PERGAMON

Vision Research 43 (2003) 867–877

Vision Research

www.elsevier.com/locate/visres

# Remodeling of second-order neurons in the retina of rd/rd mutant mice

Enrica Strettoi [a,*], Vincenzo Pignatelli [a], Chiara Rossi [a], Vittorio Porciatti [a,b], Benedetto Falsini [a,c]

[a] *Laboratorio di Neurofisiologia, Istituto di Neuroscienze del CNR, Area della Ricerca, Via G. Moruzzi 1, 56100 Pisa, Italy*
[b] *Bascom Palmer Eye Institute, 900 N.W. 17th Street, 33136 Miami, USA*
[c] *Clinica Oculistica, Università Cattolica del Sacro Cuore, Largo S. Vito 1, 00168 Roma, Italy*

Received 31 July 2002; received in revised form 5 November 2002

**Abstract**

This is a brief review of data obtained by analyzing the morphology and the physiology of the retinas in rd/rd and normal, wt mice, aged 10–90 days. Second-order neurons of the rd/rd show abnormalities that start with the anomalous development of rod bipolar cells around P10 and culminate with the atrophy of dendrites in cone bipolar cells, mostly evident at P90. Horizontal cells remodel considerably. Cone-mediated ERGs, (recorded between 13 and 16 days of age) have reduced a-wave and b-wave amplitudes and longer b-wave latency and duration. B-wave abnormalities indicate specific postreceptoral dysfunction. Morphological and ERG changes in rd/rd retinas are consistent with substantial inner retinal remodeling associated to photoreceptor degeneration.


*Abbreviations:* rd/rd mouse: retinal degeneration mouse, also known as rd1; wt: wild-type mouse; P: postnatal day; opl, onl, ipl, gcl: outer plexiform, outer nuclear, inner plexiform, ganglion cell layer of the retina; PKC: protein kinase C; ERG: electroretinogram

*Keywords:* Bipolar cell; Dendritic atrophy; Horizontal cells; ERG; Retinitis pigmentosa

## 1. Introduction

The Central Nervous System of mammals undergoes remodeling during physiological processes, such as development, plasticity and learning, as well as in response to traumatic injuries or diseases. In the case of pathological or experimental conditions causing the death of sensory receptors, secondary effects upon postsynaptic cells usually occur. Structural effects of de-afferentation range from the change in the distribution of various proteins, to the rewiring, atrophy and transneuronal degeneration of target neurons (see, for instance, Baekelandt et al., 1994; Gilbert, 1998; Milam, Li, & Fariss, 1998; Rubel & Fritzsch, 2002; Sernagor, Eglen, & Wong, 2001).

Retinal neurons of individuals affected by inherited photoreceptor degeneration are confronted with the death of a very large population of afferent nerve cells, namely rods and cones. The retina of a typical laboratory mouse contains over 4,000,000 photoreceptors that converge upon approximately 600,000 bipolar cells (roughly 200,000 rod bipolar and 400,000 cone bipolar cells) and 17,000 horizontal cells (Jeon, Strettoi, & Masland, 1998; Strettoi & Pignatelli, 2000; Strettoi & Volpini, 2002). An obvious question to ask is how these cells react, when they are deprived of their major input neurons, because of one of the many mutations causing retinal degeneration in the mouse (reviewed in Chang et al., 2002).

The identification of the effects of photoreceptor abnormal development and/or degeneration upon other retinal cells can contribute to a deeper knowledge of the normal biology of the retina. In addition, understanding the impact of photoreceptor loss upon inner retinal structure and function, is a prerequisite for most of the approaches aimed at restoring vision in retinal degeneration: cellular and retinal transplant (Lund et al., 2001), implant of prostheses (i.e. Humayun, 2001; Peachey & Chow, 1999; Zrenner, 2002), gene therapy (Bennett, 2000).

* Corresponding author. Tel.: +39-50-315-3213; fax: +39-50-315-3220.
*E-mail address:* strettoi@in.pi.cnr.it (E. Strettoi).

0042-6989/03/$ - see front matter 
doi:10.1016/S0042-6989(02)00594-1

Some of the restorative approaches cited above are already in the phase of clinical trials; it is reasonable to assume that the efficacy of others will soon reach the levels necessary for experimentation on human patients (Acland et al., 2001). The chances of such approaches to lead to functional recovery of the retinal function could be limited in those pathological conditions in which the inner retinal layers reorganized severely because of photoreceptor death.

The number of published papers addressing the issue of inner retina preservation in retinal degeneration is not very high when compared to the publications dedicated to study the genetics of diseases and the morphology and biochemistry of degenerating photoreceptors (around 400 over a total of 13.000 citations in a Medline search). In the past, this has lead to the quite general conclusion that major modifications occur only at late stages of photoreceptor degeneration, when thinning of inner retinal layers becomes very evident in various pathologies (see Milam et al., 1998, for changes associated to retinitis pigmentosa).

Particular attention has been paid to survival and death of photoreceptors (see, for instance, Adler, Curcio, Hicks, Price, & Wong, 1999) and, to some extent, of other retinal cells. Survival is usually evaluated by counting cells stained with assorted methods, including basic dyes or DNA-binding molecules. These techniques, however, are rarely suitable to reveal cell-type specific abnormalities.

An increasing number of reports, based on more selective analysis of retinal cells and networks, has recently brought to light remarkable changes in the morphology (i.e. Fariss, Li, & Milam, 2000), synaptic arrangement (Peng, Hao, Petters, & Wong, 2000) and histochemical features (i.e. de Raad, Szczesny, Munz, & Remè, 1996; Lund et al., 2001) of cells of the inner layers, in both human and animal retinas with photoreceptor degeneration. Selective alterations of the ERG have been described as well (Falsini et al., 1994; Hood & Birch, 1996). The nature and entity of inner retinal alterations vary according to the kinetics of photoreceptor degeneration (i.e., fast or slow), the molecular defect, the onset-time, the age of the individual etc. Hence, it seems important to identify, for each specific type of disease leading to photoreceptor degeneration, a temporal window, during which the inner retina might still be receptive to successful therapeutic intervention.

We addressed the issue of inner retinal remodeling by starting an analytical study, based on morphological techniques and ERG analysis, of the retina of the rd/rd mutant mouse, perhaps the best-known animal model of retinitis pigmentosa (RP). In rd/rd mice, a mutation of the beta subunit of the rod-specific phosphodiesterase leads to the rapid death of rod photoreceptors starting from the second week of life (Bowes et al., 1990; Farber & Lolley, 1974). Thus, the first phase of rod death overlaps partially with retinal synaptogenesis (Blanks, Adinolfi, & Lolley, 1974). At one month of age, rods are virtually lost. Secondary death of cones follows the onset of rod death and proceeds slowly over a period of several months (Carter-Dawson, LaVail, & Sidman, 1978).

The present paper represents a brief review of the work carried on by our laboratory on the retina of rd/rd mice in the last three years, with the addition of the recently developed visualization of retinal cells, labeled by gene-gun delivery of fluorescent dyes. Selective staining and electrophysiological recordings show major changes in the rd/rd retina, that go along with the loss of photoreceptors.

Our analysis focuses on second order neurons, in which changes are very evident, and is obviously incomplete. Still, it can be extended to other cellular types in future and provide a framework for the study of other animal models of retinopathies.

## 2. Methods

Experimental procedures were done in agreement with the ARVO Statement for the use of animals in ophthalmology and vision research and the rules for animal experimentation of the Italian Ministry of Health that follow the European Community Council Directive of 1986.

Animals were C3Hpdeb$^{rd1}$ mice, homozygous for the rd mutation (rd/rd) and C57Bl6J, wild type (wt) mice. All animals were born and maintained under controlled ambient illumination on a 12 h light/dark cycle with the illumination level below 60 photopic lux.

### 2.1. Morphology

The numbers of animals used for morphological analysis, as well as the methods used for immunocytochemistry (ICCH), cell counting and electron microscopy, have been given in detail in Strettoi and Pignatelli (2000) and Strettoi, Porciatti, Falsini, Pignatelli, and Rossi (2002). The latter reference provides the types and the sources of the antibodies used for specific labeling of retinal cells. ICCH was performed on animals ranging from 10 days of age (P10) to P90, both on retinal whole mounts and on retinal sections. Secondary antibodies were conjugated with Oregon Green 488, Alexa 568 (from Molecular Probes, Netherlands) or with Cy3 (Sigma). Fluorescent retinal preparations were examined with a Leica TCS-NT confocal microscope equipped with a krypton–argon laser.

### 2.2. Gene-gun labeling

Six additional animals (three rd/rd and three wt, 3–5 months old) were used for gene-gun labeling of cells

with lipophilic dyes (DiI and DiO), according to the protocol described in Gan, Grutzendler, Wong, Wong, and Lichtman, 2000. A Bio-Rad helium gene-gun was loaded with dye-bullets (kindly donated by Dr. R. Wong) and set up at a pressure of 60–80 psI. Retinas were dissected from quickly enucleated eyes of wt and rd animals, 3–5-months old, and immersed in ice-cold, oxygenated saline solution for 5 min. Each retina was then moved to the stage of an automatically advanced tissue chopper (McIlwain; 2Biological, Italy) and cut in radial slices, 150 μm thick. Retinal slices were collected in saline, partially dried and exposed to one or two shots of dyes from the gun. Upon successful labeling of cells, retinal specimens were fixed for 30 min in 2% paraformaldheyde, rinsed in buffer and counterstained with DAPI for visualization of nuclei. During the chopping and the subsequent mounting, retinal slices assumed a semirandom orientation, so that labeled cells became visible in radial as well as in tangential view. Images were collected both with a Zeiss Axiocam digital color camera, attached to a Zeiss Axioscope fluorescence microscope, and with the confocal microscope.

### 2.3. Electrophysiology

ERGs were conventionally recorded in response to light flashes of different intensity (0.2–20 cd/m$^2$ s$^{-1}$) both under dark- and light-adaptation (12 cd/m$^2$). The interval between repeated flashes was set to allow complete recovery of the b-wave between flashes, except for the experiments where the effect of repetition rate (0.1 and 20 Hz) was specifically evaluated. A- and b-waves were quantified in their amplitude (baseline to negative a-wave peak, a- to positive b-wave peak) and time-to-peak (from stimulus onset to negative a-wave through, from stimulus onset to positive b-wave peak). For b-wave analysis, oscillatory potentials were removed by digital filtering (Lyubarsky, Falsini, Pennesi, Valentini, & Pugh, 1999). Light-intensity dependence of the a- and b-wave amplitudes was determined (Robson & Frishman, 1995, 1996; Rohrer, Korenbrot, LaVail, Reichardt, & Xu, 1999). The numbers of animals used are given in Strettoi et al. (2002).

## 3. Results

### 3.1. Morphological changes observed in the rd/rd retina

In adult rd/rd mice, nuclear staining of vertical retinal sections (Fig. 1) fails to reveal major abnormalities in inner layers. Similarly, a survey of semithin sections obtained from well-preserved material prepared for electron microscopy and stained with basic dyes, suggests considerable preservation of inner retinal cells (Fig. 2). Only the reduction or the absence of the photoreceptor layer is evident. Selective staining reveals changes in the morphology of rod bipolar and horizontal cells first, followed by similar modifications in cone bipolar cells. Similar to the degeneration of

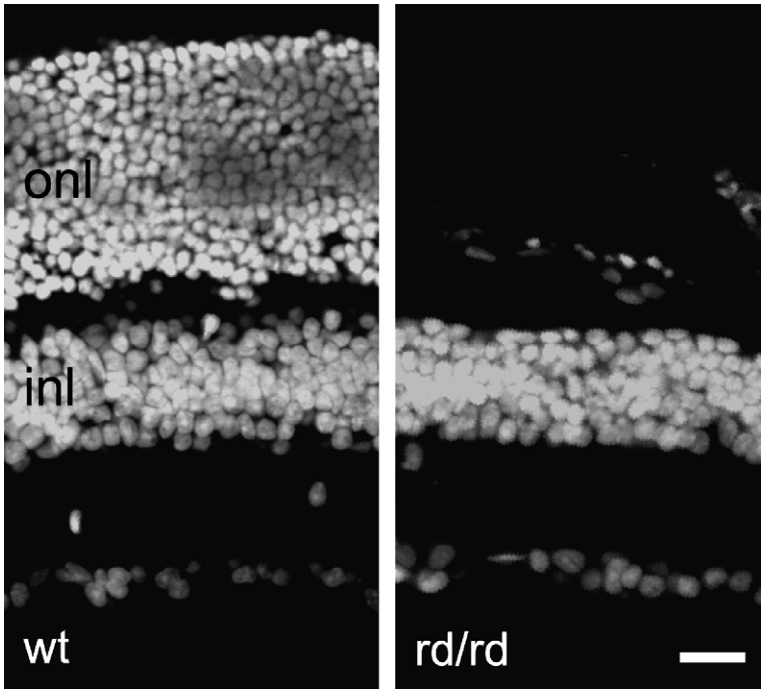


Fig. 1. Fluorescence nuclear staining of vertical sections of adult wt and rd/rd retinas. Age of animals and retinal eccentricities match. Only the absence of photoreceptors is evident in the mutant. Bar is 20 μm.

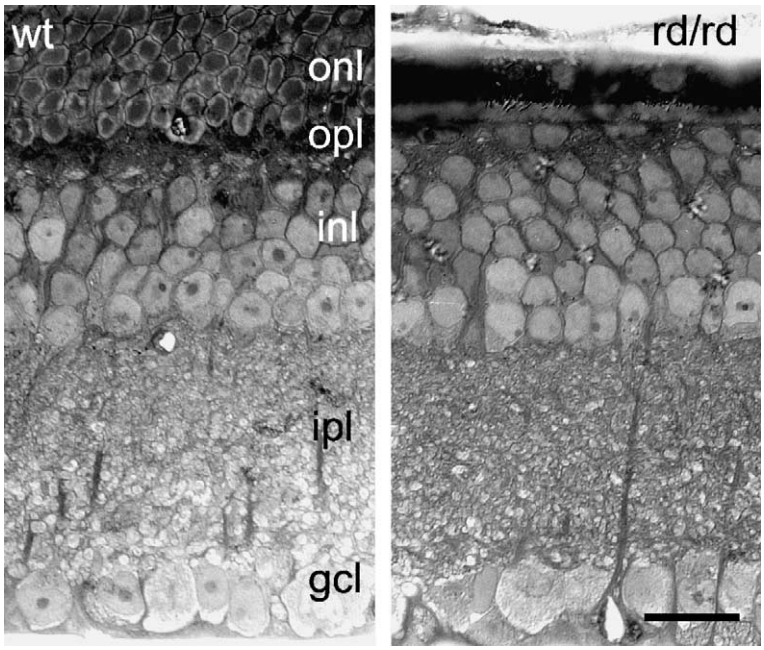


Fig. 2. Vertical semithin sections from wt and rd/rd retinal tissue, obtained after glutaraldheyde fixation and plastic embedding. Conventional toluidine blue staining. Examination with high power light microscopy and interference contrast optics shows good structural preservation of inner retinal layers in the rd/rd at three months. Large differences between wt and mutant are not obvious. Bar is 20 μm.

photoreceptors, morphological abnormalities tend to follow a center-to-periphery gradient.

### 3.2. Changes in rod bipolar cells

Rod bipolar cells stain with antibodies against the alpha isoform of protein kinase C (PKC). ICCH reveals that both dendritic arbors and axonal endings of rod bipolar cells in the rd/rd retina fail to develop normally. Between the second and third postnatal week, dendrites appear evidently shorter and spatially disordered as compared to their wt counterparts (Strettoi & Pignatelli, 2000). While the latter grow regularly and assume the normal, bushy morphology, obvious at one month of age, dendrites of rd/rd rod bipolar cells remain atrophic, disorganized and poorly oriented, as shown by confocal analysis of both vertical sections (Fig. 3A and B) and retinal whole mounts (Fig. 3C–E). There is never a uniform plane formed by rod bipolar dendrites, as it is present in the opl of the normal retina. Staining with other bipolar-specific antibodies (such as L7 and PKC-beta), alone or in combination, confirms this observation.

Atrophy of dendrites goes along with decreased immunoreactivity and spatial misplacement of mGluR6, the major glutamate receptor responsible for synaptic transmission between photoreceptors and depolarizing bipolar cells (rod bipolar and ''on-center'' cone bipolar cells). Labeling for mGluR6 show clusters of the receptor in the apical parts of bipolar cell bodies and also in their axons, in the inner nuclear layer (Strettoi & Pignatelli, 2000).

Axonal arborizations of rod bipolar cells are also anomalous in the rd retina, for single varicosities seem to stop growing at P10, and most of them remain smaller than the average size of their wt counterparts (see Figs. 3A, B and 4). They also exhibit ultrastructural features typical of immature endings, such as the presence of tubular profiles and abnormally small and round synaptic ribbons (Strettoi et al., 2002).

At three months of age, the number of rod bipolar cells in the central retina has dropped by 30% (Strettoi & Pignatelli, 2000). In the same period and in the following months, single cell staining with the gene-gun confirms the poor morphology of dendritic arbors in surviving rod bipolar cells (Fig. 4A and B).

### 3.3. Changes in horizontal cells

Antibodies against calbindin D stain horizontal cells entirely, while antibodies against neurofilaments label their axonal complexes (Peichl & Gonzalez-Soriano, 1994). ICCH with these two antibodies reveals sprouting of processes from horizontal cells, mostly originating from their axonal complexes (postsynaptic to rods) and oriented radially toward the inner nuclear layer, in which they run for short tracts. Sprouts become clearly visible around the end of the second postnatal week; at that time they are eight times more frequent than in their wt counterpart, in which they are encountered occasionally.

From around P15 on, the main branches of axonal complexes grow to be progressively thicker, at the same

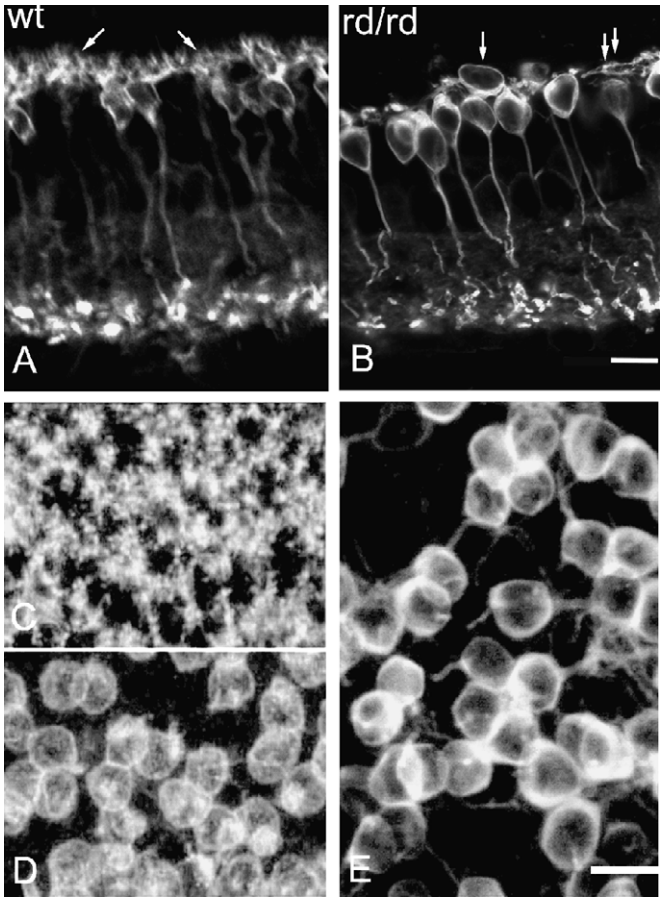


Fig. 3. Changes in rod bipolar cells revealed by PKC staining. A and B shows retinal vertical sections obtained from animals of approximately 30 days. Arrows point to well developed dendrites of rod bipolar cells in A, and to cell bodies with no processes (single arrow) or few dendrites with radial orientation in B (double arrows). C–E shows whole mounts of wt (C and D) and rd/rd (E) retinas. C corresponds to the focal plane of rod bipolar dendrites and D to the focal plane of cell bodies. In the rd/rd (E), the equivalent of C is not detectable; rod bipolar cells appear carrying few, disorganized processes. Bars are 10 μm.

time loosing thin processes (Fig. 5A and B). Covering of the retinal surface becomes poor, as primary branches appear more spaced. This series of events progresses continuously, until, at three months of age, individual axonal arbors of horizontal cells show striking remodeling: they have become wider, with thicker branches but poor of thin processes (Fig. 5C).

Cell bodies of horizontal cells remodel following a slower time scale. Up to four weeks of age, no major changes are obvious. During the following month, cell bodies become hypertrophic and spaced irregularly across the retina, while their thin dendrites are lost progressively. By three months, there is an 18% decrease in the overall retinal number of horizontal cells (Strettoi & Pignatelli, 2000); morphological changes in the central retina are obvious (compare Fig. 5D and E).

### 3.4. Changes in cone bipolar cells

The analysis of cone bipolar cells in the mouse retina is limited by technical reasons: cone bipolar dendrites are hard to visualize in retinal sections stained with PKC (labeling rod bipolar cells) and Goalpha (that stains cell bodies, dendrites and axons of both rod bipolar and depolarizing cone bipolar cells; Vardi, 1998). This difficulty increases in a pathological preparation such as the rd/rd retina. Other antibodies, that allow a more favorable study of dendrites of these cells (i.e., NK3 receptor antibodies: Casini, Brecha, Bosco, & Rickman, 2000; caldendrin antibodies: Haverkamp & Wassle, 2000), stain fractions of the varieties of cone bipolar cells that are present in the retina of the mouse (see, for instance, Tsukamoto, Morigiwa, Ueda, & Sterling, 2001). Combining several of the markers available for cone bipolar cells at this time, we start detecting major morphological alterations in these cells between one and two months of age. Cells stained with Goalpha do develop dendrites that are still *visible* at P20 and at P30, when dendrites of rod bipolar cells have already undergone major changes (Fig. 6A and B). As is the case of horizontal cells, in the following period, dendrites of cone bipolar cells undergo a process of progressive retraction, while their plexus in the opl becomes increasingly discontinuous (compare Fig. 6C and D). Fig. 7A

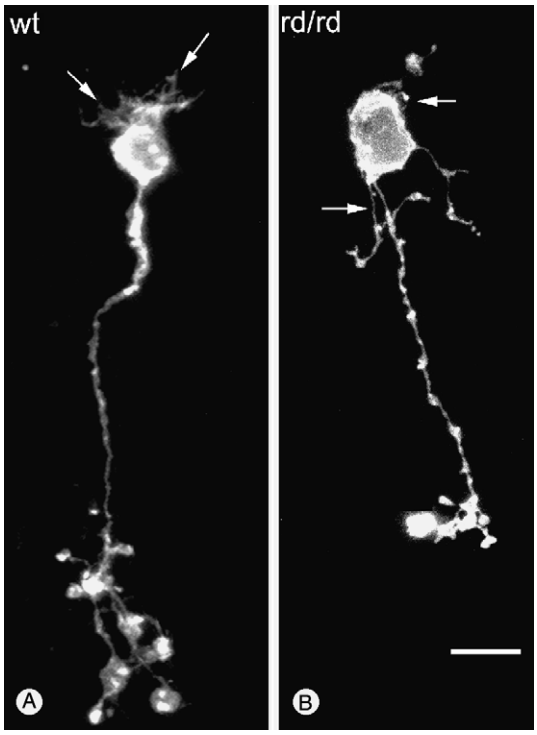


Fig. 4. (A) and (B) Wt and rd/rd rod bipolar cells, individually labeled by gene-gun delivery of lipophilic dyes on retinal slices, from retinas of three and five months, respectively. Differences in dendritic number and orientation (arrows), as well as in the sizes of axonal arbors are very evident. Bar is 10 μm.

shows individual cone bipolar cells, labeled in a five-month old preparation. Short stumps have replaced dendrites, clearly visible in single cone bipolar cells of a normal retina (Fig. 7B).

### 3.5. Other cell types

#### 3.5.1. Muller cells

As other authors have described in various forms of retinopathies (de Raad et al., 1996; Rungger-Brandle, Dosso, & Leuenberger, 2000; Sheedlo, Jaynes, Bolan, & Turner, 1995), we observe a typical sign of glial reaction in the form of increased immunoreactivity for glial fibrillary acidic protein (GFAP) in Muller cells (Fig. 8). This is best visible from approximately P18/P20 to P30, and attenuates thereafter (Strettoi et al., 2002). Major morphological abnormalities in Muller cells (labeled by glutamine synthetase) are not evident, except for the expected shortening of their apical processes, associated to the decrease in thickness of the photoreceptor layer.

#### 3.5.2. Amacrine cells

We studied three well know amacrine cell types, namely cholinergic, dopaminergic, and type AII amacrines. Each of them can be labeled as a population by specific antibodies, respectively against Choline acetyltransferase, Tyrosine hydroxylase and disabled-1 molecules (Rice & Curran, 2000). The overall morphology of these three types of cells is definitely more preserved than the morphology of bipolar and horizontal cells, at least up to the age of three months. The total retinal number of cholinergic and dopaminergic cells does not change during the same time interval (Strettoi et al., 2002). More detailed studies on amacrine cells are in progress in our laboratory.

### 3.6. Electrophysiological changes

Rod-mediated ERGs cannot be recorded from rd/rd mice of any age. A comparison between wt and rd ERGs is possible for cone-mediated responses, which we obtained reproducibly in a short time window comprised between P13 and P16.

The major differences observed in the rd/rd ERG, at all stimulation intensities, consist in an overall amplitude reduction associated to an evident change in the waveform. As shown in Fig. 9, the change in the waveform is mainly due to specific alterations of the b-wave, delayed and of abnormally long duration. At low flash rates, the ERG of the rd/rd mouse, as compared to the wild type, is dominated by the negative component (a-wave). By increasing the flash rate, the sluggish positive ERG component (i.e. the b-wave) of the rd/rd mouse becomes relatively more attenuated, resulting in a progressive "smearing out" of the ERG waveform. Decreasing the stimulus intensity in the wild-type mouse in order to mimic the subnormal a-wave amplitude of the rd/rd mouse does not normalize the b-wave abnormalities. This indicates specific defects in the kinetics of the b-wave.

## 4. Discussion

Even though our study is limited to some morphological and electrophysiological analysis of one specific animal model, some conclusions on retinal remodeling can be drawn. Indeed, the rd/rd postreceptoral retina undergoes a major anatomical and functional rearrangement induced by the loss of photoreceptors. The changes we describe follow a clear temporal trend: they appear first in cells connected to rods, that degenerate first, and than in those connected to cones. Interestingly, horizontal cells, which connect to both, show the first signs of remodeling at the side associated to rods. The kinds of remodeling that we observe are a combination of various cellular responses: sprouting (evident in horizontal cell axonal endings), hypertrophy (again in processes and bodies of horizontal cells) and atrophy, of axonal endings and dendrites. There is also cellular loss (which we quantified, so far, only for rod bipolar and horizontal cells). Similar responses (sprouting, cellular

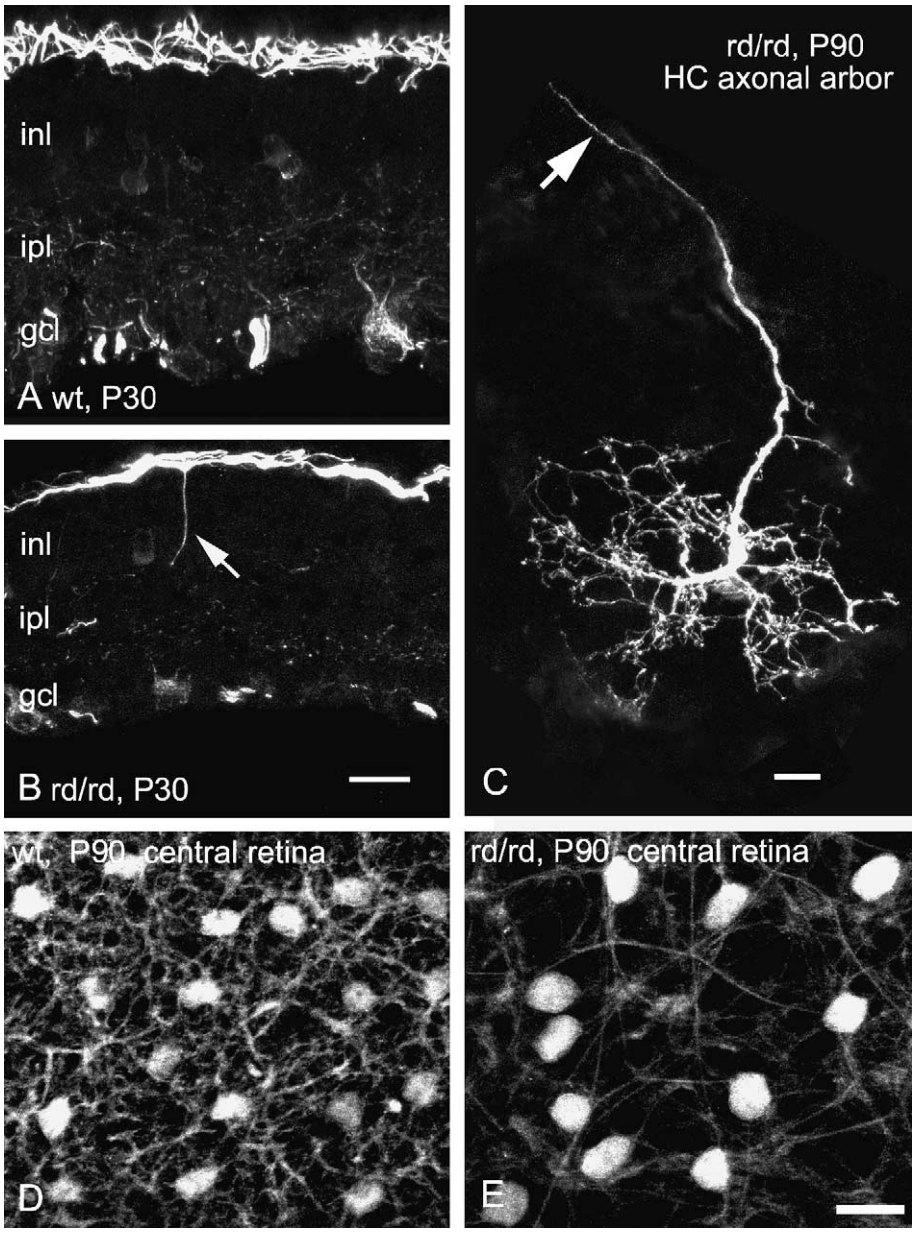


Fig. 5. Changes in horizontal cells. A and B shows neurofilament staining of vertical sections in wt (A) and rd/rd (B) retinas shows hypertrophy and moderate sprouting (arrow) of axonal branches of horizontal cells in the mutants at P30. The plane of section in A is slightly oblique. C shows individual axonal arbor of horizontal cell stained with the gene-gun and viewed as a retinal whole mount. The diameter of the axonal arborization is three times as wide as a normal one. Arrow points to the axon. D and E shows Calbindin staining in whole mount preparations reveals cellular loss, hypertrophy of bodies and absence of thin dendrites in horizontal cells from central retinal areas of rd/rd mice. Bars are 20 μm.

loss) have been described in RP (Fariss et al., 2000; Santos et al., 1997). Sprouting from horizontal cells has been described in other retinas with photoreceptor degeneration (i.e. Park et al., 2001) and in experimental retinal detachment (Lewis, Linberg, & Fisher, 1998). Disorganization in the morphology and in the distribution of horizontal cells has been described in the retina of RCS rats (Chu, Humphrey, & Constable, 1993).

Note that some of the changes we observed could be very deceiving. For instance, losses of thin processes from horizontal and bipolar cells, and the concomitant hypertrophy of larger axonal branches in horizontal cells, produce the effect of an apparently unchanged size of the opl. At the same time, while immunocytochemistry clearly shows a great loss of dendrites in rod bipolar cells, gene-gun labeling reveals few, anomalous, processes in some cells. These could be the dendrites establishing ectopic connections with cones, described by Peng et al. (2000) in young rd/rd mice; alternatively, they could be newly formed dendrites with aberrant orientation. Further analysis will be necessary to find

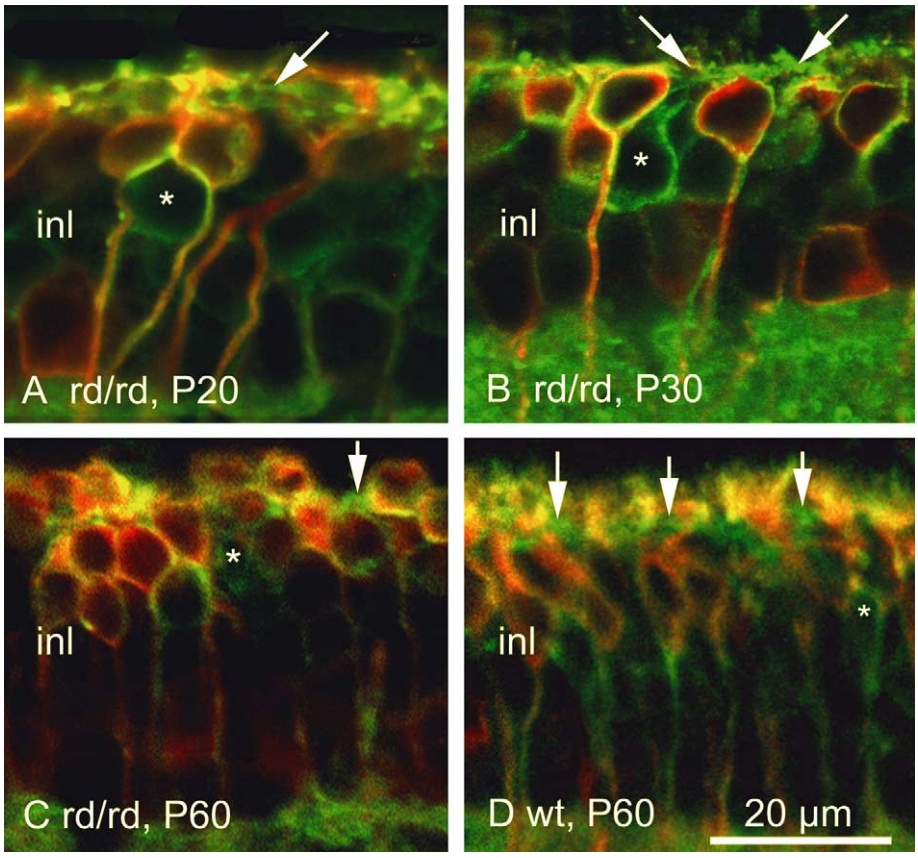


Fig. 6. Goalpha (green staining) labels rod bipolar cells and depolarizing cone bipolar cells; PKC staining (revealed in red) shows rod bipolar cells, which then appear yellow. Cell bodies (asterisks) and dendrites (arrows) of depolarizing cone bipolar cells are green only. A, B and C shows the growth and then the progressive impoverishment of green dendrites in the rd/rd retina. D shows a wt retina, in which green dendrites appear as clusters in the opl (arrows), underlying unlabelled cone pedicles.

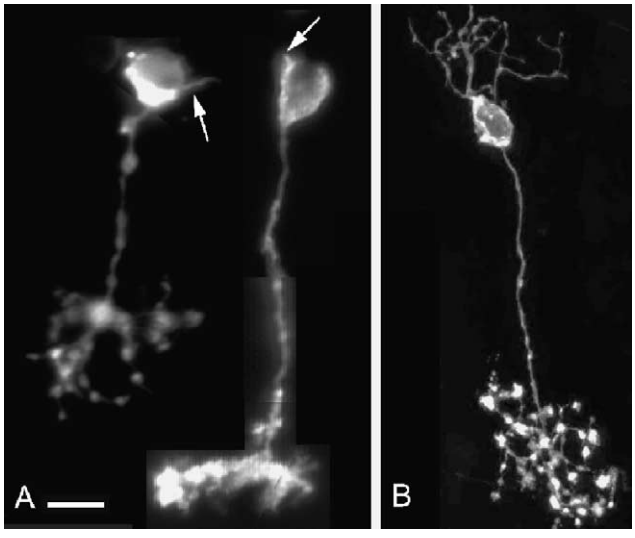


Fig. 7. Montage of cone bipolar cells, labeled with the gene-gun, from slices of one rd/rd retina (A) and one wt retina (B), aged five months. Cell identification relies on the morphology and level of stratification of axonal arbors in the ipl. The outer retinal margin corresponds to the upper border of the picture. Arrows point to residual dendrites.

out dynamic changes of dendrites in space and time. Ultimately, remodeling of rod bipolar cells is so impressive, that these neurons, in the retina of a two-month old, rd/rd mouse, are essentially unipolar, with scant dendrites, abnormal axonal endings, and very little mGluR6. Their electrical response to glutamate is greatly reduced as well (see Varela, Igartera, De la Rosa, & De la Villa, 2003).

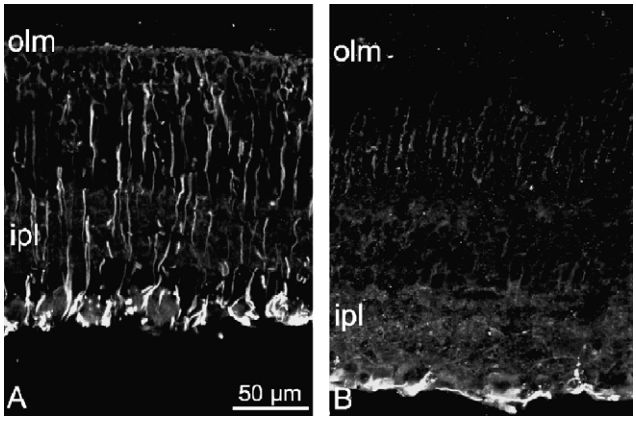


Fig. 8. Increased immunoreactivity for GFAP in Muller cells of the rd/rd retina (A), at the age of 18 days. In the matched wt retina (B), only processes from astrocytes are GFAP positive. Both sections are from the central part of the retina.

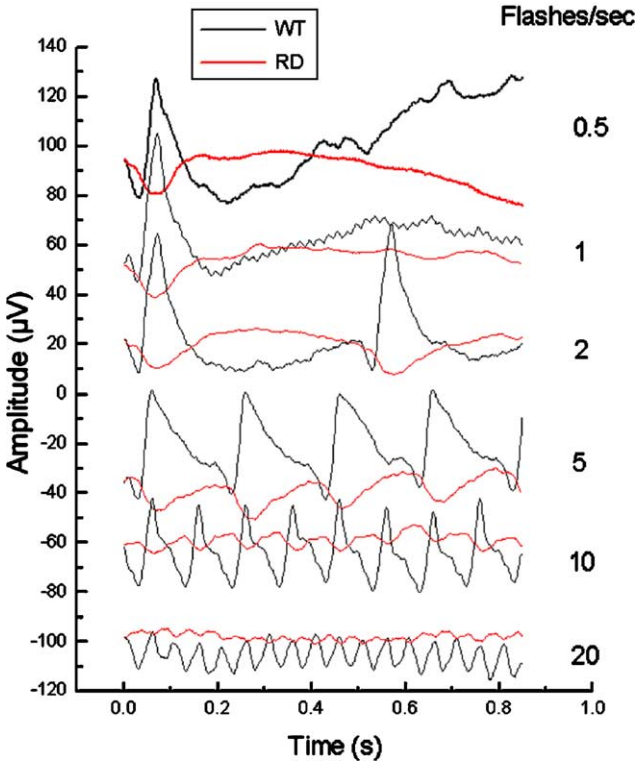


Fig. 9. Cone-mediated ganzfeld electroretinograms recorded at different flash frequencies between 0.5 and 20 $s^{-1}$ from a wt (black tracings) and an rd/rd mouse (red tracings) of 14 days of age (P14). Flash intensity is 2 $cd/m^2$ $s^{-1}$. Background intensity 12 $cd/m^2$. Note the marked b-wave attenuation and delay in the rd/rd compared to the WT mouse at all flash rates. The a-wave of the rd/rd animal is relatively preserved. However, the delayed b-wave onset results in an increased a-wave time-to-peak, and an overall "smearing out" of the rd/rd ERG waveform.

Already in 1978, Blanks et al. described the lack of connections between rods and rod bipolar dendrites during postnatal development of the rd/rd retina, in classical EM studies. We can confirm now that rod bipolar dendrites, for reasons we do not know yet, fail to grow appropriately in the outer plexiform layer. Abnormal development of dendrites is linked to an arrest in growth of rod bipolar axonal endings. An obvious hypothesis is that rod bipolar cells are missing a signal, necessary to develop successfully and possibly associated to the synaptic terminal of healthy photoreceptors. Of course, intervening glial cells and/or other factors could also be very important.

Dendritic underdevelopment and atrophy in rod bipolar cells and loss of thin processes from axonal arbors of horizontal cell are predictive of the late retraction of dendrites from cone bipolar cells. Similarly, the latter phenomenon could be predictive of the changes one can expect in animal models of slow retinal degeneration. Indeed, in the retina of crx-null mice (Furukawa, Morrow, Li, Davis, & Cepko, 1999), in which photoreceptors die over a slow time scale, we observe that dendrites of rod bipolar cells are fully developed at first, and subsequently retract; hence, at late stages of photoreceptor degeneration, they are similar to rod bipolar cells of an earlier rd/rd retina (Pignatelli, Cepko, & Strettoi, in preparation). Thus, progressive dendritic atrophy could be a common avenue for retinal neurons deprived by their input cells, similarly to what found in central auditory nuclei after de-afferentation (Deitch & Rubel, 1984). This might be of relevance in uncovering appropriate time windows for therapeutic strategies: interventions that rely upon the integrity of second order neurons have to be designed early for fast-degenerating forms of photoreceptor diseases, particularly in the instance that death of photoreceptor and synaptogenesis overlap. The reason for which restoration of vision in the rd/rd mice is possible only upon early intervention (Bennett et al., 1996; Kwan, Wang, & Lund, 1999; Radner et al., 2001) could be the extensive and early remodeling observed in this mutant.

With the exposed technical limits described above, it is somewhat encouraging that cone bipolar cells start to show dendritic atrophy later than rod bipolar cells. They are a very large population of second order neurons in the retinal of mammals and therefore a potential platform for therapeutic intervention (i.e., based on photoreceptor transplant).

However, cone-mediated ERG recordings performed near P14 show abnormalities of the b-wave that appear to depend upon specific deficits in the retinal cone-pathway. At this early stage, morphological analysis is unable to uncover changes clearly linked to the cone pathway alone.

A decreased sensitivity to glutamate in depolarizing cone bipolar cells (which give a large contribution to the b-wave in photopic conditions) could explain these abnormalities. This goes along with the observed decrease in immunoreactivity for mGluR6.

An increased sensitivity to GABA in cone bipolar cells of the rd/rd mouse (similar to that found in rod bipolar cells by Varela et al., 2003) could also result in a suppressive effect of their depolarizing response and altered kinetic, ultimately reflected in the b-wave of the ERG. In the human GABA-associated retinal dysfunction (Krauss, Johnson, & Miller, 1998), ERG recordings show reduced cone-mediate responses (b-waves and oscillatory potentials), with increased b-wave duration.

It is to note that changes in cone-mediated flicker ERGs are reminiscent of the ERG changes found at least in some RP patients. Massof, Johnson, Sunness, Perry, and Finkelstein (1986) described progressive "smearing out" of the RP flicker ERG waveforms with increasing temporal frequency. The differences in time-to-peak between the positive and negative ERG components were longer in RP patients than in the normal, suggesting that the temporal anomalies in the flicker ERG are due to changes in both amplitude and time

constants of the ERG components. Hood and Birch (1996) observed similar changes. Falsini et al. (1999) evaluated the fundamental and second harmonic components of flicker ERGs, as a function of temporal frequency, in human patients with typical RP. They reported that both components showed temporal frequency-dependent abnormalities in both amplitude and latency, with increasing response delays at higher temporal frequencies. Taken together, these data suggest that the abnormalities found in rd mice and in human RP patients share a similar mechanism, involving an abnormality of the temporal response properties of postreceptoral generators subtending the origin of ERG b-wave.

Several techniques are now available to study retinal cell types individually or as homogeneous populations: selective staining methods, mosaic analysis, electrophysiological recordings from single units, single-cell PCR, DNA-arrays, to mention some of them. It is likely that in the next few years the associated efforts of different research groups to apply these techniques to retinal pathology will contribute to gain a deeper view of the whole concept of remodeling in retinal disease, which should be considered as the rule more than the exception.

## Acknowledgements

We like to thank the colleagues who donated antibodies for this and previous studies (C. Cepko; T. Curran; B. Howell; S. Nakanishi; D. Rice; R. Wong), as well as all the colleagues of the Institute of Neuroscience in Pisa for administrative, technical and scientific support. Funded by the Italian CNR, TeleThon Project E833, NIH-R01EY12654.

## References

Acland, G. M., Aguirre, G. D., Ray, J., Zhang, Q., Aleman, T. S., Cideciyan, A. V., Pearce-Kelling, S. E., Anand, V., Zeng, Y., Maguire, A. M., Jacobson, S. G., Hauswirth, W. W., & Bennett, J. (2001). Gene therapy restores vision in a canine model of childhood blindness. *Nature Genetics, 28*, 92–95.

Adler, R., Curcio, C., Hicks, D., Price, D., & Wong, F. (1999). Cell death in age-related macular degeneration. *Molecular Vision, 3*, 5–31.

Baekelandt, V., Arckens, L., Annaert, W., Eysel, U. T., Orban, G. A., & Vandesande, F. (1994). Alterations in GAP-43 and synapsin immunoreactivity provide evidence for synaptic reorganization in adult cat dorsal lateral geniculate nucleus following retinal lesions. *European Journal of Neuroscience, 6*, 754–765.

Bennett, J. (2000). Gene therapy for retinitis pigmentosa. *Current Opinions in Molecular Therapy, 2*, 420–425.

Bennett, J., Tanabe, T., Sun, D., Zeng, Y., Kjeldbye, H., Gouras, P., & Maguire, A. M. (1996). Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. *Nature Medicine, 2*, 649–654.

Blanks, J. C., Adinolfi, A. M., & Lolley, R. N. (1974). Photoreceptor degeneration and synaptogenesis in retinal-degenerative (rd) mice. *The Journal of Comparative Neurology, 156*, 95–106.

Bowes, C., Li, T., Danciger, M., Baxter, L. C., Applebury, M. L., & Farber, D. B. (1990). Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature, 347*, 677–680.

Carter-Dawson, L. D., LaVail, M. M., & Sidman, R. L. (1978). Differential effect of the rd mutation on rods and cones in the mouse retina. *Investigative Ophthalmology and Visual Science, 17*, 489–498.

Casini, G., Brecha, N. C., Bosco, L., & Rickman, D. W. (2000). Developmental expression of neurokinin-1 and neurokinin-3 receptors in the rat retina. *The Journal of Comparative Neurology, 42*, 275–287.

Chang, B., Hawes, N. L., Hurd, R. E., Davisson, M. T., Nusinowitz, S., & Heckenlively, J. R. (2002). Retinal degeneration mutants in the mouse. *Vision Research, 42*, 517–525.

Chu, Y., Humphrey, M. F., & Constable, I. J. (1993). Horizontal cells of the normal and dystrophic rat retina: a wholemount study using immunolabelling for the 28-kDa calcium-binding protein. *Experimental Eye Research, 57*, 141–148.

de Raad, S., Szczesny, P. J., Munz, K., & Remè, C. E. (1996). Light damage in the rat retina: glial fibrillary acidic protein accumulates in Muller cells in correlation with photoreceptor damage. *Ophthalmic Research, 28*, 99–107.

Deitch, J. S., & Rubel, E. W. (1984). Afferent influences on brain stem auditory nuclei of the chicken: time course and specificity of dendritic atrophy following deafferentation. *The Journal of Comparative Neurology, 10*, 66–79.

Falsini, B., Iarossi, G., Fadda, A., Porrello, G., Valentini, P., Piccardi, M., & Scullica, L. (1999). The fundamental and second harmonic of the photopic flicker electroretinogram: temporal frequency-dependent abnormalities in retinitis pigmentosa. *Clinical Neurophysiology, 110*, 1554–1562.

Falsini, B., Iarossi, G., Porciatti, V., Merendino, E., Fadda, A., Cermola, S., & Buzzonetti, L. (1994). Postreceptoral contribution to macular dysfunction in retinitis pigmentosa. *Investigative Ophtalmology and Visual Science, 35*, 4282–4290.

Farber, D. B., & Lolley, R. N. (1974). Cyclic guanosine monophosphate: elevation in degenerating photoreceptor cells of the C3H mouse retina. *Science, 186*, 449–451.

Fariss, R. N., Li, Z. Y., & Milam, A. H. (2000). Abnormalities in rod photoreceptors, amacrine cells and horizontal cells in human retinas with retinitis pigmentosa. *American Journal of Ophthalmology, 129*, 215–223.

Furukawa, T., Morrow, E. M., Li, T., Davis, F. C., & Cepko, C. L. (1999). Retinopathy and attenuated circadian entrainment in Crx-deficient mice. *Nature Genetics, 23*, 466–470.

Gan, W. B., Grutzendler, J., Wong, W. T., Wong, R. O., & Lichtman, J. (2000). Multicolor "DiOlistic" labeling of the nervous system using lipophilic dye combinations. *Neuron, 27*, 219–225.

Gilbert, C. D. (1998). Adult cortical dynamics. *Physiological Reviews, 78*, 467–485.

Haverkamp, S., & Wassle, H. (2000). Immunocytochemical analysis of the mouse retina. *The Journal of Comparative Neurology, 424*, 1–23.

Hood, D. C., & Birch, D. G. (1996). Abnormalities of the retinal cone system in retinitis pigmentosa. *Vision Research, 36*, 1699–1709.

Humayun, M. S. (2001). Intraocular retinal prosthesis. *Transactions of American Ophthalmological Society, 99*, 271–300.

Jeon, C. J., Strettoi, E., & Masland, R. H. (1998). The major cell populations of the mouse retina. *Journal of Neuroscience, 18*, 8936–8946.

Krauss, G. L., Johnson, M. A., & Miller, N. R. (1998). Vigabatrin-associated retinal cone system dysfunction: electroretinogram and ophthalmologic findings. *Neurology, 50*, 614–618.

Kwan, A. S., Wang, S., & Lund, R. D. (1999). Photoreceptor layer reconstruction in a rodent model of retinal degeneration. *Experimental Neurology, 159*, 21–33.

Lewis, G. P., Linberg, K. A., & Fisher, S. K. (1998). Neurite outgrowth from bipolar and horizontal cells after experimental retinal detachment. *Investigative Ophthalmology and Visual Science, 39*, 424–434.

Lund, R. D., Kwan, A. S., Keegan, D. J., Sauve, Y., Coffey, P. J., & Lawrence, J. M. (2001). Cell transplantation as a treatment for retinal disease. *Progress in Retinal and Eye Research, 20*, 415–449.

Lyubarsky, A. L., Falsini, B., Pennesi, M. E., Valentini, P., & Pugh, E. N., Jr. (1999). UV- and midwave-sensitive cone-driven retinal responses of the mouse: a possible phenotype for coexpression of cone photopigments. *Journal of Neuroscience, 19*, 442–455.

Massof, R. W., Johnson, M. A., Sunness, J. S., Perry, C., & Finkelstein, D. (1986). Flicker electroretinogram in retinitis pigmentosa. *Documenta Ophthalmologica, 62*, 231–245.

Milam, A. H., Li, Z. Y., & Fariss, R. N. (1998). Histopathology of the human retina in retinitis pigmentosa. *Progress in Retinal and Eye Research, 17*, 175–205.

Park, S. J., Kim, I. B., Choi, K. R., Moon, J. I., Oh, S. J., Chung, J. W., & Chun, M. H. (2001). Reorganization of horizontal cell processes in the developing FVB/N mouse retina. *Cell and Tissue Research, 306*, 341–346.

Peachey, N. S., & Chow, A. Y. (1999). Subretinal implantation of semiconductor-based photodiodes: progress and challenges. *Journal of Rehabilitation Research and Development, 36*, 371–376.

Peichl, L., & Gonzalez-Soriano, J. (1994). Morphological types of horizontal cell in rodent retinae: a comparison of rat, mouse, gerbil, and guinea pig (1994). *Visual Neuroscience, 11*, 501–517.

Peng, Y. W., Hao, Y., Petters, R. M., & Wong, F. (2000). Ectopic synaptogenesis in the mammalian retina caused by rod photoreceptor-specific mutations. *Nature Neuroscience, 3*, 1121–1127.

Radner, W., Sadda, S. R., Humayun, M. S., Suzuki, S., Melia, M., Weiland, J., & de Juan, E., Jr. (2001). Light-driven retinal ganglion cell responses in blind rd mice after neural retinal transplantation. *Investigative Ophthalmology and Visual Science, 42*, 1057–1065.

Rice, D. S., & Curran, T. (2000). Disabled-1 is expressed in type AII amacrine cells in the mouse retina. *The Journal of Comparative Neurology, 424*, 327–338.

Robson, J. G., & Frishman, L. J. (1995). Response linearity and kinetics of the cat retina: the bipolar cell component of the dark-adapted electroretinogram. *Visual Neuroscience, 12*, 837–850.

Robson, J. G., & Frishman, L. J. (1996). Photoreceptor and bipolar cell contributions to the cat electroretinogram: a kinetic model for the early part of the flash response. *Journal of Optical Society of America A, 13*, 613–622.

Rohrer, B., Korenbrot, J. I., LaVail, M. M., Reichardt, L. F., & Xu, B. (1999). Role of neurotrophin receptor TrkB in the maturation of rod photoreceptors and establishment of synaptic transmission to the inner retina. *Journal of Neuroscience, 19*, 8919–8930.

Rubel, E. W., & Fritzsch, B. (2002). Auditory system development: primary auditory neurons and their targets. *Annual Review of Neuroscience, 25*, 51–101.

Rungger-Brandle, E., Dosso, A. A., & Leuenberger, P. M. (2000). Glial reactivity, an early feature of diabetic retinopathy. *Investigative Ophthalmology and Visual Science, 41*, 1971–1980.

Santos, A., Humayun, M. S., Juan, E. J., Greenburg, R. J., Marsh, M. J., Klock, I. B., & Milam, A. H. (1997). Preservation of the inner retina in retinitis pigmentosa. A morphometric analysis. *Archives of Ophtalmology, 115*, 511–515.

Sernagor, E., Eglen, S. J., & Wong, R. O. (2001). Development of retinal ganglion cell structure and function. *Progress in Retinal and Eye Research, 20*, 139–174.

Sheedlo, H. J., Jaynes, D., Bolan, A. L., & Turner, J. E. (1995). Mullerian glia in dystrophic rodent retinas: an immunocytochemical analysis. *Brain Research Developmental Brain Research, 85*, 171–180.

Strettoi, E., & Pignatelli, V. (2000). Modifications of retinal neurons in a mouse model of retinitis pigmentosa. In: *Proceedings of the National Academy of Sciences of the USA*, Vol. 97, pp. 11020–11025.

Strettoi, E., Porciatti, V., Falsini, B., Pignatelli, V., & Rossi, C. (2002). Morphological and functional abnormalities in the inner retina of the rd/rd mouse. *Journal of Neuroscience, 22*, 5492–5504.

Strettoi, E., & Volpini, M. (2002). Retinal organization in the bcl-2-overexpressing transgenic mouse. *The Journal of Comparative Neurology, 446*, 1–10.

Tsukamoto, Y., Morigiwa, K., Ueda, M., & Sterling, P. J. (2001). Microcircuits for night vision in mouse retina. *Journal of Neuroscience, 21*, 8616–8623.

Vardi, N. (1998). Alpha subunit of Go localizes in the dendritic tips of ON bipolar cells. *The Journal of Comparative Neurology, 395*, 43–52.

Varela, C., Igartera, I., De la Rosa, E. J., & De la Villa, P. (2003). Functional modifications in rod bipolar cells in a mouse model of retinitis pigmentosa. *Vision Research*.

Zrenner, E. (2002). Will retinal implants restore vision? *Science, 295*, 1022–1025.