Emergence of Sustained Spontaneous Hyperactivity and Temporary Preservation of OFF Responses in Ganglion Cells of the Retinal Degeneration (rd1) Mouse

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Submitted 8 February 2007; accepted in final form 14 January 2008

Stasheff SF. Emergence of sustained spontaneous hyperactivity and temporary preservation of OFF responses in ganglion cells of the retinal degeneration (rd1) mouse. J Neurophysiol 99: 1408–1421, 2008. First published January 23, 2008; doi:10.1152/jn.00144.2007. Complex alterations in the anatomy of outer retinal pathways accompany photoreceptor degeneration in the rd1 mouse model of retinitis pigmentosa, whereas inner retinal neurons appear relatively preserved. However, the progressive loss of photoreceptor input likely alters the neural circuitry of the inner retina. This study investigated resulting changes in the activity of surviving ganglion cells. Multielectrode recording monitored spontaneous and light-evoked extracellular action potentials simultaneously from 30 to 90 retinal ganglion cells of wild-type (wt) or rd1 mice. In rd1 mice, this activity evolves through three phases. First, normal spontaneous “waves” of correlated firing are seen at postnatal day 7 (P7) and last until shortly after eye opening. Second, at P14, full-field light flashes evoke reliable responses in many cells, with preferential preservation of OFF responses. These diminish as photoreceptor degeneration progresses. Third, once light-evoked responses have disappeared in early adulthood, surviving rd1 ganglion cells fire at a much higher spontaneous frequency than normal, sometimes in rhythmic bursts that are distinct from the developmental “waves.” This hyperactivity is sustained well into adulthood, for weeks after photoreceptors have disappeared. Thus striking alterations occur in inner retinal physiology as retinal degeneration through three phases. First, normal spontaneous “waves” of correlated firing are seen at postnatal day 7 (P7) and last until shortly after eye opening. Second, at P14, full-field light flashes evoke reliable responses in many cells, with preferential preservation of OFF responses. These diminish as photoreceptor degeneration progresses. Third, once light-evoked responses have disappeared in early adulthood, surviving rd1 ganglion cells fire at a much higher spontaneous frequency than normal, sometimes in rhythmic bursts that are distinct from the developmental “waves.” This hyperactivity is sustained well into adulthood, for weeks after photoreceptors have disappeared. Thus striking alterations occur in inner retinal physiology as retinal degeneration progresses in the rd1 mouse. Blindness occurs in the face of sustained hyperactivity among ganglion cells, which remain viable for months despite this activity. ON and OFF responses are differentially affected in early stages of degeneration. While the source of these changes remains to be learned, such features should be considered in designing more effective treatments for these disorders.

INTRODUCTION

Inherited retinal degenerations such as retinitis pigmentosa are the most common hereditary cause of severe visual loss for which no effective treatment yet exists (Bessant and Hameed 2001). Many proposed approaches to treatment of these diseases have emphasized replacing photoreceptor function, a strategy the success of which relies critically on the preservation of a morphologically and functionally intact inner retina (Berson and Jakobiec 1999; Delyfer et al. 2004; Loewenstein et al. 2004; Margalit et al. 2002; Woch et al. 2001). However, significant morphological changes occur in the inner retina even at early stages of photoreceptor degeneration. For example, although ganglion cells of the rd1 (previously rd) mouse in the early postnatal period appear comparatively well preserved on a simple light microscopic level, detailed histology reveals significant aberrations of horizontal and bipolar cell processes and glutamate receptor redistribution (Strettoi et al. 2003). Later, parallel changes occur in elements of the cone pathway together with multiple ectopic synapses and “microneuromas.” Eventually neurons migrate to ectopic sites and glial seal form. Similar synaptic reorganization occurs in various other animal models of retinal degeneration (Cuenca et al. 2005; Marc et al. 2003; Peng et al. 2000) and in humans with retinitis pigmentosa (Fariss et al. 2000).

Less is known about the physiological consequences of these changes. How do more proximal elements of the retina behave after the loss of photoreceptor input? Changes in the electroretinogram (ERG) parallel the histological loss of first rod, then cone photoreceptors (Berson 1996; Chang et al. 2002; Phelan and Bok 2000), but even specialized versions such as pattern and multifocal ERG do not assess individual cell responses; such responses may persist well past the loss of ERG signals (Drüger and Hubel 1978; Keating et al. 2002).

Modeling of the ERG in retinal degeneration has suggested that compensatory changes occur in rod sensitivity and in synaptic efficacy between rods and bipolar cells (Aleman et al. 2001), but direct and accurate dissection of ganglion, amacrine, and bipolar cell contributions is difficult and complex (Hood et al. 1999; Kondo and Sieving 2002; Robson and Frishman 1999). Recordings of superior colliculus neurons in a mouse strain containing the rd1 mutation and in the Royal College of Surgeons (RCS) rat have demonstrated increased spontaneous activity and burst firing that likely originated in the retina (Drüger and Hubel 1978; Sauvé et al. 2001). Few investigations have directly recorded the physiologic function of individual bipolar or ganglion cells in retinal degenerations (Rader et al. 2002; Varela et al. 2003). In this study, I use multielectrode recording to investigate changes in retinal ganglion cell activity that accompany the progressive loss of photoreceptors in the rd1 mouse, a widely studied animal model of retinal degeneration.

METHODS

Tissue preparation

Wild-type (C57BL/6J strain) and rd1 mice (C3H/HeJ strain, or B6.C3-Pde6brd1 Hps4le strain) Hps4le/le strain for additional controls with the rd1

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Spike waveform analysis

Action potential (spike) waveforms accepted for further analysis were ≥60 μV in amplitude, and >1.85 times the RMS of the background signal. To distinguish responses from different cells that might appear on the same electrode, a component of the data-acquisition software (Bionic Technologies) or a similar freeware (PowerNAP, Neuroshare, http://neuroshare.sourceforge.net/index.shtml) was used for supervised automated sorting of action potential profiles according to a principle components analysis (PCA) paradigm. For each electrode, the software displays a random sample of ≥2,000 spike waveforms (“training set”) along with all the two-dimensional projections of each waveform in the space defined by the first three principle components (PCs, or eigen vectors computed from the correlation matrix for this data subset) (Wheeler 1999).

The individual waveforms were partitioned iteratively into one to five clusters according to an automated K-means paradigm (Wheeler 1999). For each channel in a recording, ≥200 (typically 10–50) different clustering solutions were observed; one solution was chosen from among these, to maximize the similarity among waveforms within a cluster; minimize the degree of overlap between clusters, and maximize the distance between cluster centers and edges. In cases where an optimal solution was not immediately distinguished on this basis, the data initially was segregated into a greater number of clusters than seemed the likely final solution, for subsequent analysis of the corresponding spike trains (described in the following text), to determine which of these signals were generated by the same or distinct sources. The mean waveform for each of the clusters was displayed; if these mean waveforms appeared nearly identical among two or more clusters, these were joined manually. The mean waveform for each of the clusters chosen at this stage then served as a series of templates, and all remaining waveforms from that electrode were joined by the software to the cluster closest to it in PC space. In the cases with broad and overlapping clusters, individual waveforms were considered outliers and excluded if their projected point in PC space was distant from the closest cluster’s center by >2.5–4.0 times the SD of the data within that cluster.

Appropriate assignment of individual waveforms to distinct cells was confirmed further by analysis of the corresponding spike trains. Interspike interval (ISI) histograms were computed for each spike train by measuring the intervals between spikes in the train for all possible spike pairs, then distributing these values in bins of 0.2-ms width. ISI histograms from accepted data demonstrated a refractory period of >1 ms (typically 2–5 ms) and did not reflect any of several patterns of recognizable noise: 60 Hz, very high-frequency (>10 kHz) transients, or waveforms distinct from those of extracellular action potentials (e.g., sinusoidal oscillations). Cross-correlograms were computed in like fashion, measuring all intervals between each spike in one train and all spikes of the other train, binning at 5 ms. In cases where two or more templates from the same electrode appeared similar, the waveform clusters ultimately were assigned pair-wise to either one or two cells, based on their separate and combined ISI histograms and the cross-correlogram between their spike trains. Thus if the ISI histograms from the two putative cells were of clearly distinct shape, and/or if the ISI histogram formed by combining the two spike trains eliminated the refractory period observed in either separate ISI histogram, these spike trains were considered to have originated from two separate cells (Segev et al. 2004). Likewise if a prominent peak was observed near the origin in the cross-correlogram of the two spike trains, these spikes must have originated from two separate cells because both were recorded nearly simultaneously on the same electrode. These procedures generally resulted in one to three (occasionally four) cells being isolated from a single electrode.

Spike train analysis

The spontaneous mean firing rate for each recorded cell was computed as the total number of spikes divided by the length of the

{mutation on a C57BL/6J background} were bred within a local colony established from purchased breeding pairs (Jackson Laboratories, Bar Harbor, ME). Animals were cared for in accordance with institutional guidelines of the Massachusetts General Hospital and the APS Guiding Principles in the Care and Use of Animals. Animals were dark-adapted for ≥30 min prior to being anesthetized with intraocular or intramuscular injection of xylazine (10–40 mg/kg) and ketamine (50–200 mg/kg) sufficient to extinguish tail pinch and corneal reflexes. Under infrared illumination to minimize exposure to visible light, using a dissecting microscope (Leica Microsystems, Bannockburn, IL) with infrared image intensifiers (BE Myers, Redman, WA), the retina was dissected from the retinal pigmented epithelium, placed ganglion cell layer down onto a multielectrode recording array (10 μm contacts spaced 200 μm apart; Multichannel Systems, Reutlingen, Germany), and perfused with warm (36–37°C), oxygenated Ringer medium at a rate of 2.5–4 ml/min (Meister et al. 1994; Tian and Copenhagen 2001). Ringer medium included (in mM) 124 NaCl, 2.5 KCl, 2 CaCl2, 2 MgCl2, 1.25 NaH2PO2, 26 NaHCO3, and 22 glucose. Presented data are from a total of >900 wt cells from 27 retinas, >1,400 rd1 cells from 22 retinas, and >900 cells from 20 retinas with the rd1 mutation on a C57BL/6J background.

Multielectrode recording

A 60-channel amplifier (Multichannel Systems, Reutlingen, Germany) mounted on a microscope stage (Zeiss Axioplan, Göttingen, Germany) interfaced with digital sampling hardware and software (Bionic Technologies, Salt Lake City, UT) for recording and analyzing spike trains from each of the electrodes in the array. Digitized data initially were streamed onto the computer’s hard drive and further analyzed off-line. After transfer of the retina to the recording chamber, recordings were allowed to stabilize for ≥1 h as evidenced by stable action potential amplitudes, number of cells recorded, frequency of spontaneous firing, and consistency of light-evoked responses (where obtainable). Twenty-minute epochs of continuous recording were obtained from typically 30–90 ganglion cells per retina at various intervals over several hours. Data presented are from the first 1–3 h of recording, unless otherwise specified.

Visual stimulation

In experiments with light stimulation, a miniature computer monitor (Lucivid, MicroBrightField, Colchester, VT) projected visual stimuli through a ×5 objective, and these were focused via standard microscope optics (Zeiss Axioplan) onto the photoreceptor layer of the retina. Luminance was calibrated via commercial software (VisionWorks, Vision Research Graphics, Durham, NH), using a photometer (Minolta, Ramsey, NJ) and photodiode (Hamamatsu S1133-11, Hamamatsu City, Japan) placed in the tissue plane. The refresh rate of the monitor (66 Hz) was chosen to avoid entrainment of retinal ganglion cells that might contaminate light responses (Tremain and Wong 1983; Wollman and Palmer 1995). The same software controlled and recorded stimulus parameters, passing synchronization pulses to the data acquisition computer via a parallel interface with ~10-μs precision.

Full field flash stimuli (illuminance: 355 cd/m2, retinal irradiance: 0–3,400 μW/cm2, 500 ms, P43 phosphor with peak emission at 545 nm) extended beyond the dimensions of the recording array (stimuli: 2,100 × 2,800 μm; array: 1,700 × 1,700 μm). They were displayed at 5-s intervals, and responses averaged over 10 trials. These parameters are known to evoke a reliable ERG response and to allow separation of on- and off-pathway responses in individual ganglion cells (Balkema and Pinto 1982; Stone and Pinto 1993; Strettoi et al. 2002).
recording period. Cross-correlation functions were computed for cell pairs after spike trains had been assigned to particular cells, in the same manner as the cross-correlograms computed during spike train assignment procedures (see preceding text). For comparison among these cross-correlograms (i.e., any cell pair vs. other pairs), each cross-correlogram was normalized by the number of spikes in the longer of the two spike trains. The power spectral density function for any spike train was computed via fast Fourier transformation of the train’s autocorrelation function (the cross-correlation function computed from all intervals between each spike of the train and every other spike in the train).

Parameters of spontaneous developmental waves of ganglion cell activity were computed in accord with previous investigators (Torborg et al. 2004, 2005; Wong et al. 1993), from recording segments of 20–30 min: mean firing rate (total number of spikes divided by the length of the recording period), instantaneous firing rate (inverse of interspike interval within bursts), burst duration and frequency (as interburst interval), and percent of the entire recording period spent firing at >1 Hz or <10 Hz. Here a burst was defined as a group of at least three spikes in which all ISIs were <1 s. In addition, the correlation index (CI) was computed as described by Wong et al. (1993)

\[ C_I = \frac{N_{AB}^{-0.1s} \cdot T}{N_{A(0,T)} \cdot N_{B(0,T)}^{-0.2s}} \]

where \( N_{AB} \) is the number of instances in which a spike from neuron A occurs within ±0.1 s of a spike from neuron B, \( T \) is the total length of the recording (here, 20 min.), and \( N_A \) and \( N_B \) are the numbers of spikes in each neuron’s spike train over the total recording period.

Light-evoked responses were quantified as the total number of action potentials occurring within 500 ms following a light transition (ON or OFF), after subtracting the background spontaneous firing rate over the 1 s preceding the stimulus. These responses were averaged over 10 trials, and thus response amplitude was expressed as a net mean firing rate (spike/s). The proportion of each response attributed to light onset was estimated as the ratio of the ON response (calculated as in the preceding text) to the total (ON + OFF) response. Response latency was estimated by identifying the first instance following an ON or OFF light transition in which there were three consecutive 20-ms time bins with at least one action potential, preceded by at least two empty bins; the time at the leading edge of the first filled bin was taken as the latency of this response. For calculating population parameters of light-evoked responses, ganglion cells were included if the net response to the maximum stimulus (3,400 \( \mu \text{W/cm}^2 \) retinal irradiance) was ≥1 spike/s (average of 1 spike every other trial).

Statistical analysis

Given the nonnormal distribution of the various parameters in the preceding text, central values were expressed as medians ± first and third quartiles, and these values compared using the Wilcoxon-Mann-Whitney U test; the shape of distributions was compared further in some cases using the Kolmogorov-Smirnoff test for the difference of two distributions. The Kruskal-Wallis test was employed for comparisons across multiple experimental groups (e.g., animal age).

RESULTS

Emergence of spontaneous hyperactivity in rd1 ganglion cells

I recorded spontaneous and light-evoked extracellular action potentials in wt and rd1 ganglion cells over the first several postnatal weeks using a multielectrode array. During this period, virtually all photoreceptors are lost progressively in the rd1 mouse (Bennett et al. 1996; Bowes et al. 1990; Chang et al. 2002; Strettoi et al. 2002). Representative raster plots in display spontaneous activity in wt and rd1 ganglion cells at various postnatal ages. Firing rates are mildly elevated in rd1 cells at postnatal days 7–8 (P7-8), then progressively increase through P28, and remain elevated thereafter.

Whereas spontaneous firing rates varied among individual ganglion cells of both wt and rd1 retinas, the frequency of spontaneous activity was higher overall among rd1 ganglion cells than among wt ganglion cells. Figure 2A plots the distribution of firing rates for all rd1 cells relative to those for wt cells at each of the postnatal ages sampled. The increased rate of activity in a larger proportion of the rd1 ganglion cell population as compared with wt may be seen as a rightward shift and decreased initial slope in the cumulative frequency histograms for each age.

Figure 2B quantitatively compares the overall level of spontaneous activity in rd1 and wt ganglion cells over the first several postnatal weeks (median and quartiles of all cells’ firing rates, estimated by averaging over a 20-min sample period). This demonstrates the progressive increase in spontaneous activity that is sustained at least through P115 (Kruskal-Wallis test across age groups, \( P < 0.001 \), with the greatest increase between P14-15 and P21).

Additional controls

To assure that the preceding changes in spontaneous activity were not attributable simply to a difference in background strain between rd1 (C3H/HeJ) and wt (C57BL/6J) mice, additional data were collected from retinas of a strain with the rd1 mutation back-bred onto a C57BL/6J background (B6.C3-Pde6brd1 Hps4e/J, hereafter abbreviated rd1/C57). Figure 2 shows that firing rates were nearly identical to those of wt cells at P7-8 and suggests that the elevation in spontaneous discharge frequency may peak later in rd1/C57 mice. Ultimately, in both rd1 strains, spontaneous activity is markedly elevated over that in wt ganglion cells.

Detailed analysis of this activity revealed distinctive features in each of three stages of retinal degeneration: prior to eye opening or significant photoreceptor loss; during the period of active photoreceptor degeneration but partially preserved light responsiveness; and of increasing spontaneous activity; and after loss of virtually all rod photoreceptors.

Developmental waves

Others previously have described early developmental spontaneous waves of activity correlated among multiple ganglion cells and migrating across broad regions of the retina (Feller et al. 1997; Wong 1999). In such investigations, a variety of dynamic parameters have been used to characterize these waves and to compare them among various animal strains and/or experimental conditions (Torborg et al. 2004, 2005; Wong et al. 1993). At this developmental stage, the eyes are still closed and photoreceptors have yet to complete synaptic contacts, so the activity is believed to originate among amacrines and ganglion cells without participation of the outer retina (Firth et al. 2005).

Recordings from rd1 retinas at P7-8 exhibited retinal waves that appear typical of wt ganglion cells at this developmental
FIG. 1. Spontaneous activity increases with age, and with photoreceptor degeneration, in rd1 ganglion cells. Representative raster plots display extracellular recordings of spontaneous action potentials occurring in wild type (wt, left) and rd1 (right) retinas at various postnatal ages (P7–P115). Each panel displays the activity recorded over a 5-min period in a sample of 30 ganglion cells (1 per row) from a single retina. Blank rows (without raster tick marks) indicate cells from which no activity was recorded during this 5-min epoch, but light-evoked and/or spontaneous activity was recorded during other epochs. Prior to eye opening (P7), spontaneous waves of correlated activity are seen in both wt and rd1 ganglion cells, as described previously by others in the wt (see text). By P14-15 (after eye opening), retinal waves have disappeared in both strains, and spontaneous activity is modest in the wt retina but increases dramatically and progressively in rd1 ganglion cells.
FIG. 2. Quantification of increasing spontaneous activity in rd1 ganglion cells. A: cumulative frequency histograms display the distribution of mean firing rates among all ganglion cells recorded in normal and degenerating retinas (wt, black; rd1, gray; rd1/C57, dotted gray; bin size: 0.2 Hz; total number of retinas and cells sampled under each condition are given in B). For each retina, firing rates of individual ganglion cells were estimated over a single 20-min epoch 2–3 h into a recording session. Each panel represents the proportion of the ganglion cell population firing at a rate up to the frequency on the x axis. A greater proportion of cells is active at higher rates in rd1 and rd1/C57 retinas, seen as a rightward shift and lower slope of the histogram curve. 

B: median spontaneous firing rates (middle line in each bar) and 1st and 3rd quartiles (ends of bars) are indicated for each of 6 age groups (P7–P115; wt, black; rd1, white; rd1/C57, gray). rd1 firing rates increase progressively, most dramatically between P14-15 and P21, whereas wt firing rates increase only slightly between P14-15 and P110-115. Statistically significant differences exist among wt, rd1, and rd1/C57 ganglion cells at each age (Wilcoxon-Mann-Whitney U test, P < 0.01, except P = 0.012 for rd1 vs. rd1/C57 at P14-15), although these differences are of small magnitude between wt and rd1 at P7-8 and among all 3 strains at P14-15 (see text). Differences among age groups within a given strain also are statistically significant (Kruskal-Wallis test, P = 0.05, Tukey’s criterion), indicating a progressive increase in firing rate with age. The distributions of firing rates become increasingly distinct as photoreceptor degeneration progresses. Data are from the same recordings as in A. The number of cells in each sample is indicated above the bar, with the number of retinas in parentheses. Each retina was from a different animal.
FIG. 3. Spontaneous retinal “waves” in wt and rd1 mouse ganglion cells at P7. A: pseudo color maps of ganglion cell activity in representative retinas at serial snapshots in time (left, wt; right, rd1). Firing rate is indicated by a color change at the location(s) on each map that correspond to the positions of array electrode(s) at which activity was recorded (electrode positions identified by gold squares at t = 0; colors at positions between these sites indicate interpolated values for firing rates). This series of maps illustrates periodic bursts of correlated activity that migrate across several electrodes (from middle right to center bottom) over 4 s (t = 0 to t = 4 s). These waves appear similar between the 2 strains and to those reported previously by other investigators for wt mice and other mammals (Demas et al. 2006; Torborg et al. 2004, Torborg et al. 2005; Wong et al. 1993). B: individual raster plots from the same recordings show in greater detail the involvement of 41 cells in a number of waves traversing the retina during a representative 2.5-min epoch. Rasters are arranged according to electrode position: bottom to top rasters follow electrodes down each column, from left to rightmost column, of the activity maps in A. Variability is evident in the particular cells participating in each burst, the initiation site and spatial extent (number of cells) of individual waves, and the direction and duration of wave propagation. These patterns are similar between strains. C: peak in cross-correlogram provides evidence of correlated firing for 3 example cell pairs of each strain within the region of retina participating in these waves (cell numbers identify corresponding raster plots labeled in B). D: the Correlation Index (CI, see METHODS) calculated for all ganglion cell pairs in these recordings (wt, black line, 5,356 pairs among 6 retinas; rd1, gray line, 10,123 pairs among 4 retinas), as a function of the distance between cells of a pair. Cell activity is correlated to a similar degree among rd1 as among wt cells. Line symbols indicate median values for CI, and error bars indicate 1st and 3rd quartiles. Values are comparable to those reported by others (cited in the preceding text).
stage (Fig. 3). These waves were quantitatively compared between the two strains using several dynamic parameters used in previous studies: mean firing rate, median burst duration and interburst interval, instantaneous firing rate, percent of time spent firing at >1 Hz and at >10 Hz, and correlation index (Figs. 3D and 4). This analysis revealed small but statistically significant differences between wt and rd1 ganglion cells in most of these population measures (medians: Wilcoxon-Mann-Whitney U test, and distributions: Kolmogorov-Smirnov test, \( P < 0.001 \)). Burst duration in particular was not significantly different between the two strains (Kolmogorov-Smirnov test, \( P = 0.084 \)), but firing rate within bursts was slightly higher among rd1 cells (reflected in the bottom 6 panels of Fig. 4). Values for the correlation index are similar to those in previous reports at equivalent distances (Table 1) (Wong et al. 1993). By this developmental stage, in the rd1 retina there has been minimal loss of photoreceptors (Farber et al. 1994; Firth et al. 2005), yet even in the wt synaptic input from photoreceptors to the inner retina has not yet developed (Demas et al. 2003; Feller et al. 1997; Wong et al. 1998; Zhou and Zhao 2000).

**Phase of increasing spontaneous activity**

Consistent with previous studies, soon after eye opening (P12-13) these spontaneous waves disappear in both wt and rd1, although rare bursts of spikes continue in both strains (Demas et al. 2003; Feller et al. 1997; Wong et al. 1998; Zhou and Zhao 2000). Thus as synapses form between photoreceptors and bipolar cells, rd1 ganglion cells exhibit progressively more frequent spontaneous firing, yet without the structured waves of burst firing seen prior to eye opening. In parallel, during this period (P14–P28) consistent light-evoked re-
Features of sustained spontaneous hyperactivity

I examined in greater detail the vigorous spontaneous activity present by 1 mo postnatal age. Two representative cells from a P29 wt mouse and two from a P29 rd1 mouse are illustrated in Fig. 5A. The average spike rate over a similar 20-min epoch was greater for the rd1 retina (cell 1 = 11.9 Hz, cell 2 = 15.6 Hz) than for the wt (cell 1 = 1.29 Hz, cell 2 = 0.93 Hz). These two rd1 cells also exemplify a regular rhythmic fluctuation in firing rate seen in ∼2/3 of ganglion cells in rd1 preparations. The rhythmic activity is easily visualized as a secondary peak in the ISI histograms for each cell (Fig. 5B) at ∼150 ms (∼7 Hz). It is also evident in the power spectral density (PSD) functions computed for the rd1 population (Fig. 5C), as a pair of peaks at ∼6 and ∼12 Hz. There are no corresponding peaks in the ISI plot or PSD for the wt cells, which do not fire in this rhythmic fashion. Note that although both of these rd1 cells modulate their firing rate rhythmically at ∼7 Hz, they do not do so synchronously. This is evident in the lack of a peak in the normalized cross-correlogram plotted for their two spike trains (Fig. 5D; compare with peaks in cross-correlograms for cells participating in developmental “waves,” Fig. 3C), as well as in a correlation index near unity (CI = 0.87 for the wt pair, ∼630 μm apart; CI = 1.03 for rd1, ∼850 μm apart).
FIG. 6. Light-evoked responses in wt and rd1 ganglion cells at P15. Representative recordings from a variety of broad response groups. A: ON ganglion cells. Raster plots and peristimulus time histograms (PSTHs) display the brisk, transient response of a P15 wt ganglion cell (rasters of 10 trials, averaged in ■) and a P15 rd1 cell (□ and accompanying rasters) to a full field flash (3400 µW/cm², on during the shaded interval, ■). B: OFF ganglion cells. Similar traces from P15 wt and rd1 cells show responses primarily to light offset. C: ON-OFF ganglion cells that respond transiently to light onset and offset. D: “ON-sustained” cells display sustained responses predominantly to light onset. E: delayed OFF responses. F: sustained ON-OFF responses. For each response group and each strain, the number of cells in that group, of the total number of cells with readily categorized responses, is indicated. Other response types (ON sluggish, ON-OFF sluggish) were encountered rarely (not shown). Thus light-evoked responses of various types are partially preserved in rd1 ganglion cells at this stage of degeneration. (Note differences in scale for response amplitude among various response groups but equal scale between wt and rd1 examples of each response type.)
in each strain appear in Fig. 6. A similar variety of basic response characteristics may be seen among both wt and rd1 populations: those responding predominantly to light onset (ON responses), to light offset (OFF), or to both (ON-OFF); brisk or sluggish response onset; transient or sustained response duration. Estimates are given of the relative proportion of cells that fall into each broad grouping. It is important to recognize that this categorization does not represent a precise classification of ganglion cell response types (see Discussion).

The median amplitude of total responses was lower among rd1 than wt cells (Fig. 7A). In accord with findings in the superior colliculus (Dräger and Hubel 1978; Sauvé et al. 2001) and with the more severe loss of photoreceptors centrally (Dräger and Hubel 1978), there was a trend for this decrease in response amplitude to be more prominent in central than peripheral retina, although the collected data did not reach statistical significance (data not shown). Separate examination of ON and OFF responses shows that ON responses had degenerated disproportionately to OFF responses, which remained relatively preserved (Fig. 7A). However, this feature was not seen in rd1/C57 cells, in which the decrease of response amplitude generally was more severe.

These findings might result from a global decrease in ON responses of all ganglion cells or from selective effects on particular ganglion cell subpopulations that have strong ON pathway input. Although currently there is no widely recognized taxonomy of physiologic response types for mouse ganglion cells, recent studies have delineated 10–12 morphological classes (Kong et al. 2005; Sun et al. 2002), and a similar degree of diversity in physiologic characteristics (Balkema and Pinto 1982; Carcieri et al. 2003; Deans et al. 2002; Pang et al. 2003; Stone and Pinto 1993; Volgyi et al. 2004). As a first step to determine whether the decrease in ON responses is selective for particular ganglion cell subpopulations, the cells in this study were divided according to the proportion of their total response that consisted of an ON or OFF response. The distribution of recorded cells among such subgroups reveals that a greater fraction of the entire population is dominated by OFF responses.

**FIG. 7.** Light-evoked ON responses in rd1 ganglion cells at P14-15 are decreased more than OFF responses. A: amplitude of responses to maximal full field stimulus (3400 μW/cm²) among 165 wt (●) and 243 rd1 (○) ganglion cells. Ends of bars represent 1st and 3rd quartiles, horizontal line within each bar indicates the median value, and numbers above bars indicate total number of responses of the given subtype. The decrease among rd1 cells in total response amplitude is in large part attributable to decreased ON responses, whereas OFF responses are relatively preserved. In the additional control strain, rd1/C57, responses are reduced overall to a greater degree and more evenly between ON and OFF subtypes. Differences are statistically significant among all strains for both response types (Wilcoxon-Mann-Whitney U test, P < 0.03), except between wt and rd1 OFF and total responses. The final group of bars (far left) demonstrates the lack of light-evoked responses in rd1 ganglion cells by P28. Data are from 5 wt, 5 rd1, and 3 rd1/C57 retinas at P14-15, and from 3 wt and 1 rd1 retina at P28. B, bottom axis: distribution of the same ganglion cells according to the relative proportion of ON vs. OFF response amplitude making up the total response. ON-dominant cells (ratio >0.5) are less common, and OFF-dominant cells (ratio <0.5) more common, in rd1 retinas. Top axis: median spontaneous firing rates do not differ systematically among the categories of ON vs. OFF response proportion (Kruskal-Wallis test, n = 121 wt, 209 rd1 cells; see text for details). Vertical lines indicate 1st and 3rd quartiles. D: latency of light responses also is increased in rd1 cells, disproportionately in ON responses but more severely and relatively evenly between ON and OFF responses in rd1/C57 cells. Differences are statistically significant among all strains for both response types (Wilcoxon-Mann-Whitney U test, P < 0.01), except between wt and rd1 OFF responses.
responses (on/total ratio \(\leq 0.5\)) in the rd1 retina than in the wt (Fig. 7B, bottom). That is, either subgroups of ganglion cells with on-dominated responses are preferentially lost and off-dominated cells preserved or on-dominated cells convert to being off-dominated as rd1 degeneration progresses. There was no systematic difference in the spontaneous firing rate of cells in any of these subgroups (Fig. 7B, top. The Kruskal-Wallis test indicates a significant difference for wt only between the 1.0 fractional on response subgroup and the 0.6 or 0.8 fractional on subgroups, \(p < 0.01, n = 121\) cells; and for rd1 between 0.9 on and 0.2 or 0.3 on subgroups, \(P = 0.01, n = 209\).

Response latency also was increased in many rd1 ganglion cells, though to a variable degree. The time of response onset following a light transition was estimated separately for on and off stimuli in the subpopulation of cells in which these response components were clearly separable (see METHODS). Latency was increased significantly only for on responses in rd1 retinas (Fig. 7C), consistent with the relatively greater loss of on than off response amplitudes. However, overall greater delays were seen in rd1/C57 responses, equally so for both on and off components. More refined interpretation of this feature will require detailed study of ganglion cell stimulus intensity-response relationships and responses to complex stimuli.

No reliable light-evoked responses were obtained in any of 263 ganglion cells in four rd1 preparations at P21 or 217 ganglion cells in six rd1 retinas at P28-29 or in 363 rd1/C57 cells at P28. Robust responses remained in wt retinas (\(n = 11\)) at these ages (data not shown). This is consistent with the degeneration of virtually all rod photoreceptors and a majority of cones (Mohand-Said et al. 1998) as well as with the minimal ERG responses in the rd1 mouse by P28 (Strettoi et al. 2002, 2003).

DISCUSSION

This study identifies marked alterations in the physiologic activity of retinal ganglion cells accompanying outer retinal degeneration in a well-recognized animal model. Here I report several features of this activity not previously described. First, spontaneous hyperactivity originates in the retina, with rhythmic bursting in many ganglion cells. Second, at least three distinguishable phases of activity may be identified, from an initially normal pattern to one of sustained hyperactivity that does not compromise ganglion cell viability. Third, the temporary preservation of light-evoked responses that accompanies this hyperactivity may be selective for specific response types.

Increased spontaneous activity in rd1 ganglion cells

I show that retinal ganglion cells display increased spontaneous activity as retinal degeneration progresses in the rd1 mouse. Both the number of cells with ongoing spontaneous activity and the mean firing rate of the population increase. The present results show for the first time that spontaneous hyperactivity originates at the level of the retina, directly identifying a source for increased firing among superior colliculus cells that Dräger and Hubel previously demonstrated in rd1/C57 mice (Dräger and Hubel 1978) and that Sauvé et al. found in RCS rats (Sauvé et al. 2001).

This hyperactivity is not simply due to increased susceptibility of the rd1 retina to injury during tissue preparation or to decreased viability under in vitro recording conditions. First, these elevated firing rates, as well as light-evoked responses (when present), remained stable for 3–8 h. Second, the finding is reproducible from animal to animal. Third, it is consistent with the in vivo superior colliculus studies mentioned in the preceding text. The hyperactivity also is not simply due to a difference in background strain because similar hyperactivity (albeit developing with a slower time course) occurred in ganglion cells of the additional control strain (rd1/C57). It might be hypothesized that the hyperactivity results from increased \(pO_2\) in the face of decreasing oxygen consumption by rod photoreceptors, but inner retinal oxygenation is normal in cats with retinal degeneration (Padnick-Silver et al. 2006). Instead the increase in ganglion cell activity suggests a significant alteration either in intrinsic ganglion cell electrophysiological properties or in the organization of the neural circuitry presynaptic to the ganglion cells.

Three phases of ganglion cell activity

A key finding of this study is that rd1 ganglion cell activity passes through at least three phases. In the first phase (P7-8), prior to complete photoreceptor differentiation and synapse formation (Demas et al. 2003; Feller et al. 1997; Wong et al. 1998; Zhou and Zhao 2000), the overall architecture of spontaneous activity is relatively normal. As for wt mice, periodic waves of correlated activity are seen and later disappear as synapses first form between photoreceptors and bipolar cells. Qualitatively the waves appear very similar to those of wt retinas, although statistical measures do show that several parameters are slightly greater among rd1 cells (particularly the firing rate within bursts). The meaning of this difference is not yet clear; larger differences in these parameters have been reported among other genotypes or experimental manipulations (Demas et al. 2006; Torborg et al. 2004, 2005; Wong et al. 1993).

By P14, most rods have disappeared and the ERG has much diminished amplitude and increased latency (Strettoi et al. 2002, 2003). In this second phase, I found that many ganglion cells retain robust responses to light stimulation. Dräger and Hubel noted similarly robust evoked responses in the superior colliculus in the face of minimal ERG responses (Dräger and Hubel 1978). This may be expected from the relatively small contribution that ganglion cell activity makes to the full-field ERG (Hood et al. 1999; Robson and Frishman 1999). The present results thus add knowledge of inner retinal function in the rd1 mouse that has not been easily accessible by prior ERG studies. Also in line with these authors, I find nonetheless that response amplitudes have decreased overall. Interestingly, on responses are affected disproportionately to off responses, in the original rd1 strain. This may be seen both in the median response amplitudes for the population as a whole, and as a shift in the proportion of the population that is dominated by on versus off responses (Fig. 7).

The significance of the relative preservation of off responses at this intermediate stage of retinal degeneration is not yet clear. First, a distinction should be drawn between on versus off responses and the well-known, distinct on versus off signal processing pathways of the inner retina. The full-field stimuli

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used here do not selectively activate either the dominant center response of the ganglion cell or direct isolated input to either ON or OFF dendritic laminae. Thus while the net responses here reported may reflect specific differential effects on the ON and OFF pathways, it is as likely that they reflect a combination of direct and indirect inputs from these pathways. Possible explanations for the different degree of change in ON versus OFF responses include preferential degradation of rod and ON cone bipolar cells; decreased excitatory drive to the AII amacrine cell, resulting in disinhibition of throughput from OFF cone bipolar cells (Taylor and Smith 2004; Wu et al. 2004); enhancement of surround inhibition mediated by various amacrine cells; or new cone input to rod bipolar cells [as seen in the congenital absence of rod photoreceptors (Strettoi et al. 2004)]. Notably, loss of ON responses has been reported recently in a subset of ganglion cells of the RCS rat (Pu et al. 2006).

The full-field stimuli used here have identified differential effects of retinal degeneration on distinct inner retinal responses but are suboptimal for dissecting such mechanisms or for classifying ganglion cells into physiologic response types (Sagdullaev and McCall 2005). Furthermore, no widely accepted physiologic classification scheme yet exists for the mouse. It will be interesting to characterize the full range of dynamic and receptive field properties in greater detail using more complex stimuli and to apply pharmacologic tools to dissect the relative contributions of ON and OFF pathways to both light-evoked and spontaneous activity.

Also during this second phase, ganglion cell spontaneous hyperactivity begins to emerge. By early adulthood (P28), rd1 ganglion cells have entered a third phase, in which light-evoked responses have disappeared and spontaneous hyperactivity has increased further. This hyperactivity then is sustained for at least several months, outlasting the loss of the great majority of photoreceptors. This is consistent with previous recordings in the superior colliculus (Dräger and Hubel 1978). Histochemical measures of glutamate receptor activation (Marc et al. 2003) indicate that a high level of activity persists even into the advanced stages of retinal degeneration characterized by large-scale distortions of retinal architecture (but see also Radner et al. 2002). Because photoreceptors have disappeared by this stage, the hyperactivity must originate in the inner retina.

The stepwise changes outlined in the preceding text suggest that there may be an optimal time window to which potential treatments should be targeted to be most effective.

**Potential mechanisms**

Possible mechanisms leading to the hyperactivity may be divided into two principal categories: changes in intrinsic cellular function versus reorganization of synaptic circuitry. The first category would include alterations such as denervation hyperexcitability of ganglion or bipolar cells, with altered membrane properties such as seen in other sensory systems (Varela et al. 2003; Waxman et al. 2000). The second class of mechanisms includes various rearrangements of inner retinal circuitry presynaptic to the ganglion cells—for example, increased excitatory bipolar cell activity or decreased inhibitory amacrine cell input (Cuenca et al. 2005).

Although such reorganization might occur in mature circuitry, the fact that rapid photoreceptor degeneration occurs during an early developmental period in rd1 mice suggests that normal mechanisms of developmental plasticity may be perturbed by the concurrent photoreceptor loss. For example, ON and OFF ganglion cell dendrites may not segregate normally (Tian 2004; Tian and Copenhagen 2001). Actively forming inner retinal synaptic contacts (Wong et al. 1998) may favor increased excitatory bipolar cell or decreased inhibitory amacrine cell input to ganglion cells, or the period of increased glutamatergic transmission (Wong and Wong 2001) might be prolonged. Any of these might result in an abnormal excitatory/inhibitory balance. To begin to sort out the possible contribution of such developmental mechanisms, it may help to compare rd1 ganglion cell activity with that in other retinal degeneration models with later onset and slower progression.

**Effects of background strain differences**

The major findings in the preceding text are confirmed in large part by the additional experiments in rd1/C57 mice, but several differences may be noted. First, spontaneous hyperactivity appears to develop at a more rapid rate in the rd1 than rd1/C57 strain, although maximal firing rates ultimately reach a similar level in the two strains (Fig. 2). In contrast, light-evoked responses are more severely decreased in amplitude and delayed in latency and are more evenly so between ON and OFF responses in rd1/C57 retinas (Fig. 7). On the other hand, these data indicate that developmental retinal waves of rd1/C57 retinas are even more similar to those of the wt than those of the rd1 strain, and the overall structure of these waves is highly similar in all three strains (Fig. 2 and Table 1). Care should be taken not to overinterpret these differences because factors such as simple as differential sampling of the 10–12 functional mouse ganglion cell classes among the strains may influence these measures (Balkema and Pinto 1982; Carcieri et al. 2003; Deans et al. 2002; Pang et al. 2003; Stone and Pinto 1993; Vogli et al. 2004). As noted in the preceding text, more complex methods may be needed to differentiate the physiologic behavior of these subtypes.

**Sustained viability of the inner retina**

Finally, it is remarkable that even as the animal is going blind, ganglion cells do not become quiescent but rather sustain a high level of activity (~3–10 times normal). Furthermore, this activity continues for many weeks, without the cells “wearing out” or dying of neurotoxicity from accompanying increased neurotransmitter release. The effects may be wide-ranging: altered and/or persistent retinal activity with abnormal temporal structure can cause abnormal patterning of synaptic connections in downstream targets such as the lateral geniculate nucleus (Demas et al. 2006; Hooks and Chen 2006). Future treatments that aim to replace photoreceptor function should take into account this abnormal hyperactivity and the mechanisms of neural reorganization that support it. The present findings do indicate at least that surviving ganglion cells are viable for an extended period (Bush et al. 1995). This provides hope that the inner retina may remain receptive to input from rejuvenated or transplanted photoreceptors, stem cells, or direct electrical stimulation.
Acknowledgments

The author thanks M. Bell, S. Mamun, A. Molins, and M. P. Andrews for assistance in data analysis.

Grants

This work was supported by National Institutes of Neurological Disorders and Stroke Grant NS-01701-09 and by the Lewis Rhodes Charitable Trust.

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