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Differential intrinsic firing properties in sustained and transient mouse alpha RGCs match their light response characteristics and persist during retinal degeneration

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Conflict of interest

The authors declare no competing interests.

Abstract

Retinal ganglion cells (RGCs) are the neuronal connections between the eye and the brain conveying multiple features of the outside world through parallel pathways. While there is a large body of literature how these pathways arise in the retinal network, the process of converting presynaptic inputs into RGC spiking output is little understood. In this study, we show substantial differences in the spike generator across three types of alpha RGCs in female and male mice, the αON sustained, αOFF sustained and αOFF transient RGC. The differences in their intrinsic spiking responses match the differences of the light responses across RGC types. While sustained RGC types have spike generators that are able to generate sustained trains of action potentials at high rates, the transient RGC type fired shortest action potentials enabling it to fire high-frequency transient bursts. The observed differences were also present in late-stage photoreceptor-degenerated retina demonstrating long-term functional stability of RGC responses even when presynaptic circuitry is deteriorated for long periods of time. Our results demonstrate that intrinsic cell properties support the presynaptic retinal computation and are, once established, independent of them.

Significance Statement

Spiking output from retinal ganglion cells (RGCs) has long been thought to be solely determined by synaptic inputs from the retinal network. We show that the cell-intrinsic spike generator varies across RGC populations and therefore that postsynaptic processing shapes retinal spiking output in three types of mouse alpha RGCs (αRGCs). While sustained αRGC types have spike generators that are able to generate sustained trains of action potentials at high rates, the transient αRGC type fired shortest action potentials enabling them to fire high-frequency transient bursts. Computational modeling suggests that intrinsic response differences are not driven by dendritic morphology but by somatodendritc biophysics. After photoreceptor degeneration, the observed variability is preserved indicating stable physiology across the three αRGC types.

Introduction

The primary task of the visual pathway is to manipulate and convey information between the eye and the brain. Conceptually, this information pipeline can be divided into early retinal processing and higher order processing taking place in the brain. From a retinal perspective, many processing steps are performed along the way from photoreceptors to retinal ganglion cells (RGCs), however action potentials first generated in RGCs are the only and therefore critical output to the brain. Over the last decade, classification of RGCs has made substantial progress leading to classification schemes comprising 20-40 RGC types, depending on species and classification methods (Baden et al., 2016; Bae et al., 2018; Goetz et al., 2022).

Historically, RGC output was mainly thought to be determined by presynaptic inputs and the conversion of these (analog) inputs into (digital) spiking output has not been in the focus of research. Within the last two decades, however it became clear that also the intrinsic properties, i.e., the spike generating machinery including ion channels, and how they affect the conversion of presynaptic inputs into postsynaptic outputs in RGCs play an important role in retinal signal processing. Different mechanisms were identified to contribute to this postsynaptic shaping of signals, starting from dendritic processing (Fohlmeister and Miller, 1997; Velte and Masland,

1999; Oesch et al., 2005; Ran et al., 2020), to somatodendritic biophysics (O'Brien et al., 2002; Wong et al., 2012; Emanuel et al., 2017; Milner and Do, 2017) as well as processing in the proximal portion of the axon, the so-called axon initial segment (AIS) (Werginz et al., 2020a; Wienbar and Schwartz, 2022).

Alpha RGCs (αRGCs) are a subgroup of RGCs in mammalian retina which are well described in terms of their responses to light, presynaptic partners, anatomical features and genetic identity (Peichl et al., 1987; Feng et al., 2000; Bleckert et al., 2014; Baden et al., 2016; Krieger et al., 2017). αRGCs can be divided into two types, transient and sustained cells, for both the ON and OFF pathway (Krieger et al., 2017). In the mouse retina, αRGCs are characterized by their large somas, their large dendritic arbors and their brisk responses to spots of light; especially soma size and light responses have been used regularly to identify αRGCs without performing time-consuming anatomical or genetic analyses. Although αRGCs have been in the spotlight of mouse retinal research for two decades, only little research exists that compared the spike generator of αRGCs systematically. For example, Margolis et al. (Margolis and Detwiler, 2007) compared αON sustained (αONs), αOFF sustained (αOFFs) and αOFF transient (αOFFt) RGCs and found differences in their spontaneous activity and rebound excitation which could be attributed to different biophysical properties in their sodium and calcium channels. A follow-up study of the same group confirmed that general spiking properties were roughly conserved in a retinal degeneration model, however no in-depth analysis of action potential generation and spike properties was performed (Margolis et al., 2008). More recently, Wienbar and Schwartz (Wienbar and Schwartz, 2022) found distinct differences between one type of non-αRGCs and αRGCs that transform similar synaptic inputs into highly different spiking outputs.

Based on previous studies on the spike generator of RGCs (Dhingra and Smith, 2004), we investigated the spiking output of mouse αRGCs and compared their intrinsic spiking properties. We were interested in whether the intrinsic properties are tuned to the responses generated by visual stimulation, e.g., are the biophysical features of sustained αRGCs optimized to process the more sustained synaptic inputs they receive? By using patch-clamp electrophysiology, imaging of cell morphology and computational modeling, we demonstrate distinct intrinsic differences between αONs, αOFFs and αOFFt RGCs. The observed differences can be used to cluster cell types solely based on their intrinsic firing patterns. After long periods of photoreceptor degeneration, we still found distinct firing patterns in αRGCs indicating that even strongly altered presynaptic inputs do not lead to substantial changes in RGC physiology.

Methods

Electrophysiology

The experimental procedures for preparation of the *ex-vivo* retinae were approved by the Center for Biomedical Research, Medical University Vienna. All breeding and experimentation were performed under a license approved by the Austrian Federal Ministry of Science and Research in accordance with the Austrian and EU animal laws (BMWFW-66.009/0403-WF/V/3b/2014). We used either wild-type (C57BL/6) or retinal degeneration 10 (rd10, (Cojocaru et al., 2022)) mice with parvalbumin-positive (PV^+) RGCs expressing the Channelrhodopsin-2-Enhanced Yellow Fluorescent Protein (ChR2-EYFP). EYFP expression allowed us to narrow down the ~40 RGC types in the mouse retina to 8 types including three out of the four αRGCs (αONs, αOFFs, αOFFt) (Farrow et al., 2013), the αON transient type was not in the focus of this study. 18 wild-

type retinae from mice aged p48-p143 (mean p97; 10 females, 8 males) and 8 rd10 retinae from mice aged p193-p227 (mean p206; 4 females, 4 males) were used in this study.

Retinal dissections were performed following previously described protocols at Medical University of Vienna (Corna et al., 2024). In brief, after cervical dislocation a small mark was made at the dorsal pole of the eyeball and subsequently eyeballs were harvested. After removal of the cornea the lens was extracted and eyeballs were transported to TU Wien in oxygenated Ames medium buffered to pH 7.4 (Sigma-Aldrich or US Biological). The eyeball was cut into a nasal and temporal portion using the visible mark at the dorsal pole. Subsequently the retina was isolated and the vitreous carefully removed. After dissection, the retina was mounted photoreceptors side down on a filter paper in a petri dish and perfused with oxygenated Ames medium at ~33-35° Celsius for the duration of the experiment.

αRGCs were targeted by their large somas (>~14µm), identified by their characteristic light responses and clustered into αONs, αOFFs and αOFFt cells (Pang et al., 2003; Margolis and Detwiler, 2007; van Wyk et al., 2009; Krieger et al., 2017; Warwick et al., 2018). In a subset of recordings, we used fluorescence imaging to confirm that targeted RGCs were PV^+. Cell location on the retinal surface was tracked as either nasal or temporal, RGCs were targeted in the mid-periphery to avoid axon bundles close to the optic nerve head. Small holes were made in the inner limiting membrane to obtain access to RGC somas. Spiking responses were obtained using whole-cell patch recordings. Intracellular solution consisted of 125mM K-gluconate, 10mM KCl, 10mM Hepes, 10mM EGTA (all Carl Roth), 4mM Mg–adenosine 5′-triphosphate, and 1mM Na–guanosine 5′-triphosphate (all Sigma-Aldrich).

Data were recorded by an EPC10 USB amplifier (HEKA) at a sampling rate of 100kHz, stimulus control and data acquisition were performed in PatchMaster Next (HEKA). After break-in, the pipette series resistance was compensated with the bridge balance circuit of the amplifier. Recorded membrane voltage was corrected for the liquid junction potential (−8mV). Current injections into the soma of RGCs were performed at a membrane voltage of approximately -65mV which was controlled by the low frequency voltage clamp mode of the amplifier; the time constant of the procedure was set to 100 seconds to avoid interference with the spiking activity (in the range of milliseconds) of the cell. Stimuli consisted of short (3ms) and long (500ms) current injections; stimulus amplitude ranged from 10 to 400pA for threshold search experiments and from -500 to 1000pA for experiments investigating repetitive spiking. Spike-triggered average (STA) was determined by injecting white noise current into RGC somas. The standard deviation of the white noise was adjusted to elicit spiking activity in the range of 2-6Hz and ranged between 25 and 200pA. The amplitude of the white noise was updated every 0.2ms. Recording sampling rate for STA sequences was 40kHz. 400-1200 action potentials were used for STA computations.

Visual stimulation of the retina

Light stimuli were projected onto the retina by a micro-OLED display (Sony, 1920x1200px) through the 40x objective of the microscope (Olympus BX51WI). Bright and dark spots, 225µm in diameter, at 100% Weber contrast were projected onto the retina from a uniform gray background (~0.135mW/mm^2). Stimulus duration was 1 second, each stimulus was repeated 5 times and breaks between stimuli were 1.5 seconds long. Light stimuli were controlled via GEARS (Szécsi et al., 2017), to guarantee correct alignment of multiple trials the precise timing of the on- and offset of the stimulus was tracked by the recording system. Light power during

visual stimulation was multiple orders of magnitude lower than necessary to optogenetically activate RGCs.

Immunohistochemistry, confocal imaging and anatomical tracings

During patch-clamp experiments RGCs were filled with Neurobiotin Tracer (Vector Laboratories), retinas were fixed in 4% PFA (Invitrogen) for 30 minutes at room temperature and washed 3x with PBS (10x, Gibco) for 15 minutes. For permeabilization, the retina was treated with 0.5% PBS-TX (10x PBS with 0.5% Triton X-100, Sigma-Aldrich) for 20 minutes at room temperature. Afterwards, the retina was blocked with 3% Bovine Serum Albumin (BSA, Sigma-Aldrich) + 0.5% TX for 4 hours at 4°C. After removing the blocking solution, the retina was stored in 1% BSA+0.5% TX at 4°C for one overnight. The next day, the retina was washed 3x with 0.5% PBS-TX for 15 minutes. A reconstituted Streptavidin Alexa Fluor 405 conjugate (Invitrogen) was 1:200 diluted in 3% BSA+0.5% TX solution and added to the retina for one overnight incubation at 4°C. The next day, the retina was washed with 3x with 0.5% PBS-TX for 15 minutes and fixed with 4% PFA for 10 minutes at room temperature. PFA was washed out 3x with 10x PBS twice for 15 minutes. The retina was finally mounted and coverslipped with ProLong Glass Antifade Mountant (Invitrogen).

RGCs were imaged with a confocal laser scanning microscope (Leica Stellaris 5) at 40x magnification using the Leica Application Suite X (LAS X). The full RGC morphology including soma, dendrites, and axon was imaged at a resolution of 0.38x0.38x0.3µm (x/y/z).

Accurate 3-dimensional tracings of dendritic trees were either obtained from previous studies (Raghuram et al., 2019; Werginz et al., 2020a) (available on www.neuromorpho.org (Ascoli et al., 2007)) or by tracing confocal images of filled RGCs in the TREES toolbox (Cuntz et al., 2010) in Matlab (Mathworks).

Computational modeling

Multi-compartment models of αRGCs were based on 3-dimensional anatomical tracings. The NEURON simulation environment version 8.0 (Hines and Carnevale, 1997) controlled via Python was used to solve the arising system of differential equations of the multi-compartment model. Spatial discretization of dendrites, soma, proximal and distal axon was set to 5, 1, 2 and 10µm, respectively; simulation time step was set to 10µs. While dendritic tree geometries varied between model RGCs, a standard somatic and axonal anatomy was used in all cells. The soma was modeled as a sphere 20µm in diameter, the axon was segmented into an axon hillock (L=24µm, d=3-1µm taper), an AIS (L=25µm, d=1-0.6µm taper) and a distal axon (L=1000µm, d=1µm). Ion channel dynamics were based on the RGC membrane model from Fohlmeister (2010) with the addition of a hyperpolarization-activated current (Guo et al., 2016) to more accurately resemble responses arising from hyperpolarizing pulses. Model temperature was set to 30° Celsius similar to experimental conditions. Intracellular resistivity and specific membrane capacitance were set to 140Ω*cm and 1µF/cm^2, respectively. Ion channel densities can be found in Table 1. Additionally to ion channels an Ornstein-Uhlenbeck noise term with zero mean was added to the model. This allowed to compute modeled thresholds in the same way as in experiments, i.e., 10 repetitions of increasing amplitude and threshold was defined as the amplitude at which 66% of trials resulted in an action potential.

Data analysis & statistics

Recorded data were loaded and analyzed in Matlab (Mathworks). Spike timing was detected as the depolarization (positive) peak at least crossing -25mV and having a minimum depolarization rate of 25mV/ms as spikes at small depolarization rates were shown not to be propagated along the axon of RGCs (Wienbar and Schwartz, 2022). Firing rate was computed by pooling responses from multiple trials (≥3) and subsequent convolution with a 50-ms sliding window. In order to determine membrane polarization levels without spiking activity, we filtered the data with a 3rd order Butterworth filter with a cut-off frequency of 20Hz.

Statistical analyses were performed in Matlab (Mathworks) or Python. We measured linear correlation between two variables using Pearson's correlation coefficient. We used a two-sample t test, Welch's t test, or Wilcoxon rank sum test for comparison of two groups depending on the distribution of the data. For comparison of multiple groups One-way ANOVA used Tukey's honestly significant difference post hoc test. Significance levels were set as follows: * $p<0.05$, ** $p<0.01$, and *** $p<0.001$. Numerical values are presented as mean ± one standard deviation except otherwise noted. Box plots use standard notation (first quartile, median, and third quartile).

Principal component analysis (PCA) and k-means clustering were performed in Matlab (Mathworks). PCA was performed on 12 parameters extracted from electrophysiological recordings: current threshold (pA), voltage threshold (mV), spike amplitude (mV), spike width (ms), maximum rate of hyperpolarization (mV/ms), maximum rate of depolarization (mV/ms), input resistance (MOhm), peak firing rate (Hz), maximum sustained firing rate (Hz), rebound firing rate (Hz), break amplitude (pA) and break voltage (mV). Similarity between ground truth data, i.e., cell type determined by visual stimulation, and clustering based on intrinsic spiking properties was determined by the Adjusted Rand Index with a value of 1 indicating a perfect match (Rand, 1971).

Results

Intrinsic responses of αRGCs match their light responses

It has long been thought that light responses of RGCs are mainly driven by presynaptic inputs (Roska and Werblin, 2001), e.g., more sustained inputs in αONs and αOFFs RGCs lead to more sustained spiking outputs than the transient inputs in αOFFt RGCs (Bleckert et al., 2014; Warwick et al., 2018). An increasing body of recent work shows that αOFFt RGCs exhibit significant differences in their intrinsic spiking responses which matched the range of transient to sustained light responses in the ventral and dorsal retina, respectively (Werginz et al., 2020a). The surprising result of differential postsynaptic processing of synaptic inputs within a single cell type (Werginz et al., 2020a) led us to investigate spiking properties in three different types of αRGCs to explore the possibility of different spiking properties in αONs, αOFFs and αOFFt RGCs.

We used whole cell patch-clamp recordings to measure light responses and responses to somatic current injections to identify potential differences in the spike generator of αRGCs. In Figure 1A (left) representative light responses of an αONs, αOFFs and αOFFt RGG are shown to illustrate sustained versus transient responses. While αONs cells were stimulated by a bright spot αOFFs and αOFFt RGCs were stimulated by a dark spot. Overlays of the normalized firing

rates over time for the three cell types (Figure 1A, right) summarize the pronounced differences between sustained and transient αRGCs as well as previously reported more subtle differences such as decay time constant of the firing rate across αONs and αOFFs RGCs (Krieger et al., 2017).

To investigate the spiking responses of αRGC, including the possibility of intrinsic spiking matching the light responses in the three αRGC types, we injected current into the soma for 500ms at stimulus amplitudes ranging from -500-1000pA (Figure 1B, left; stimulus amplitude is color-coded, stimulus timing is indicated by gray bars at the bottom of each plot). Baseline membrane voltage was held at approximately -65mV by the low frequency voltage clamp mode of the amplifier (see Methods).

For hyperpolarizing current amplitudes, we found visible differences between αRGCs. For example, as reported previously (Margolis and Detwiler, 2007), both αOFFs and αOFFt RGCs responded to membrane hyperpolarization to levels of ~-85mV with rebound spiking after offset of the pulse while αONs RGCs did not substantially increase their firing rate above spontaneous spiking after hyperpolarization offset. Peak rebound firing rates were >60Hz for all but two αOFF cells while all recorded αONs cells had peak rebound firing rates below 40Hz.

Spiking responses to depolarizing current injections are shown in Figure 1B (left), with small current injections (100pA) leading to firing rates slightly higher than baseline firing rates while larger amplitudes resulted in both higher peak and sustained firing rates (dark red responses in Figure 1B, left). At high levels of depolarization, failure of spiking is a result of depolarization block indicating that the normally fine-tuned interplay between mainly sodium and potassium channels during action potential generation is impaired (Bianchi et al., 2012; Kameneva et al., 2016; Milner and Do, 2017). In order to explore the maximum and sustained spiking properties of the three different αRGC types, we extracted the response at the depolarizing stimulus amplitude which led to maximum sustained firing rates. An increase of the stimulus (+100pA) from this amplitude led to a failure of action potential generation (breakdown) as we described in our previous work (Werginz et al., 2020a). Plotting the normalized maximum firing rates at this amplitude over time revealed peak firing rates at the onset of the current injections followed by a decrease to a plateau level within 300-500ms (Figure 1B, right). While αONs and αOFFs cells declined to sustained spiking levels that were slightly higher than half of the peak firing rate, the sustained-to-peak ratio in αOFFt RGCs decreased to levels of roughly 25-45% of peak firing rate (gray traces in Figure 1B, right). Figure 1C compares responses to light stimulation and current injections between αONs, αOFFs and αOFFt RGCs. Overlays of the mean normalized maximum firing rates over time from Figures 1A and B are plotted in the top and bottom panels of Figure 1C (colored shadings indicate ± one standard deviation) revealing similarities; sustained (αONs & αOFFs) cells were both more sustained during light stimulation as well as during current injections than αOFFt RGCs. This was quantified by comparing the sustained-to-peak ratio between the three cell types (Figure 1C, middle panels). Thereby, pronounced differences between sustained and transient cells were found for light stimulation (0.42±0.11 / 0.55±0.12 / 0.14±0.10 for αONs, αOFFs and αOFFt RGCs) as well as current injections (0.57±0.06 / 0.49±0.06 / 0.32±0.07 for αONs, αOFFs and αOFFt RGCs). While light and intrinsic responses did not match perfectly, a general trend towards more sustained intrinsic responses in sustained αRGCs was evident while αOFFt RGCs responses in a more transient fashion to intrinsic current injections.

Breakdown of spiking occurs at different stimulus amplitude and membrane voltage in αRGCs

Our finding that intrinsic spiking responses approximately match the light responses in three types of αRGCs led us to examine which stimulus amplitudes and membrane depolarizations resulted in spiking failure. Figure 2A illustrates a representative spike train in an αONs RGC at an amplitude of 800pA. The early phase of spiking, as shown in the firing rates in Figure 1, shows the highest firing rates followed by a phase of increasing inter-spike-intervals (ISI) while later the response plateaus at a steady firing rate and ISIs. When the stimulus amplitude was increased by 100pA the sustained spiking phase was interrupted leading to a breakdown of spiking (Figure 2A, inset).

To better understand the relationship between stimulus amplitude and voltage that led to breakdown of spiking we plotted the average sustained firing rate during the late phase of the 500ms stimulus (indicated in panel A) versus the applied stimulus amplitude, the normalized stimulus amplitude (I_{Stim}*R_M) and the resulting level of depolarization ΔV_m (Figure 2B, see Methods). During each experiment the increase of the stimulus amplitude was stopped when spike breakdown was apparent and therefore we did not record each cell at amplitudes up to the maximum amplitude (1000pA). Comparing break amplitude and break voltage between the three αRGC types shows pronounced differences (Figure 2C). On average, αONs RGCs could maintain spiking up to stimulus amplitudes of 792±132pA while spiking failed at significantly lower stimulus levels in αOFFs (369±63pA) and αOFFT RGCs (560±154pA). Similarly, αONs RGCs could be depolarized to higher levels than the two other αRGC types before spiking ceased (-37.9±3.8 / -43.2±3.0 / -45.3±3.8mV for αONs, αOFFs and αOFFt RGCs). We expected an inverse relationship between break amplitude and break voltage, i.e., that cells which could maintain spiking up to higher stimulus amplitude should also have a more depolarized break voltage. However, while αOFFt RGCs had significantly higher break amplitude than αOFFs cells, the break voltage of αOFFs RGCs was more depolarized (Figure 2C, left and middle). We used small (-100pA) hyperpolarizing current injections to determine input resistance as the ratio between the steady-state membrane voltage deflection and applied current amplitude. As can be inferred from the exemplary cells in Figure 1B (left, blueish traces), input resistance varied across the three cell types with αOFFt RGCs having lowest input resistance (80±18 / 108±30 / 60±16MΩ for αONs, αOFFs and αOFFt RGCs, Figure 2C, right). The observed differences in input resistance can explain the apparent discrepancy in the relationship between break amplitude and break voltage, with higher stimulus amplitudes resulting in lower levels of membrane depolarization in αOFFt versus αOFFs RGCs. The relationship of membrane depolarization and stimulus amplitude was further investigated by plotting the amount of depolarization from holding membrane voltage (ΔV_m) versus the normalized stimulus amplitude just before breakdown to account for the observed differences in input resistance. The normalized stimulus amplitude can be seen as a predictor for depolarization in a passive neuron (once the capacitor is fully charged). Figure 2D shows that while αOFFs and αOFFt RGCs almost fall on the prediction line, αONs cells are shifted to the right; such a deviation from unity indicates differential intrinsic processing of depolarization in the αON population.

In sum, our experiments show pronounced differences in the intrinsic spiking responses across αRGCs. These differences are likely to shape the conversion of presynaptic inputs from bipolar and amacrine cells into spiking output of the retina.

Rapid action potential kinetics allow fastest action potentials in αOFFt RGCs

A second set of experiments aimed to reveal potential differences in action potential kinetics in the three αRGC types. Cells were again held at ~-65mV before onset of each stimulus to adapt the spike generator to a predefined membrane voltage (in contrast to the different resting membrane voltage of recorded cells). Brief (3ms) current injections were used to determine threshold, we stimulated each cell with a battery of stimuli increasing in amplitude (8-10 repeats at each amplitude). Threshold was defined as the stimulus amplitude at which more than 66% of trials led to a spiking response (Figure 3A).

We compared action potential shape and other spike-related properties at threshold voltage allowing us to examine potential differences in action potential dynamics between the three αRGC types. Figure 3B shows action potentials over time as well as phase plots, i.e., the temporal derivative of membrane voltage versus the membrane voltage. All action potentials analyzed in this study consisted of the initial segment-somadendritic (IS-SD) break (Coombs et al., 1957; Fohlmeister et al., 2010) followed by the somatic action potential. If no apparent IS-SD break could be observed we reasoned that we axotomized the cell during the opening of the inner limiting membrane (Werginz et al., 2020a) and therefore cells were removed from further analysis. While αONs and αOFFs RGCs had similar action potential kinetics in both plots of Figure 3B, αOFFt RGCs appeared to fire action potential that had a shorter duration (Figure 3B, left) which was accompanied by higher rates of de- and hyperpolarization (Figure 3B, right, arrowheads). We extracted maximum de- and hyperpolarization rates for all cells and created a scatter plot; from this plot it became clear that, on average, αOFFt RGCs not only had higher rates of maximum de- and hyperpolarization (boxplots in Figure 3C) but also that the ratio between de- and hyperpolarization was different in αOFFt RGCs as the slopes of the two linear fits to sustained and αOFFt RGCs were not equal (compare the slopes of the two solid lines in Figure 3C). This finding mirrors the qualitative finding of faster repolarization of the representative αOFFt RGC action potential in Figure 3B (left, green trace). Quantification of the ratio between hyper- and depolarization is shown in Figure 3D (top); while sustained αRGCs had maximum hyperpolarization rates approximately half of the maximum depolarization rate this ratio was significantly higher for αOFFt RGCs (0.49±0.04 / 0.48±0.05 / 0.59±0.03 for αONs, αOFFs and αOFFt RGCs). The observed higher maximum rates of de- and hyperpolarization furthermore resulted in significantly shorter action potentials in αOFFt RGCs as well (0.31±0.03 / 0.28±0.03 / 0.21±0.02ms for αONs, αOFFs and αOFFt RGCs, Figure 3D, bottom).

Morphological and biophysical parameters modulate RGC spiking responses

In order to disentangle potential mechanisms responsible for the observed differences in spiking properties between sustained and transient αRGCs, we employed multi-compartment modeling using morphologically- and biophysically realistic models of αRGCs (see Methods). Aside from their characteristic light responses, mouse αRGCs can be identified morphologically by their large somas and dendritic arbors. Recent studies have furthermore reported variability in dendritic field diameter dependent on retinal location with a nasal-temporal gradient in sustained RGCs (Bleckert et al., 2014) and a ventral-dorsal gradient in αOFFt RGCs (Warwick et al., 2018; Werginz et al., 2020a). Upon visual inspection, no obvious differences between the dendritic arbors of αRGCs was observed (Figure 4A) and comparing dendritic field diameter and total dendritic length between the three αRGC types showed no differences (Figure 4B), however we found a significant difference in the number of dendritic branches which was higher

in αOFFt RGCs (Figure 4B). Based on these findings we were interested in whether the morphological differences between sustained and transient αRGCs are responsible for the cell type-specific spiking responses reported in Figure 3.

We repeated the short current injections in our models (10 repetitions at increasing amplitude, 66% threshold level; also see Methods) and found high agreement in modeled versus experimental results (Figure 4C & D). Total surface area of traced αRGCs ranges between ~8000-16000µm^2 (Raghuram et al., 2019); modeled input resistance ($R_M = \frac{1}{g_L * A_M}$, with g_L=0.1mS/cm^2, see Table 1) was in the range of 62.5-125MΩ similar to experimentally determined values (Figure 2C, right). Modeled action potentials typically had a visible IS-SD break in their phase plots indicating an axonal origin of spiking (compare phase plots in Figure 4D, left with Figure 3B, right). In a first set of simulations we kept somatic and axonal anatomy and biophysics the same and compared responses when dendritic tree anatomy was varied. We found no difference in spike amplitude and maximum hyperpolarization rate but spike duration was slightly shorter and maximum depolarization rate was slightly higher in modeled αOFFs RGCs (Figure 4D, right) demonstrating that dendritic morphology in αRGCs is not likely to be the driving factor leading to the observed strong response differences in sustained versus transient cells. To estimate the influence of differences in dendritic tree size and anatomy on spiking responses we compared the coefficient of variation (CV) for experimental and modeled responses. CV in experimental data was larger than in modeling results indicating a minor role of dendritic processing in the observed spiking differences but other factors to be involved (Figure 4E). Therefore, we next sought to determine whether the ion channel complement and densities are substantially different in the different types of αRGCs. The spike-triggered average (STA) is an estimate for the preferred linear receptive field of a neuron which can be recovered by white noise analysis (Figure 4F, left) (Chichilnisky, 2001; Arcas and Fairhall, 2003; Yunzab et al., 2022)). Comparison of STAs between αONs and αOFFt RGCs demonstrated highly similar input filters indicating similar ion channel complements in both cell types (Figure 4F, right) and therefore we reasoned that ion channel density but not ion channel dynamics are the underlying factor for the observed differences in action potential shape.

Somatodendritic ion channel density is difficult to estimate as standard immunohistochemical methods can only detect regions of densely packed ion channels such as in the AIS of RGCs (Boiko et al., 2003; Raghuram et al., 2019; Werginz et al., 2020a). Potential differences between αRGC types can be inferred from phase plots as shown on Figure 3 as a higher maximum rate of de- and hyperpolarization is likely to be the result of a larger sodium and potassium channel density (e.g., maximum $\frac{dV_m}{dt} = \frac{I_{Na,max}}{C_M}$ during depolarization). As a consequence, we hypothesized that up- and downregulation of the somatodendritic sodium and potassium channel density can lead to the observed differences in spike dynamics. As shown for a representative model cell in Figure 4G (left), we found several changes when sodium and potassium channel density were increased or decreased by 15% from their base value. Higher channel density not only led to higher rates of de- and hyperpolarization but also increased action potential amplitude (Figure 4G, left). An opposite trend was observed for lower ion channel density with smaller and wider action potentials. Modifying somatodendritic ion channel density in all model RGCs confirmed the trend observed for the exemplary cell with strong correlations towards larger and shorter action potentials when sodium and potassium channel density was increased (Figure 4G, right). In two control experiments we modified AIS length and AIS distance from soma and found only slight changes in somatic action potential properties.

Based on our findings we conclude that both the dendritic anatomy as well as biophysical parameters have an influence on spiking output. While dendritic architecture only has a minor influence on most action potential features, changes in somatodendritic biophysics were able to replicate experimental results from sustained and transient αRGCs.

αOFFt RGCs respond with highest peak firing rates at low membrane depolarization

We hypothesized that the significantly shorter action potential duration in αOFFt RGCs might be a feature to generate higher rates of peak firing rates in transient cells. Therefore, we examined peak firing rates for given membrane voltage as extracted from the long current injection experiments. Figure 5A shows the initial (0-14ms) response of an αONs, αOFFs and αOFFt; in these representative traces the αOFFt RGC fires spikes at a higher rate as indicated by the spike timings on top of Figure 5A. We found strong differences for sustained versus αOFFt RGCs, with αOFFt RGCs showing significantly higher peak firing rates than their sustained counterparts already at low levels of depolarization (V_m≥-60mV, Figure 5B). Even when comparing peak firing rates at breakdown, αOFFt RGCs were able to generate transient trains of action potentials at significantly higher rates than αONs and αOFFs RGCs (278±37 / 270±53 / 346±44Hz for αONs, αOFFs and αOFFt RGCs, Figure 5B, right).

Intrinsic spiking properties allow clustering of αRGCs

Based on the pronounced differences in the intrinsic spiking responses across the three αRGC types in the mouse retina, we were interested in how responses from the αRGC population compare to non-αRGC responses. The same experiments as described before, i.e., long (500ms) and brief (3ms) current injections into the soma of different types of non-αRGCs were performed. Non-αRGCs were targeted based expression of EYFP in their soma as labeled in the used EYFP mouse line (see Methods), however in order to avoid αRGCs only small soma RGCs were targeted. We observed obvious differences between non-αRGCs and αRGCs, for example, some non-αRGCs had significantly higher input resistance (Figure 6A), significantly slower de- and hyperpolarization rates (Figure 6B) or only transient spiking responses which declined to zero without a clear sustained spiking phase (Figure 6C). In general, non-αRGCs generated peak and sustained firing rates at lower stimulus amplitudes than αRGCs and fired action potentials which were wider than spikes in αRGCs (compare Figures 6A-C to Figure 6D). We performed principal component analysis followed by k-means clustering to determine whether non-α and αRGCs can be separated solely by their intrinsic spiking properties and found an almost perfect clustering of the two classes (Figure 6E). Only 3 out of 119 analyzed cells were classified incorrectly, resulting in an Adjusted Rand Index (see Methods) of 0.9 (Figure 6G, top).

We next explored the possibility of clustering αRGCs into three types solely by their intrinsic properties. Similar to the non-αRGC / αRGC clustering, principal component analysis followed by k-means clustering resulted in only a small number of incorrectly classified cells (Figure 6F) and an Adjusted Rand Index of 0.8. Interestingly, incorrectly classified cells were mostly found between the two sustained cell types whereas only one αOFFt RGCs was misclassified as an αOFFs RGC (Figure 6G, bottom). This can also be inferred from the 95% confidence interval ellipses shown in Figure 6F with only a small overlap between αOFFt and αOFFs clusters and no overlap between αOFFt and αONs clusters. In sum, our results show that the differences in intrinsic spiking properties are sufficient for clustering αRGCs into their three subtypes.

Differences between intrinsic spiking properties in sustained and transient αRGCs are not affected by retinal degeneration

In a final set of experiments, we examined the robustness of the observed differences between sustained and transient αRGCs when presynaptic inputs are altered for a long period of time. We used retinae from the same mouse line as for previous experiments, however with an additional genetic mutation leading to photoreceptor degeneration (rd10) (Chang et al., 2002). In these mice, during the progression of degeneration, light responses vanish and after approximately 60 days no photoreceptors are left (Chang et al., 2007). We repeated the experiments performed in wild-type retina in 200 days old rd10 retina. As expected, none of the tested cells exhibited substantial light responses to stationary flashes of bright and dark spots (Figure 7A). The clustering approach introduced in Figure 6 allowed us to determine cell type in recorded RGCs that did not respond to light and therefore enabled us to study cell type-specific differences in rd10 retina (Figure 7B & C). A difference between wt versus rd10 clustering was a similar input resistance of αONs and αOFFs RGCs in rd10 cells indicated in the biplots in Figure 7C. Comparison of input resistance in the sustained RGCs in rd10 retina (74±23 / 80±29MΩ, $p>0.05$ for αONs and αOFFs RGCs, see Figure 2C, right for comparison to wt retina) confirmed the observation which was most likely due to altered synaptic inputs in αOFFs RGCs. Because of the small number of rd10 αOFFs (n=10) cells we pooled both cell types and compared sustained (αONs+αOFFs) to transient (αOFFt) RGCs. A potential explanation for the relatively small number of αOFFs versus αONs RGCs in the rd10 dataset might be the way we targeted cells. By targeting large-soma cells (visible by eye in the microscope) the data might be biased towards αONs cells as these have been shown to have larger somas than αOFFs RGCs (Krieger et al., 2017; Werginz et al., 2020b).

First, we compared response properties of sustained and transient αRGCs between wild-type and rd10 retina (Figure 7D). Statistical comparison of spike amplitude, spike duration as well as peak firing rate at breakdown amplitude/voltage showed similar means in healthy and degenerated retina indicating that basic functionality in rd10 cells is similar to wild-type αRGCs (Margolis et al., 2008). As reported previously in mouse models of retinal degeneration (Stasheff, 2008; Goo et al., 2011; Menzler et al., 2014), we observed oscillations of the membrane voltage in the majority (~70%) of αRGCs (Figure 7E, left). We investigated the oscillations in more detail by filtering the membrane voltage to remove high-frequency spiking activity and then performed a fast Fourier transform to determine the oscillation frequency (Figure 7E, right). Oscillation frequencies were in the range of 4-10Hz with similar frequencies in sustained and transient αRGCs (inset). We found similar differences in firing rates between sustained and transient RGCs as in wild-type retina, with lower sustained firing rates in αOFFT RGCs (161±47 / 93±42Hz for αONs+αOFFs and αOFFt RGCs, Figure 7F), and therefore higher sustained-to-peak ratios in sustained cells (0.55±0.13 / 0.30±0.11 for αONs+αOFFs and αOFFt RGCs). A comparison of action potential dynamics confirmed results from wild-type retina showing faster rates of de- and hyperpolarization in αOFFt RGCs resulting in shorter spike duration (0.29±0.05 / 0.22±0.05ms for αONs+αOFFs and αOFFt RGCs, Figure 7G). Taken together, our findings show that long periods of altered presynaptic inputs and vanishing light responses do not change intrinsic spiking properties in αRGCs.

Discussion

Retinal output, i.e., spiking activity of RGCs, has been attributed mainly to the interplay of excitatory and inhibitory inputs from the presynaptic retinal network (Murphy and Rieke, 2006; Bleckert et al., 2014; Warwick et al., 2018). Postsynaptic processing as the procedure of converting analog synaptic inputs into proper spiking output, however is a rarely studied topic. This study expands our understanding in how αRGCs in the mouse retina affect retinal signal processing by their intrinsic spiking properties. Based on previous findings that intrinsic response properties across the same (Werginz et al., 2020a) and different (Wienbar and Schwartz, 2022) types of RGCs can vary substantially, we found that mouse αRGCs exhibit distinct response characteristics which roughly match their network-induced light responses (Figure 1). Breakdown of sustained spiking was different across the three cell types and dependent on stimulus amplitude and the resulting level of depolarization (Figure 2). Differences in sustained spiking responses were paralleled by differences in the rate of de- and repolarization as well as action potential duration (Figure 3). Computational modeling shows that the observed differences in action potential properties are not dependent on dendritic tree architecture but may be attributed to differences in somatodendritic ion channel conductance (Figure 4). We used PCA followed by k-means clustering to group αRGCs into their three types solely based on intrinsic firing properties (Figure 6) which allowed us to study cell type-specific firing properties in photoreceptor-degenerated retina revealing no changes in intrinsic differences between sustained and transient αRGCs, even after extended periods of blindness (Figure 7).

Intrinsic spiking responses have similar characteristics than network-mediated light responses

Recent evidence suggests tailored intrinsic properties of RGCs to allow conversion of presynaptic inputs to spiking outputs (Werginz et al., 2020a; Wienbar and Schwartz, 2022). Our findings show that both sustained RGC types (αONs, αOFFs) are able to respond to sustained depolarization with high-frequency spiking (Figure 1). Previous studies (Milner and Do, 2017; Wienbar and Schwartz, 2022) identified depolarization block as a mechanism in RGCs contrast response function, our findings here that the membrane voltage at which cells enter depolarization block is different across αRGCs is another piece of evidence that the spike generator in RGCs has an influence on retinal signal processing.

In contrast to sustained αRGCs, the transient type (αOFFt) showed a pronounced transient behavior during current injections (Figure 1). αOFFt RGCs were further characterized by fastest action potential kinetics resulting in shortest spike durations (Figure 3), as well as their tendency to fire transient burst of action potentials at higher rates than their sustained counterparts (Figure 5). αOFFt RGCs were found to be looming detectors important in triggering innate defensive responses (Münch et al., 2009; Wang et al., 2021). While the circuit for looming detection has been shown to originate in presynaptic retinal neurons, our findings here that αOFFt RGCs fire shortest action potentials as well as bursts of action potentials at highest rates (Usrey et al., 1998) support αOFFt RGCs in signaling thread detection from the eye to the brain at high speed.

While none of the three αRGC types tested here is a particularly transient cell type (see light responses in Figure 1) other cell types in the retina were shown to generate only short bursts of action potentials in response to light input (Farrow et al., 2013). Our preliminary results from

non-α RGCs indicate diverse spike generators with some cell types being highly transient also in their intrinsic response (Figure 6A-C). Future efforts are needed to reveal the diversity in intrinsic RGC firing properties across the full range of light responses.

Biophysical foundations for differences in action potential dynamics

Our experimental results strongly suggest different spike generators in αONs, αOFFs and αOFFt RGCs. The axon initial segment (AIS) has been proposed to be a crucial part in the signaling cascade, both with its task of being the site of spike initiation (Stuart et al., 1997; Kole et al., 2008) which origins from a high density of low-voltage activated $Na_v1.6$ sodium channels (Van Wart and Matthews, 2006; Kole et al., 2008; Werginz et al., 2020a; Wienbar and Schwartz, 2022). However, as we show in this study, differential spiking responses can also be a result of differential expression of (somatodendritic) ion channels, their conductance as well as multiple other biophysical and anatomical properties. Based on electrophysiological results that αONs and αOFFt RGCs have similar linear input filters (STAs, Figure 4) we assumed similar ion channel complements in all three types of alpha RGCs. Larger rates of de- and hyperpolarization in αOFFt RGCs indicate a larger sodium and potassium channel density in the somatodendritic compartments. Whereas our anatomically- and biophysically realistic αRGC models showed a relatively minor impact of dendritic tree size and AIS architecture on spike dynamics we show that a modest (15%) increase in sodium and potassium conductance results in action potential dynamics similar to those observed in αOFFt recordings (Figure 4). In sum, somatodendritic biophysical properties and a smaller contribution of cell anatomy lead to cell type-specific action potential properties in the αRGC population. We did not aim to study the influence of AIS composition in this study which has been shown to underly specific response features such as sustained spiking (Werginz et al., 2020a; Wienbar and Schwartz, 2022). The observed differences in sustainedness may also be attributes to differential AIS properties between sustained and transient αRGC types. There is also the possibility that other ion channels which do not affect the linear input filter can affect spiking output of αRGCs. Additional studies combining immunohistochemical and electrophysiological methods are needed to clarify on the specifics of ion channel complements in αRGCs.

Intrinsic responses were recorded from a common holding voltage of approximately -65mV. While this guarantees a fair comparison of the spike generator across cells having different resting membrane voltage it does not capture the fact of differential synaptic input into RGCs. The resting membrane will be shifted in different RGC types to varying depolarization levels. This will affect the contrast level eventually leading to depolarization block as some cells will be closer to their break voltage than other cells, depending on multiple factors such as ambient luminance. Our aim here was to investigate differences in the spike generators of αRGCs, the impact of these differences on retinal signal processing *in-vivo* is an open question for upcoming research.

Spiking properties in αRGCs are robust in response to photoreceptor degeneration

By using responses from short and long somatic current injections and ground truth cell type identification based on light responses, we were able to reliably cluster αRGC types solely based on their intrinsic spiking responses (Figure 6). This allowed us to study cell type-specific responses in photoreceptor-degenerated retina which does not allow for identification of cell type by light stimulation (Figure 7). Similar to previous results (Margolis et al., 2008), our findings suggest no physiological adjustments to the strongly altered presynaptic inputs in

mature rd10 mice. In contrast, in an anatomical study Schlueter et al. (Schlüter et al., 2019) reported a significant increase in AIS length when animals were visually deprived during development of the retina, however no physiological recordings were performed to measure neuronal activity. There is additional evidence that dendritic trees of αRGC become smaller during the progression of photoreceptor degeneration; this is further accompanied by a shortening of the AIS (M. Yunzab, S. Fried personal communication, unpublished data). Undersized dendritic trees have also been reported in a subset of unidentified RGCs in the rd1 retina (Damiani et al., 2012). Taken together, there is a growing body of literature showing a remarkable physiological stability in RGCs during photoreceptor-degeneration, at least if degeneration starts after the visual system has developed normally. This is surprising as anatomical findings indicate that RGCs undergo significant remodeling of their morphology during degeneration indicating multiple parallel processes that enable a stable spike generator in RGCs.

Retinal degenerative diseases such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD) lead to a loss of photoreceptors thereby ultimately resulting in total blindness. Aside from approaches trying to restore the conversion of light inputs to neuronal signals, e.g., photoreceptor transplantation (Gasparini et al., 2022), restoration of vision in patients suffering from photoreceptors degeneration can be achieved by electrical or optogenetic stimulation of the remaining retinal neuronal populations (Humayun et al., 2003; Zrenner et al., 2011; Sahel et al., 2021; Palanker et al., 2022). For strategies targeting RGCs for vision restoration it is of utmost importance to understand if RGCs change their physiological properties upon photoreceptor degeneration and if intrinsic RGC differences are preserved. Margolis et al. (2008) reported previously that RGCs in a mouse model of early degeneration onset (rd1) maintain certain spiking characteristics (e.g., rebound firing) similar to those found in wild-type retina. The inferred RGC stability is corroborated by our results. Furthermore, we demonstrate that intrinsic spike properties can be used to separate cell types – an important step towards cell-type specific activation in artificial vision. In a retinal implant, however the only possibility to record from RGCs is extracellular sensing of action potentials which is different from the patch-clamp approach used in this study. Studies in primate retina were able to successfully discriminate between four types of RGCs by intrinsic properties such as spike propagation velocity and autocorrelation function (Zaidi et al., 2023), Our results point to potential additional properties in RGC that might be useful for cell type identification in blind retina.

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	Dendrites	Soma	Soma-AIS	AIS	Axon
g_{Na}	75	75	75	1300	65
g_{K}	48	48	48	800	40
g_{Ca}	1	1	1	1	1
$g_{K,Ca}$	0.1	0.1	0.1	0.1	0.1
g_{H}	0.56	0.43	0.43	0.43	0.43
g_{L}	0.1	0.1	0.1	0.1	0.1

Table 1: **Ion channel densities along the neural membrane** – Values are based on Fohlmeister et al. (Fohlmeister et al., 2010) with minor modifications. An additional hyperpolarization-activated channel (i_H) was added to the base model. Values are given in mS/cm^2.

Figure 1: **Intrinsic responses of sustained and transient αRGCs are different and approximately match their light responses** – (**A**, left) Representative light responses for an αONs (top), αOFFs (middle) and αOFFt (bottom) RGC. Stimulus timing and polarity are indicated by dashed vertical lines and schematic on top of each panel. (**A**, right) Normalized maximum firing rate over time for αONs (n=31), αOFFs (n=19) and αOFFt (n=23) RGCs in response to light stimulation. Thick colored lines indicate population means; thin gray lines indicate single cell responses. (**B**, left) Representative responses to 500ms long current injections into the soma for an αONs (top), αOFFs (middle) and αOFFt (bottom) RGC. Stimulus amplitude is indicated by colors ranging from -500 (dark blue) to 1000pA (dark red). (**B**, right) Normalized maximum firing rate (see main text for details) over time for αONs (n=31), αOFFs (n=19) and αOFFt (n=23) RGCs in response to current injections. Thick colored lines indicate population means; thin gray lines indicate single cell responses. (**C**) Population means ± one standard deviation indicated by shadings of the normalized maximum firing rate for αONs, αOFFs and αOFFt RGCs during light stimulation (top) and current injections (bottom). (**C**, middle) Sustained to peak ratio is compared across the three populations of αRGCs during light stimulation (left, One-way ANOVA: $F(2,71)=57.27$, $p<0.001$) and current injections (right, One-way ANOVA: $F(2,71)=90.04$, $p<0.001$). The sustained phase of the response was defined as the period 350-450ms after pulse onset (black arrows).

Figure 2: **αRGCs enter depolarization block at different levels of depolarization** – (**A**) Membrane voltage over time for a current injection just below break amplitude (800pA) in an αONs RGC. Timing for the phase of sustained spiking is indicated in violet (350-450ms after pulse onset). The inset shows the response to a stimulus of 900pA with break voltage indicated by the horizontal arrow. (**B**, left) Average sustained firing rate is plotted for αONs, αOFFs and αOFFt RGCs for the full range of positive stimulus amplitudes. (**B**, middle) Average sustained firing rate is plotted for αONs, αOFFs and αOFFt RGCs for normalized positive amplitudes ($I_{Stim}*R_M$). (**B**, right) Average sustained firing rate is plotted for αONs, αOFFs and αOFFt RGCs for different levels of depolarization (same data as in (B, left), V_m was extracted by filtering, see Methods). Shadings indicate the standard error of the mean.(**C**) Comparison of break amplitude (left, One-way ANOVA: $F(2,71)=66.41$, $p<0.001$), break voltage (middle, One-way ANOVA: $F(2,71)=24.83$, $p<0.001$) and input resistance (right, One-way ANOVA: $F(2,71)=35.95$, $p<0.001$) between αONs, αOFFs and αOFFt RGCs. (**D**) ΔV_m, i.e., depolarization from holding membrane voltage (-65mV), is plotted versus normalized stimulus amplitude at break voltage for αONs, αOFFs and αOFFt RGCs. The dashed line represents the unity line representing the passive prediction.

Figure 3: **Fastest action potential kinetics in αOFFt RGCs** – (**A**) 8-10 repetitions of 3ms current pulses at increasing amplitude were injected into αRGCs to determine spiking probability for each amplitude (blue 'x'). A sigmoid response curve (dark blue) was fitted to the raw data to extract current thresholds at 66% response probability. (**B**) Representative action potentials over time (left) and phase plots (right) at threshold amplitude for an αONs, αOFFs and an αOFFt RGC. Arrowheads indicate maximum rates for de- and hyperpolarization, the arrow indicates the IS-SD break. (**C**) Maximum hyperpolarization rate is plotted versus maximum depolarization rate for αONs, αOFFs and αOFFt RGCs. Boxplots for maximum de- and hyperpolarization rates are shown on the top and on the right, respectively. Linear regression fits are shown for sustained (αONs+αOFFs) and αOFFt RGCs. (**D**) The ratio between maximum hyper- and depolarization (top, One-way ANOVA: $F(2,64)=76.43$, $p<0.001$) and action potential duration (bottom, One-way ANOVA: $F(2,64)=41.00$, $p<0.001$) are compared between αONs, αOFFs and αOFFt RGCs.

Figure 4: **A computational model of αRGCs shows the influence of dendritic morphology and somatodendritic ion channel density on action potential dynamics** – (**A**) Representative dendritic morphologies of an αONs (left), αOFFs (middle) and αOFFt (right) RGC. (**B**) Comparison of dendritic field diameter (DFD, left, One-way ANOVA: $F(2,64)=0.92$, $p>0.05$), dendritic length (middle, One-way ANOVA: $F(2,64)=1.70$, $p>0.05$) and number of branches (right, One-way ANOVA: $F(2,64)=39.54$, $p<0.001$) between αONs (left, n=16), αOFFs (middle, n=8) and αOFFt (right, n=43) RGCs. (**C**) Experiments from Figure 3 were repeated by simulating 3ms long current injections into the soma of each model RGC. At each stimulus amplitude 10 repetitions were performed and spiking responses (red) or subthreshold

responses (gray) were recorded to compute threshold based on a sigmoidal fit (inset). (**D**, left) Overlays of phase plots of three model RGCs which were based on an αONs (red), αOFFs (yellow) and αOFFt (green) tracing. While dendritic trees were different in the three cells, the soma and axon of the model cell were the same (see Methods). (**D**, right) Population statistics for spike amplitude, spike duration, maximum hyperpolarization rate and maximum depolarization rate for αONs (n=7), αOFFs (n=3) and αOFFt (n=12) model cells. (**E**) Comparison of experimentally determined ('o') and modeled ('x') coefficient of variation (CV) for five action potential features in αONs, αOFFs and αOFFt RGCs. (**F**, left) Spike triggered average (STA) was computed by averaging over multiple stimulus ensembles (red) preceding an action potential (black). (**F**, right) Normalized STA is plotted for αONs (n=5) and αOFFt (n=6) RGCs; thin lines indicate single STAs, thick lines indicate population means. (**G**, left) Overlays of phase plots of the same αOFFt model RGC when somatodendritic sodium and potassium channel density was varied to -15% (black) and +15% (orange) of its base value (turquoise). (**G**, right) Population summary for spike amplitude, spike duration, maximum hyperpolarization rate and maximum depolarization rate when somatodendritc sodium and potassium channel density was varied. Thin gray lines connect responses from the same model morphologies.

Figure 5: **αOFFt RGCs are tuned to fire high-frequency bursts of action potentials** – (**A**) Representative early responses for an αONS, αOFFs and αOFFt RGC for a membrane depolarization to approximately -48mV (black horizontal line). First spikes (gray arrowhead) were peak aligned for better visualization of different firing rates. Action potential timings are indicated by colored arrowheads at the top. (**B**, left) Peak firing rate is plotted versus membrane voltage for αONS, αOFFs and αOFFt RGCs. Error bars indicate population means ± one standard deviation, thin colored lines indicate responses from single cells. The brown and green shadings indicate the mean break voltage ± one standard deviation for αONS+αOFFs and αOFFt RGCs, respectively. (**B**, right) Comparison of peak firing rate at breakdown amplitude/voltage between αONS, αOFFs and αOFFt RGCs (One-way ANOVA: $F(2,70)=20.62$, $p<0.001$).

Figure 6: **Intrinsic spiking properties allow for identification of αRGCs** – (**A-C**) Spiking responses to 500ms long current injections for different stimulus amplitudes in three exemplary non-αRGCs. (**D**) Spiking responses to 500ms long current injections at different amplitudes in an exemplary αONs RGCs. Stimulus amplitude is color-coded, see color bar. (**E**) K-means clustering results for αRGCs (n=68) versus non-αRGCs (n=51). Colors show the predicted labels solely based on intrinsic spiking properties, centroids are indicated by colored 'x', green arrowheads indicate non-αRGCs which were misclassified as αRGCs. The ellipses indicate 95% confidence intervals. (**F**) K-means clustering results for the three αRGC types. Colors show the predicted labels solely based on intrinsic spiking properties, centroids are indicated by colored 'x', arrowheads indicate classification errors, colors indicate the correct cluster. The ellipses indicate 95% confidence intervals. (**G**) Confusion matrices for αRGCs versus non-αRGCs (top) as well as αONs, αOFFs and αOFFt (bottom) clustering. Percentages in the main diagonal indicate correct classification, off-diagonal entries indicate classification errors.

Figure 7: **Retinal degeneration does not affect intrinsic differences between sustained and transient αRGCs** – (**A**) Comparison of visual responses in αONs (left), αOFFs (middle) and αOFFt (right) RGCs in wild-type and rd10 retina. (**B**) K-means clustering results for the three αRGC types in rd10 retina. Colors show the predicted labels solely based on intrinsic spiking properties; centroids are indicated by colored 'x'. The ellipses indicate 95% confidence intervals. (**C**) Matlab biplot for wild-type (left) and rd10 (right) data visualizing the magnitude and sign of each variable's contribution to the first two principal components. (**D**) Comparison of action potential amplitude (left), action potential duration (middle) and peak firing rate at breakdown amplitude/voltage (right) for wild-type (black) and rd10 (blue) retina in sustained and transient αRGCs. (**E**, left) Representative recording of an αONs rd10 RGC with the filtered membrane voltage indicated in blue (vertically shifted for better visibility). (**E**, right) The filtered membrane voltage was used to extract the oscillation frequency by FFT (right). Inset: Comparison of the oscillation frequency of rd10 αONs+αOFFs (n=22) and αOFFt (n=10) RGCs. (**F**) Population means ± one standard deviation (indicated by shadings) of firing rate over time for rd10 αONs+αOFFs (n=34) and

αOFFt (n=14) RGCs during long (500ms, indicated by gray bar) current injections. (**G**) Phase plots of rd10 αONs+αOFFs and αOFFt RGCs (left) as well as bar plots of maximum depolarization rate and maximum hyperpolarization rate (right).