths of the epithelium as the basal cells, which give rise to replacements of the other two, differentiated cells throughout the life of the organism.
In the adult olfactory epithelium the sustentacular cells are arrayed in a sheet continuous with the ordinary pseudo-stratified columnar epithelium of the upper nasal passages (Morrison & Costanzo 1990). The ordinary columnar cells are ciliated, but the sustentacular cells of the olfactory sensory epithelium have only microvilli extending from their apices into the mucus overlying the free surface (Fig. 2). The nuclei of the sustentacular cells lie in the middle and upper thirds of the epithelium. The apical cytoplasm displays a thick terminal web and prominent desmosomes and tight junctions that bind neighbouring cells together. Axial intermediate filaments are prominent in the cytoplasm beneath the nuclei. The sustentacular cells, like epithelial cells elsewhere, have a brief lifespan, but their turnover rate is slower than that of the receptor cells in the same olfactory epithelium (Costanzo & Graziadei 1987).
The sensorireceptor cells, as mentioned, are true nerve cells. Their light, vesicular nuclei lie in the middle and lower thirds of the epithelium, below those of the sustentacular cells. The two cell types form a columnar unit with sustentacular cells in the centre and receptor cells arranged in a circle around them (Graziadei & Monti-Graziadei 1979, Costanzo & Graziadei 1987). Basal cells cluster at the bottoms of the columns. The receptor cell body is globular and contains a meagre complement of the usual cytoplasmic organelles, including some neurofilaments. The apical process, or dendrite, is a slender extension bound with junctional complexes to the sustentacular cells at the level of the terminal webs of the latter. Beyond this level, the dendrite protrudes as a rounded expansion, the so-called olfactory vesicle or dendritic knob, from which extend numerous cilia. In frogs (Reese 1965), each cell bears six to eight cilia, which start out as conventional cilia with basal bodies and the usual 9+2 array of axial microtubules. But, a short distance from their origin, the cilia suddenly narrow into slender tubes containing eleven single, parallel microtubules. The cilia are non-motile and splay into the mucous layer from the top of the olfactory vesicle like limp flower petals. In the human olfactory epithelium (Morrison & Costanzo 1990), each olfactory receptor cell can bear ten to thirty cilia, which taper gradually from their origins to their tips without alteration in their internal microtubular structure. The basal process of the cell is an unmyelinated axon, which extends through the basal lamina of the epithelium to be bundled together with its congeners into the filaments of the olfactory nerve. These axons are ensheathed by Schwann cells and terminate in the glomeruli of the olfactory bulb, where they synapse upon mitral cells.

In addition to receptor cells and sustentacular cells, a microvillar cell has recently been identified in the human olfactory epithelium (Moran et al 1982, Morrison & Costanzo 1990). This cell resembles the receptor cell, but displays only a dense brush of microvilli on its apical surface and no dendritic knob or ciliary crown. It is thought to have some receptor function.

Basal cells are pyramidal cells wedged among the basal processes of the sustentacular cells and the exiting axons of the receptor cells. They lack extensions that reach the free surface of the epithelium. Because mitoses are sometimes found among them, they have long been considered as stem cells for replacing the other cells in the olfactory epithelium. Studies with tritiated thymidine have confirmed this opinion (Hinds et al 1984). Stages in the life history of the receptor cells can be recognized in the epithelium, extending from the precursor basal cell through a cell resembling immature neurons in the central nervous system to the mature elongated receptor with its crown of peculiar cilia (Fig. 3; Graziadei & Monti-Graziadei 1978). Labelling with tritiated thymidine also shows that the distribution of dividing cells throughout the epithelium is uneven, with patches of mitotically active regions containing many immature receptors and large unlabelled areas containing mostly mature receptors (Graziadei & Monti-Graziadei 1979). The labelled regions are considered to be
FIG. 3. Developmental stages in the life history of olfactory receptor cells. Arrows indicate the locations of the free surface (s) of the epithelium and the basal lamina (b) on which it rests. bc, basal cell; m, basal cell in mitosis; n, neuroblast; lr, immature receptor; r, mature receptor with dendritic knob and cilia; dr, degenerating receptor. (From Costanzo & Graziadei 1987, with permission.)

actively neurogenic whereas the others are thought to be quiescent. In addition, the numbers of basal cells vary according to their location in the olfactory epithelium. They are much more numerous in the active, neurogenic zones than in the quiescent ones.

The organ of Corti

As an example of a neuroepithelium concerned with the transduction of mechanical stimuli, I have chosen the mammalian organ of Corti, the peripheral organ of hearing. The organ of Corti is a specialized part of the cochlear duct, which courses through the spiral osseous cochlear canal. A thin fibrous sheet, the basilar membrane, stretches across the lumen of this osseous canal, dividing it along its entire length into two parallel tubular chambers. Another sheet, Reissner’s membrane, further subdivides the upper chamber. Thus the lumen of the osseous canal is divided into three parallel spiral compartments, each lined by squamous or specialized epithelium and filled with fluid. The upper compartment is known as the scala vestibuli, the lower one as the scala tympani, and the one in between, bounded by Reissner’s membrane and the basilar
membrane, is the scala media or the cochlear duct. The scala vestibuli and the scala tympani communicate with each other at the apex of the cochlea. They are filled with perilymphatic fluid, whereas the cochlear duct contains endolymphatic fluid and is continuous with the semicircular canals and associated structures of the vestibular portion of the labyrinth.

The wall of the cochlear duct consists of several differentiated and highly developed areas, but I shall confine my description to the architecture of the organ of Corti, as it is the only part that is considered to be a neuroepithelium. The organ of Corti is a complex specialization appearing as a ridge of cells in the epithelium of the cochlear duct lying upon the basilar membrane (Fig. 4). It consists of rows of sensorireceptor cells, the hair cells, and a variety of supporting cells. All of the supporting cells extend from the basilar membrane to the free surface of the organ, but they are separated from one another by large intercellular spaces except at their apices. The largest of these spaces is the inner tunnel, which runs the length of the organ of Corti and is bounded by the inner and outer pillar cells (rod cells) leaning toward each other. The pillar cells contain dense arrays of microtubules collected together with 6 nm thick microfilaments into axial bundles that course vertically through the cells from base to apex. The intercellular spaces between the pillar cells communicate with the intercellular spaces between the other supporting cells, particularly the neighbouring space of Nuel and the outer tunnel. A row of inner phalangeal cells lies on the inner side of the inner pillar cells and completely surrounds the

![Diagram of a transverse section through the organ of Corti. The separation between the tectorial membrane and the tips of the stereocilia on the inner and outer hair cells is an artifact. (From Warwick & Williams 1973, with permission. ©Longman Group UK Ltd. Published by Churchill Livingstone.)](image)
single row of inner hair cells, except at their apical surfaces. Unlike the outer phalangeal cells, the inner ones are closely apposed and are not separated by enlarged intercellular spaces. The outer phalangeal cells (cells of Deiters) are columnar cells standing on the basilar membrane and enclosing the lower thirds of the outer hair cells in their cuplike apices. From each outer phalangeal cell a thin process reaches the surface of the organ where it expands into a flat plate attached to the adjacent hair cells. Both the outer phalangeal cell and its slender process contain an axial bundle of microtubules.

The sensorireceptor cells are hair cells arranged in regular rows on either side of the inner tunnel. The inner hair cells form a single row of columnar cells, each one lifted off the basilar membrane and enclosed in an inner phalangeal cell. The free apical surface of each inner hair cell is fitted with a cluster of microvilli arranged in the form of an arc with its concavity facing away from the inner pillars. The microvilli contain large numbers of axial filaments that extend deep into the thick terminal web at the apex of the cell. The outer hair cells line up in three parallel rows running the length of the organ of Corti. Each cell rests in the cuplike top of an outer phalangeal cell and a W-shaped cluster of microvilli extends from its apical surface. The concavities of the W face the outer pillar cells.

The microvilli of the hair cells are known as stereocilia, so named from the mistaken notion that they were non-motile cilia. But they do not have the internal fine structure of cilia, although there is a basal body and an associated centriole in the apical cytoplasm under the peak of the W or the arc of microvilli. The microvilli of the hair cells stretch out toward the tectorial membrane, into which their tips are embedded. The tectorial membrane is an extracellular proteinaceous material secreted by the cells covering the limbus at the inner angle of the scala media. Vibrations induced in the basilar membrane by sound waves transmitted to the fluid in the scala vestibuli and scala tympani distort the stiff stereocilia with a resultant depolarization or hyperpolarization of the hair cell, depending upon direction. The change in potential results in the release of transmitter from the base of the cell onto terminals of afferent nerve fibres, which are activated to carry impulses to the central nervous system.

The afferent fibres consist of two types originating from different types of cell bodies in the spiral ganglion (Simmons & Liberman 1988a). About 95% of the cells are Type I cells, which give rise to large, heavily myelinated fibres, the radial fibres, that contact individual inner hair cells at their bases and around their sides with large boutons containing few vesicles. The radial fibres ramify only sparsely and individual ones end on a single inner hair cell. Any one hair cell may receive afferent endings from several Type I cells. In contrast, Type II cells give rise to unmyelinated, thin nerve fibres, the spiral fibres, that enter the organ of Corti with the radial fibres but then cross the floor of the inner tunnel and turn to run along the rows of outer phalangeal (Deiters) cells. After ramifying, the spiral fibres give off short digitiform processes that end in small
boutons on the basal surfaces of the outer hair cells. Each fibre contacts as many as a hundred outer hair cells, largely in a single row. Furthermore, each outer hair cell receives the endings of several different spiral fibres (Liberman 1980, Simmons & Liberman 1988a, b, Liberman et al 1990).

In addition to this afferent innervation, there is an efferent system, originating in the superior olivary complex. Olivocochlear fibres coming from the medial periolivary cells terminate as large vesicle-filled endings on the outer hair cells, whereas efferent fibres coming from the lateral periolivary cells terminate as small boutons mostly on the endings of radial fibres around inner hair cells (Brown et al 1988). This efferent system is thought to inhibit activation of the afferents.

Like the olfactory epithelium, the membranous labyrinth originates from a placode, a thickening of the ectoderm. This placode forms dorsal to the first branchial groove and, invaginating, sinks into the mesenchyme between the myelencephalon and the metencephalon of the brain, as the otic vesicle. The epithelium lining this vesicle enlarges and differentiates into the membranous labyrinth with its several vestibular and cochlear neuroepithelial specializations.

In mammals the cochlear epithelium is apparently not capable of regenerating after destruction of the organ of Corti. Hair cells that are killed by toxic substances or by excessive stimulation are permanently lost.

The retina

Of the neuroepithelia to be considered in this symposium, the retina is the most complicated. It is also different from all the others in its origin directly from the rostral end of the neural tube instead of from an ectodermal placode. It really is a peripherally situated part of the central nervous system. It starts out as an evagination from the prosencephalon attached to the brain by the optic stalk. Later local invagination transforms it into the optic cup, a bilaminar epithelium turned in on itself. The inner lamina differentiates into the neural retina, while the outer lamina remains fairly simple and becomes the pigment epithelium. The invagination practically obliterates the lumen of the vesicle and accounts for the fact that the photosensitive elements, the rods and cones, of the neural retina face into the pigment retina instead of toward the light. The optic stalk becomes transformed into the optic nerve by the growth of axons from the innermost layer of retinal nerve cells toward the brain through intercellular spaces in the ependymal lining of the stalk.

The complexity of the retina is such that I shall confine my remarks to its general character in relation to the topic of this symposium. Detailed descriptions are available in numerous textbooks and monographs, the most recent being the book by Dowling (1987). The retina is a conspicuously laminated structure. The outer layer of rods and cones and the alternating layers of nuclei and interwoven nerve cell processes form one of the images of the nervous system that is most familiar to students of biology (Fig. 5). Although the retina can be
described in the same terms as the other neuroepithelia—that is, as an assemblage of receptor cells and sustentacular cells resting on a basal lamina—its additional components, the vast numbers of nerve cells and their processes, give it an altogether different aspect of complexity. This is a structure that does not simply transduce external energy into nerve impulses, but also contributes to the analysis and refinement of those impulses so that the brain can construct a coherent and integrated visual image from them.

The outermost layer of the retina is the pigment epithelium, which derives from the outer lamina of the embryonic optic cup. This epithelium consists of a single layer of pigment-containing cuboidal cells connected to each other by typical junctional complexes. The apical surface of the cells is thrown up into folds that surround the outer segments of the receptor cells and into numerous
slender microvilli. Although there are no structural connections between the photoreceptor layer and the pigment epithelium, the intimate contact between the two layers is necessary for the normal function of the photoreceptor cells. In addition to absorbing stray light, the pigment cells phagocytose the membranous tips of the photoreceptors, which are continually being cast off, and they also store vitamin A, which is a necessary precursor of the photosensitive pigments in the rods and cones.

The sustentacular cells of the neural retina, the Müller cells, correspond to the neuroglial cells of the central nervous system. They are radially oriented cells that extend through the whole thickness of the neural retina. Their processes are velate and filiform, insinuating themselves among the neural elements like the protoplasmic astrocytes of the brain. Müller cells contain vimentin filaments and form junctional complexes with the photoreceptor cells and one another at the outer limiting membrane, and again with one another at the inner limiting membrane, in effect defining the neural retina.

The photosensitive elements in the neural retina are the terminal projections of cell bodies densely packed into the outer nuclear layer. The apical process of each of these cells extends into the pigment epithelium and is divisible into two parts, an outer and an inner segment. The outer segment contains the photosensitive pigment. On the basis of the shape of the outer segment, the photoreceptor cells can be classified into two types, rods and cones. The difference in shape is, however, not always easy to discern, and the inner segments are often an easier criterion. The outer segment of the rod is a slender threadlike process of uniform calibre containing a stack of collapsed membranous discs. The inner segment, which is slightly thicker than the outer segment, contains numerous longitudinally oriented mitochondria in its outer part (the ellipsoid) and the other cytoplasmic organelles in the inner part (the myoid). The two segments are connected by a short, very narrow stalk containing nine pairs of longitudinal microtubules extending from a basal body in the tip of the inner segment. This connecting stalk is the vestige of the ciliary process out of which the outer segment derived.

The inner and outer segments of cones have an architecture similar to those of the rods, but there are certain differences of shape and detailed structure. Cone outer segments tend to taper toward their outer tips and the inner segment is usually broader and more obviously conical than that of the rods. In the fovea of primates, however, the cones are packed so closely together and are so attenuated that there is hardly any noticeable morphological difference between them and rods elsewhere.

The cell bodies of the photoreceptors give rise to axonal processes that extend inward to the outer plexiform layer, where they terminate in characteristic presynaptic expansions. Both the rods and the cones synapse with bipolar cells and with horizontal cells in complex synaptic formations. In addition, the terminal expansions of the rod and cone axons may themselves be interconnected by punctate or elongated gap junctions.
The perikarya of the bipolar cells make up the inner nuclear layer. Their dendrites reach into the outer plexiform layer to synapse with the terminals of the photoreceptor cells, whereas their axons descend into the inner plexiform layer to synapse with the dendrites of retinal ganglion cells and amacrine cells. The ganglion cells make up the innermost cellular layer of the retina, and they give rise to the optic nerve fibres, which course in a fibre layer on the innermost surface of the retina to the optic disc, where they penetrate through the thickness of the retina and, becoming myelinated, constitute the optic nerve.

The simple three-neuron chain described in the retina—photoreceptor to bipolar to ganglion cell—is a caricature of the circuitry in this neuroepithelium (Fig. 6). Not only are there many different kinds of each one of these primordial

FIG. 6. Diagram of the principal synaptic interconnections in the vertebrate retina. Synapses are represented by thickened lines and associated synaptic vesicles, sometimes together with synaptic ribbons. RT, receptor terminal; H, horizontal cell, IB, invaginating bipolar cell; FB, flat bipolar cell; A, amacrine cell; G1-3, ganglion cells with various kinds of input from bipolar and amacrine cells. (From Dowling 1987, with permission.)
types of cell, but there are also the horizontal cells lying in the outer rows of the inner nuclear layer and the amacrine cells lying in the innermost inner nuclear layer and even in the ganglion cell layer. And besides these elements, there are the interplexiform cells, which lie in the inner nuclear layer and send dendritic processes into the inner plexiform layer and axonal processes into the outer plexiform layer. Furthermore, one must remember the efferent fibres emanating from the brain (for example, the isthmo-optic nucleus in birds) and terminating on amacrine cells of the inner nuclear layer. All of these varieties of cells and their complex interactions, both morphological and physiological, lead to an intricate circuitry that is only now beginning to yield its secrets.

More to the point of this symposium, it is important to emphasize that this neuroepithelium has a distinctly different potential for plasticity and regeneration in different species and classes of vertebrates. In mammals and birds damage to the retina cannot be repaired by regeneration of the lost neural elements. In fishes and certain amphibians the retina continues to grow during the life of the animal; it can be restored after injury, and even, in some experimental situations, completely re-formed after being destroyed.

Conclusion

Neuroepithelia range in complexity from simple clusters of specialized cells to complex interconnected neural assemblies. In all cases their integrity depends upon the existence of connections with the nervous system, whether they give rise to the connections, as in the olfactory epithelium and the retina, or are merely innervated by peripheral sensory nerves. The capacity of neuroepithelia for regeneration varies not only according to the species of animal but also according to the age, structure, and natural history of the organs themselves.

References

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Raymond: Dr Palay, you mentioned that basal cells in taste buds of certain vertebrate species receive synapses. Could you elaborate on that?

Palay: In the mouse, Kinnamon and colleagues have looked in light and electron microscope preparations for synapses, because they wanted to define the gustatory receptor cell. They found in the mouse that all three of the intragemmal cell types, including basal cells, are innervated, whereas in the rabbit, this is not so (Kinnamon et al 1988, Royer & Kinnamon 1988).

Rubel: How sure are they that it's not true in the rabbit?

Palay: Kinnamon does say that it's possible that there are varieties of the innervated cell in the rabbit that may be closer to the basal cell in the mouse, so that as the intragemmal cell is developing from a basal cell to the definitive receptor cell, it may be innervated. This is not commonly found, however, in the rabbit.

Raymond: The basal cells are usually thought to be a mitotic population. Do mitotic cells receive synapses?
**Palay:** Apparently the basal cells in mitosis were not innervated, in rabbits.

**Raymond:** So the basal cell population could include not only a mitotic cell population, but also recently postmitotic cells which are beginning to differentiate?

**Palay:** Yes. With a cell that is in the process of differentiating, there is a threshold at which you can recognize when it has really begun to differentiate. So some of the basal cells may be further along in this process than others.

**Rubel:** As with psychophysics, as our judgment gets better, or as different markers become available, we see earlier signs of when a cell’s fate is distinguishable.

**Margolis:** Are there adequate biochemical or immunohistochemical markers with which to distinguish the various stages of receptor cells in the taste system?

**Palay:** There are some markers, namely whether electron-dense granules are present or not, and whether the gustatory receptor cells have what appear to be monoamine granules.

**Farbman:** Studies on cell division in taste buds show that $[^3H]$thymidine is taken up mainly by epithelial cells just lateral to the taste bud. Epithelial cells, then, divide outside of the taste bud; then postmitotic cells move into the taste bud and become basal cells (Beidler & Smallman 1965). There have been occasional observations of mitotic figures within the taste bud proper, but the usual situation is that taste bud precursor cells are outside the bud and migrate in.

**Raymond:** Do they have to cross the basal lamina to reach the taste bud?

**Farbman:** No. They are the basal cells of the stratified squamous lingual epithelium lateral to the taste bud.

**Raymond:** What is the capsule around the taste bud?

**Farbman:** The ‘capsule’ consists of non-gustatory epithelial cells that are flattened against the periphery of the taste bud. They may be a special kind of glia-like cell, but so far as I know there are no markers that distinguish these cells from other non-gustatory epithelial cells nearby. The taste cell precursors would have to move laterally along the basement membrane to enter the taste bud. Whether they must cross a ‘capsular’ barrier or whether they must pass through a stage of differentiation as a peripheral or capsular cell is not known. In any event, it is apparent in electron micrographs where the non-gustatory epithelium ends and the taste bud epithelium begins.

**Fernald:** So are the basal cells intercalated among existing differentiated cells? And where do you find the other member of the pair of cells produced by mitosis?

**Farbman:** One daughter cell remains at the periphery, outside the bud, and the other enters the bud.

**Rubel:** Do the postmitotic cells slide across the stem cells?

**Farbman:** That isn’t known. By Day 2 after $[^3H]$thymidine injection, the label is within the bud. That is all we can say.