Published in final edited form as:

J Neural Eng. 2016 April ; 13(2): 025001. doi:10.1088/1741-2560/13/2/025001.

Changes in ganglion cell physiology during retinal degeneration influence excitability by prosthetic electrodes

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Abstract

Objective—Here we investigate ganglion cell physiology in healthy and degenerating retina to test its influence on threshold to electrical stimulation.

Approach—Age-related Macular Degeneration and Retinitis Pigmentosa cause blindness via outer retinal degeneration. Inner retinal pathways that transmit visual information to the central brain remain intact, so direct electrical stimulation from prosthetic devices offers the possibility for visual restoration. Since inner retinal physiology changes during degeneration, we characterize physiological properties and responses to electrical stimulation in retinal ganglion cells of both wild type mice and the rd10 mouse model of retinal degeneration.

Main results—Our aggregate results support previous observations that elevated thresholds characterize diseased retinas. However, a physiology-driven classification scheme reveals distinct sub-populations of ganglion cells with thresholds either normal or strongly elevated compared to wild-type. When these populations are combined, only a weakly elevated threshold with large variance is observed. The cells with normal threshold are more depolarized at rest and exhibit periodic oscillations.

Significance—During degeneration, physiological changes in retinal ganglion cells affect the threshold stimulation currents required to evoke action potentials.

Keywords

retina; ganglion cell; prosthetic; physiology; retinal degeneration; rd10

1. Introduction

Retinitis pigmentosa and age-related macular degeneration are two common outer retinal diseases for which there are currently no cures. Progressive photoreceptor death ultimately

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leads to significant blindness when input signals to retinal ganglion cells (RGCs) cease to convey visual meaning. The inner retina is less affected by disease (Lin 2013, Strettoi 2008, Medeiros 2001), and as a result prosthetic retinal stimulation can still convey meaningful visual information to the brain (Humayun 2012, Zrenner 2013, Ayton 2014). Clinical trial results have been promising and have shown that patients implanted with these devices perform better at various visually-guided tasks when using the prosthesis (Humayun 2012, Dorn 2013, da Cruz 2013). The next step is to improve the performance of bioelectronic-based vision restoration therapy by understanding how patterns of electrical stimulation produce patterns of RGC firing in diseased cells that broadly resemble firing patterns in healthy cells.

RGCs can reliably respond to electrical stimulation in normal retina (Jepson 2014, Fried 2011, Ahuja 2008) and diseased retina (Margolis 2008, Stasheff 2008, Sekirnjak 2009), though degeneration generally elevates stimulation thresholds (Chan 2011, Rizzo 2009, Jensen 2009). There are at least 20–25 anatomically and physiologically distinct types of RGCs, whose axons form the optic nerve. In wild type mouse retina, we have found that RGC stimulation threshold increases with RGC soma size (Cho et al 2011), suggesting a possible anatomical correlate for threshold increase. Evidence of altered physiology can be found in both rd1 and rd10 mice. In rd1, oscillatory network activity has been measured in the outer retina (Haq *et al* 2014) and the inner retina (Choi *et al* 2014). The inner retinal oscillations are thought to generate the oscillatory RGC spiking that is prominent in the rd1 model (Margolis *et al* 2014). In the rd10 model, intrinsic oscillations are also observed and are thought to result from similar mechanisms (Biswas *et al* 2014). It is not known how these physiological changes, elevated threshold and oscillatory activity, during degeneration affect RGC susceptibility to electrical stimulation.

Here we characterize spontaneous RGC activity in WT and rd10 retina by measuring resting membrane potential, baseline spontaneous spike rate, and temporal patterns of spiking activity. We compare relationships between these physiological properties within and across mouse models, and assess their effects on stimulation threshold. Our results suggest that stimulation thresholds increase during degeneration, and that the magnitude of the change likely depends on RGC type.

2. Methods

2.1 Animals

Wild-type (WT) and rd10 mice were purchased from Jackson Laboratories (Bar Harbor, Maine) and bred into a common C57BL/6J background. The age of WT and rd10 mice used for physiological recordings ranged from P39–80 ($P68 \pm 14$, mean $\pm$ SD) and P42–P77 ($P62 \pm 18$), respectively. The rd10 mouse carries a missense mutation on the gene encoding for the PDE- β subunit, an integral component of the phototransduction cascade (Chang 2007). In rd10 mice, photoreceptor death peaks around P21 with almost complete loss of rods and cones by the end of 2 months (Chang 2007, Strettoi 2008). The animals were housed in facilities on a 12 h light/dark cycle, and were dark-adapted briefly prior to physiological recordings.

2.2 Tissue preparation and visualization

WT and rd10 mice were euthanized in accordance with protocols approved by the IACUC of the University of Southern California. Flattened retina was trimmed into a 2-mm square section and mounted onto Whatman filter paper with the ganglion cell side facing up. The retina was superfused with heated and oxygenated bicarbonate-buffered Ames media (35–37°C; 95% O₂/5% CO₂) at a rate of 4–5 ml/min. A glass pipette was used to tear a section of the inner limiting membrane (ILM) to expose several RGC bodies for electrophysiological recordings. The ILM of rd10 retinas was more difficult to remove so a weighted horseshoe was used to secure the retina during tearing. A Nikon 40× water-immersion objective (0.75 NA) was used to visualize cells under infrared (IR) illumination. Mouse ganglion cell somas typically range from 10–20 µm in diameter. Since smaller ganglion cells dominate numerically, we sometimes targeted larger somas to obtain a representative population. Prior to recording, each cell was identified and the length of the soma was measured along its major and minor axes.

2.3 Physiological recordings

Whole-cell current clamp recordings were made using patch electrodes with tip resistances ranging from 6–10 MΩ; signals were amplified using an Axopatch 200B amplifier and were acquired through an ITC-16 interface using software written in Igor Pro (Wavemetrics, Lake Oswego, Oregon; Okawa et al 2010). Mice were dark adapted for several hours and euthanized in accordance with protocols approved by the IACUC of the University of Southern California. The pipette internal solution contained (in mM): 125 K-Aspartate, 10 KCl, 10 HEPES, 5 NMG-HEDTA, 0.5 CaCl₂, 1 ATP-Mg, 0.2 GTP-Mg with pH of ~7.3 and osmolality of ~280 mOsm; measured liquid junction potential was approximately –10 mV. Prior to electrical stimulation, the resting membrane potential and baseline activity were recorded for each cell. The resting potential was also recorded between stimulus trials to ensure consistency of evoked responses; if baseline potential deviated permanently from its initial value, the recording was stopped and no further data was collected from that cell.

2.4 Electrical stimulation and response characterization

The external electrode used for stimulation was a 75 µm diameter Pt-Ir disk electrode positioned approximately 50 µm from the targeted RGC soma. A Sutter MP-285 micromanipulator allowed precise positioning of the RGC soma relative to the stimulating electrode. Relative position was set by moving the edge of the stimulating electrode until it aligned with the center of the RGC, then using the micromanipulator to move the stimulating electrode 50 µm laterally. Thus, there was a 50 µm separation between the edge of the electrode and the center of the cell. Using a custom recording chamber (figure 1), the return electrode was placed beneath the retina on the photoreceptor side while the extracellular electrode was positioned above the ganglion cell layer. The placement of the ground was chosen to maximize the amount of current flowing through the retina rather than allowing current to shunt through the solution to ground. This ground placement best simulates current clinical devices that have current return outside the eye. Multi-Channel Systems (Reutlingen, Germany) stimulus software was used to deliver current pulses through the extracellular electrode. Charge-balanced biphasic current pulses (cathodic-phase

first) were delivered at 10 Hz (interpulse period = 100 ms) for 4 different pulse durations: 100 μ s, 200 μ s, 500 μ s, and 1 ms; stimulus amplitudes were randomized within pulse duration groups.

Responses to all delivered stimuli were fit with a strength-duration curve unique to each cell. Threshold for each cell was defined as the current level at which a spike was evoked in at least 50% of delivered pulses. The physiological measurements recorded both evoked action potentials to stimulation and the stimulus artifact (biphasic square pulse). In most cases, the stimulus artifact did not interfere with detection of the evoked spike; however, in some cells, the spike was partially hidden in the stimulus artifact. To remove the stimulus artifact, raw traces where a spike was not evoked were averaged, then the average trace was subtracted from individual traces that did evoke an action potential. Since baseline spontaneous rates varied between cells, the strength-duration curves were adjusted to include each cell's spontaneous rate. For cells with spontaneous rate > 0 , the strength-duration baseline was a non-zero value that represented the resting spike rate; threshold was defined as the midpoint between [100% probability of evoking a spike - baseline spontaneous rate %]. An evoked action potential was defined as a spike whose peak occurred within 3 ms of the stimulus onset. Strength-duration curves were fit using Lapicque's equation (Lapicque 1931):

$$I_{th} = \frac{I_{rh}}{1 - \exp(-\frac{PD}{\tau_{SD}})}, \quad (1)$$

where I_{rh} represents the rheobase current, PD is the pulse duration, and τ_{SD} is the strength-duration time constant.

2.5 Data analysis

Statistical analysis was performed using SAS (Statistical Analysis System) software and Matlab. WT and rd10 parameters included for analyses were stimulation threshold at pulse widths ranging from 100 μ s to 1 ms, soma diameter, response latency, resting membrane potential (V_{rest}), baseline spontaneous rate, membrane potential periodicity. Interspike interval data were fit to exponential distributions using maximum likelihood estimation. Data points were identified as outliers using SAS residual analysis by comparing the jackknife residual significance to the newly adjusted significance level α/n ; any data points that were statistically significant outliers were excluded from the analysis. One WT RGC was excluded from our analyses based on this criterion.

3. Results

The aim of this study was to determine how ganglion cell physiology influences threshold for electrical stimulation in WT versus rd10 retinas. To compare stimulation thresholds in different mouse lines and among cells with different intrinsic physiological properties, we first established that our method for measuring threshold was consistent from trial-to-trial.

3.1 Similar mechanisms of action potential generation in WT and rd10 mice

Action potentials were observed following electrical stimulation as described in Methods. We classified an action potential as stimulus-evoked if it occurred within 3 ms of stimulus

onset. An action potential occurring outside the 3 ms window, even if it had been evoked consistently at the same time point after stimulus onset, was not considered a threshold response. Such delayed responses can occur if the cell fires spikes in bursts, or if the cell is not stimulated directly but rather through network activation (Boinagrov et al 2014). Our main interest was direct stimulation, which should predominate given the strength and duration of our stimuli, and our electrode placement. We first determined whether stimulus amplitude affected the timing precision of action potentials generation. Figure 2 shows representative traces from WT and rd10 retina stimulated using different current amplitudes. The two peaks observed in each trace reflect the timing of the stimulus artefact (first peak) and the action potential (the second peak). The timing of the action potential (denoted by *) appeared similar across stimulus amplitudes. Based on these results, we conclude that response latency does not depend on stimulus amplitude.

Since electrodes are designed and tuned clinically to deliver current pulses near RGC stimulation threshold, we asked whether WT and rd10 RGCs displayed differences in response latency at threshold. To control for the possibility that different response artefacts in rd10 and WT retina could obscure differences in the timing of evoked action potentials, we compared responses at threshold after stimulation artefacts were removed (Figure 3). Stimulus artefacts were removed individually for each cell by subtracting traces without an action potential from traces with an action potential. Specifically, 15–20 AP-free traces were averaged, resulting in a single 'average AP-free trace', which was then subtracted from each trace that evoked a response at that stimulus amplitude. A separate average AP-free trace was created for each stimulus level. The tight clustering of time-to-peak for evoked action potentials suggests that near a cell's stimulation threshold, action potentials were likely evoked by similar mechanisms. We explored the possibility that differences in genotype could lead to differences in spike timing, but instead the average response latencies for WT and rd10 RGCs were not significantly different from each other (Table 1).

3.2 Comparison of intrinsic physiological properties and stimulation thresholds in WT and rd10

Prior to evaluating the potential effects of retinal degeneration on RGC response, it was essential to assess the response properties of normal RGCs to stimulation and to evaluate the effects of any structural or physiological properties on threshold. In a pilot study, we investigated the effect of RGC soma diameter on stimulation thresholds in WT mice (Cho *et al* 2011). The results showed a negative correlation between WT threshold and soma size, where larger diameter cells required lower threshold currents. Applying this same analysis to rd10 cells revealed no correlation between threshold and soma diameter (figure 4A). However, stimulation thresholds in rd10 RGCs were highly variable and elevated compared to WT cells (figure 4B).

Since the response latencies of rd10 RGCs were identical to WT cells (Fig 3, Table 1), we concluded that passive conduction of electrical current was not a significant source of physiological difference between these two populations. To study the mechanisms behind the physiological differences we have observed, we examined several other intrinsic

properties of RGCs. Resting membrane potential V_{rest} and baseline spontaneous rate were measured by recording passively from the cell for ~30 seconds without stimulation.

Stimulation thresholds followed similar trends in both WT and rd10 RGCs, where more depolarized cells had decreased thresholds (Fig 5A); this correlation was statistically significant for rd10 cells ($p = 0.0114$). WT and rd10 RGCs did not display significantly different V_{rest} values (figure 5A). There was also no significant correlation between threshold and spontaneous rate for either WT or rd10 cells, or in mean differences in spontaneous action potential rate between the two groups (figure 5C,D). A noteworthy observation was the wide range of baseline spontaneous rates observed in WT and rd10 cells; this range included cells that were very quiet at rest as well as cells that were quite active.

3.3 Differences in threshold and V_{rest} between WT and rd10 RGCs arise when categorized by spontaneous rate

Due to the appreciable variability in spontaneous firing rate, WT and rd10 RGCs were categorized into two groups based on their spontaneous rate. Cells with baseline rates greater than their respective group means were classified as 'high rate' cells while those with rates below the mean were classified as 'low rate' cells. Differences in threshold, V_{rest} , and spontaneous rate between the two rate groups for WT and rd10 cells are shown in table 2. In WT retina, stimulation thresholds and resting membrane potential values were comparable for low ($n = 12$) and high rate cells ($n = 6$). However, rd10 thresholds were lower for cells displaying higher rates of spontaneous activity; these high rate cells ($n = 19$) also displayed membrane potentials that were more depolarized than low rate cells ($n = 40$).

Comparisons of threshold, V_{rest} , and spontaneous rate between low rate and high rate WT and rd10 RGCs are shown in figure 6. The range of spontaneous rates between WT and rd10 cells in both rate groups were comparable and average rate values were not significantly different within groups (figure 6C). In low rate RGCs, stimulation thresholds in rd10 cells were considerably higher than thresholds in normal retina, though resting potentials were not different (figure 6A1,B1). However, in high rate cells, thresholds were comparable between WT and rd10 cells even though rd10 resting potentials were more depolarized (figure 6A2,B2).

3.4 Rd10 RGCs display distinct modes of baseline spontaneous behavior

Classification of cells by spontaneous rate group revealed differences in threshold and V_{rest} in both groups that were not apparent during a combined analysis of these same parameters. In particular, threshold differed significantly between WT and rd10 groups before separation by spontaneous rate, but only differed significantly for low rate groups after separation. Conversely, resting membrane potential did not differ between WT and rd10 groups before separation, but differed significantly for high rate WT versus rd10 cells after separation by spontaneous rate.

We further examined the characteristics of each cell's baseline spontaneous behavior using their interspike interval (ISI) histograms. The resting spontaneous action potential rate and corresponding ISI histograms for four representative ganglion cells - one WT and three rd10

- are shown in figure 7. The ISI histogram for the high rate WT cell (figure 7A) peaked near zero and declined monotonically toward a long tail. This ISI distribution was well fit by an exponential distribution, which would result if the cell fired spikes randomly with a constant probability (i.e., a Poisson process). All WT RGCs displaying high spontaneous rates had ISI histograms similar to that shown in figure 7A2.

Some rd10 RGCs were well fit by an exponential distribution, but others deviated from this pattern due to a second peak in the ISI distribution near 100 ms, suggesting a mode of periodic firing at around 10 Hz. There is no standard statistical test for periodicity, but it has a characteristic appearance and is a known property of the disease model (Margolis, 2014). As such we applied a quantitative criterion by which cells could be classified as periodic: the ISI distribution of a periodic cell contained a second peak between 50 and 150 ms, at least 10 ms wide, and at least twice the amplitude of the best-fitting exponential distribution. Non-periodic spiking rd10 RGCs were high rate cells that did not have any apparent patterns in spontaneous activity and had ISI histograms similar to high rate WT cells ($n = 6$) (fig. 7B). A subset of high rate rd10 RGCs displayed periodicity in their baseline spiking behavior ($n = 10$) (fig. 7C). Additionally, we observed cells with low spontaneous spike rate and apparently periodic oscillations in subthreshold membrane voltage.

To assess whether this low-rate population of rd10 cells exhibited the same periodicity as the high-rate population, we assigned “spike times” for these recordings based on the peaks in membrane potential (figure 7D). Although the spontaneous spike rates for these cells were low, there was periodicity in their membrane fluctuations that resulted in secondary peaks similar to those seen in periodic spiking RGCs (fig. 7D2). Secondary peaks were not present in the ISI histograms of non-periodic high rate cells in both WT and degenerate retina. These results suggest that periodic oscillations occur in only a subset of rd10 cells.

Rd10 cells exhibited mainly three types of baseline activity: non-periodic spiking cells, periodic spiking cells, and low rate cells displaying subthreshold periodic membrane fluctuations (fig. 7B–D). There were also several rd10 RGCs with periodic membrane fluctuations that did not necessarily generate action potentials (fig. 7D) and were thus classified as low rate cells.

3.5 Periodicity and higher spontaneous activity contribute to decreased stimulation thresholds in rd10 RGCs

We compared the properties of spontaneous activity in rd10 retina in two distinct populations of RGCs - cells exhibiting periodicity in spontaneous spike rates and cells exhibiting no apparent patterns in spiking activity or subthreshold membrane fluctuations (non-periodic). To assess how periodicity in resting activity relates to excitability and intrinsic RGC properties, we compared thresholds, V_{rest} , and spontaneous rates between non-periodic and periodic rd10 ganglion cells (table 3). Rd10 cells exhibiting periodicity ($n = 10$) displayed significantly lower stimulation thresholds, more depolarized membrane potentials, and higher spontaneous rates than non-periodic RGCs.

The cells with the lowest stimulation thresholds among rd10 RGCs exhibited high spontaneous rates and periodic firing. When rd10 RGCs with these properties were

compared to all WT data, there was no significant difference in stimulation thresholds (figure 8A). However, despite having comparable thresholds to normal retina, these high rate periodic rd10 cells remained intrinsically different from WT cells, with depolarized membrane potentials and elevated spontaneous activity.

4. Discussion

Our results are the first to investigate the physiological mechanism for increased electrical stimulation threshold in the degenerated retina. We found that RGCs in rd10 retina will have increased threshold if the RGCs are not spontaneously active. However, RGCs that spike at high rate or have periodic membrane fluctuations have thresholds similar to wild-type RGCs, although the baseline activity of the RGCs is different from wild-type.

Elevated and highly variable stimulation thresholds in degenerate retina have been observed both in clinical and animal studies (Ahuja 2013, Jensen 2012, Keseru 2012, Chan 2011). These observations may be influenced by a number of factors including the extent of retinal circuitry reorganization, changes in the cell density, differences in measurement methodology, and possibly cortical reorganization. As examples, de Balthasar et al (2008) noted that electrode proximity to the retina correlated with threshold and Chan et al (2011) correlated threshold increase with a decrease in RGC density. In contrast, a study in P23H rat by Sekirnjak et al. using multi-electrode array recordings found that thresholds between degenerate and normal RGCs were not significantly different (Sekirnjak 2009). However, the average spontaneous firing rates for P23H RGCs were considerably higher compared to normal retina, similar to our findings. It is possible that the subset of P23H cells studied were similar to the high rate RGCs we found in rd10 and thus resulted in equivalent thresholds in P23H and WT rat RGCs. Collectively, these findings reinforce the need to understand better the physiological basis for elevated thresholds in degenerate retina to inform continued advances in sight restoration.

We found that thresholds in rd10 RGCs not only were significantly elevated compared to normal RGCs but also were highly variable. Investigating the source of this variability led to the finding that rd10 RGCs threshold elevation was strongly dependent on the physiological properties of the RGC. Specifically, rd10 RGCs that exhibited either increased spiking or periodic membrane fluctuations tended to not have elevated thresholds. The physiology of RGCs during degeneration has gained increasing attention in recent years. Multiple models of retinal degeneration demonstrate an increase in spontaneous activity of RGCs (Yee 2013, Stasheff 2011, Sekirnjak 2011, Margolis 2008, Stasheff 2008). Various groups have reported RGC membrane oscillations as a prominent feature of retinal degeneration (Trenholm 2012, Sagdullaev 2012, Menzler 2011, Stasheff 2011, Margolis 2008, Ye 2007). For rd10 RGCs in our study, we observed membrane periodicity in ~22% (13/59) of recorded cells, where 10/13 were categorized as high rate periodic RGCs. Periodicity in membrane fluctuations was measured in a subset of high rate rd10 cells and not every high rate RGC displayed periodic behavior. Although the source of aberrant activity in RGCs may differ, it is evident that the loss of photoreceptors causes considerable changes to ganglion cell physiology. WT RGC threshold was largely unaffected by differences in spontaneous activity (Fig 5C and Table 2).

The age of rd10 mice used in this study and condition of the retina represent an intermediate stage of retinal degeneration. By P45 a single row of aberrant cones remains, and bipolar dendrites are modified; by P60, most cones are gone (Gargini et al., 2007). As such, tissue of this age range appropriately models the retina of patients that receive retinal implants (typically patients with light perception vision at best). From a clinical and engineering perspective, the decreased thresholds observed in high rate rd10 RGCs maybe promising. However, it is unknown whether this increased spontaneous activity is stable and persists through later stages of degeneration. If spontaneous activity eventually decreases, will thresholds for these cells subsequently rise? When comparing spontaneous activity with age in rd10 mice, Stasheff et al. observed an initial increase in baseline rate, peaking around P50, followed by a slower decline in spontaneous activity in older animals. Similarly, in P23H rat, Sekirnjak et al. observed an increase followed by slow decrease in spontaneous rate with age in OFF RGCs. The periods of increased activity in both studies coincided with intermediate stages of degeneration for the respective rodent models.

Our findings suggest that degenerate high rate RGCs in this intermediate stage of degeneration will have decreased thresholds which are preferential from a clinical perspective. These experiments were conducted in rd10 mice within a narrow age window so investigating the influence of spontaneous activity on thresholds for different age groups would provide additional insight as to how these properties are interrelated. If spontaneous activity and thresholds are indeed correlated, treatments to slow the progression of inner retinal degeneration and thus maintain excitability could potentially be highly beneficial for prosthesis patients. Animal studies using various retinal neuroprotective therapies, including neurotrophic factors and pharmacological targeting of ion channels, have shown varying degrees of success in preserving ganglion cells and inner retinal neurons (Yamazaki 2002, LaVail1992, Sieving 2006, Husain 2012). These neuroprotective therapies might not necessarily be treating the source of degeneration but may help prolong inner retinal health to improve the effectiveness of sight restoration therapy.

5. Conclusions

RGC physiology is altered by the degeneration process and plays a critical role in determining the response of the retina to electrical stimulation. Aberrant RGC physiology, characterized by higher spontaneous spike rate and/or periodic membrane fluctuations, maintained electrical stimulation threshold at a level comparable to wild type RGCs, in spite of the altered physiology. Retinal prosthesis system design must account for altered and varying physiological conditions of the retina as degeneration progresses.

Acknowledgments

This work was supported by the National Eye Institute (R01EY022931), National Science Foundation (EEC-0310723), and Research to Prevent Blindness.

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Figure 1. Schematic cross-section of the recording setup. Whole mount retina with external electrode positioned above RGC layer and ground electrode below photoreceptor layer. Whole-cell patch clamp RGC recordings were made both before and during electrical stimulation. Retinal cell types: amacrine cell (AC), bipolar cell (BC), ganglion cell (GC). Image not to scale.

Figure 2.

Evoked action potentials at various current levels for representative WT and rd10 RGCs.

Threshold for each cell (the current required to generate action potentials in 50% of trials)

denoted by black arrows. Stereotyped waveform of spikes evoked by both subthreshold and suprathreshold stimulation (denoted by asterisks) centered within 3 ms window of stimulus onset.

Scale bars: 50 mV vs. 2 ms (WT); 100 mV vs. 2 ms (rd10).

Figure 3.

Response latency of evoked action potentials to extracellular 500 μ s biphasic (cathodic-first) pulse stimulation (denoted by gray arrows) at stimulation threshold. (A,B) Overlaid traces (N=11) showing response latencies for representative WT and rd10 RGCs at threshold for each cell (stimulus artefact subtracted to reveal evoked spikes). (C) Traces from panel B with before artifact removal (D) Traces of artifact only, used to make an average trace for artefact subtraction. The same scale applies to both C and D. Thresholds for these cells were 20 μ A (WT) and 36 μ A (rd10)

Figure 4.

Relationship between threshold and soma diameter in WT and rd10 RGCs. (A) Statistically significant correlation between threshold and soma size in WT cells ($p = 0.0309$, $n = 14$) but not rd10 cells ($p = 0.5871$, $n = 27$). Slope of linear regression for WT correlation was $-0.95 \mu\text{A}/\mu\text{m}$. (B) Rd10 thresholds significantly higher than WT (** $p < 0.001$).

Figure 5.

Relationship between stimulation threshold and intrinsic RGC properties. (A) Correlation between threshold and resting membrane potential (V_{rest}) for WT and rd10 cells, statistically significant only for rd10 cells ($p = 0.0114$). Slope of linear regression for rd10 correlation was $-1.8 \mu\text{A}/\text{mV}$. (B) No significant difference in V_{rest} between WT and rd10 cells. (C) No significant correlation between threshold and baseline spontaneous rate for either WT or rd10 cells. (D) No significant difference between WT and rd10 spontaneous rates.

Figure 6.

Comparisons of RGC threshold and intrinsic properties, categorized by rate group. WT and rd10 cells classified into groups of low spontaneous spike rate (left column) and high spontaneous spike rate (right column). (A) Significant difference in thresholds only between WT and rd10 cells with low spontaneous activity (A1). (B) V_{rest} significantly more depolarized in rd10 cells with high spontaneous activity (B2) ($p = 0.0290$). (C) Comparable range of spontaneous rates between WT and rd10 RGCs in both low rate and high rate groups. Significance: (**) $p < 0.001$ and (*) $p < 0.05$. Error bars show standard deviation.

Figure 7.

Baseline activity and interspike interval (ISI) histograms. (A) Representative high rate WT RGC and corresponding ISI histogram. Total of $n = 6$ high rate WT RGCs. (B-D) Physiologically distinct rd10 RGCs and corresponding ISI histograms. Total of $n = 59$ rd10 RGCs. (B) Rd10 cells with no apparent patterns in spontaneous firing (B2, $n = 6$), similar to WT cells (A2). (C) Rd10 cells with periodic baseline firing illustrated by secondary peak at ~100 ms in the ISI histogram (C2, $n = 10$). (D) Rd10 cells with periodic membrane fluctuations and spike rates too low to generate reliable ISI distributions ($n = 3$);

corresponding “ISI” histogram generated from peaks in membrane fluctuations; secondary peak ~120 ms. Time bin size 5 ms.

Figure 8.

Comparison between WT and high rate, periodic rd10 RGCs. (A) Comparable stimulation thresholds ($p = 0.89$). (B) Membrane potential (V_{rest}) significantly more depolarized for high rate, periodic rd10 cells ($p = 0.00070$). (C) Significantly higher spontaneous rates for high rate, periodic rd10 cells ($p = 0.0015$).

Table 1

Mean, standard deviation, and Student's t-test values for WT ($n = 19$) and rd10 ($n = 59$) RGC parameters: stimulation threshold, response latency, resting membrane potential, and baseline spontaneous rate.

	threshold (μ A)	response latency (msec)	Vrest (mV)	spontaneous rate (Hz)
WT	mean $\pm$ SD	1.8 $\pm$ 0.31	-63 $\pm$ 4.7	3.6 $\pm$ 5.2
Rd10	41 $\pm$ 19.4	1.8 $\pm$ 0.27	-60 $\pm$ 6.5	4.4 $\pm$ 7.6
	p-value	0.81	0.16	0.74

Table 2
Mean, standard deviation, and Student's t-test values for WT and rd10 RGC parameters, categorized by rate group.

Rate group		threshold (μA)	Vrest (mV)	spontaneous rate (Hz)
WT	Low	25 ± 7.5	-64 ± 3.3	0.61 ± 1.1
	High	21 ± 9.1	-59 ± 5.9	10 ± 4.2
	p-value	0.41	0.059	0.0023
Rd10	Low	49 ± 17.2	-62 ± 5.7	0.72 ± 1.1
	High	28 ± 15.1	-55 ± 3.8	12 ± 9.5
	p-value	0.0021	< 0.0001	< 0.0001

Table 3

Mean, standard deviation, and Student's t-test values for threshold, resting potential, and spontaneous rate between non-periodic and periodic rd10 RGCs.

		threshold (μ A)	Vrest (mV)	spontaneous rate (Hz)
Periodicity	Absent	50 $\pm$ 17	-62 $\pm$ 4.8	2.8 $\pm$ 5.4
	Present	26 $\pm$ 15	-57 $\pm$ 3.8	9.2 $\pm$ 5.2
			p-value	0.0069