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2

Comparative Aspects of Visual System Development

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2.1 Introduction

Teleological thinking in evolution tends to worsen the closer the animals we compare come to ourselves. It is not difficult to objectify a shark or a bat – to examine the niche they inhabit and the problems they must solve, and begin the exploration of how their nervous systems combine species-general neural structure with species-typical adaptations. When we examine other primates, however, we often fail to view them in the large evolutionary context that makes sense of their design features, viewing them as the 'model systems' so favored by biomedical funding agencies. In this chapter, we will attempt to place primate visual system development in the larger context of vertebrate visual system evolution. We will argue that only an evolutionary context makes sense out of the developmental mechanisms that are deployed to generate eyes and connect them up.

A concept useful for understanding the nature of developmental mechanisms found in all present-day animals is 'evolvability' (this concept borrowed and modified from the sense used by Gerhart and Kirschner (1997) in *Cells, Embryos and Evolution*). Every extant animal is the descendant of animals going back to the beginning of evolutionary time, the successful survivor of multiple mass extinctions, climatic shifts, niche invasions, diseases, disasters, and mishaps. These forces alone predispose to powerful, redundantly organized developmental strategies. Not only disastrous change, however, but also persistent kinds of changes seen over evolutionary time should be considered in the context of evolvability. Changes in body size are a change of this kind. Consider

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the case where competition favors the survival of animals of larger body size. If individuals varied in the degree to which their developmental programs coordinate the size and structure of body parts, in this case the organization of the retina, and vision was seriously compromised by eye enlargement consequent to body enlargement in a number of animals (e.g. as we will discuss later, their visual sensitivity became poor in the dark), these animals will not fare well. Only the animals with good coordinating programs will be with us today. Scaling up and scaling down are commonplace in evolutionary time, and it is thus reasonable to examine present-day developmental mechanisms for the details of their scaling properties, stabilized by repeated challenges of the kind just described.

Similarly, every present-day primate is the descendant of species which at minimum has made the transitions from a diurnal niche to a nocturnal niche and again back to a diurnal niche, beginning from the first vertebrates. A second structural feature of evolution to consider in contexts like these is how the genome is translated to variable morphology. Duplications and accretions characterize evolving developmental networks, as opposed to substitutions of new pathways (Gerhart and Kirschner, 1997; Wilkins, 2001). These two phenomena together – the repetition of movement between nocturnal and diurnal niches, and genetic change that build on redundancy and duplication – suggest we should see the evidence of successful transitions in building eyes suited to both low- and high-light levels in the developmental programs of present-day primates.

In the present comparative analysis of visual system development in primates, we will compare two things – present primates to non-primates, and primates to each other. We will first describe the commonalities of fundamental retinogenesis, and the development of ocular morphology of primates compared with other vertebrates. We will then describe variations within primates in retinal size, color vision, and nocturnality and diurnality, and what is known about their development. This review will concentrate on the retina and the eye, though we will note a few features of central nervous system organization of particular developmental interest in primates, such as the pattern of crossing of the optic nerves at the optic chiasm. Finally, we will discuss the development of a feature unique to primates (among mammals), the fovea, with its high central concentration of photoreceptors coupled with centripetal displacement of photoreceptor cell bodies and the rest of its associated retinal processing architecture.

2.2 Fundamental organization and development of the retina

The basic organization of the vertebrate retina has been fixed at least since the first divergence of the jawless vertebrates, over 400 million years ago, insofar as we can judge from the retinas of the current representatives of the major vertebrate radiations, a rather remarkable fact. 'Basic organization' in fact refers to virtually all functional and structural features of importance, including the image-forming eye with its components of cornea, lens, and retinal conformation; the method of phototransduction, the presence of rod and cone photoreceptors; multiplication of opsin types allowing the possibility

of color vision; the remaining classes of retinal cells, including horizontal cells, bipolar and amacrine cells, and retinal ganglion cells and their pattern of layering; lateral inhibition as realized both in the photoreceptor layer and in interactions between photoreceptors, horizontal cells, and bipolars, and again in the interactions of bipolar and amacrine cells with retinal ganglion cells; many transretinal classes of neuromodulators, and finally oculomotor organization relevant to eye stabilization (Ramón y Cajal, 1972 (translation date); Rodieck, 1973; Braun, 1996). A discussion of the evolution of the vertebrate retina, and secondarily, the primate retina, is about the adaptation of the eye to the broad nature of the environment (e.g. eye-in-water versus eye-in-air), to size (eye diameters range over approximately a hundredfold, from the smallest fish to the largest whale eye), and to niche (nocturnal versus diurnal, predator versus prey, and so on). Niche adaptations may have many facets, for example, the characteristic chromaticity of the environment, the specialization of the eye for central vision and associated ocular motility, characteristic patterns of whole-body motion, the use of the eyes as social signaling devices, and so on. Resulting variable features of the eye and visual system include the number and spectral features of photopigments (Chapters 3 and 4), the number and topological arrangement of photoreceptors, bipolar cells and ganglion cells (Chapter 5); the presence of neural machinery capable of producing eye movement (capable of overriding ancestral eye stabilization mechanisms under vestibular control; see Chapter 10) and various mechanisms for the central modulation of retinal function.

In broad strokes, the development of the eye is similarly conserved, and in fact, the fundamental mechanism of the embryonic positioning of the eye through the action of the conserved developmental patterning gene, *PAX6*, antedates vertebrates. The nature of this conserved function is the subject of much current debate (Callaerts *et al.*, 1997; Fernald, 2000). We will not discuss here the early morphogenesis of the eye (Robinson, 1991), though we will consider some aspects of early gene expression when we discuss the development of the primate fovea. At this point, we will discuss retinal development following from the time that the primordium of the eye has evaginated, contacted the tissue that will give rise to the lens, and folded back in to give the bilaminar cup-shaped organ that will give rise to the retina in the ventricular lamina adjacent to the prospective lens, and the choroid and supporting tissue in the ventricular region forming the outside of the cup.

2.3 Neurogenesis

As in all neural tissue, the retina arises from the cells of the ventricular zone directly adjoining the ventricle. The onset of retinogenesis is signaled by the interaction of 'symmetry-breaking' cell surface molecules and receptors that take the population of undifferentiated neural precursor cells, and drive some proportion of them toward their terminal, differentiating division. After cells exit the precursor pool, a second step determines what sort of cell the postmitotic neuroblast becomes (Cepko, 1999; Dyer and Cepko, 2001). The order of retinogenesis is conserved in all mammals (Clancy *et al.*, 2001), though not in all vertebrates (Beazley *et al.*, 1989). Ganglion cells are produced first, then cones, then horizontal cells. A slight decrement in cell production follows, and then

amacrine, Müller cells, and bipolar cells, and finally rods are produced. This pattern of neurogenesis as it is seen in the macaque retina, schematized for retinal ganglion cell, cone, bipolar, and rod cell classes from LaVail *et al.* (1991), is shown in Figure 2.1. In this representation, the amount of each cell class that is produced on each postnatal day is represented as a percentage of its own total population, with area under each curve roughly approximated; in subsequent representations, the absolute numbers of cells of each type will be emphasized instead. This pattern of neurogenesis has many intrinsic

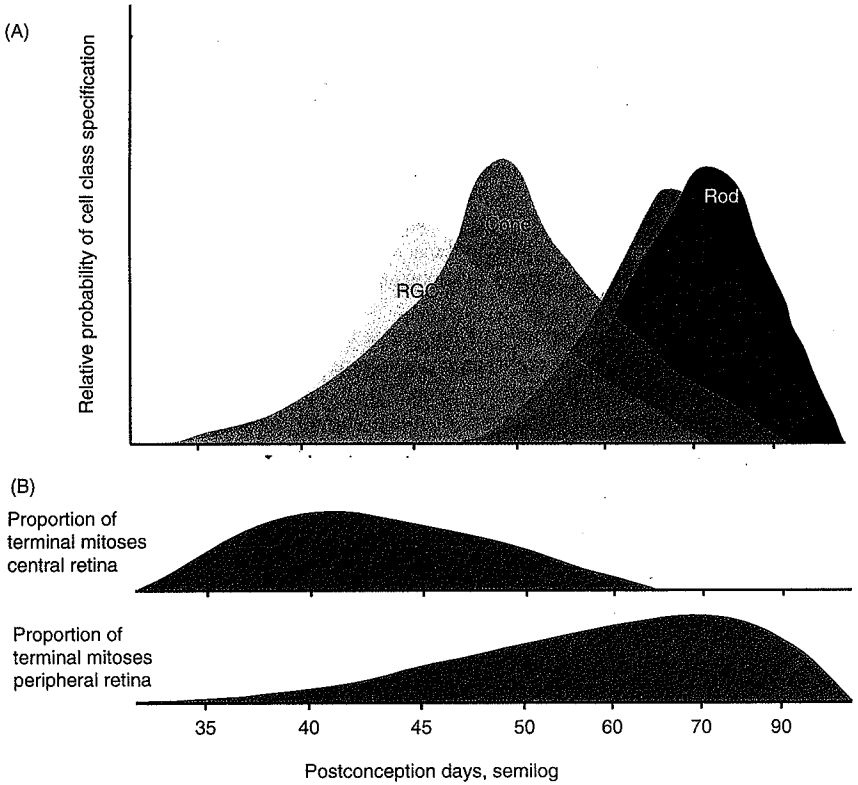

Figure 2.1 (A) Order of production of retinal ganglion cells, cones, bipolar cells, and rods in the retina of rhesus macaque. For the two types of neurons omitted from this map, horizontal cells virtually overlap cones; amacrine cells lead bipolars slightly. The ordinate shows the postconceptional day of terminal cell division, the abscissa percent of each cell class produced on that day. (B) The temporal gradient in the probability of specification of cell of a particular class from an undifferentiated cell (top graph) versus the spatial gradient in the probability of exit of precursor cells in central retina (middle graph) versus peripheral retina (bottom graph). Replotted and schematized from Rapaport *et al.*, 1996

features of interest for understanding retinal variation in primates – scaling of the retina to different eye sizes and for nocturnality – and we will return to it several times more. At this point, however, we will turn to general features of retinal maturation.

2.3.1 Gradients of maturation

The mammalian retina differs from the non-mammalian retinas that have been the most studied – the goldfish (Raymond, 1991), the cichlid fish (Fernald, 1989, 1991), and the 'workhorse' of developmental biology, the zebra fish (Easter and Nicola, 1996), in that the mammalian retina is produced in a single bout in early embryogenesis, while most teleost retinas have indeterminate growth, setting up a basic retinal structure, but adding cells to it throughout the lifespan. Retinas with indeterminate growth must essentially solve scaling problems on the fly as they grow. As in mammals, a separate strategy for the genesis of cones versus rods exists, to solve the distinct scaling required to maintain acuity in high-light conditions versus sensitivity in low-light conditions (Fernald, 1991). The solution to this same problem appears in a different form in the mammalian retina, which we will discuss in some detail.

Primates, compared generally with mammals, have long periods of retinogenesis (Clancy *et al.*, 2001) and a relatively large retina (Ross, 2000). This extended time and large space allow probably the most distinctive feature of neurogenesis in primates to be seen, which is the presence of major gradients in the onset and cessation of neurogenesis across the retinal surface (LaVail *et al.*, 1991; Rapaport *et al.*, 1992, 1996). The nature of the retinal gradient with respect to control of cell specification is somewhat counterintuitive. Cells exit the precursor pool first in the central retina to begin their terminal maturation, and the specification of cell type is determined by a complex interaction of cell lineage and the extracellular environment (Alexiades and Cepko, 1997; Livesey and Cepko, 2001; Ohnuma *et al.*, 2002). One could imagine that this process could overall be best described by each retinal location in turn proceeding through the cascade of events that first statistically favors retinal ganglion cells, then cones, and so forth. The process, however, seems better described by two gradients, one spatial and one temporal (Figure 2.1A and B). The exit of the precursor population to terminal neurogenesis has a central-to-peripheral spatial gradient (Figure 2.1B), while cell specification appears best described as a transretinal clock (of unknown kind) without a strong spatial gradient (Figure 2.1A). Experimental support for this conjecture comes from the manipulation of time of exit from the cell cycle in the rat (Austin *et al.*, 1995; Bao and Cepko, 1997) and in *Xenopus* (Chang and Harris, 1998; Ohnuma *et al.*, 2002). Production of excessive terminal cells in early retinogenesis produces larger numbers of retinal ganglion cells and cones, while exit of precursor cells later produces mostly rods – if a local count of divisions advanced the specification clock, we would not see this pattern. Extrapolating these findings to the particular case of the primate retina, it is likely that at the time that the majority of cells in the central retina become postmitotic, cell specification mechanisms produce principally retinal ganglion cells and cones and their attendant horizontal cells and some bipolars, producing the very low convergence ratio from photoreceptors to ganglion cells seen in the central primate retina. By the time most peripheral retina cells enter terminal mitoses, principally bipolars and rods are specified, but fewer ganglion cells, automatically producing

the high convergence ratio of the peripheral retina. In smaller retinas with flatter gradients, the disparities in cell type between central and peripheral retinas are not so pronounced. The statistical probabilities of producing each cell class are broad enough that no retinal location totally lacks any cell type, with one exception, the primate fovea (hypotheses for the development of this primate feature will be discussed subsequently). A possible cause of the absence of rods in the fovea is that neurogenesis is terminated, or central precursor pools are exhausted, before the progression of cell-type specification reaches the period when rods are specified, leaving a central rod-free zone.

Across species, this gradient of maturation appears to have immediate consequences for the rest of retinal maturation, as the gradient becomes realized in the mechanical properties of the retina. The retina grows in diameter after initial cytogenesis principally by passive stretch – the 'balloon' model (Coulombre, 1957; Mastronarde *et al.*, 1984; Kelling *et al.*, 1989; Robinson *et al.*, 1989; Reichenbach *et al.*, 1991). The early 'area centralis' becomes relatively inelastic compared with the rest of the retina and remains relatively fixed at its embryonic dimensions and, most important, embryonic cell density. The cause of the lesser central elasticity could simply be the direct result of features of early maturation, i.e. greater thickness, absence of cell interpolation, and growth of connective processes between cells, or might involve additional directed changes in the cytoskeleton or cell adhesion. Thus, the peripheral retina reduces cell density per unit visual angle substantially during early development, even as it adds cells, due to its greater elasticity during the period when ocular dimensions are growing most rapidly; in several species, including humans, there is evidence for excess cell loss in the peripheral retina as well (Sengelaub and Finlay, 1982; Provis, 1987). Extrapolating from several species of which the relationship of embryonic cell density, elasticity, and retinal stretch have been studied (chick, cat, and rabbit), it is likely that the unusually steep spatial gradients of neurogenesis in the primate produce very steep gradients of cell density by this biomechanical process.

2.4 Topology and specification of cell-type subcategories

The prior discussion has lumped all variation in cell categorization to the seven large retinal cell classes. There is a second aspect of cell deployment, which is the spatial arrangement of cell subclasses across the tangential aspect of the retina. These include the different subclasses of photoreceptors and their associated bipolars; ON- and OFF-bipolars and ganglion cells; the very wide diversity of amacrine cells; and the various subtypes of ganglion cells. With two exceptions, there are few studies in primates that would suggest any unusual variations on vertebrate-general mechanisms that order the array of subclasses of cells across the retinal surface. Research to date suggests that any identifiable cell class 'tiles' the retina completely (Cook and Chalupa, 2000). Work on ON- and OFF-bipolar cells and the dendritic development of retinal ganglion cells suggests that the initial distribution of cells is more random than the terminal distribution, which becomes hyperdispersed due to the combined effects of competition

with like cells for synaptogenesis, some cell death, and biomechanical factors (Reese and Galli-Resta, 2002).

2.4.1 Cone photoreceptors

All primates, with the exception of two cases of nocturnal cone monochromats, the New World monkey *Aotus*, the owl monkey, and the bush baby *Otolemur crassicaudatus* (Jacobs *et al.*, 1996) possess at least two cone types, a short- and a middle-wavelength sensitive cone (Ross, 2000; Heesy and Ross, 2001; Figure 2.2). A large fraction, including all Old World monkeys and the great apes (Jacobs and Deegan, 1999), the New World howler monkey *Alouatta* (Jacobs and Deegan, 2001), and female New World monkeys of the tamarin and cebid groups (Jacobs, 1998), further differentiates the middle-wavelength cone into long- and medium-wavelength-sensitive classes (i.e. are trichromatic). We will first discuss the disposition of the first two cell classes in dichromats, those that express the 'blue' (S) opsin versus those that express any one of the family of intermediate opsins (historically named R/G or M/L), which can be distinguished immunocytochemically. Work in fetal rhesus monkey shows that cones are initially laid out in a regular array, particularly one precocious cone that leads its neighbor cones in the expression of opsins, an arrangement that appears similar to the early arrangement of photoreceptor mosaics in *Drosophila* (Wikler and Rakic, 1994). Eventually, the S and M/L groups form independent mosaics, with about 10 times as many M-/L- cones as the S class; different primate species differ distinctly in the regularity of their cone mosaics, but the significance of this observation is unclear (Wikler and Rakic, 1996). In addition to the opsin expressed, these two cone photoreceptors (S versus. M/L, Figure 2.2) differ from each other in a number of dimensions, including number, retinal distribution, size of cell body, and connectivity at maturity.

The duplication and differentiation of two opsins (M, L) from the single M-/L-opsin appear to have occurred at least three times in the primate lineage, producing obligatory trichromacy in all Old World monkeys and their descendants, and at least once in the ateline line of New World monkeys (demonstrated only in *Alouatta*, the howler monkey). In other New World monkeys, non-obligate trichromacy appears in females only, as the gene for opsin in the yellow range takes two forms and is linked to the sex chromosome (Jacobs, 1998; Jacobs and Deegan, 1999, 2001). In only those females who happen to carry two different alleles of the gene, trichromacy is possible, as only one opsin will be expressed per photoreceptor, the other silenced at random (Chapters 3 and 4). In all these cases, however, the only difference between the L- and M-opsin-expressing photoreceptors lies in the opsins themselves, in the substitution of a few amino acid groups that cannot be distinguished immunocytochemically. In fact, there is no known feature of cell type – size, distribution, or dendritic appearance – that can as yet distinguish the L and M photoreceptors. Recent work shows highly variable foveal distributions of these photoreceptors, in no way mosaic in appearance (Roorda and Williams, 1999; Roorda *et al.*, 2001). The likelihood is that in the absence of other cell-type specification or intrinsic mosaic organization, it is only the one-to-one (or better) photoreceptor to ganglion cell convergence in central retina that allows the L/M chromatic distinction to be conveyed to the central nervous system (Sjostrand *et al.*, 1999; see Chapter 5). The generic activity-dependent organizational capabilities of the

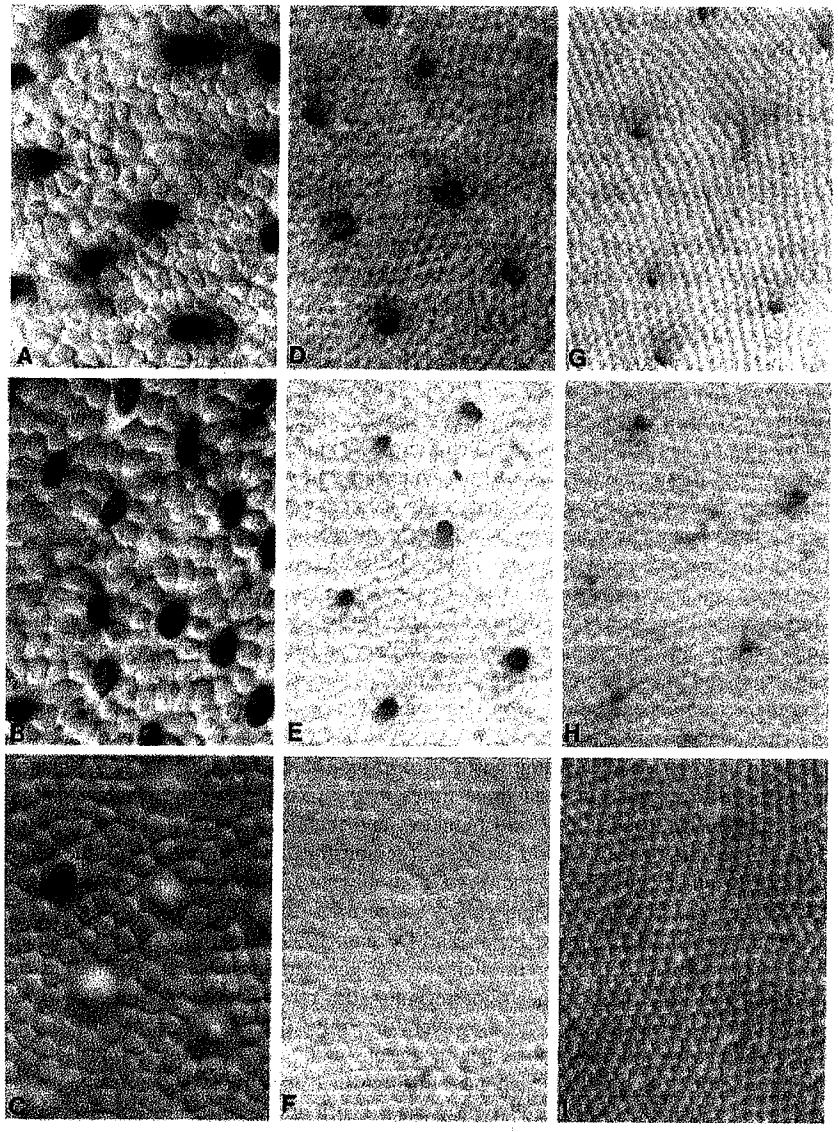

Figure 2.2 Photomicrographs of CSA-I-labeled (A, D, G) and 4942A-labeled (B, E, ZZ) red-green-sensitive cones and 108B-labeled blue-sensitive cones (C, F, Z) in rhesus monkey (first column), owl monkey (middle column), and bush baby retina (third column) (Wikler and Rakic, 1990). Note the regular and periodic absence of labeling of putative blue-sensitive cones in the CSA-1- and 4942A-labeled rhesus monkey retinas. Magnification: 1900× for E, F, H, and I; 1350× for A, B, C, D, and G. Reproduced by permission of The Society for Neuroscience

central nervous system rather than molecularly mediated cell recognition mechanisms may then permit the development of a three-dimensional color space. This Hebbian 'fire together, wire together' mechanism is already employed in such basic developmental visual functions as sharpening topographic maps and organizing ocular dominance layers and columns, and could also be employed in the segregation of central chromatic pathways. In addition to Hebbian mechanisms, existing dichromatic color vision has mechanisms available potentially useful to implement trichromatic vision. In particular, the ordinary contextual dependency of color computations, whether the context is immediate ambient illumination, or the relative number of photoreceptors of a particular type, could be used to extract a signal from the apparently random expression of L- versus M-opsins for useful perceptual distinctions (Golz and MacLeod, 2002; Neitz *et al.*, 2002).

2.4.2 Retinal ganglion cells

The retinal ganglion cells of both New and Old World monkeys come in several subclasses, 'PC' ('midget', or the small-celled classes that project to the parvocellular layers of the lateral geniculate nucleus (LGN)), 'MC' (also parasol, projecting to the magnocellular layers of the LGN), and several other smaller groups (Silveira *et al.*, 2003; see Chapters 5 and 6). In development, the PC class is produced first, and MC second (Rapaport *et al.*, 1992). The relative order of the final precursor divisions producing these two cell types, and the spatial distribution of rods and cones and the order of their final divisions may allow convergence to remain proportional in eyes of different sizes. Though both cell classes get rod input, the greater rod input is to MC cells; in addition, there is greater convergence of all bipolar input, cone and rod, to MC cells compared with PC cells. As will be discussed in the following text, the similar position of MC cells and rods in the relative order of neurogenesis will allow these functional pathways to scale similarly in eyes of different sizes.

2.4.3 Other dimensions of cell-type specification within broad classes

One dominating feature of the historical retinal literature is the classification of cells into types (e.g. nine types of bipolar cells in rats; multiple classes of amacrine cells), where the process is usually carried to maximal distinctiveness, sorting neurons on every possible dimension of function (ON/OFF; temporal and spatial selectivity; chromatic response) and morphology (size of arbor; branching pattern and lamination pattern – e.g. Wässle *et al.*, 1987, 1991). A developmental classification system might produce a more condensed taxonomy. Considering activity-dependent organization and other aspects of competition in early development, many aspects of 'cell type' are revealed to be epigenetic rather than intrinsic, depending on the pattern of interaction of the cell with its neighbors, or the relative densities of cells (Bodnarenko and Chalupa, 1993; Bodnarenko *et al.*, 1995; Reese and Galli-Resta, 2002). The rapid growth of genomic approaches to cell specification should ultimately give us a better taxonomy of 'type' – how many rules exist for arbor growth, receptor expression, and so forth. For now, however, the

understanding of the details of adult typologies in terms of specific developmental growth rules is only beginning to be established.

2.5 Lamination; synaptogenesis; axon outgrowth; and cell death

Extension of processes, and expression of the metabolic processes associated with the production and degradation of neurotransmitters, begins rapidly following the final division of precursors producing retinal neurons and photoreceptors. The first evidence of processes are found in the inner plexiform lamina nearest the retinal ganglion cells (Robinson, 1987). Development of processes spreads to the peripheral retina following the gradient of cessation of neurogenesis (Robinson, 1991). Slightly later differentiation of the outer plexiform layer begins and the connections between photoreceptors, bipolar cells, and horizontal cells develop in a similar manner. The lamination in the adult retina is highly precise, with bipolars and ganglion cells, respectively, sorting themselves into laminae according to their ON or OFF response to light, to their chromatic responses (Rodieck, 1973). While no experimental demonstrations of the activity dependence of development of cell processes have been done in primates, from work with cats and ferrets, we may infer three possible organizing roles or manifestations of activity dependence in early retinal organization. If ganglion cell spiking activity is experimentally eliminated, the normal developmental course of dendritic spine reduction of ganglion cells is altered (Wong *et al.*, 1991). In initial development, ganglion cells extend dendrites in all laminae, while bipolar axons are restricted in their lamination. In cats, if retinal activity is blocked by glutamate blockers, the ganglion cells retain their overlapping and uniform arborization to adulthood (Bodnarenko *et al.*, 1995), much in the way the axons of the lateral geniculate neurons representing the right and left eyes retain their overlapping and uniform distribution if their activity is blocked. However, it should be noted that virtually all activity-dependent retraction effects that have been described in the central nervous system involve the retraction of axons to stabilize their pattern of connectivity on dendrites, while in the case of the retina, the *dendrites* of retinal ganglion cells segregate according to the lamination of the bipolar 'axons' which appears to be fixed by some other source of information, yet unknown (Katz and Shatz, 1996).

Finally, in a number of species (Wong, 1999) in the time preceding eye opening, organized activity is produced in the retina in the form of retinal waves, which sweep across the retina in spatially organized fronts. The spatial correlation of neuronal activity that these wavefronts represent may provide some of the statistical structure required for activity-dependent organization of retinal projections in the central targets of the retina before actual visual experience. The locally correlated activity in each eye could initiate the organization of a number of important aspects of visual system circuitry, for example, the oriented receptive fields of the visual cortex, the topographic mapping of retinal projections on any central array, or the sorting of projections that are decorrelated, as the two eyes are before they begin to view the external world.

2.5.1 Connecting with central targets

In the rhesus monkey, axons appear in the optic stalk at approximately postconception day 35 (PCD 35), and reach the chiasm about five days later, which occurs before the peak of retinal ganglion cell genesis occurs in the retina, reminder of the ongoing and overlapping nature of cytogenesis and development of connectivity in retinal development. The optic nerve reaches its principal central targets (LGN and superior colliculus) at about PCD 48 and progressively invades them over the next 10 days (Clancy *et al.*, 2001). The peak of optic axon nerve number is reached at PCD 65, and is reduced by more than half by PCD 100, due to the apoptotic death of retinal ganglion cells (Rakic, 1983; Provis, 1987). For comparison, eye opening occurs in rhesus at PCD 126 and birth at PCD 165. Comparative studies of the timing of such events showed that the order of these events is highly conserved and predictable across species including primates (Clancy *et al.*, 2001).

At the optic chiasm, the hemi-decussating pattern of contralateral and ipsilateral retinal projections in primates is quite precise, and while it follows the general vertebrate strategy of aligning congruent representations of the visual field in the central targets of the retina, some interesting details vary (Godement *et al.*, 1990; Chalupa and Dreher, 1991; Chalupa and Lia, 1991). In particular, in most mammals at least one cell class in the retina projects contralaterally from all parts of the retina, temporal and nasal, while in primates all cells from the temporal retina project contralaterally and cells from the nasal retina project contralaterally. The molecules mediating midline pathway choice, including the optic chiasm, appear to be highly conserved across invertebrates and vertebrates (Erskine *et al.*, 2000). The question of how some retinal neurons and their axons from the nasal retina come to carry different labels to dictate their midline choice is not known.

2.5.2 Cell death

Cell death in the retina has been the subject of many past reviews that have summarized its incidence and topographic distribution (Finlay, 1992), and current work concentrates upon the developmental context of cell survival (de la Rosa and de Pablo, 2000), the characterization of the molecular communication between interconnecting populations of neurons, and the nature of the pathways that initiate the orchestrated cell death termed apoptosis (Nijhawan *et al.*, 2000). Cell death occurs in all retinal layers (Georges *et al.*, 1999; Linden *et al.*, 1999; de la Rosa and de Pablo, 2000). In nervous system development, two-way signaling between most interconnected populations, like motoneuron and muscle, or retinal ganglion cells and their targets, is required for the growth and often survival of both populations. Not all apoptotic cell death is regulated by such trophic interactions between neurons – sometimes a period of neuronal apoptosis will occur in development entirely independent of the connections of cells. Retinal ganglion cells, however, which are highly dependent on trophic support for their survival, are the best-studied cells of the visual system and thus we know the most about this aspect of early cell death. Retinal ganglion cell survival requires that axons establish connections, so there is an absolute requirement for trophic support mediated by target contact (Finlay and Pallas, 1989). The absolute amount of cell loss in regions of the retinal ganglion cell layer can be extremely high in normal development (e.g. 90 percent in the human retina (Provis, 1987)) and varies between species. The pattern of cell loss

also is variable with topographic position in the retina, with excess ganglion cell loss in the retinal periphery, and is thus a potential contributor to retinal organization and topography.

2.6 Emmetropization

In primates, much retinal maturation is still to occur after birth, though all cells have been generated, all central connections are generally stabilized, and lamination is adult-like. The eye grows substantially in size from birth to adulthood with proportional growth of the retina, principally by stretching in the non-uniform manner described previously. The fovea is beginning its development, which will continue in the first year of life, to be described later in this chapter. In early development, the growth of the eye is in part under the control of experience. 'Deprivation myopia' was first described in primates (Wiesel and Raviola, 1977), noting the unusual growth of the eye after early eye suture in infant macaques; and early "runaway myopia' is a well-known clinical syndrome. While most of the detailed work on mechanism has been done in the chick (Troilo, 1992), work in marmosets (Troilo and Judge, 1993), rhesus monkey (Hung *et al.*, 2000), and human clinical syndromes make extrapolation from the work in chick retina quite plausible (Wallman *et al.*, 1987a).

'Emmetropization' refers to the process of matching the length of the eye to the power of the eye's optics (principally contributed by lens and cornea). While most primates are born with a general match achieved, early acuity is very poor compared with the adult, and there may be very substantial astigmatic errors of refraction – that is, the refracting power of the eye may be quite different on its horizontal and vertical axes (Howland and Sayles, 1985). During the first year of life, the optics undergo much modification, under the control of eye activity. Defocus can be measured directly by the retina and alter retinal growth, even if the optic nerve is severed (Troilo *et al.*, 1987). 'Defocus' can be represented crudely as the relative amount of retinal activity, as a blurred image is a poor stimulus to photoreceptors, and other features of image movement can be used to infer sign of defocus (Schaeffel and Diether, 1999). In chicks, a high-contrast image signals that the length of eye and optics match, and growth of the eye is checked. A blurred image is taken as evidence that the eye is too small, and the eye continues to grow, which can result in the positive feedback condition of 'runaway myopia' if the eye has made the wrong guess about the direction of defocus and the eye is already too long (Wallman, 1995). This activity must be initially mediated by cones, though it is thought that the signal that modulates eye growth directly probably goes through the amacrine pathway, causing changes in choroidal thickness and scleral elasticity (Wallman *et al.*, 1994). In particular, the glucagonergic amacrine cells have been shown to respond to defocus in retinal image and even to its sign, by changed expression of the immediate-early gene product, ZENK (Feldkaemper and Schaeffel, 2002).

Anthropocentrism may have caused us to somewhat mischaracterize this process, as what was described above is the sequence of events necessary to properly focus an eye in the day. A nocturnal animal will want a different control regime – although it is always useful for the image to be focused on the retina, linking focus to inhibition of growth is a poor strategy for a nocturnal animal, as a much larger eye will produce a

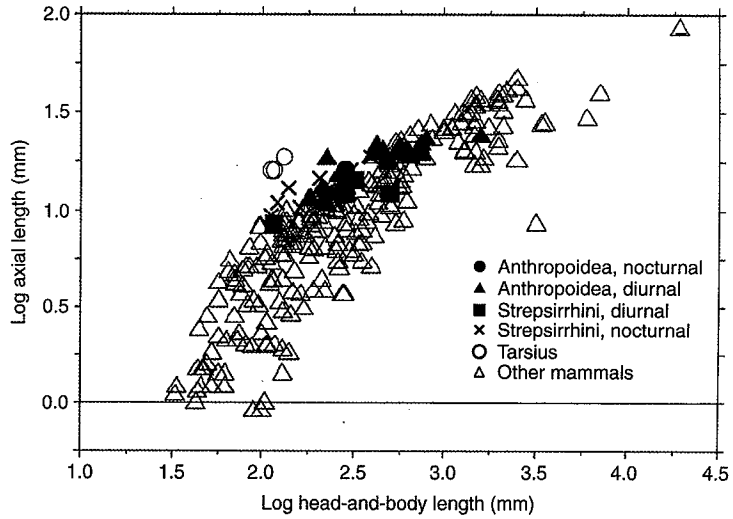

Figure 2.3 Log–log plot of eyeball axial diameter against head-and-body length (HBL). Redrawn from Ross (2000). Reduced major axis slope = 0.815. Best-fit line is axial diameter = $-2.353 + 1.97$ HBL -0.244 HBL^2; $R^2 = 0.775$

much greater photon capture. Most nocturnal animals do have larger eyes than diurnal ones; such is the case, for example, for the owl monkey compared to other New World monkeys of similar body size, as well as other nocturnal primates (Heesy and Ross, 2001; Figures 2.3 and 1.3). We hypothesize that mammalian eyes may have two competing control regimes available, one rod-dominated and one cone-dominated. In fact, it is known that the 'emmetropization' signal is gated by circadian rhythms in the very diurnal chick (Schaeffel *et al.*, 1995) – in a diurnal animal, the absence of cone activity at night should not be taken as evidence of inadequate focus. However, if there is a great deal of rod activity at night, or an absence of cone activity during the day, this could conceivably be transduced as a signal to produce the optimally large nocturnal eye, an adaptation, not a 'failure of emmetropization'. Later, we will consider how employing this proposed developmental route might produce the coordinated changes seen in the conformation of the eye in the owl monkey, and in part account for the curious pattern of cone monchromacy they have evolved.

2.7 Scaling the eye

The eyes of diurnal primates are absolutely large compared with most mammals and scale allometrically with body size (Ross, 2000; see also Chapter 1). Curiously, however, given the fact that diurnal primates are thought to be visual specialists, the eye of diurnal

primates scales with a rather flat slope, particularly notable if you consider the human case – if we scaled at the general mammalian slope, our eye would be considerably larger in diameter than it is (Figure 2.3). We have proposed that one unusual, non-scaling feature of the primate eye could account for it. In all primates, regardless of overall eye size, the fovea is approximately 0.5 mm in diameter, and it is possible that it can be no larger due to various physiological and metabolic constraints (Franco *et al.*, 2000). The high metabolic activity of the fovea coupled with the absence of vasculature, and the drawn-out fibers of Henle from photoreceptor processes to cell body may limit its size to the size observed. As the fovea probably first arose in a primate of relatively small eye and body size (Heesy and Ross, 2001), retention of the fovea would have put a substantial brake on further enlargement of the eye with brain and body size, so as not to have the fovea subtend an excessively small visual angle.

Even with the constraint of the fovea, primate eyes do scale with body size, ranging from around 10 mm in diameter in the smallest monkeys to around 30 mm in various anthropoid apes (Heesy and Ross, 2001). Scaling an organ (made of cells of constrained size) which has several geometrical features under different constraints is an interesting construction puzzle – not all parts of the eye may scale the same and retain function. The overall conformation of the optics of the eye scale up linearly – for example, the eye of the rat and the eye of the mouse, appropriately scaled, are superimposable (Remtulla and Hallett, 1985). Within the eye, however, retinal thickness may not vary much, due to the constraints of perfusion and light passage, and stays close to a thickness of some 200 μm or so; as we have already discussed, the size of the fovea appears to be constrained to approximately 0.5 mm.

Rods and cones must scale at different slopes with eye size, in order to hold constant their particular functions. If an eye becomes twice as large in diameter, no change is necessary in the number of cones to retain the same visual acuity – since the retina is flooded with photons in diurnal vision, a single cone will have no difficulty encountering a photon in the visual angle it represents regardless of the angle the cone itself subtends. More cones could of course be added, to improve acuity, but we are discussing here what is required to maintain equivalent, not improved, function over different eye sizes.

The same solution will not work for rods – working at low-light levels and low photon numbers, a single rod located in a larger absolute retinal area even if retinal angle is unchanged will detect proportionately fewer photons, even allowing for biologically plausible increases in the size of a single rod. Rods must tile the surface of the retina to maintain sensitivity, increasing in number approximately at the square of change in retinal diameter. The observed scaling of rods and cones in diurnal primates conforms closely to this functional necessity, where cones increase in number by less than a factor of 2 between marmosets (*Callithrix jacchus*) and humans, while rods increase by more than a factor of 10 (Figure 2.4).

How is this consistent within- and across-species scaling necessity executed in the schedule of neurogenesis of the retina? While the precise kinetics remain to be worked out, the schedule of neurogenesis in the retina is arranged such that extension of the period of embryogenesis (the principal way mammals get bigger) should automatically produce the desired differential scaling. In much prior work, for the central nervous system in general, we have determined that components of the nervous system scale very

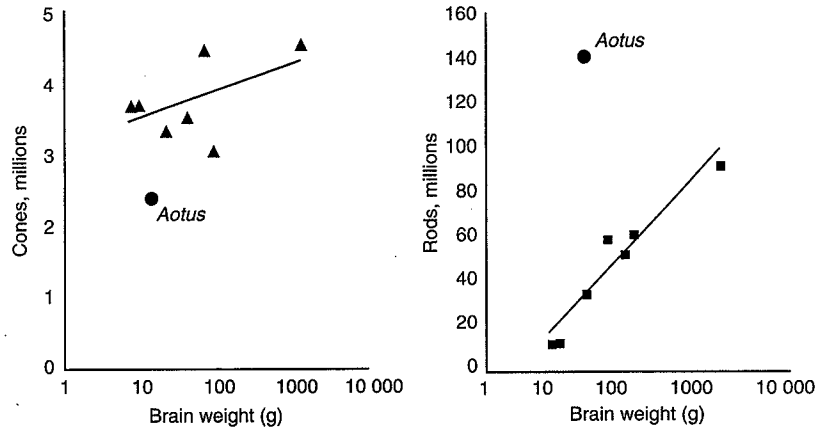

Figure 2.4 Rod and cone numbers versus brain weight in seven diurnal primates (in order, *Callithrix jacchus, Saguinus midas niger, Saimiri sciureus, Alouatta caraya, Cebus apella, Macaca mulatta,* and *Homo sapiens*) and one nocturnal primate (*Aotus azarae*). Data for *Macaca* and *Homo* from Curcio *et al.* (1987, 1990) and Curcio and Hendrickson (1992) and the remainder from Snow *et al.* (1997), Franco *et al.* (2000), Kaskan *et al.* (2005). Regression equation, cones, diurnal primates only, $y = 0.167 \ln(x) + 3.159$; $R^2 = 0.27$; regression equation rods, diurnal primates only, $y = 0.16.137 \ln(x) - 16.96$; $R^2 = 0.92$

predictably with brain size, but with different slopes – for example, as mammalian brains become larger, they are composed of predictably and proportionately more neocortex, but proportionately less medulla. The pattern of neurogenesis, conserved across mammals, accounts for which structures will grow disproportionately large in neuron number – 'late equals large' (Finlay and Darlington, 1995; Finlay *et al.*, 2001). That is, if a schedule of neurogenesis is extended, for example, from 15 days in one species to 30 days in another, groups of neurons go into terminal neurogenesis at roughly proportionate times within the entire period. However, the precursor pools from which these neurons are derived are increasing at an exponential rate in absolute time, and the last-differentiating cell groups (like the cortex and cerebellum) will be launched from a disproportionately larger pool. Such is the case for the relative timing of cone and rod neurogenesis in the retina, as modeled for marmoset versus human (Figure 2.5). Those cell types that must change in number exponentially with eye diameter, rods and their attendant bipolar cells, are located last in order of differentiation, and those that need not change are produced first.

This obligatory, coordinated scaling of retinal cell classes to match functional requirements is an example of the idea of 'evolvability' with which we began this chapter. Those potential ancestors with perhaps a reversed order of neurogenesis in the retina who might have had a selective advantage at a larger body size, but who unfortunately became blind in the dark as a result, presumably would enjoy less reproductive success. Common selection points, such as body size, will naturally select on coordinating features

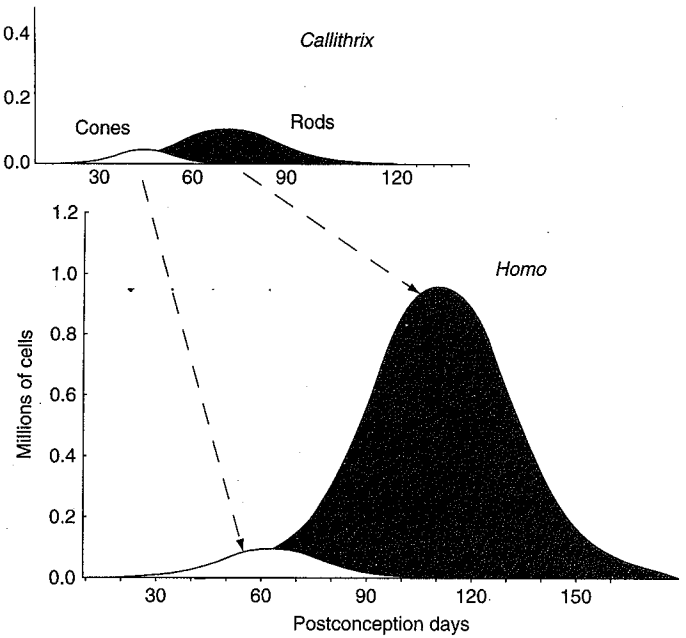

Figure 2.5 Schematic to demonstrate how extension of the period of retina neurogenesis may disproportionately increase the numbers of later-generated cell groups by allowing disproportionate increase in the precursor pool from which later cell groups are drawn. Neuron numbers and developmental durations drawn from Finlay *et al.* (2001) and Clancy *et al.* (2001)

in embryogenesis, and the retina is a nice example of order of neurogenesis adapted to two contrasting functional constraints.

2.8 Producing the nocturnal eye

Nocturnal eyes, overall, have a number of features that are different from diurnal eyes. Underscored at the outset should be the fact that most eyes are duplex, with the ability to function at both night and day, but most eyes have features that optimize one or the other niche. Eyes specialized for nocturnal vision have design features which maximize photon capture – they are often frontal to double light catch by focusing two eyes on the same scene, each eye is larger in size, with larger pupils, relatively more rods, and an absence of central specializations for high-acuity vision which would be wasted, and often nocturnal eyes have a reflective tapetum behind the retina to double again the chances of photon capture. Diurnal eyes often contain central specializations where cones are packed at the maximal density possible, an area often accorded special chromatic

sensitivity or special processing, in combination with a more spatially distributed set of lower density over the rest of the retina (Walls, 1963).

We will discuss the particular case of the owl monkey, *Aotus*, the only nocturnal representative of the anthropoids, with some additional observations from nocturnal strepsirrhine primates and the tarsier. The owl monkey, thought to be derived from originally diurnal monkeys, possesses all of the specializations listed above with the exception of a tapetum and has a variable partial appearance of a fovea, usually only an increased cone density containing many rods (Figure 2.6D). Its retinal diameter is strikingly large, and it has fewer cones and many more rods that would be expected compared to diurnal primates of similar brain and body size (Figures 2.3 and 2.4). Interestingly, it is a cone monochromat – the photopigment which is found in the S cones is not expressed (Figure 2.2; Wikler and Rakic, 1990). How is development altered to produce these new constellations of features? We propose a hypothesis here whose predictions we are presently investigating.

As described earlier, the production of retinal photoreceptors and retinal neurons is a two-stage process: First, the uniform population of multiplying precursor cells is driven to a terminal division by the symmetry-breaking actions of Notch/Delta signaling. Second, cells are specified as to type with respect to some as-yet-unidentified feature of the retinal environment in combination with lineage (Cepko, 1999). A single biasing event could shift the numbers of terminally differentiating neurons early in development, when cones and retinal ganglion cells are being specified, or later, when rods are produced (Figure 2.7). Such an alteration may be produced in a developing rat retina in tissue culture; it is also essentially the same mechanism already described to produce the different cell constituents of the central and peripheral retina (Austin *et al.*, 1995; Alexiades and Cepko, 1997). This single change in developmental timing alters the relative proportions of all retinal cells, and the production of rods in central retina may disable the means by which the fovea is normally produced, which is to be discussed later.

The larger eye of the owl monkey may be a secondary consequence of alteration of the diurnal mechanism of 'emmetropization' – recall that a high-contrast image, which must be transduced through the cone pathway in the daylight hours, is what checks the growth of the eye. In the owl monkey, the dose of the growth-limiting cone signal is reduced in two ways – not only is the overall production of cells during the cone-generating period limited, but also the S photopigment is not expressed, as described earlier (Figure 2.2). One explanation for the lack of cones is to make maximal room for rods, optimizing sensitivity. However, the removal of the cone signal may have an active developmental function as well, to allow the rapid growth of the large nocturnal eye; the fact that the cone lost is the one widely distributed across the retina would support this argument, as the growth of the eye appears to be controlled locally, not globally (Wallman *et al.*, 1987b). Interestingly, another nocturnal prosimian, the bush baby *Otolemur crassicaudatus*, is also a cone monochromat (Figure 2.2; Jacobs *et al.*, 1996).

The tarsier is a particularly interesting, and confusing, case. Tarsiers are classed with Haplorrhini (the group that includes monkeys and apes, but not lemurs and bush babies), a separate branch from the New and Old World monkeys we have been discussing. The stem haplorrhine primate is thought to have been diurnal, and the tarsier has a peculiar mixture of diurnal and nocturnal features, perhaps having become secondarily nocturnal like the owl monkey (Ross, 2000; see Chapter 1). It has a fovea, variable in morphology,

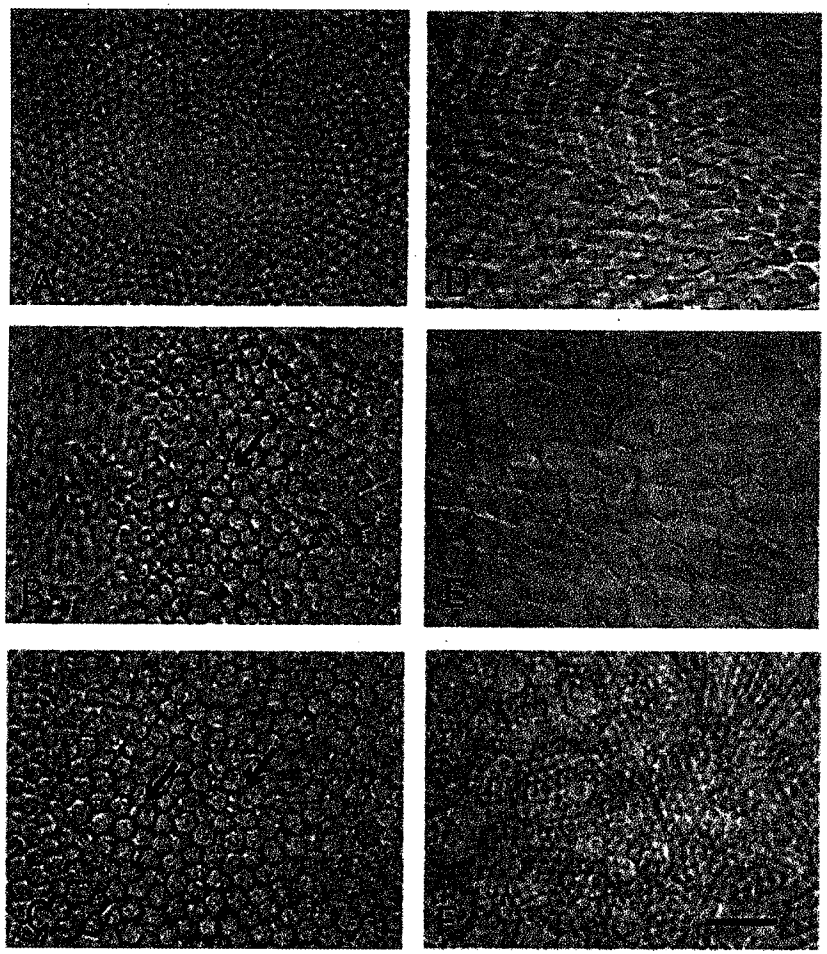

Figure 2.6 Foveae in *Alouatta caraya*, the howler monkey (A, B, C); *Cebus apella* (D, E), and *Aotus azarae* (D), showing the very reduced cone size and high cone density of *Alouatta* compared with *Cebus* (whose fovea is typical of most New and Old World monkeys) and central rods in *Aotus*. Scale bar 10 μm for all photomicrographs. (A) Center of fovea of *Alouatta caraya*. (B) Location 0.123 mm from foveal center of *Alouatta*; arrows point to two of a number of rods interposed among the larger cones. (C) Location 0.168 mm from foveal center of *Alouatta*; arrows point to two of a number of rods interposed among the larger cones. Other morphological and distributional evidence that these are rods are outlined by Franco *et al.* (2000). (D) Center of fovea, *Cebus apella*. (E) Outer fovea, *Cebus apella*. (F) Center of 'area centralis' in *Aotus azarae* focused on the internal segments of cones; principally rods are visible

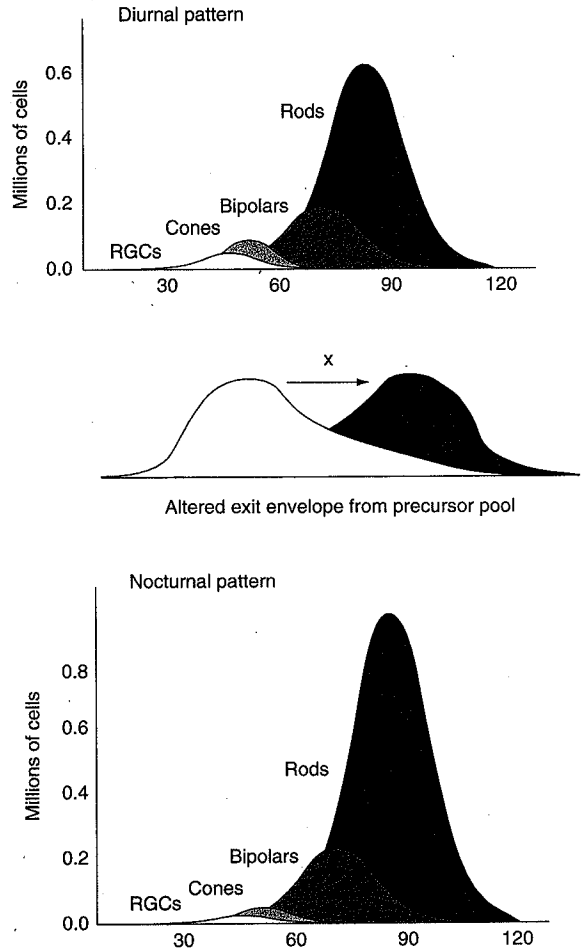

Figure 2.7 Schematic of the hypothesis of how precursor-pool exit may be biased to select principally the period of diurnal cell specification, cones and ganglion cells (diurnal precursor pool) or nocturnal cell specification (rods, and fewer ganglion cells improving convergence). Rod and cone numbers and durations from Franco *et al.* (2000) and Clancy *et al.* (2001)

but also has the largest eyes per body size seen in mammals (Figure 2.3). Nothing is known about the development of its retina, which might reveal another mechanism of decoupling eye growth from cone production.

The order of neurogenesis in the retina seems optimized for the production of a coordinated day-to-night niche transition, poised for 'exaptation' through its fundamental permissive organization into a number of adaptive, niche-specific varieties (Gould and

Vrba, 1982). The eye of the owl monkey elaborates on a feature of variability already present in primate eyes between the central and peripheral retinas – essentially, the owl monkey has an all-peripheral retina. The dose of cones (either activity or gene expression) may be the functional link that coordinates eye morphology with receptor complement, and possibly, as we will discuss, the foveal specialization. This coordinating linkage thus minimizes the number of separate features that must be selected upon to produce an eye appropriately configured for the light level of its environment.

2.9 Mechanisms of the genesis of the fovea centralis in primate retina

We now turn to a feature of ocular morphology unique to primates. It would be highly desirable to understand the ontogenetic mechanisms, which generate the fovea centralis, the small but crucial retinal area that provides the vast majority of our visual input (Figure 2.8). Since among mammals the occurrence of a fovea is restricted to primates,

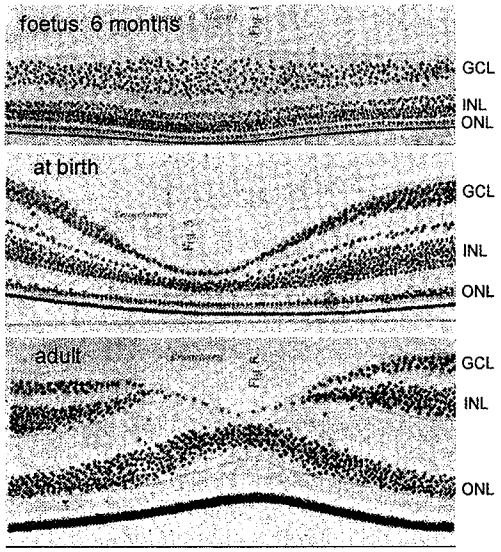

Figure 2.8 Ontogenetic development of the human fovea (modified after Bach and Seefelder, 1914; from Mann, 1950). Note that at 6th month of gestation, the ganglion cell layer (GCL) of the future fovea is even thicker than that of the neighboring retina, whereas it is completely missing in the mature fovea; by contrast, the foveal outer nuclear layer (ONL) consists of only one row of cone nuclei at 6th month but displays several layers in the adult. The inner nuclear layer (INL) undergoes similar developmental changes as the GCL

the amount of available data on its ontogenetic development is limited, and experimental approaches are hardly possible. Moreover, it should be pointed out that although foveae (meaning 'pit') may occur in the retinas of all vertebrate classes (with the probable exception of the amphibians), the structure of the primate fovea is very distinct, and differs from that of other known foveae (Figure 2.9) so that non-mammals cannot serve as model systems. Thus, our ideas about the make up of the primate fovea (Provis *et al.*, 1998) largely originate from 'experiments of nature' (such as albinism) and from mechanistic interpretations of histological data (Springer, 1999). This section is aimed at an attempt to critically review the available data, and to draw some cautious conclusions about the underlying mechanisms.

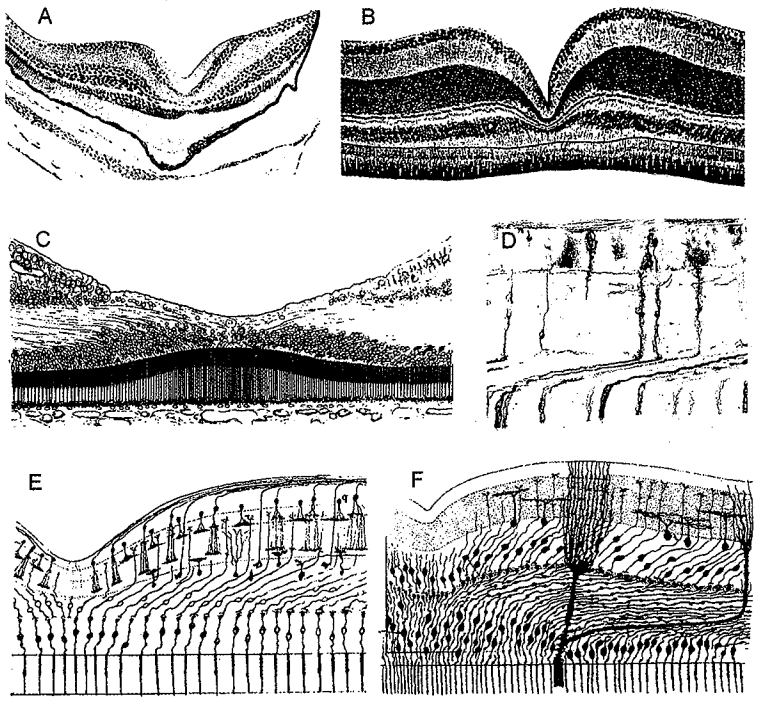

Figure 2.9 Comparison between primate (C, D) and non-mammalian foveae (A, B, E, F). (A–C) General structure of the fovea; (A) fish retina (*Evermanella indica*, Scopolidae; modified after Brauer from Franz, 1913); (B) avian retina (European bank swallow; modified after Rochon-Duvignaud from Walls, 1963); (C) human retina (modified after Polyak, 1941). (D–F) Retinal cells; (D) original microphotograph of a Golgi-labeled rhesus monkey retina (preparation of B.B. Boycott, courtesy of H. Wässle); (E) drawing of a Golgi-labeled Greenfinch retina (*Fringilla chloris*; modified after Ramón y Cajal, 1972); (F) drawing of a Golgi-labeled chameleon retina (*Chamaeleo vulgaris*; modified after Ramón y Cajal, 1972)

It has been shown that the morphogenesis of the primate fovea begins rather late in ontogenesis, i.e. *after* cytogenesis has ceased, and that the future foveal area at this time differs greatly from the mature conditions (Bach and Seefelder, 1914). The main differences are that in the immature or future fovea (i) the inner retinal layers are thicker rather than thinner than the adjacent retina, and (ii) the outer nuclear layer consists of a single layer of nuclei of cone photoreceptor cells which are thick and short rather than long and thin as in the adults (Figure 2.8). This means that – as no new cells are generated, and only a few cells die (Robinson, 1991; Georges *et al.*, 1999) – the available cells must undergo considerable changes in their shape and even in their location in order to achieve the adult configuration. These mechanisms may differ from those involved in the generation of other vertebrate foveae. As shown in Figure 2.9B, the foveae of birds may be smaller in diameter but their walls may be much steeper (i.e. they possess a 'convexiclivate' shape, enabling them to act as 'magnification lenses'). This difference prompted Walls (1963) to differentiate between the 'well-developed' avian fovea (Figure 2.9B) and the 'poorly developed' human fovea (Figure 2.9C). However, the foveae of other vertebrates, including birds, usually contain continuous inner nuclear and ganglion cell layers within their center (Figure 2.9A, B, E, F) where these layers are missing in the foveola of humans (Figure 2.9C) and most monkeys; thus, it should be kept in mind that the lateral displacement between a foveolar cone and its circuit partners (i.e. bipolar and ganglion cells) is much larger in the 'flat' primate fovea (up to $>300\,\mu m$ in humans: Yuodelis and Hendrickson, 1986; Reichenbach, unpublished data) than in the 'steep' avian fovea (up to $40\,\mu m$, as estimated from drawings of Ramón y Cajal, 1972). This means that unusual mechanisms may be required to generate the primate design. In particular, a characteristic feature of the typical primate fovea is the 'Z course' of the photoreceptor cell axons (the so-called Henle fibers) and the accompanying Müller cell processes (Figure 2.9D), suggesting that much of the re-location of retinal cells during foveogenesis occurs by an 'en block' counter-shift of the inner retina (inner part of the outer plexiform layer up to ganglion cell layer) against the outer retina (outer nuclear layer), using the (outer part of the) outer plexiform layer, together with the developing Henle fiber layer, as a 'compliant zone'. This 'Z course' is much less distinct (if at all present) in the foveae of lower vertebrates (where much of the transversal cone-ganglion cell distance is bridged by obliquely oriented bipolar cells in the inner nuclear layer: Figure 2.9E, F), and will thus be used here as a key feature of the typical mature primate fovea.

In order to search for an initial signal required to switch on the development of the fovea at the right place and time, a promising way is to look for conditions that are associated with foveal hypoplasia. It has long been known that several forms of albinism are accompanied by foveal hypoplasia or even aplasia (Elschnig, 1913; Naumann *et al.*, 1976; Fulton *et al.*, 1978). More recently it has been discovered that missense mutations of the 'major ocular control gene', *PAX6* (Azuma *et al.*, 1996, 2003), as well as Trisomy 2p(p23→pter) (Al-Saffar *et al.*, 2000), and other hitherto unknown conditions (Oliver *et al.*, 1987) may also be involved in foveal hypoplasia.

At least for albinism-associated foveal hypoplasia it has been shown that the central retina (where the fovea is supposed to be located) is not devoid of rod photoreceptor cells (Naumann *et al.*, 1976; Fulton *et al.*, 1978) as is always the case in the normal foveolar region from the very beginning of cytogenesis (Mann, 1950; Hollenberg and

Spira, 1973; Rhodes, 1979; Yuodelis and Hendrickson, 1986). Furthermore, the retina of *Aotus*, which is the only monkey species without a fovea (Kolmer, 1930), neither displays a central rod-free area (Odgen, 1975; Wikler and Rakic, 1990; Franco *et al.*, 2000; Figure 2.6). This suggests that the presence of a rod-free area in the embryonic retina may be necessary to trigger (at least, some aspects of) the generation of the fovea. This is of particular interest since it may be causally related to albinism as a well-established factor in foveal hypoplasia. In many forms of albinism, the underlying gene defect causes impairments of the availability of dopamine and related metabolites. One of these metabolites, 3,4-dihydroxyphenyl-alanine (DOPA), has been shown to stimulate the proliferation of early retinal progenitor cells but to inhibit the proliferative activity of late progenitor cells (Jeffery, 1997; Jeffery *et al.*, 1997). As described previously, early progenitor cells produce cone photoreceptor cells, retinal ganglion cells, and horizontal cells, and the late progenitor cells predominantly generate rod photoreceptor cells (for reviews, see Robinson, 1991; Reichenbach and Robinson, 1995). A deficiency in dopamine and/or DOPA may prevent both the enrichment of cones and ganglion cells and the lack of rods which are characteristic (and probably essential) for the future fovea (Figure 2.10). In other terms, a growth factor-induced block of late retinal cell generation may be a precondition to generate a rod-free area, and, thus, a fovea, well before any fovea in the morphological sense is present. As discussed earlier when describing the severe gradients of neurogenesis, one possibility raised for the absence of cones was the shifting of the time course of exit for terminal neurogenesis with respect to the clock of specification of cell determination in the retina, such that the precursor pool for the area of the retina producing the fovea was depleted before the time period for specification of rods was reached.

Both possibilities must result in an absence of cones centrally, and may be discriminated by the examination of gene activation patterns and transcription factors present in the central region at the time when the above-mentioned distinct growth pattern is generated at the place of the future fovea but nowhere else. Homeobox genes could play a crucial role in this determination, and in fact, missense mutations of *PAX6* were found to be associated with foveal hypoplasia (Azuma *et al.*, 1996, 2003). There are quite a few signaling molecules that have been shown to display a locally restricted expression pattern in the embryonic retina (for review, see Reichenbach and Pritz-Hohmeier, 1995); for example, there is a nasal-to-temporal gradient in the expression of the homeodomain factors, SOHo-1 and GH6 (Deitcher *et al.*, 1994; Schulte and Cepko, 2000), and the homeodomain transcription factor *VAX2* was shown to control the patterning of the eye dorso-ventral axis (Barbieri *et al.*, 1999). A particular combination of these, or another hitherto unidentified key molecule, may determine the location of the future fovea from the very beginning of retinal development (Figure 2.11). Once this location has been specified, the same signal and/or its downstream signals must trigger (at least) two fovea-specific events, viz. (i) either the failure to reach or a direct block of rod cell generation, and (ii) a repelling activity directed onto the growing axons of the retinal ganglion cells which thus grow *away* from the center of the future fovea even if this means (with the exception of those located at the side of the fovea directed toward the optic nerve head) that they also initially grow away from the optic nerve head (Figure 2.12, top); this latter event may be indirectly triggered by a high local density of retinal ganglion cells (Leventhal *et al.*, 1989). It may be of interest for a comparative view that both features

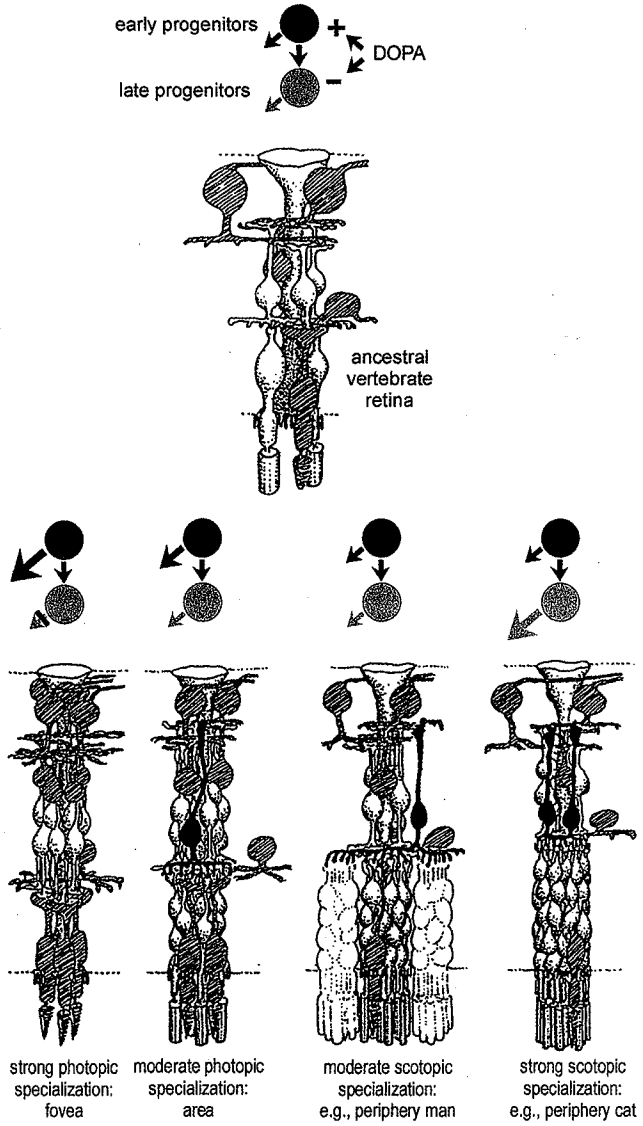

Figure 2.10 Hypothesis of the generation of (local) photopic/scotopic retina specialization by differential activation of early and late progenitor cells. Modified after Reichenbach and Robinson (1995). Early progenitors (black circles and arrows) mainly generate cells of the photopic pathways (hatched cells in the drawings: cones, ganglion cells, horizontal cells, and a subpopulation of amacrine cells) and the late progenitors (grey circles and arrows) then generate the rest (white cells in the drawings: rods, bipolar cells, another subpopulation of amacrine

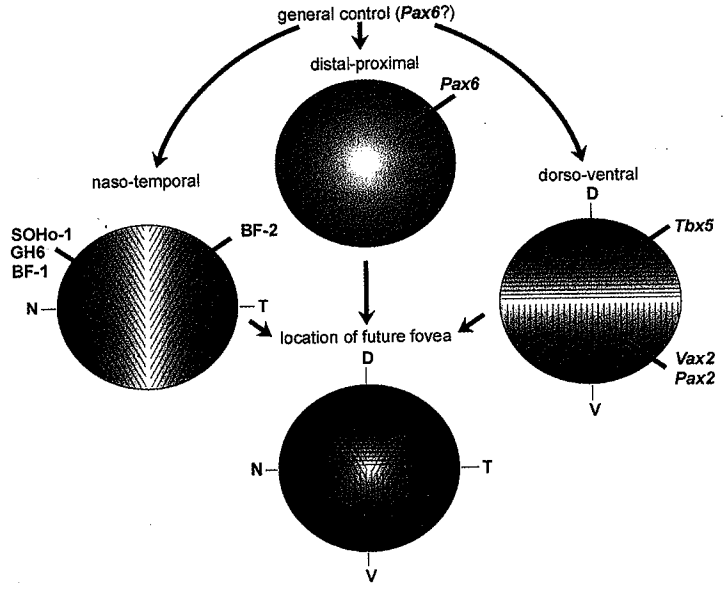

Figure 2.11 Possible mechanisms of the determination of the (site of the) future fovea by homeobox genes. The 'major ocular control gene', *PAX6*, may determine the future retina as a whole (for review, see e.g. Reichenbach and Pritz-Hohmeier, 1995), organize a peripheral-to-central pattern directly (Bäumer *et al.*, 2002), and be (indirectly?) involved in the organization of naso-temporal and dorso-ventral patterns (Bäumer *et al.*, 2002), and thus, finally, in the location of the future fovea (Azuma *et al.*, 1996, 2003). The establishment of naso-temporal and dorso-ventral patterns involves several other homeodomain transcription factors and related molecules (Deitcher *et al.*, 1994; Barbieri *et al.*, 1999; Schulte and Cepko, 2000; Bäumer *et al.*, 2002; and references therein)

Figure 2.10 (*continued*)
cells, and one type of Müller cell). A more or less equal proliferative activity of both types of progenitors will generate what is called here the 'ancestral vertebrate retina', typical e.g. for lampreys and most amphibians, and apparently capable of both scotopic and photopic vision. The preconditions for a future foveal specialization may be achieved by an enhanced proliferation of the early progenitors (generating e.g. a high density of cones and ganglion cells) and a decreased proliferation of the late progenitors (resulting in a local lack of rods). By contrast, the strong scotopic specialization of the retina of nocturnal mammals may be generated by an enhanced activity of the late progenitors, leading to a high density of rods. It is noteworthy in this context that DOPA was shown to stimulate the proliferation of early progenitors but to inhibit that of the late progenitors (Jeffery, 1997; Jeffery *et al.*, 1997; for review, see Reichenbach *et al.*, 1998). The black cells represent rod bipolar cells

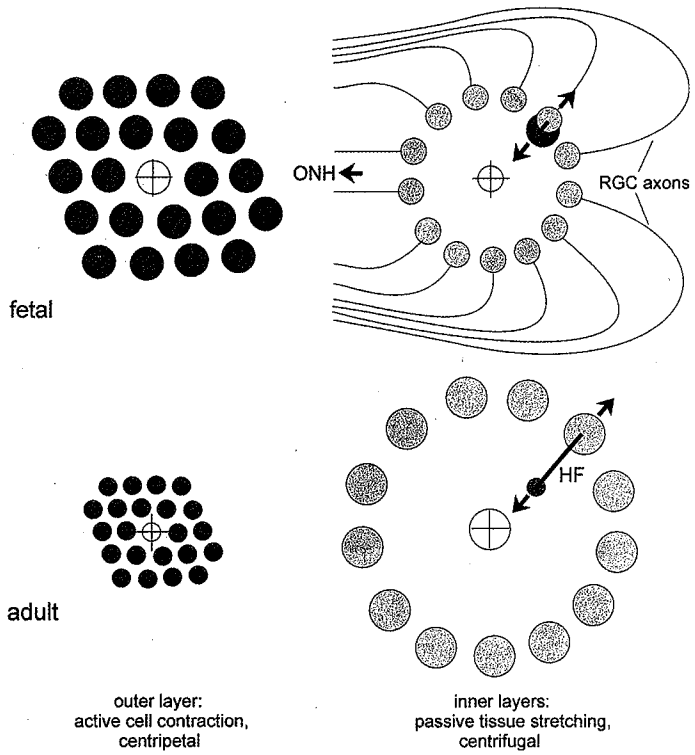

Figure 2.12 Schematic presentation of the differential developmental dislocation of cells in the inner and outer retinas during foveogenesis. Original. In the fetal retina, the thick cone cells (dark) are not very densely arranged, and retinal ganglion cells (RGC, bright) are still present in the future fovea, such that the (bipolar cell-mediated) coupling between a cone and 'its' ganglion cell(s) is arranged straightforward (i.e. 'vertical' if viewed from inner retinal surface), as exemplified by the couple of cells at right top. During further development, the cone cells become more slender and move up closer, whereas the ganglion cells (together with their partners in the inner nuclear layer) are drawn apart from foveal center along (and by?) their axons. This generates a dislocation within the 'cone-ganglion cell pairs' that must be bridged by the Henle fibers (HF) which elongate parallel to the retinal surface (i.e. 'horizontal' rather than 'vertical'), as exemplified by the couple of cells at right bottom. ONH, optic nerve head

may not apply to the fovea of the fish retina, as some deep sea fish possess a pure rod fovea (Walls, 1963), and as many nerve fibers are present within the fish foveal pit (Figure 2.9A).

The first of these 'secondary fovea-specific events' (i.e. the generation of a rod-free area) may stimulate morphological alterations of the local cone cells (perhaps, via extensive cone–cone contact signaling, by missing near-field signals secreted by

rods, or by activation of the fibroblast growth factor receptor, FGFR-4, on cones (Cornish *et al.*, 2004)). Anyway, the most probable response of the central cones to their pure cone environment seems to be a contraction of their actin filaments such that the cells become more slender; within the next 11–15 months of development in humans, the diameter of the central cone cells is reduced from about 7.5 to 2 μm (Yuodelis and Hendrickson, 1986; Curcio *et al.*, 1990). As the cones (and their accompanying Müller cells) are tightly glued together by zonulae adherentes, a wave of contraction compacts the cones within the prospective fovea (Diaz-Araya and Provis, 1992). As a result of this 'snuggling up', the local density of cones increases from about 20 000 cones/mm^2 in the neonate to up to more than 150 000 cones/mm^2 in the adult (Yuodelis and Hendrickson, 1986; Curcio *et al.*, 1990). Among other morphological changes, this leads to a characteristic stacking of the cone cell nuclei (Ahnelt *et al.*, 2004) which eventually form several rows in the adult after they have been arranged in a monolayer in the fetus (Figure 2.8).

It is remarkable that the inner retinal layers fail to follow this centripetal movement; rather, they undergo a centrifugal dislocation. This latter dislocation is the reason for the generation of the central pit, which is (almost) devoid of secondary and tertiary retinal neurons (and which is responsible for the appropriateness of the term 'fovea'). The mechanism of this centrifugal dislocation is difficult to understand. There are two reasons to assume that there is no active migration of ganglion cells: (i) these cells establish very complex dendritic trees and synaptic contacts before this dislocation (one can hardly imagine these complex 'adnexes' to be dragged behind), and, more importantly, (ii) there is no apparent movement of the ganglion cells relative to their local environmental 'landmarks' such as their neuronal partners in the inner nuclear layer or the neighboring Müller cells (Figures 2.9D and 2.12). This suggests the action of forces generated outside the foveal cells. The most probable of these is the 'passive growth' or expansion of the retinal as a whole, driven by the inner ocular pressure (and following the enlargement of the sclera; Mastronarde *et al.*, 1984; Kelling *et al.*, 1989; Reichenbach *et al.*, 1991). In mammalian retinas without a fovea such as those of cat and rabbit, this mechanism causes a flattening and areal enlargement of the retinal tissue which is more pronounced in the periphery than in the center of the retina. Owing to different mechanical properties of the tissue, the (developmentally more advanced, and thicker) central retina displays more resistance against tangential stretching than the periphery (Kelling *et al.*, 1989; Reichenbach *et al.*, 1991). The mechanical properties of the fetal (future) foveal retina have not yet been measured; probably, however, its resistance against tangential stretching forces is relatively low rather than high since there are no nerve fibers traversing the inner surface of the tissue, at this place (all ganglion cell axons grow away from the future foveolar center such that there exists a central nerve fiber-free area (Figure 2.12)). During further development and thinning of the future foveal tissue (Figure 2.8), its mechanical resistance must even further decrease, as the thickness of both the tissue and, particularly, the nerve fiber layer are important determinors of retinal tensility (Reichenbach *et al.*, 1991). It may thus be concluded that the future foveal area is subjected to much tangential stress and stretching during the period of 'passive retinal growth'.

These 'passive stretching forces' all seem to act centrifugally possibly mediated by the specific centrifugal arrangement of the ganglion cells axons. The 'turning points' of the axons move together with the surrounding retinal tissue such that the force is transmitted to the ganglion cells (and probably to their synaptic partners, as well) in a

strictly centrifugal direction (Figure 2.12). Because there is no tangentially connecting network of neurites in the center of the future foveola (the dendritic trees of central neurons are small – e.g. Figure 2.9E – and ganglion cell axons are missing), the cells of this area are then drawn away from the center where eventually a cell-free area arises. This hypothesis requires, in the case of the primate fovea, that each ganglion cell forms a rather stiff 'tissue bloc' together with its synaptic partners in the inner nuclear layer, such that small columns of neural tissue are dragged behind the stretched ganglion cell axons. Although this hypothesis remains to be proven, it is consistent with the developmental changes that can be observed when the Müller (radial glial) cells are used as markers of retinal 'columnar units' (Reichenbach *et al.*, 1993; Reichenbach and Robinson, 1995; Germer *et al.*, 1997). Figure 2.13 shows unpublished data obtained from a neonatal and an adult baboon retina. In the neonate, the fovea was not yet established. Along the straight central Müller cells, specific clusters of retinal neurons were identified for central and peripheral regions of the retina. Virtually identical clusters were found in the adult, but now the (para-) foveolar Müller cells displayed the typical 'Z- course' which indicated the course of the Henle fibers and, thus, of the 'wiring' of the retinal columnar units.

Following the above argumentation the genesis of the fovea may be the result of a centripetal movement of the outer nuclear layer and a centrifugal movement of the inner retina including all tissue from nerve fiber layer down to the inner part of their outer plexiform layer. In order to enable such a counter-movement, a 'compliant zone' must be interposed between the two layers. Such a zone should be characterized by initially a very low resistance against shear forces and subsequently an elongation of the fibers. What do we know about the border between the outer nuclear and outer plexiform layers (beginning) and the Henle fiber layer (later) which constitutes this zone? It consists of a mixture of only two elements, the axons of the cone photoreceptor cells, and the outer processes of Müller cells. In general, axons are known to be able to grow behind the surrounding growing embryonic/fetal tissue (for instance, after the connection between spinal nerves and a muscle has been established, the nerve may grow from a few micrometers up to a length of about a meter). In the case of rabbit Müller cells, it has been shown that they express cytoskeletal elements along much of their length but not in that part of the outer stem process which is located within the outer plexiform layer (Magalhães and Coimbra, 1973; Reichenbach *et al.*, 1988; Reichenbach, 1989). Although this feature remains to be demonstrated on fetal primate Müller cells, Figure 2.14 shows how it may contribute to the function of the outer plexiform layer as a 'compliant zone'.

2.9.1 The unusual fovea of the howler monkey

The retina of *Alouatta* presents a very interesting deviation from the foveae described for nearly all anthropoids, and may eventually provide a clue to the cellular mechanisms of foveal production. As the retina of other Atelidae have not been systematically examined for this feature yet, we do not know whether the howler monkey fovea is a singularity, or a characteristic of the entire family, which includes spider and woolly monkeys. Since *Alouatta* appears to be a singularity among New World primates in its obligatory trichromacy (Chapters 3 and 4), however, it may also be a singularity in foveal organization.

The fovea of *Alouatta* is of the same size as all other primate foveae, about 0.5 mm in diameter, but has twice the number of cones, which are reduced in diameter (Figure 2.6A,

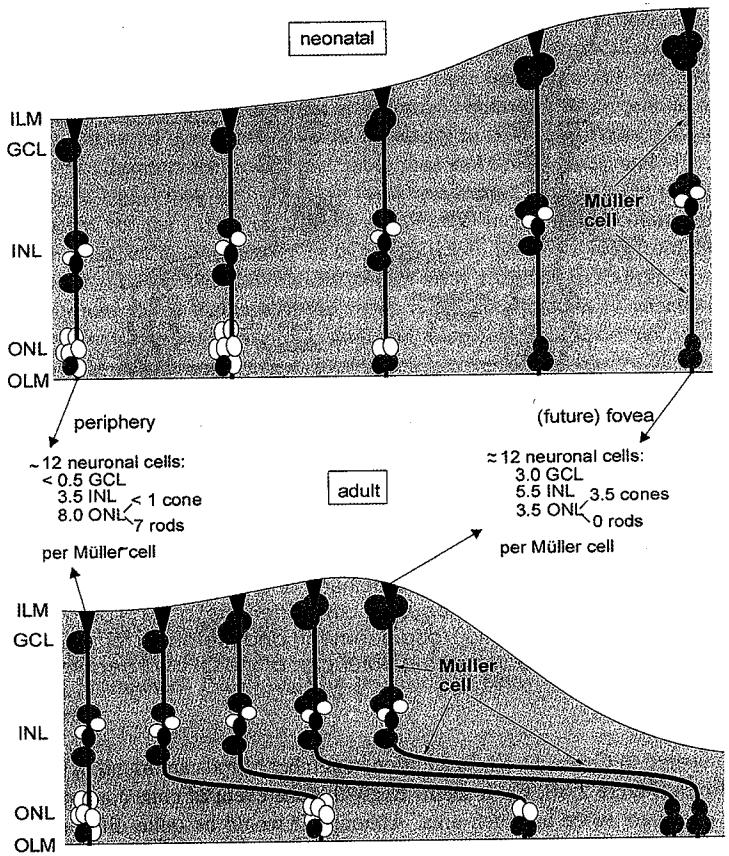

Figure 2.13 Comparison of a newborn and an adult baboon retina. Schematic drawing of the columnar units as estimated from cell counts (Müller cells were labeled by vimentin immunocytochemistry, and cell nuclei were counter-stained by hematoxylin; local densities of Müller cells and neuronal cell nuclei were counted along the course of Müller cell processes). The grey cells represent early born neurons, whereas white cells are the progeny of the late progenitors; Müller cells are drawn in black. Original (H. Kuhrt, unpublished results). Note that (i) the cellular constituents of foveal and peripheral columnar units differ although a total of 12 neurons per Müller cell was counted at both places, and (ii) the number and specification of cells within the foveal units remain unchanged during the large morphological alterations between neonatal and adult stages. The foveal data represent units whose cones are located outside the very center of the foveola. ILM, inner limiting membrane; GCL, (cells within the) ganglion cell layer; INL, (cells within the) inner nuclear layer; ONL, (cells within the) outer nuclear layer; OLM, outer limiting 'membrane'

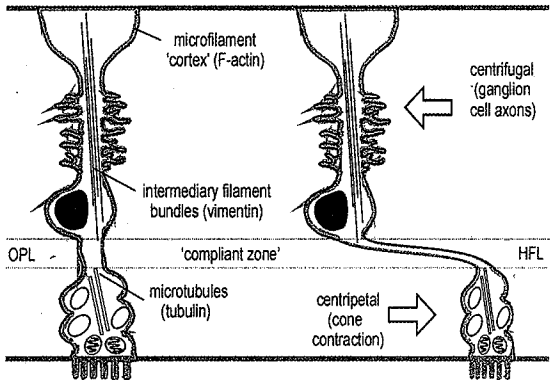

Figure 2.14 Hypothetic view of the contribution of the subcellular specifications of the Müller cell cytoskeleton to the function of the outer part of the outer plexiform layer (OPL) as a 'compliant zone' during foveogenesis. Original. In their inner stem process and soma (i.e. the 'inner part') the Müller cells contain densely packed bundles of intermediate filaments, and the distal outer Müller cell process contains microtubuli; only in the proximal outer process (located within the OPL) longitudinal cytoskeletal elements are missing (Magelhães and Coimbra, 1972; Reichenbach *et al.*, 1988). This may generate a '*locus minoris resistentiae*' against both shear forces (beginning cone cell contraction/displacement) and stretching forces (during later stages of development) such that the outer part of the OPL will become the Henle fiber layer (HFL)

B, C, compared to *Cebus*, D, E). In addition, a number of rods are found in the outer half of this fovea (Figure 2.6B, C). It seems likely that the event of cone contraction is twice as long or covers twice as much area. Although it would be quite difficult to procure retinas in this species during the time of foveal development, it is possible that close inspection of the details of foveal architecture with respect to remaining cell classes may illuminate the early events that produce this unusual cone density.

2.10 Summary

In this chapter, we have attempted to place the development of the primate retina in the larger context of vertebrate retinal evolution. We have also described four interesting dimensions of primate variation: scaling of the entire retina with respect to the different scaling requirements of rods and cones, the development of trichromacy in some primates, adaptation of the retina for nocturnal and diurnal niches, and finally, the unique case of the primate fovea. In all these cases, it is interesting to see how a very few genetic changes in timing or expression of opsin variation could be coordinated by existing developmental programs to produce adaptive variations in the eye and retina to produce a large number of secondary, epigenetic alterations. In the case of retinal scaling, extension of neurogenesis to produce a larger eye may automatically have the feature of generating

the required many more rods than cone. In the development of trichromacy, it is possible that the sole change is the alteration of several amino acids in a duplicated opsin; generic mechanisms for silencing the expression of more than one opsin per cell and generic features of the nervous system allow this signal to be employed for perceptual decisions. To produce a nocturnal eye, it may only be necessary to shift the envelope of precursor-pool exit later in development to produce more rods and fewer cones, and after that, the loss of the cone signal may allow the eye to become larger, and halt the production of a fovea. Finally, for the fovea, creation of an all-cone location and contraction of the cone segments can set in motion a series of events which get a free ride from the normal balloon expansion of the eye, moving cell bodies away from the foveal pit.

Particularly unusual in the case of the retina is the coordination of genetic changes, generic features in information-processing and biomechanical qualities of the eye. All serve to seat new primate adaptations into cross-vertebrate functional constraints. Evolutionary and developmental approaches have so far been very successfully integrated to understand the organization of the basic vertebrate plans, and we have argued in this chapter that we may profit by extending the approach to the design of complex organs and complex information-processing problems.

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3

The Genetics and Evolution of Primate Visual Pigments

David M. Hunt, Gerald H. Jacobs, and ***James K. Bowmaker***

3.1 Introduction

In vertebrates, four different cone visual pigments form the ancestral complement that first appeared at the base of the vertebrate lineage around 540 million years ago (mya) (Collin *et al.*, 2003). Amongst eutherian mammals, however, this complement has been reduced to two and it is only in the primates that routine trichromacy has evolved to partially reverse this loss. Color vision in primates is superior therefore to that in other eutherian mammals but still falls short of the tetrachromacy that is not uncommon in other vertebrate classes.

3.2 Structure of visual pigments

Visual pigments are formed by a seven-transmembrane (TM) opsin protein and a chromophore that is covalently attached to a lysine residue via a Schiff base. In mammals, the chromophore is invariably 11-*cis*-retinal derived from vitamin A1, so the peak spectral absorption (λ_{max}) of a visual pigment is determined not by the chromophore but by the amino acid sequence of the opsin protein, with certain residues tuning the pigment to particular spectral locations. Opsin proteins are members of the super-family of G protein-coupled receptors that function through the activation of a guanine nucleotide binding protein (G protein) and an effector enzyme that changes the level of a second messenger in the cell cytoplasm.

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