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1 **Spatio-temporal characteristics of retinal response to network-mediated**
2 **photovoltaic stimulation**

3 Elton Ho^{2,†}, Richard Smith^{1,†}, Georges Goetz², Xin Lei³, Ludwig Galambos³, Theodore I.
4 Kamins³, James Harris³, Keith Mathieson⁵, Daniel Palanker^{2,4}, Alexander Sher¹

5
6 ¹Santa Cruz Institute for Particle Physics,
7 University of California, Santa Cruz, CA 95064, USA

8 ²Hansen Experimental Physics Laboratory;

9 ³Department of Electrical Engineering;

10 ⁴Department of Ophthalmology, Stanford University, CA 94305, USA

11 ⁵Institute of Photonics, University of Strathclyde, Glasgow, Scotland, United Kingdom

12 [†]these authors contributed equally

13
14 **Correspondence:** Alexander Sher (email: sasha@scipp.ucsc.edu).

15
16 **Running head:** Characteristics of RGC responses to subretinal stimulation

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19 electrophysiology, neural prosthesis, electrical stimulation.

22

Abstract

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Subretinal prostheses aim at restoring sight to patients blinded by photoreceptor degeneration using electrical activation of the surviving inner retinal neurons. Today, such implants deliver visual information with low-frequency stimulation, resulting in discontinuous visual percepts. We measured retinal responses to complex visual stimuli delivered at video rate via a photovoltaic subretinal implant and by visible light. Using a multielectrode array to record from retinal ganglion cells (RGCs) in the healthy and degenerated rat retina ex-vivo, we estimated their spatio-temporal properties from the spike-triggered average (STA) responses to photovoltaic binary white noise stimulus with 70 μ m pixel size at 20Hz frame rate. The average photovoltaic receptive field size was 194 \pm 3 μ m (S.E.M.), similar to that of visual responses (221 \pm 4 μ m), but response latency was significantly shorter with photovoltaic stimulation. Both visual and photovoltaic receptive fields had an opposing center-surround structure. In the healthy retina, ON RGCs had photovoltaic OFF responses, and vice versa. This reversal is consistent with depolarization of photoreceptors by electrical pulses, as opposed to their hyperpolarization under increasing light, although alternative mechanisms cannot be excluded. In degenerate retina, both ON and OFF photovoltaic responses were observed, but in the absence of visual responses, it is not clear what functional RGC types they correspond to. Degenerate retina maintained the antagonistic center-surround organization of receptive fields. These fast and spatially localized network-mediated ON and OFF responses to subretinal stimulation via photovoltaic pixels with local return electrodes raise confidence in the possibility of providing more functional prosthetic vision.

New and noteworthy: Retinal prostheses currently in clinical use have struggled to deliver visual information at naturalistic frequencies, resulting in discontinuous percepts. We demonstrate modulation of the retinal ganglion cells (RGC) activity using complex spatio-temporal stimuli delivered via subretinal photovoltaic implant at 20Hz in healthy and in degenerate retina. RGCs exhibit fast and localized ON and OFF network-mediated responses, with antagonistic center-surround organization of their receptive fields.

53 Retinal degenerative diseases, such as retinitis pigmentosa and age-related
54 macular degeneration, cause a gradual loss of photoreceptors in millions of patients
55 worldwide, and are the leading cause of incurable blindness in the developed world (Smith
56 et al. 2001). However, most of the inner retinal neurons survive in these diseases, despite
57 some changes in the wiring of the retinal circuitry (Jones and Marc 2005; Marc and Jones
58 2003). Retinal prostheses aim at restoring sight by reintroducing information into the visual
59 system using electrical stimulation of the remaining retinal neurons (Goetz and Palanker
60 2016; Yue et al. 2016). Epiretinal prosthetic devices primarily target the retinal ganglion
61 cells (RGCs) – spiking neurons which represent the output cascade of the retinal signal
62 processing. A major difficulty with this approach is that bundles of axons from distant RGCs
63 passing under the epiretinal electrodes are also stimulated, which results in arcuate
64 percepts distorting the retinotopic map of the image (Nanduri et al. 2012). Avoiding this
65 effect while using sub-millisecond pulses is very difficult since stimulation thresholds of the
66 axons in the nerve fiber layer are similar to those of RGCs. Such a distortion can be
67 avoided by applying much longer (~25ms) pulses (Weitz et al. 2015), which are more likely
68 to activate inner retinal neurons while avoiding direct ganglion cell activation since these
69 non-spiking neurons in the inner nuclear layer have significantly lower stimulation
70 thresholds at long pulse durations than RGCs (Boinagrov et al. 2014; Freeman et al. 2010).
71 Subretinal implants are closer to the inner nuclear layer and activate these neurons
72 (Lorach et al. 2015b; Mathieson et al. 2012) with lower thresholds than the ganglion cells
73 (Boinagrov et al. 2014), thereby reducing the likelihood of axonal activation. Both epiretinal
74 (ARGUS II, Second Sight Inc. and IRIS 2, Pixium Vision Inc.) and subretinal (Alpha IMS,
75 Retina Implant AG) prostheses currently approved for clinical use require a transscleral
76 cable for transfer of signals and/or power to the stimulating array (Ho et al. 2015; Humayun
77 et al. 2012; Stingl et al. 2013b). This requirement leads to difficult surgical procedures and
78 increases probability of post-implantation complications.

79 We therefore developed a prosthetic system where both power and information are
80 delivered optically to a subretinal array of photovoltaic pixels (Mathieson et al. 2012;
81 Palanker et al. 2005). A video stream is projected onto the implant from video goggles
82 using pulsed near-infrared light (NIR) (Goetz et al. 2013). The implant converts light pulses
83 into charge-balanced pulses of electric current in each pixel (Boinagrov et al. 2015), which
84 stimulate the nearby inner retinal neurons. The use of NIR light (880-915nm wavelength)
85 avoids both photophobic and phototoxic effects associated with intense illumination
86 (Lorach et al. 2016).

87 We demonstrated previously that photovoltaic subretinal stimulation can elicit retinal
88 and cortical responses in healthy animals (Long-Evans, LE rats) and in animals with
89 degenerate retina (Royal College of Surgeons, RCS rats) at safe illumination levels (Lorach
90 et al. 2015a; Mathieson et al. 2012), (Lorach et al. 2015b). We characterized the response
91 properties of RGCs using high frequency (20Hz) stimulation, while the amplitude envelope
92 of this carrier frequency was modulated at a lower frequency (1 Hz), resulting in slow full-
93 field changes in intensity. Using this paradigm, we assessed contrast sensitivity and spatial

94 resolution with alternating gratings (Goetz et al. 2015; Lorach et al. 2015b). We found that
95 only the first few stimulation pulses following the increase in intensity elicited an increase in
96 spiking of the RGCs, demonstrating that the network-mediated response to subretinal
97 electrical stimulation exhibits flicker fusion and adaptation to static images (Lorach et al.
98 2015b), (Goetz et al. 2015). These observations suggested that flicker-fused prosthetic
99 vision might be possible, even though clinical implants currently use a much lower
100 frequency (<7Hz) in patients (Stingl et al. 2013a). It remains unknown, however, whether
101 RGCs can respond to complex spatio-temporal photovoltaic stimulation at naturalistic
102 frequencies, and how their response properties compare to the normal visual responses to
103 such stimuli.

104 The goal of this study was to investigate RGC responses to complex spatio-
105 temporal electrical activation patterns, and compare them to natural visual responses in the
106 healthy retina. We used a custom-made transparent extracellular microelectrode array
107 (MEA) (Litke et al. 2004) and spatio-temporal binary white noise to jointly characterize the
108 spatial and temporal response properties of RGCs to photovoltaic subretinal and visual
109 stimulation in the healthy (LE) and degenerate (RCS) rat retina. Spike-triggered average
110 (STA) responses of RGCs to white noise stimulation (Chichilnisky 2001) have been
111 extensively used to measure response properties of the healthy retina (Chandler and
112 Chichilnisky 2001; Devries and Baylor 1997; Field et al. 2010; Field et al. 2007; Sher and
113 DeVries 2012). Measurements of the spatial receptive fields and response dynamics of
114 individual RGCs enable their classification into functional types, representing parallel retinal
115 pathways that extract various features of the visual scene. Two major RGC types are ON-
116 and OFF-center cells that respond to the onset and offset of light, respectively, in their
117 receptive field centers, and have opposing wider surrounds.

118 We show that the hallmark RGC visual properties, such as fast response time,
119 spatially-localized receptive field and opposing surround, are present with subretinal
120 photovoltaic stimulation of both healthy and degenerated retina. This indicates that spatial
121 and temporal characteristics of prosthetic vision, mediated by a subretinal photovoltaic
122 array, may closely resemble the normal visual responses.

123

Methods

124 Implant fabrication

125 Photovoltaic arrays were manufactured on silicon-on-insulator wafers using a six-
126 mask lithographic process. Different versions of the devices were fabricated with either 2 or
127 3 diodes in series per pixel, with anodic-first polarity on active electrode. The arrays
128 consisted of 70- μm -wide pixels, separated by 5- μm trenches (Figure 1A,B,C). Details of the
129 fabrication process were described previously for pixels of the opposite wiring polarity
130 (Wang et al. 2012).

131 Electrophysiological Recording

132 Retinal responses were recorded from 4 adult healthy Long-Evans (LE) (ages: p60
133 to p100 days) and 7 degenerate Royal College of Surgeons (RCS) rats (ages: p120 to
134 p360 days), all of which were kept in accordance with the institutional guidelines and

135 conformed to the guidelines of the Association for Research in Vision and Ophthalmology
136 (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Only one
137 retinal recording was obtained from each rat. Retinal tissue was mounted according to
138 previously described procedures (Goetz et al. 2015). In summary, the eyes were
139 enucleated from a euthanized (390 mg/kg pentobarbital sodium, 50 mg/mL phenytoin
140 sodium) rat. After vitrectomy an approximately 3mm x 3mm piece of the isolated retina was
141 placed ganglion cell side down on the 512-electrode MEA (Litke et al. 2004). The retina
142 was constantly perfused with Ames' medium at 29.4 °C and bubbled with a mixture of 95%
143 O₂ and 5% CO₂. The photovoltaic array was placed carefully on top of the retina and
144 pressed onto the retina and underlying MEA with a 100µm cell size nylon mesh. The
145 voltages on the 512 electrodes were amplified and digitized with 20kHz sampling frequency
146 using custom-made readout electronics and data acquisition system (Litke et al. 2004). The
147 stimulation was delivered to the photoreceptors or the photodiode array from below through
148 the transparent MEA and the retina (Figure 1D). In a typical preparation, RGCs had stable
149 responses to stimulation for several hours.

150 Retinal Stimulation

151 Light sourced from either a NIR (880 nm) diode laser for photovoltaic stimulation
152 (4ms pulses at 20Hz, 9mW/mm² peak power), or a yellow (591nm) LED for visual
153 stimulation (continuous illumination), was coupled into the same optical path. Images were
154 formed by an amplitude modulation in a transmissive LCD screen (Holoeye HEO-0017), as
155 described previously (Goetz et al. 2015; Lorach et al. 2015a). The 8bit LCD panel had a
156 60Hz native frame rate, 1024x768 resolution with a square pixel layout, a white-to-black
157 intensity ratio of 10000:1 at 520nm, and of 200:1 at 880 nm. The pixel size projected onto
158 the retina was 6 microns.

159 A spatio-temporal binary white noise stimulus was used to characterize spatio-
160 temporal response properties of the RGCs (Chichilnisky 2001). Each pixel in each frame
161 had a 50% chance to be white or black, independently from others and from frame to frame.
162 The white noise for visual stimulation was shown at 30Hz frame rate and consisted of
163 square pixels of 60µm in size focused on the photoreceptor layer. The white noise for
164 photovoltaic stimulation had 20Hz frame rate and consisted of hexagonal pixels that were
165 matched in size and location to the 70µm hexagonal pixels of the implant, resulting in each
166 hexagonal image pixel illuminating one pixel on the implant (Figure 1E). The duration of
167 each white noise stimulus recording was 30 minutes. Photovoltaic stimulation was applied
168 at low rate to minimize problems caused by the electrical artifact elicited on the recording
169 electrodes by the stimulation pulses. The lower frame rate resulted in lower temporal
170 resolution of the RGC response to photovoltaic, compared to the visual stimulation.

171 Full-field flashes were also used to measure RGC responses to photovoltaic
172 stimulation. The train of 4ms NIR pulses repeated at 20Hz was modulated in intensity at
173 1Hz frequency (Goetz et al. 2015). Each alternate full-field image was presented for 500ms
174 and had an irradiance level of 10mW/mm², while the other full-field image was dark,

175 resulting in +100% and -100% contrast transitions. The contrast steps were presented a
176 total of $n = 100$ times.

177 Neuron Finding

178 Photovoltaic stimulation produces a large electrical artifact unique to each of the 512
179 recording electrodes and different for each frame of the white noise movie. We fitted the
180 artifact using a difference of two Gaussians. The fitted function was then subtracted from
181 the raw voltage trace. This procedure was repeated for each artifact on each of the
182 individual electrodes. The artifact was too large during the first 8.25ms after the laser pulse
183 for this procedure to work, therefore we replaced this 8.25ms period with randomly
184 generated noise that matched the noise level of the electrode in question. As a result, any
185 action potentials that occurred within 8.25ms from the start of the laser pulse were lost. We
186 expect that the omission of some of the elicited spikes might result in an underestimation of
187 the strength of the RGC response. Figure 1F shows an example voltage trace from one of
188 the electrodes before and after the subtraction. The artifact-subtracted raw data was then
189 used to find and sort action potentials (spikes). Spikes were defined as an event where the
190 negative voltage deflection amplitude exceeded 3 times root-mean-squared noise on each
191 electrode. Custom-made software was used to perform spike sorting as described in (Field
192 et al. 2010; Goetz et al. 2015; Litke et al. 2004). In short, to identify spikes of individual
193 RGCs, all waveforms underwent dimensionality reduction by noise-whitened principal
194 component analysis, and spike trains of putative neurons were obtained by expectation-
195 maximization clustering. For each candidate neuron, an estimate of the fraction of spikes
196 coming from other neurons (“contaminating” spikes) was obtained from the number of
197 refractory period violations in the spike train. We excluded from our analysis contaminated
198 neurons that had over 10% of their spikes coming from another cell. Furthermore, we
199 excluded from the analysis putative neurons that had abnormal electrophysiological images
200 (EIs) (e.g. EIs showing backward propagation of the axonal signal). Each of these selection
201 criteria removed less than 10% of the cells with good responses to the stimulus as defined
202 in the next subsection. The electrophysiological image is the average electrical signal
203 measured on all of the recording electrodes within 10ms of the RGC spike, and typically
204 shows both soma location and the axonal trajectory of the RGC (Li et al. 2015; Petrusca et
205 al. 2007).

206 Spike sorting was performed separately for retinal responses to each stimulus. We
207 used each neuron’s unique EIs to match the individual cells across multiple stimulus
208 conditions (Li et al. 2015; Sher and DeVries 2012). This match was performed between
209 RGCs identified in the visual and photovoltaic stimulation runs in each LE retina and
210 between the RGCs identified in the visual and photovoltaic stimulation of the retina in the
211 pharmacology experiment. For these experiments, only the cells that were successfully
212 matched between the stimulation conditions were retained for analysis. The fraction of
213 RGCs with significant photovoltaic responses (see the next subsection) that were matched
214 to the visually responding cells varied from 90% to 50% between preparations.

215 Characterization of the RGC responses

216 Each cell's spatio-temporal response properties was estimated by calculating the
217 spike-triggered average response (STA) of each RGC to the white noise stimulus
218 (Chichilnisky 2001). Short white noise movies (typically 20 frames) that preceded each of
219 the detected spikes of an RGC are averaged over the recording to obtain the STA of the
220 RGC (Figure 2A). The spatial sensitivity profile of the RGC (receptive field) corresponds to
221 the STA regions with significant deviations from the average gray level. We quantified the
222 spatial extent of the receptive field by the $1-\sigma$ contour of the 2-dimensional Gaussian fitted
223 to the STA frame with the largest deviation from grey (Figure 2B). The receptive field size
224 is estimated as the diameter of a circle with the area equivalent to that of the ellipse. The
225 time course shows the STA intensity within the receptive field as a function of time
226 preceding the spike (Figure 2B). In a fully linear system, convolution of the time course with
227 the full-field step in illumination provides the predicted response of the cell to such a step.
228 Therefore, the sign of the first peak preceding the spike in the time course determines if the
229 RGC increases its spiking rate in response to the ON- or OFF-set of light (Chichilnisky and
230 Kalmar 2002). We used the time courses of individual RGCs to distinguish between the two
231 major RGC types: ON- and OFF-center (Chichilnisky and Kalmar 2002; Sher and DeVries
232 2012). STAs of example ON- and OFF-center RGCs are shown in Figure 2B. The
233 spatiotemporal white noise is not well suited for classifying ON-OFF cells. ON and OFF
234 parts of an ON-OFF receptive field would be averaged by the STA resulting in either (1) no
235 response if they are matched exactly and cancel each other, or producing (2) a weak ON-
236 or OFF-center STA if they are not balanced exactly. We expect that most of such RGCs
237 would be excluded from the analysis by the STA significance requirements (see below), but
238 we cannot exclude the possibility that some of the classified ON and OFF cells might be
239 ON-OFF cells.

240 We quantified the response latency of the individual cells by first fitting a difference
241 of two low-pass filters to the time course and then finding the time between the spike and
242 the first fitted time course peak and the time between the spike and the first zero crossing
243 of the time course (Figure 2B). These two time intervals describe dynamics of an RGC
244 response to the light step of the preferred polarity (Chichilnisky and Kalmar 2002). For
245 some cells, the fit to the photovoltaic time course had a small peak prior to and with
246 opposite polarity with respect to the time course first peak. To avoid using this false peak,
247 we calculated both time to peak and time of the first zero crossing based on the first peak
248 of the fitted function with the deflection polarity matching that of the time course. The mean
249 intensity of some STAs exhibited slight offset from zero. We used the average STA value
250 preceding the spike by 10 to 25 movie frames to determine the offset and subtract it from
251 all of the STA intensities prior to fitting. The STAs were calculated and parameterized in
252 identical fashion for the visual and photovoltaic responses. RGCs with the time course
253 signal-to-noise (SNR) ratio below 3 were excluded from the analysis. In each preparation,
254 30 to 60% of the initially identified cells were excluded by this requirement prior to other
255 cuts described above. For the SNR calculation, the peak value of the time course was used
256 as a signal, and the root mean square value of the 10 time course values most removed
257 from the time of the action potential was used as noise.

258 Ganglion cell body location

259 Electrodes with the largest EI signal are located close to the soma and can be used
260 to estimate its position (Li et al. 2015). We estimated the RGC soma location as the center
261 of the 2-dimensional Gaussian function fitted to the EI of the cell. The fit location was
262 determined mostly by the somatic signal, which typically had an order of magnitude larger
263 amplitude than the axonal signals. The estimated location of the cell body was then
264 transformed in the stimulus coordinate system for comparison to the location of its
265 receptive field. The transformation was obtained by imaging the known stimulus pattern
266 projected onto the retina at the end of the experiment. Such images capture simultaneously
267 the stimulus pattern and the MEA electrodes, providing the relative angle between RF and
268 EI coordinates. We calculated the center of mass (centroid) location of the receptive fields
269 in the preparation and their average distance from this centroid. The calculations were
270 repeated for the EIs of the same RGCs. The relative shift and scaling between the stimulus
271 and EI coordinates were obtained by matching the centroid locations and average
272 distances from centroid calculated for the receptive fields and EIs. For healthy retinas, we
273 also compared the relative positions of prosthetic and visual RF centers by mapping both
274 stimuli coordinates to the same EI coordinate system.

275

Results

276 RGCs can respond to complex spatio-temporal patterns at high stimulation frequencies.

277 We characterized the responses of RGCs to complex visual stimuli in seven
278 degenerate (RCS) retinas by activating the subretinally-placed photovoltaic array with a
279 binary white noise movie at 20Hz frame rate. The movie had 70 μ m hexagonal pixels, which
280 were aligned with the hexagonal photodiode pixels of the implant (see Methods).

281 For 104 RGCs from seven retinas, the spike-triggered analysis of the white noise
282 stimulus yielded statistically significant responses, with SNR of at least 3 (see Methods),
283 indicating that the implant successfully elicited RGC responses despite the rapidly varying
284 spatio-temporal structure of the stimulus (Figure 3). The photovoltaic spike-triggered
285 averages (pSTAs) are the prosthetic equivalent of the classical visual spike-triggered
286 averages, which approximate the temporal characteristics and spatial localization of the
287 RGC receptive fields (Chichilnisky 2001) (Figure 2). The pSTAs were spatially localized. 72
288 RGCs had photovoltaic ON (pON) responses with the positive pSTA value of the first peak
289 preceding the spike (see Methods). 32 RGCs had pOFF responses with the negative time
290 course peak. Two example cells with the distinct pON and pOFF pSTAs are shown in
291 Figure 3A,B. The pSTAs were similar within a single preparation, although the relative
292 number of cells with pON and pOFF response properties varied between retinal
293 preparations (Figure 3C). The presence of both pON and pOFF responses in the
294 degenerated retina is surprising, given that both ON and OFF bipolar cells are expected to
295 be depolarized by the stimuli, and hence provide ON response, but no pOFF responses.
296 The observed pOFF responses might be caused by depolarization of the rod bipolar cells
297 that, in turn, relay their excitation through All amacrine cell to the ON and OFF RGCs (see
298 Discussion for more details). The average receptive field diameter in RCS retina was

299 195±6μm (standard error of the mean, S.E.M.) for pON and 170±8μm for pOFF RGCs, in
300 line with the values previously reported in the literature for low-frequency sparse binary
301 white noise stimulation of the rat retina (Lorach et al. 2015b). We estimated the average
302 response latency by measuring the time between the spike and the first peak and the first
303 zero crossing of the pSTA time course that preceded it (see Methods). In the linear-
304 nonlinear model of RGCs, the time of the first peak corresponds to the time of maximum
305 rate of increase in the spike frequency in response to the light step of preferred polarity
306 (increase in light level for an ON and decrease for an OFF RGC). In turn, the first zero
307 crossing corresponds to the moment of the maximum response (Chichilnisky and Kalmar
308 2002). On average, across seven RCS retinas, the time to first peak was 51±3ms for pON
309 and 50±8ms for pOFF RGCs. The time to first zero was measured to be 87±3ms for pON
310 and 92±3ms for pOFF RGCs.

311 In healthy retina, polarity of the ON and OFF RGC responses to photovoltaic activation is
312 reversed compared to visual stimulation.

313 To compare RGC responses to photovoltaic and visual stimulation in healthy retina,
314 we applied both the visual and photovoltaic white noise stimuli to each LE retinal
315 preparation. The photovoltaic stimulus was identical to the one used in RCS rats. The
316 visual white noise had 60μm size square pixels and was refreshed at 30Hz frame rate (see
317 Methods). Visual STAs (vSTAs) and photovoltaic STAs (pSTAs) were obtained by reverse
318 correlation analysis between the RGC spike trains we recorded and the stimuli delivered to
319 the retina (Figure 4). Average response latency, estimated from the STA time courses, was
320 shorter for photovoltaic than for visual stimulation (71±2ms vs. 168±3ms, respectively). The
321 faster response to photovoltaic stimulation is likely due to bypassing the phototransduction
322 cascade of normal vision, and is consistent with observations previously reported in the
323 literature (Chichilnisky and Kalmar 2002; Mathieson et al. 2012). The average photovoltaic
324 receptive field diameter was 194±3μm, compared to 221±4μm for the visual receptive fields
325 of the same RGCs (Table 1).

326 We classified RGCs based on their vSTAs into ON- and OFF-center types (Figure
327 4A,B). Using the unique electrophysiological images (EIs) of the RGCs (Li et al. 2015;
328 Petrusca et al. 2007), we matched cells between the visual and prosthetic stimuli (see
329 Methods). We identified 139 RGCs across four preparations that had visual and
330 photovoltaic responses. Polarity of the photovoltaic RGC responses was reversed relative
331 to the visual ones, i.e. visual ON (vON) RGCs behaved as photovoltaic OFF (pOFF), and
332 vOFF RGCs behaved as pON cells (Figure 4A,B). All of the RGCs that had both visual and
333 photovoltaic STA responses in the four LE retinas exhibited this reversal. While some of
334 the RCS timecourses had tri-phasic shapes (Figure 3C), this feature was more pronounced
335 in LE photovoltaic timecourses (Figure 4C).

336 A possible source of this reversal is the opposite response of photoreceptors to
337 electrical and light stimuli: cells are depolarized by electrical stimulation, but photoreceptors
338 hyperpolarize when illuminated by light. Depolarization of photoreceptors normally
339 corresponds to a decrease in illumination, and hence the retina interprets electrical

340 activation of the photoreceptors as a decrease in light intensity. Thus, an increase in the
341 electrical stimuli mimics a decreasing light level, while a decrease in electrical stimulation
342 has the same effect as an increase in the light intensity. Consequently, normal signaling
343 from photoreceptors to the ON and OFF-bipolar cells should lead to reversed responses
344 with photovoltaic stimulation: pOFF responses of the vON ganglion cells and pON
345 responses of the vOFF ganglion cells. Note that for this hypothesis to hold, the effect of the
346 direct activation of photoreceptors should overwhelm the direct depolarization of ON
347 bipolar cells, which would mediate pON responses in the vON RGCs.

348 To test if photoreceptors play a role in the photovoltaic responses of the healthy
349 retina, we used a mixture of 100 μ M concentration of mGluR6 receptor antagonist LY
350 341495 and 150 μ M l-AP4 mGluR6 agonist (l-2-amino- 4-phosphonobutyric acid) to
351 selectively block synaptic transmission from photoreceptors to ON-bipolar cells (Sher and
352 DeVries 2012). We then measured the photovoltaic response properties of the RGCs using
353 full-field steps of +100% or -100% contrast (see Methods). Before application of the
354 blockers, vON cells responded to negative contrast steps with 0.70 \pm 0.57 spikes per step
355 (\pm standard deviation, pOFF response), and to positive contrast steps with 1.26 \pm 0.45
356 spikes per step (pON response) (Figure 5B). vOFF cells responded to positive contrast
357 steps with 1.73 \pm 1.05 spikes per step, and did not respond to negative contrast steps
358 (0.01 \pm 0.05 spikes per step). After application of the blockers visual responses of the
359 vON RGCs to the visual white noise disappeared (Figure 5A), while the responses of the
360 vOFF cells remained largely unchanged. Blocking the signal transmission from
361 photoreceptors to the ON-bipolar cells led to the complete disappearance of the pOFF
362 photovoltaic responses initially observed in vON RGCs, consistent with pOFF responses
363 being caused by electrical depolarization of photoreceptors. At the same time, pON
364 responses of the vOFF RGCs remained, with 2.55 \pm 1.21 spikes elicited per positive
365 contrast step (Figure 5B). While these results are consistent with the photovoltaic response
366 in the healthy retina mediated mostly by photoreceptors, it leaves open the question about
367 the contribution of the direct depolarization of bipolar cell. We did not detect pON
368 responses of the vON RGCs after adding the blockers. However, we cannot say if this was
369 due to such response being negligible or due to the ON bipolar cells being driven to the
370 state of constant de- or hyper-polarization by the combination of the mGluR6 agonist and
371 antagonist used.

372 An opposing surround is present in photovoltaic responses.

373 The center-surround organization of the RGC receptive fields is one of the
374 fundamental properties of vision (Kuffler 1953). The classical surround mechanism in the
375 healthy retina is associated with negative feedback by the horizontal cells on the
376 photoreceptor terminals (McMahon et al. 2004; Werblin and Dowling 1969). Inhibitory
377 signaling from amacrine cells in the inner retina is another source of an opposing surround
378 (Flores-Herr et al. 2001; Ichinose and Lukasiewicz 2005; Taylor 1999). We investigated
379 whether the antagonistic surround is maintained under electrical stimulation, as
380 disappearance of photoreceptors and their terminals in retinal degeneration is likely to

381 eliminate the role of horizontal cells, and it is not clear how the electrical surround is
382 affected by the associated retinal rewiring (Jones and Marc 2005).

383 To test if an opposing surround is present in photovoltaic responses, we measured
384 the surround and central signals in the following way: The center signal was estimated as
385 the average (per pixel) STA time course for the pixels located within the $2\text{-}\sigma$ ellipse of the
386 2-d Gaussian fit to the receptive field. The surround signal was calculated as the average
387 STA time course for the pixels located outside the central zone, in the $(4\text{-}8)\sigma$ band for
388 visual and $(3\text{-}6)\sigma$ band for the photovoltaic STAs. The cutoff values were selected so as to
389 avoid the region where the center signal switches to the surround while maximizing both
390 center and surround signals. As expected, we observed opposing surround signals in both
391 vON and vOFF vSTAs. Figure 6A,B shows two example RGCs with visual surrounds
392 having opposite stimulus preference (sign of the time course deflection preceding a spike)
393 compared to their centers. With electrical stimulation of the same cells, we observed
394 reversal of the polarity not only in centers, but also in the antagonistic surround in the LE
395 pSTAs (Figure 6C vs. A and D vs. B). Surprisingly, the photovoltaic responses of the RGCs
396 in the degenerate RCS retina also had opposing surround signals (Figure 6E,F).

397 We quantified the strength and sign of the center and surround by measuring the
398 maximum time course deflection preceding the spike. Spatial properties of the center and
399 surround signals were characterized by calculating the STA response as a function of
400 distance from the receptive field center. Figure 6G shows that both visual and photovoltaic
401 STAs have opposing surrounds that are wider than the center and become weaker with
402 increasing distance. Photovoltaic surrounds were stronger than visual ones, except for the
403 RCS pOFF RGCs, as measured by the ratio of the maximum surround amplitude to that of
404 the center (Figure 6G). We noticed that cell-to-cell variability of the surround signal was
405 larger for the LE pOFF RGCs than for the other responses. A possible explanation is that
406 direct stimulation of the bipolar cells and photoreceptors has opposite effects on the pOFF
407 RGCs. The balance between these two mechanisms determines the strength of the
408 response, leading to larger cell-to-cell variability than in the pON RGCs in LE and RCS
409 retinas, for which both photoreceptor-mediated and bipolar cell-mediated stimulation
410 mechanisms affect the cell in the same way.

411 Subretinal electrical stimulation preserves the retinotopic mapping.

412 Retinotopic mapping between the input patterns and RGC somata is essential for
413 proper image formation in the brain. If retinotopic mapping is not preserved in prosthetic
414 vision, stimulation patterns can appear distorted to a patient, as in the case of axonal
415 activation by epiretinal prostheses (Nanduri et al. 2012; Weitz et al. 2015). As shown
416 above, the photovoltaic responses of the ganglion cells to high frequency binary white
417 noise were spatially localized, with receptive field sizes similar to those obtained with
418 visible light stimulation (Table 1). These results also matched receptive field sizes
419 previously reported using low frequency sparse white noise stimuli (Lorach et al. 2015b).

420 We verified proximity between the receptive field center and the RGC soma by
421 measuring the distance between the center of the functional receptive field and the RGC
422 cell body location estimated from its electrical image (see Methods). The average

423 displacement between the center of the receptive field and cell soma in photovoltaic
424 stimulation of the RCS retina was $52\pm 5\mu\text{m}$ and $81\pm 17\mu\text{m}$ for pON and pOFF RGCs,
425 respectively (Table 1). The average displacement between visual receptive fields and cell
426 somas in the healthy retina was measured to be $53\pm 4\mu\text{m}$. Finally, the average
427 displacement between visual and prosthetic receptive field centers was $68\pm 8\mu\text{m}$, with no
428 significant difference between the cell types. Directions of the individual RGCs
429 displacements were random. All displacements were smaller than the corresponding
430 receptive field sizes. Together with spatially localized STAs, these results suggest that
431 retinotopic mapping is preserved in the degenerate retina.

432

Discussion

433 Preservation of the spatio-temporal response properties of individual RGCs in
434 prosthetic vision is important for successful restoration of sight to patients blinded by retinal
435 degeneration. Natural vision relies on multiple parallel pathways in the retina, each
436 corresponding to its own RGC type. While each of these pathways has its unique spatio-
437 temporal and sometimes chromatic response properties, the following three features have
438 been found to be almost universal among different types of the RGCs: (1) fast (fraction of a
439 second) response; (2) spatially localized receptive fields and (3) antagonistic center-
440 surround organization of the receptive fields.

441 We find that RGCs in both healthy and degenerate retinas respond to photovoltaic
442 spatio-temporal binary white noise at 20Hz frame rate. The spatial localization of the
443 response is preserved by subretinal photovoltaic stimulation. At the same time, the
444 response is significantly faster. Antagonistic center-surround organization of the
445 photovoltaic receptive fields is present in both healthy and degenerated retinas.
446 Photovoltaic stimulation in healthy retina leads to distinct responses of the ON- and OFF-
447 center RGCs, opposite to their responses to visual stimulation. Both pON and pOFF STAs
448 are present in degenerated retina, although it is not clear which RGC types exhibit these
449 distinct responses. These findings and their implications are discussed below.

450 It has been shown previously that spatially simple (full-field or 1-dimensional
451 reversing gratings) and temporally slow (2Hz) amplitude modulation of high frequency (20
452 to 40Hz) trains of subretinal photovoltaic pulses resulted in transient responses of the
453 retinal ganglion cells to slow changes in light intensity (Goetz et al. 2015; Lorach et al.
454 2015b). These results indicated that subretinal photovoltaic stimulation preserves flicker
455 fusion and adaptation to static images. It was also reported that retinal network-mediated
456 responses can be elicited by epiretinal stimulation at 25Hz with static spatial distribution,
457 but stochastic temporal changes in amplitude, indicating that fast changes in the full-field
458 stimulation can elicit responses despite the flicker fusion (Sekhar et al. 2016).

459 In this paper, we demonstrate for the first time that retina responds to spatio-
460 temporal white noise stimulation delivered through a photovoltaic subretinal prosthesis at
461 20Hz frame rate. Retinal response to complex spatial and fast temporal patterns exhibited
462 many similarities to natural visual response.

463 Spatio-temporal properties of the response to photovoltaic stimulus.

464 Localized RGC receptive fields are essential for the transmission of spatial
465 information to the brain. We observed that the size of the receptive fields was similar
466 between photovoltaic and visual responses in the healthy retina. This size did not increase
467 in the degenerate retina, which is consistent with our previous results obtained with a slow
468 (2Hz) sparse white noise stimulus, where a single random pixel was illuminated in each
469 frame (Lorach et al. 2015b). Our current measurements demonstrate that spatial
470 localization is preserved in response to a more dynamic and complex stimulus.
471 Furthermore, we show that the photovoltaic receptive fields of individual RGCs co-localize
472 with their cell bodies, thereby preserving the topological mapping between the inputs into
473 the retina and their representation in the brain. This is an important feature of the network-
474 mediated retinal responses achieved by subretinal implants. Epiretinal implants have been
475 shown to disturb this mapping due to direct activation of axons from remote neurons, which
476 results in distorted visual percepts (Nanduri et al. 2012; Weitz et al. 2015).

477 Temporal response properties of the RGCs, as measured through the STA time
478 course, confirm that the photovoltaic response has shorter latency than the visual one
479 (Mandel et al. 2013; Mathieson et al. 2012), most likely because it bypasses the
480 phototransduction cascade in the photoreceptors. Latency of the photovoltaic responses in
481 healthy retina was somewhat shorter than in the degenerated retina (Table 1). Changes in
482 the neural circuitry of the degenerated retina do not allow for a clear interpretation of this
483 difference. Both the photovoltaic and visual STA time courses had no significant deflection
484 from the average gray level up until about a few hundred milliseconds before the spike.
485 This suggests that RGC spiking activity is affected only by the most recent changes in the
486 stimulus. Such short “memory” is another essential feature of prosthetic vision enabling
487 responses to a rapidly changing visual stimulus. It is important to note that uncertainties of
488 the response latencies in Table 1 are purely statistical. They were calculated based on the
489 cell-to-cell variability of the responses. Additional uncertainty comes from the low sampling
490 rate of the photovoltaic response measurement. The 20Hz photovoltaic white noise movie
491 allowed for 50ms sampling of the time course, likely resulting in overestimation of the
492 latencies. Thus, while we can state with certainty that the photovoltaic responses have
493 shorter latencies than the visual ones, the reported values of these latencies should be
494 used as estimates of the maximum, rather than the exact values.

495 One distinct feature of the photovoltaic STA was three and sometimes four or five
496 (Figure 3,4,6) peaks in the time course, while visual time courses most often have only two
497 peaks. The STA convolution with the stimulus predicts the linear portion of the RGC
498 response in a linear-nonlinear (LN) model of the retina (Chichilnisky 2001). Therefore, the
499 first peak before the spike determines the sign of the preferred change of light level. The
500 second peak of the opposite sign, in turn, predicts how transient the response of the cell
501 will be to a light step of the preferred polarity (Chichilnisky and Kalmar 2002). Thus, the LN
502 model predicts that the spike rate of the RGC will increase and then decrease in response
503 to the preferred direction of the light level change. More than two peaks suggest that RGC
504 will increase and decrease its spike rate more than once in response to the same stimulus.
505 One possible explanation is that flicker fusion does not happen instantaneously and the

506 response to the change in the NIR pulse amplitude persists for a few pulses following the
507 change. With the pulse frequency matching the white noise movie frame rate (20Hz), such
508 persistence might explain the multiple peaks we observe in the pSTA time course.
509 Increasing the frequency of the NIR pulses might eliminate this effect, and previous studies
510 showed that frequencies as high as 40Hz can be used (Lorach et al. 2015b). Another
511 possible explanation to multiple peaks could be that they represent the sum of the distinct
512 contributions from the bipolar cells and photoreceptors, which occur at different latencies
513 (Boinagrov et al. 2014).

514 The opposing center-surround organization we observed in the photovoltaic
515 receptive fields of RGCs in the healthy and in degenerate retinas is another important
516 feature of retinal signal processing preserved in prosthetic vision. Our result is corroborated
517 by the recent study reporting opposing surround in the degenerated mouse retina in
518 response to a subretinal electric stimulation (Stutzki et al. 2016). Receptive field surrounds
519 are thought to contribute to edge detection, and their preservation might result in better
520 prosthetic vision. Two mechanisms are thought to be responsible for the opposing wide
521 surrounds in the visual receptive fields of the healthy retina: (1) negative feedback onto the
522 photoreceptors by the network of the horizontal cells (McMahon et al. 2004; Werblin and
523 Dowling 1969) , and (2) amacrine cells providing inhibitory inputs to bipolar and ganglion
524 cells (Cook et al. 1998; Flores-Herr et al. 2001; Ichinose and Lukasiewicz 2005; Taylor
525 1999). Absence of photoreceptors in the degenerate retina eliminates the original
526 contribution of the horizontal cells to the surround in the RCS retina. At the same time, the
527 surviving horizontal cells form synapses in the inner plexiform layer (Jones and Marc 2005)
528 and we cannot eliminate a possibility of their contribution to the observed surround.
529 Amacrine cells survive the degeneration process (Jones and Marc 2005; Marc and Jones
530 2003) and can provide the opposing surround as well. Determination of the balance
531 between the two mechanisms will require further studies. Both mechanisms involve the
532 surround signal crossing at least one additional synapse compared to the center signal. We
533 see that the surround signals were indeed somewhat delayed in the visual responses. The
534 surround time course had the first peak occur earlier than the center, relative to the spike
535 (Figure 6A,B). The coarser time resolution of 20Hz frame rate, compared to 30Hz in the
536 visual stimulus, did not allow us to accurately measure this difference in the photovoltaic
537 time courses (Figure 6C-F). Intensity of the surround relative to the center in photovoltaic
538 receptive fields was stronger than in the visual responses, except for the RCS pOFF
539 RGCs.

540 Selective photovoltaic activation of ON and OFF pathways in the healthy retina.

541 The distinctly different responses of the ON- and OFF-center RGCs to photovoltaic
542 stimulation in healthy retina, opposite in polarity of their natural visual response, is
543 consistent with electrical depolarization of the photoreceptors, which overcomes the effects
544 of the direct bipolar cell stimulation and elicits responses opposite to hyperpolarization of
545 photoreceptors under visible light. This explanation is supported by elimination of the
546 photovoltaic OFF responses upon pharmacological blockade of neural transmission from
547 photoreceptors to ON-bipolar cells. Our results are consistent with previous findings that

548 ON and OFF RGCs in healthy rabbit retina can be activated by the opposite phases of a
549 sinusoidal electrical stimulus, and that the response of the ON RGCs disappears when the
550 photoreceptor to ON bipolar cells transmission is selectively blocked (Freeman and Fried
551 2011). It was also shown in the healthy mouse retina that the network-mediated component
552 of ON and OFF RGC responses to temporal Gaussian electrical white noise, delivered
553 epiretinally, have distinct STA time courses (Sekhar et al. 2017). As a result, it becomes
554 clear that electrical stimulation of the healthy retina preserves the two major retinal
555 pathways, only operating in reversed polarity: ON becoming OFF and vice-versa,
556 compared to visual responses.

557 Distinct pON and pOFF responses in the degenerated retina.

558 Presence of the distinctly different pON and pOFF responses in the degenerated
559 retina is intriguing. In the absence of photoreceptors, direct depolarization of the bipolar
560 cells with “feed-forward” excitatory signaling to the RGCs should result in all of the RGCs
561 having pON response properties. One possible mechanism for introducing the two
562 response polarities is the depolarization of the rod bipolar cells. In the healthy retina rod
563 bipolar cells relate their signals to the cone pathway through All amacrine cells that have
564 the sign-inverting glycinergic synapse with the OFF cone pathway and the sign-preserving
565 gap-junction coupling to the ON cone pathway. If this circuitry were preserved in the
566 degenerated retina, direct depolarization of rod bipolar cells and/or the All amacrine cells
567 by the photovoltaic prosthesis would lead to the visual ON RGCs being excited at the onset
568 of the photovoltaic stimulus and exhibiting pON responses. In turn, the visual OFF RGCs
569 will have inhibition removed from them at the end of the photovoltaic stimulation and exhibit
570 pOFF response properties. This hypothesis assumes that the consequences of the direct
571 depolarization of the cone ON and OFF bipolar cells are overwhelmed by the signals from
572 the rod pathway. In absence of visual responses in the RCS retina we could not verify the
573 predicted identity of the RGCs with pON and pOFF response properties. While the
574 presence of the pOFF responses in the degenerating retina is surprising, it was also
575 recently observed in the RGC responses to temporal Gaussian electrical white noise,
576 delivered epiretinally to the degenerate mouse retina (Sekhar et al. 2017).

577 An alternative explanation is that some photoreceptor cells survive degeneration
578 and the pON and pOFF responses are mediated through them, similarly to the LE retina.
579 LE rat retina has about eight layers of photoreceptor nuclei. In RCS rat, by p90 days at
580 most a single layer of photoreceptor nuclei is left, and by p180 days practically all
581 photoreceptors are gone (Sauvé et al. 2001). Our experiments were performed in p120 to
582 p360 rats, so we cannot exclude that some photoreceptor cell bodies were still present in
583 the younger animals. However, we did not observe a significant trend in the number of
584 responsive cells or in the ratio between detected pON and pOFF cells over this big range
585 of the degeneration progression. This leads us to believe that the few remaining
586 photoreceptors were not the main conduit of the RGC responses in degenerate retina.

587 Implications of the pON and pOFF responses.

588 Selectivity of the pON and pOFF responses present in the healthy retina might

589 disappear after complete photoreceptor degeneration and therefore might be useful only
590 during the limited period when patients lose outer segments, but the photoreceptor nuclei
591 are still present. However, even in this case, subretinal implants block the supply of
592 nutrients from the choroid to the retina, which quickly eliminates the remaining
593 photoreceptor somas (Lorach et al. 2015a; Lorach et al. 2015b; Lorach et al. 2015c;
594 Mandel et al. 2013). Epiretinal implants do not have such an effect. Long (≥ 25 ms) electrical
595 pulses delivered by an epiretinal implant have been shown to elicit selective network
596 responses (Weitz et al. 2015). If stimulation of photoreceptors without activation of the
597 RGCs and bipolar cells were possible, it could take advantage of the selective activation of
598 the ON and OFF retinal pathways while some photoreceptor somas are still present in
599 degenerating retina.

600 Implications of the distinct pON and pOFF responses in degenerated retina are less
601 certain because the identity of the RGCs exhibiting these responses is yet unknown. If our
602 hypothesis regarding rod bipolar cells-mediated responses is correct, selective activation of
603 the ON and OFF pathways might be possible. However, scarcity of the rod bipolar cells in
604 the center of the macula would prevent the proposed mechanism from being utilized in the
605 foveal region.

606 **Conclusions**

607 Our measurements show that spatio-temporal properties of the RGC receptive fields
608 in photovoltaic network-mediated stimulation of the degenerate retina are similar to those
609 of natural vision, with the most pronounced difference being shorter latency of the
610 photovoltaic responses. Both types of responses are spatially localized, have fast
611 dynamics, and exhibit opposing center-surround organization. Furthermore, we show that
612 not only ON, but also OFF responses to prosthetic stimulation are possible. These
613 similarities raise confidence that subretinal stimulation via small photovoltaic pixels with
614 local return electrodes can result in functional prosthetic vision.

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625 **Author Contributions**

626 AS and DP conceived and designed the research. RS, EH and AS conducted
627 experiments and analyzed the data. XL, LG, TIH, JH, and KM developed and
628 manufactured photovoltaic arrays used in the experiments. EH, RS, GG and AS prepared

629 figures for the manuscript. AS, DP, EH, RS, GG and KM wrote and revised the text of the
630 manuscript. All authors reviewed the manuscript.

631

632

633 **Figure 1**

634 *Photovoltaic array and experimental setup.*

635 **A)** A single module of the photovoltaic prosthesis is composed of 70- μm -wide pixels
636 separated by 5- μm trenches arranged in a 1-mm-wide hexagonal pattern, with the adjacent
637 rows separated by 65 μm . **B)** Close-up photograph of a 2 diode, 70- μm -wide pixel. **C)**
638 Wiring diagram: each pixel consists of two (shown here) or three photodiodes connected in
639 series between the central active (1) and surrounding return (2) electrode. **D)** Schematic
640 representation of a healthy rat retina sandwiched between a transparent multielectrode
641 array (MEA) which records from the ganglion cell layer (GCL) and the photovoltaic array
642 (PVA). Visible light stimulates the photoreceptors (PR), while much brighter pulsed NIR
643 (880–915 nm) illumination generates biphasic pulses of electric current flowing through the
644 tissue between the active and return electrodes of photovoltaic pixels. **E)** Schematic
645 representation of stimulus patterning. An LCD screen modulates the incoming pulsed laser
646 illumination. A white noise stimulus frame is shown. Each pixel in the image is aligned with
647 a pixel on the implant. **F)** Example voltage trace from one of the 512 individual electrodes,
648 before and after artifact removal. Each electrode detects action potentials of multiple cells
649 along with electrical artifacts from the activation of the photodiodes. These artifacts are
650 removed by (1) blanking a short period (~ 8 ms), during which spikes are not recovered,
651 and (2) subtracting a difference of Gaussian function from the raw trace. The parameters of
652 the function are fitted to the data for each artifact on each electrode separately.

653 **Figure 2**

654 Spike-Triggered Average (STA) response to binary white noise stimulus.

655 **A)** The STA is the frame-by-frame average of the short spatio-temporal white noise movie
656 that precedes each action potential of an RGC. The spatial sensitivity profile of the RGC
657 (receptive field) corresponds to the STA regions with significant deviation from the average
658 gray level. **B)** Visual STAs of the example ON and OFF-center rat RGCs. For each cell, the
659 STA frame corresponding to the largest deviation from gray level within the receptive field
660 is shown. The spatial extent of the receptive field is quantified by fitting a 2-dimensional
661 Gaussian to this STA frame. An elliptical $1\text{-}\sigma$ contour of the fit is overlaid on top of the
662 receptive field. The time course shows the STA intensity within the receptive field as a
663 function of time preceding the spike. Overlaid over each time course is a fitted difference of
664 low pass filters (dotted line). ON and OFF RGCs have opposite signs in the STA deflection
665 preceding the spike. The response latency is estimated as the time to the first zero
666 crossing of the fitted function.

667 **Figure 3**

668 Photovoltaic spatio-temporal response properties of the RGCs in RCS retinas.

669 **A)** and **B)** Photovoltaic responses of an example pON and pOFF RGC in RCS retina,
670 respectively. Left panel shows the receptive field and the right panel the corresponding
671 STA time course. **C)** Overlaid time courses of all of the RGCs detected in three separate
672 retinal preparations.

673 **Figure 4**

674 Visual and photovoltaic spatio-temporal response properties of RGCs in the healthy retina.

675 **A)** Responses of an example ON-center RGC. Top panels show receptive fields elicited by
676 the visual and photovoltaic stimulation of the same cell and the middle panels show the
677 corresponding STA time courses. Polarity of the photovoltaic response is opposite to that
678 of the visual response: the visual ON cell (vON) becomes photovoltaic OFF cell (pOFF).
679 The lower panels show the identical electrophysiological images of RGCs responding to
680 visual and electrical activation (see Methods) confirming that the responses of the same
681 RGC were measured. Ellipses overlaid on the receptive field panels correspond to the 1-
682 sigma contours of the 2-d Gaussians fitted to the receptive fields. **B)** Responses of an
683 example OFF-center RGC. The response polarity is again reversed with the vOFF
684 becoming the pON RGC. **C)** Overlaid time courses of all of the RGCs detected in two
685 separate retinal preparations. In each preparation the RGCs were divided into vON and
686 vOFF types according to their visual responses (blue traces on the left). The photovoltaic
687 responses of the same cells (red traces on the right) show response polarity reversal.

688 **Figure 5**

689 Effect of blockers on RGC responses.

690 **A)** STA time courses of RGCs with and without blockers. pON responses completely
691 disappeared under the influence of blockers, while pOFF cells remained active. **B)** Spike
692 counts of cells responding to +/-100% contrast steps. The sign of the step is indicated on
693 the horizontal axis with + for positive and - for negative contrast steps. Error bars
694 correspond to one standard deviation.

695 **Figure 6**

696 Center-surround organization of the receptive fields.

697 **A)** The visual STA receptive field of an ON RGC in the healthy LE retina. The center and
698 surround time courses are shown at the bottom of the panel. The center time course is
699 calculated as the average time course of the pixels located inside the red ellipse. The
700 surround time course is the average of the pixels located between the two blue ellipses.
701 Panels **B), C), D), E)** and **F)** show receptive fields as well as the center and the surround
702 time courses calculated in the same way for visual response of an vOFF LE RGC,
703 photovoltaic response of an example LE pOFF, pON, RCS pON, and RCS pOFF RGC,
704 respectively. **G)** STA response (peak time course deflection preceding the spike) vs.
705 distance from the center of the receptive field. The curves represent the average
706 responses of all the identified RGCs. The distance from the center was measured in
707 standard deviations of the 2D Gaussian fitted to the STA receptive field. The average time
708 course deflections were calculated for eight 1- σ wide bins. The average deflections in each
709 bin were normalized to the deflection in the most central bin. The markers on the RCS pON
710 response curve show centers of the bins. The bands correspond to the standard error of
711 the mean. Visual and photovoltaic OFF responses were inverted for the ease of
712 comparison.

713 **Table 1**

714 Comparison of the spatiotemporal characteristics of the visual and photovoltaic responses.
715 Row 1: Numbers of identified cells that exhibited visual and/or photovoltaic responses and
716 were used in the calculation of the averages. Row 2: Average STA receptive field sizes for
717 visual and photovoltaic responses. Row 3: Average response latency (time-to-zero
718 crossing) estimated from the photovoltaic and visual STA time courses. Row 4: Average
719 time-to-first peak estimated from the photovoltaic and visual STA time courses. Row 5:
720 Offsets between receptive field center location and cell soma. Row 6: Offsets between
721 photovoltaic and visual receptive field center locations. See Methods section for the
722 description of how the quantities in the table were calculated. Standard errors of the mean
723 (S.E.M.) are reported alongside each value. Some averages were calculated for a subset
724 of the cells. Cell counts for those measurements are shown separately.

725

726

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	RCS pON	RCS pOFF	LE pON	LE pOFF	LE vON	LE vOFF
Cell Count	72	32	93	46	46	93
Receptive Field Diameter (μm)	195 \pm 6	170 \pm 8	203 \pm 3	177 \pm 4	202 \pm 5	230 \pm 4
Response Latency/Time-to-zero (ms)	94 \pm 5	90 \pm 8	76 \pm 2	63 \pm 2	185 \pm 4	160 \pm 2
Time-to-first-peak (ms)	50 \pm 3	56 \pm 3	55 \pm 2	42 \pm 3	109 \pm 2	98 \pm 1
Distance between EI and RF centers (μm)	52 \pm 5 (n=35)	81 \pm 17 (n=10)	79 \pm 4 (n=115)		53 \pm 4 (n=115)	
Distance between photovoltaic and visual RF centers (μm)			63 \pm 5 (to vOFF) (n=76)	72 \pm 6 (to vON) (n=39)	72 \pm 6 (to pOFF) (n=39)	63 \pm 5 (to pON) (n=76)

Table 1

Comparison of the spatiotemporal characteristics of the visual and photovoltaic responses.

Row 1: Numbers of identified cells that exhibited visual and/or photovoltaic responses and were used in the calculation of the averages. Row 2: Average STA receptive field sizes for visual and photovoltaic responses. Row 3: Average response latency (time-to-zero crossing) estimated from the photovoltaic and visual STA time courses. Row 4: Average time-to-first peak estimated from the photovoltaic and visual STA time courses. Row 5: Offsets between receptive field center location and cell soma. Row 6: Offsets between photovoltaic and visual receptive field center locations. See Methods section for the description of how the quantities in the table were calculated. Standard errors of the mean (S.E.M.) are reported alongside each value. Some averages were calculated for a subset of the cells. Cell counts for those measurements are shown separately.

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