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Calcium Imaging Reveals a Network of Intrinsically Light-Sensitive Inner-Retinal Neurons

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Summary

Background: Mice lacking rod and cone photoreceptors (*rd/rd cl*) are still able to regulate a range of responses to light, including circadian photoentrainment, the pupillary light reflex, and suppression of pineal melatonin by light. These data are consistent with the presence of a novel inner-retinal photoreceptor mediating non-image-forming irradiance detection.

Results: We have examined the nature and extent of intrinsic light sensitivity in *rd/rd cl* retinae by monitoring the effect of light stimulation (470 nm) on intracellular Ca^{2+} via FURA-2 imaging. Using this approach, which does not rely on pharmacological or surgical isolation of ganglion cells from the rod and cone photoreceptors, we identified a population of light-sensitive neurons in the ganglion cell layer (GCL). Retinal illumination induced an increase of intracellular Ca^{2+} in ~2.7% of the neurons. The light-evoked Ca^{2+} fluxes were dependent on the intensity and duration of the light stimulus. The light-responsive units formed an extensive network that could be uncoupled by application of the gap junction blocker carbenoxolone. Three types of light-evoked Ca^{2+} influx were observed: sustained, transient, and repetitive, which are suggestive of distinct functional classes of GCL photoreceptors.

Conclusions: Collectively, our data reveal a heterogeneous syncytium of intrinsically photosensitive neurons in the GCL coupled to a secondary population of light-driven cells, in the absence of rod and cone inputs.

Introduction

The nature and identity of the irradiance detectors responsible for non-image-forming (NIF) functions has been the subject of intense speculation. In mammals, enucleation abolishes the synchronization of internal circadian time to the external day/night cycle; this finding implies that the origin of the light-sensitive input is ocular [1]. However, classical photoreception is not required for circadian entrainment or for a number of other irradiance-dependent responses. The complete absence of rod photoreceptors (and most cones) in mice homozygous for the retinal degeneration *rd/rd* mutation does not preclude the ability to entrain to a light/dark cycle [1]. Furthermore, the inclusion of a transgene (*cl*) leading to the additional ablation of the cone photoreceptors in an *rd/rd* background confirmed that classical photoreception is not a prerequisite for circadian light sensitivity [2, 3]. These studies suggest that novel retinal photoreceptors mediate NIF irradiance detection.

Irradiance information is relayed to the suprachiasmatic nucleus (SCN; the central circadian pacemaker) and other central brain regions via a dedicated projection, the retinohypothalamic tract (RHT) [4–6]. Electrophysiological recordings from rat retinal ganglion cells that project to the SCN determined that these neurons depolarize in response to light stimulation [7]. Light-evoked changes in membrane potential were measured in the presence of an extensive pharmacological blockade to inhibit rod/cone input or following physical isolation by microdissection, thus confirming that this population of ganglion cells was intrinsically photosensitive. These same neurons were found to express melanopsin [8], a candidate photopigment for NIF photoreception. Melanopsin-expressing cells comprise 1% of the ganglion cell population in the murine retina. Recently, it has been shown that melanopsin knockout mice display a number of functional anomalies in both circadian and pupillary responses to light [9–11], including the loss of intrinsic photosensitivity of the SCN-projecting ganglion cells [11].

Previous reports have exclusively described light sensitivity of single SCN-projecting ganglion cells, and thus the extent and diversity of intrinsic photosensitivity within the inner retina is unknown. Furthermore, both the intracellular signaling pathway and the ion fluxes associated with light activity remain unexplored. In contrast to classical phototransduction in mammalian retinae, the intrinsically light-sensitive ganglion cells depolarize in response to light stimulation [7], which would represent a unique transduction mechanism for mammalian photoreceptors.

Using fluorescent imaging, we investigated the role of Ca^{2+} in the generation of light responses by using the *rd/rd cl* mouse retina as a model system [2, 3, 12]. This preparation has several advantages. First, intrinsic light responses can be detected in a physiological state without the need for potentially toxic pharmacological treatment or physical damage from mechanical isolation. Second, no assumptions are made concerning the retino-recipient targets of the intrinsically light-sensitive cells. And, third, we are able to simultaneously image and screen several hundred neurons of the GCL, including displaced amacrine cells, permitting a comprehensive survey on the nature and extent of inner-retinal photoreception.

We show that retinal illumination at 470 nm induced a Ca^{2+} influx in a subset of the GCL neurons in the absence of rod and cone photoreception. A heterogeneous population of light-responsive neurons was identified, constituting 2.7% of the cells sampled. A propor-

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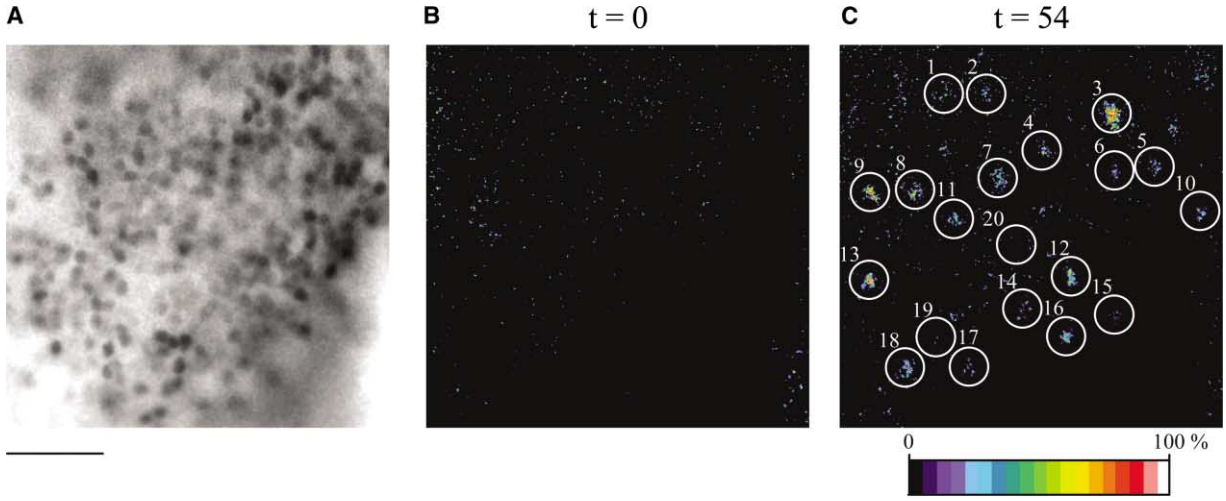

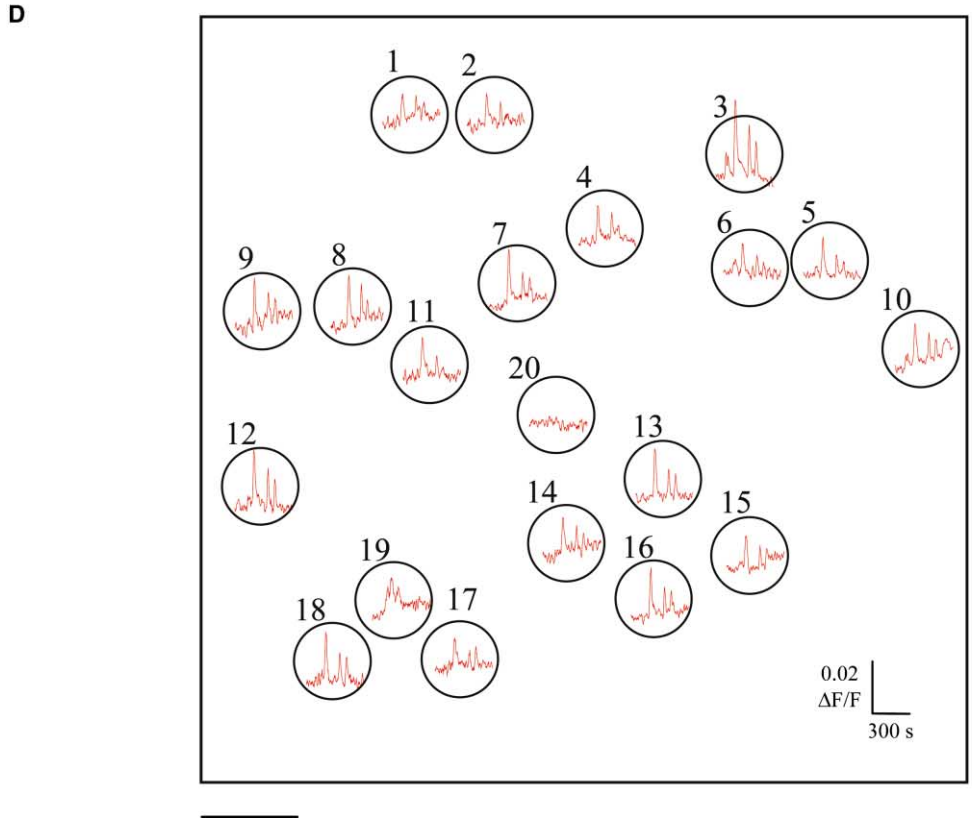

Figure 1. Light Induces Changes in Intracellular Ca^{2+} in GCL Neurons

(A) A typical fluorescence ratio image of a whole-mount *rd/rd cl* retina with the ganglion cell side facing up. Individual cells are clearly visible. Retinae were exposed to 470 nm light stimulation (1.3×10^{15} photons/cm^2/s) for 1 min. Several neurons in this preparation demonstrated light sensitivity.

(B and C) The spatial distribution of all the light-responding cells can be observed by generating images of the change in fluorescence ($\Delta F/\Delta T$). The images were processed by using rolling averaging and subtraction as described in the Experimental Procedures. (B) Before light stimulation (at t = 0 s), the change in fluorescence across the preparation was minimal. (C) During illumination (t = 54 s), several cells demonstrated increases in FURA-2 fluorescence (19 of 223 loaded cells). The scale bar represents 50 μm (see Movie 1 in the Supplemental Data).

(D) The normalized fluorescence intensity ($\Delta F/F$) over time is plotted for each of the light-sensitive cells in the same region. Cells 1–18 responded with synchronous successive transients to light stimulation. An example of the majority of neurons that do not respond is also shown (cell 20).

The scale bar represents 25 μm.

tion of the light-responsive units expressed melanopsin. The light-sensitive neurons formed an extensive network that could be uncoupled by application of the gap junction blocker carbenoxolone.

Results and Discussion

Isolated *rd/rd cl* retinae, ganglion cell side up, were loaded with the UV light-sensitive Ca^{2+} indicator FURA-2AM. Fluorescence ratio images were continuously acquired every 2 s. Following the acquisition of baseline images, retinae were stimulated with monochromatic 470 $\pm$ 20 nm light pulses (via the epifluorescent light source) interleaved between image acquisitions for a 1-min period. This wavelength was selected because spectral analysis of pupillary constriction in the *rd/rd cl* mouse [12] and intrinsically light-sensitive ganglion cells in rodents [7] independently suggested the involvement of a photopigment maximally sensitive at $\sim$480 nm. Furthermore, this wavelength is outside the excitation range of FURA-2 (250–440 nm; Molecular Probes).

Light Induces Ca^{2+} Fluxes in GCL Neurons

Light stimulation at 470 nm was associated with a marked increase in intracellular Ca^{2+} in a subset of the neurons of the GCL (e.g., Figures 1, 2A–2C, 3A, and 3C). The effect of retinal illumination on FURA-2 fluorescence is shown in a typical retinal preparation in Figure 1. Individual cells bodies in the GCL of the retina can be clearly distinguished in the fluorescent ratio image (Figure 1A). The spatial distribution of the light-responding units can be observed by generating images of the change in fluorescence ($\Delta F/\Delta T$). Nineteen out of 223 cells exhibited increases in fluorescence during application of the light stimulus (Figure 1C) (see Movie 1 in the Supplemental Data available with this article online). The light-evoked Ca^{2+} responses took several seconds to develop; following which, synchronous Ca^{2+} transients were observed (Figure 1D). In common with the vast majority of the neurons, cell 20 (as indicated) did not respond to light stimulation.

Approximately 2.7% of cells in the GCL show light-dependent increases in intracellular Ca^{2+} (142 of 5246 cells; n = 18 retinae; Figure 2D). This is significantly greater than the 1%–1.5% of ganglion cells that comprise the murine RHT and suggests that inner-retinal light sensitivity may be more widespread than previously considered. Light-induced Ca^{2+} fluxes could be blocked by application of 1 mM Cd^{2+} (12 of 14 cells), implying that the increases in FURA-2 fluorescence reflect Ca^{2+} influx rather than intracellular Ca^{2+} mobilization. The light-induced Ca^{2+} fluxes could reflect either direct gating of ion channels by light or an indirect secondary response to light stimulation.

Light activity was maintained in the presence of a general ionotropic glutamate receptor antagonist blocking synaptic input to horizontal and bipolar cells (kynurenic acid; KYN; 1 mM or cis-2,3-piperidinedicarboxylic acid; PDA; 1 mM) combined with a metabotropic glutamate receptor agonist blocking ON-bipolar cell activity (L-amino-4-phosphonobutyrate; L-AP4; 100 μM; Figure 4A). This finding confirms that the light-induced Ca^{2+} fluxes were not the result of synaptic activation of glutamate receptors (15 of 16 cells; n = 6 retinae). Extensive previous studies have established that rod and cone photoreceptors are undetectable in the *rd/rd cl* retina [2, 3, 12]. Taken together, this evidence eliminates the

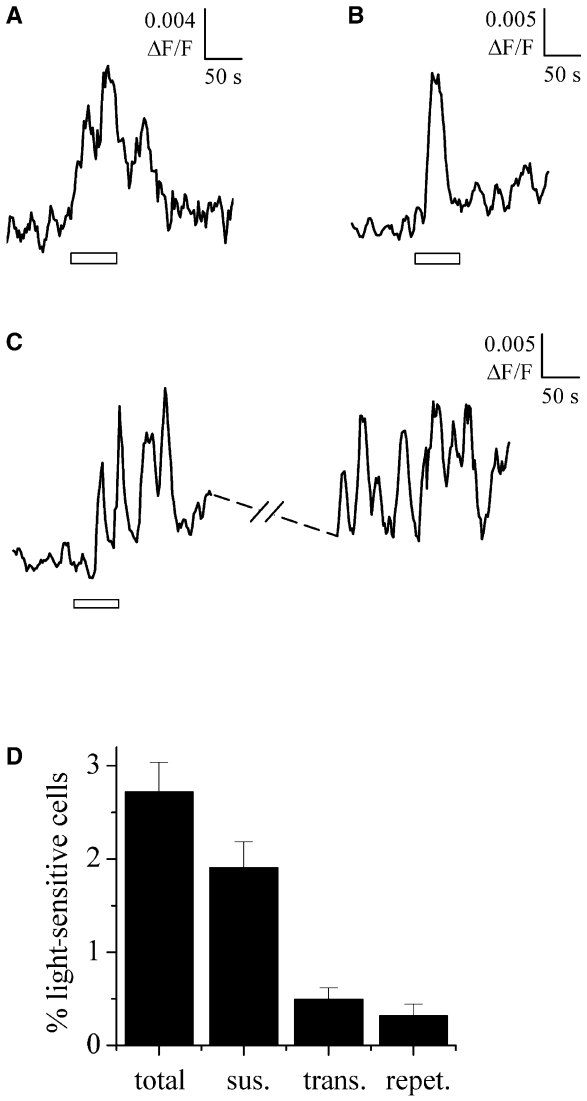

Figure 2. Multiple Types of Light-Evoked Ca^{2+} Responses

In response to 470 nm light stimulation (open bar; 6.7×10^{14}–1.3×10^{15} photons/cm^2/s), three distinct types of change in the normalized fluorescence intensity were observed.

(A) Sustained response. The increase in fluorescence was maintained up to 1 min after termination of the light stimulus.

(B) Transient response. The increase in fluorescence quickly recovered toward the baseline level, typically before the end of stimulation.

(C) Repetitive transients without full recovery. Light stimulation induced successive transients that continued following termination of the stimulus. In the absence of any stimulation for a 10-min period (dashed lines), the cell continued to demonstrate repetitive Ca^{2+} transients.

(D) Of the 5246 cells sampled in the GCL of the *rd/rd cl* retina (n = 18), 142 responded to light stimulation (2.7%). Of these, 97 cells responded in a sustained fashion, 26 were transient, and 19 showed repetitive transients without recovery.

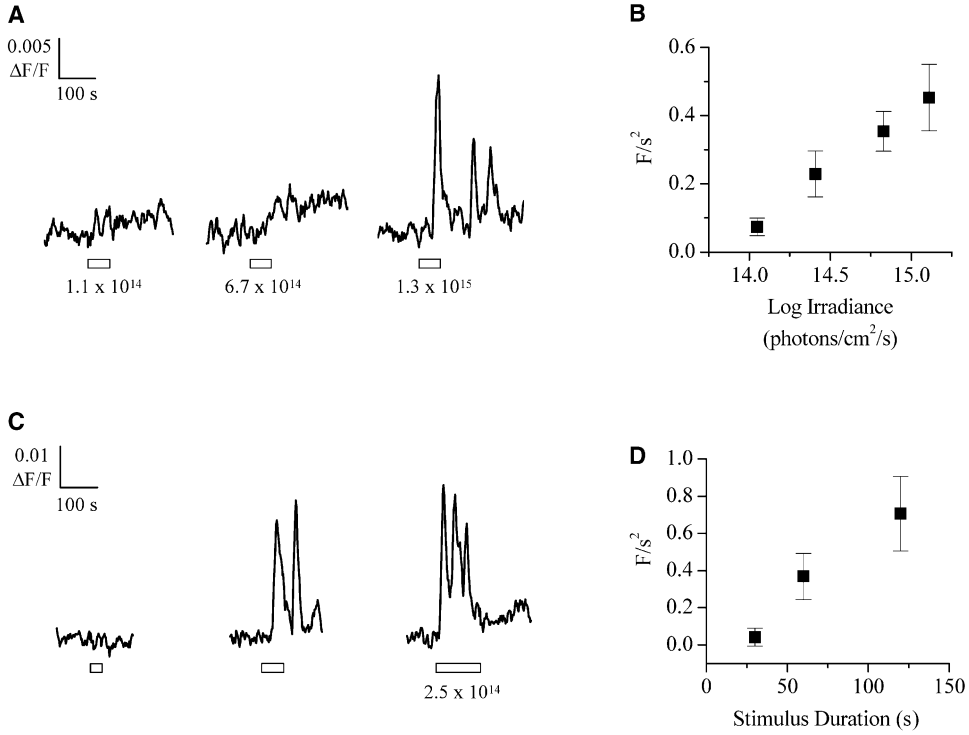

Figure 3. Effect of Varying the Stimulus Intensity/Duration on Ca^{2+} Fluctuations

(A) Increasing the intensity of 470 nm light stimulation (photons/cm^2/s) induced successively greater increases in the normalized FURA-2 fluorescence, as shown for a typical cell.

(B) A linear increase in the averaged integrated fluorescence values (F/s^2) in response to increasing intensities of light stimulation was observed.

(C) The effect of varying the duration of the light stimulus for a typical cell is shown. Calcium transients were induced by stimulus durations of 60 s and above.

(D) A linear relationship between the averaged integrated fluorescence and stimulus duration was observed. Data are expressed as mean ± SEM.

possibility that the light sensitivity of the GCL neurons originates from renegade photoreceptors in these aged *rd/rd cl* retinas.

Multiple Types of Light-Evoked Ca^{2+} Fluxes

We observed three discrete classes of light-induced Ca^{2+} change: (1) *Sustained*: cells continued to show increases in intracellular Ca^{2+} following termination of the light stimulus and generally recovered within 5 min (e.g., Figures 1D and 2A); (2) *Transient*: the increase in intracellular Ca^{2+} quickly returned to baseline levels, typically before the end of light stimulation (e.g., Figure 2B); (3) *Repetitive firing without recovery*: continuous Ca^{2+} oscillations that failed to show full recovery up to 20 min after cessation of the light stimulus were observed (e.g., Figure 2C).

The majority of the light-responsive cells were of the sustained subtype, and a smaller fraction responded transiently or repetitively (Figure 2D). All three types of intracellular Ca^{2+} fluctuation were observed in single retinal preparations, suggesting that they genuinely represent different functional populations within the inner retina. This could signify responses from different retinal cell types and/or the transmission of different types of information.

Sensitivity of Irradiance Detection in GCL Neurons

According to our current understanding, the principle function of the light-sensitive input to NIF photoreception would be to signal changes in ambient light levels. Hence, we determined if the sustained type of light-evoked Ca^{2+} fluxes were dependent on the intensity and duration of the light stimulus. A typical example of the effect of increasing the intensity of light stimulation on intracellular Ca^{2+} levels is shown in Figure 3A. A near log-linear intensity-dependent increase in the cellular Ca^{2+} levels was observed (1.1×10^{14}–1.3×10^{15} photons/cm^2/s; n = 5 retinae; Figure 3B). The threshold intensity for light sensitivity was at $\approx 1.1 \times 10^{14}$ photons/cm^2/s (at this intensity, 6 of 13 cells responded to light stimulation). This suggests that inner-retinal photoreception in the *rd/rd cl* mouse retina is geared toward the detection of high light intensities. The light sensitivity of the Ca^{2+} fluxes in the GCL neurons closely matches the reported sensitivity of the intrinsically photosensitive SCN-projecting GCs in the rat retina [7]. Indeed, the operating range of the pupillary light reflex in the *rd/rd cl* mouse also implicated the involvement of a bright light-sensitive irradiance detector [12].

The light-evoked Ca^{2+} fluxes were also dependent on the duration of the light stimulus (e.g., Figure 3C). The threshold stimulus duration for light responsiveness was

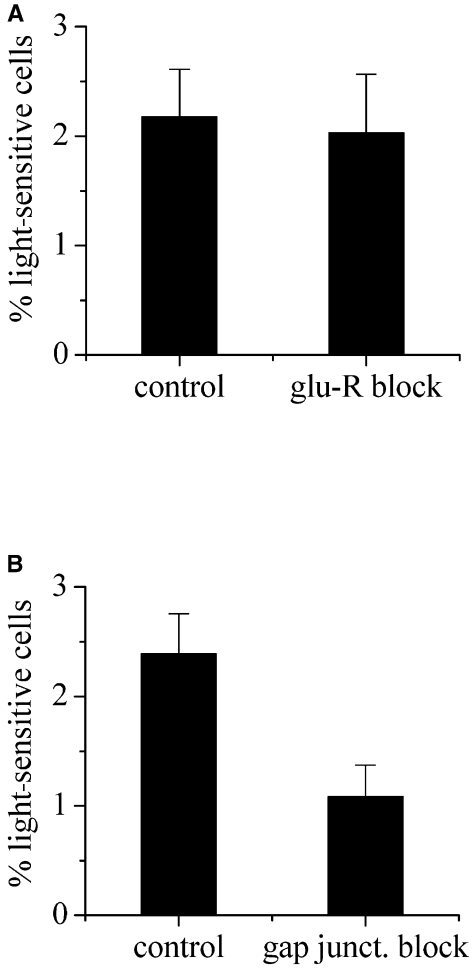

Figure 4. Pharmacological Investigation of Light-Evoked Ca^{2+} Fluxes

(A) The effect of a glutamate receptor blockade on light activity. In the control condition, 16 of 720 cells responded to light stimulation (2.2%). In the presence of Ringer solution containing the metabotropic glutamate receptor agonist L-AP4 and the general ionotropic glutamate receptor antagonist KYN or PDA, the majority of the cells continued to respond (15 of 720 cells; 6.7×10^{14} photons/cm^2/s).
(B) The effect of the gap junction blocker carbenoxolone. In normal Ringer solution, 34 of 1432 cells responded to light stimulation (2.4%), whereas, following application of the gap junction blocker, only 15 of these cells continued to respond (1.1% of the total cell population; 6.7×10^{14} photons/cm^2/s). Thus, gap junction connectivity appears to account for over 50% of the light-responding units. Data are expressed as mean ± SEM.

at ≈30 s (at this stimulus duration, 4 of 10 cells responded to light stimulation; 6.7×10^{14} photons/cm^2/s; n = 4 retinae; Figure 3D). For a 1-min stimulus duration, response latencies (measured at 50% response amplitude) ranged from 2 to 67 s depending on the stimulus intensity and previous stimulation history.

A Functional Syncytium of Light-Sensitive Cells

One compelling feature of the data in Figure 1 is the synchronous nature of the individual light-induced Ca^{2+} wavelets in cells that are some 200–300 μm apart. This is suggestive of a functional syncytium of light-sensitive neurons in the inner retina. Local clustering of light-sensitive cells, often displaying similar response waveforms, was also observed (Figure 1D). We tested whether clustering of light responsiveness might reflect gap junctional connections from intrinsically photosensitive cells to neighboring cells in the GCL.

Retinae were stimulated with 470 nm light in the presence or absence of the gap junction blocker carbenoxolone [13, 14]. In the control condition, a total of 34 of 1432 cells responded to light stimulation (2.4%; n = 5 retinae). Following application of carbenoxolone (100 μM), only 15 of these cells continued to respond (1%). Thus, carbenoxolone treatment reduced the number of light-responding cells by approximately 56% (Figure 4B).

A representative example of this phenomenon is shown in Figure 5. Two cells (as indicated) showed a transient increase in fluorescence in response to 470 nm light stimulation. The gap junction blocker was applied for 10 min before restimulating at 470 nm. In the presence of carbenoxolone, cell 2 continued to respond to the light stimulus. In contrast, cell 1 showed no change in intracellular Ca^{2+} during light stimulation. All three Ca^{2+} response phenotypes were found to survive carbenoxolone treatment. Thus, cell-to-cell coupling does not appear to underpin the diversity of light-evoked Ca^{2+} fluctuations.

Melanopsin Expression in Light-Sensitive Cells

Melanopsin is expressed in retinal ganglion cells that innervate the SCN, the intergeniculate leaflet (IGL), the olivary pretectal nucleus (OPN), and the ventral lateral geniculate [8, 15]; this expression pattern implies that this opsin-like protein is a candidate photopigment for NIF photoreception. In the absence of any biochemical characterization, the precise function of melanopsin (i.e., as a photopigment or photoaccessory protein) is yet to be defined. However, melanopsin has been implicated as a robust marker for at least one population of intrinsically light-sensitive ganglion cells projecting to the SCN [8].

Using melanopsin immunocytochemistry, we observed melanopsin-positive neurons in the GCL of the *rd/rd cl* retina (Figure 6A). In several cases, clusters of melanopsin-positive cells were identified (Figure 6B). By combining the Ca^{2+} imaging with melanopsin immunocytochemistry, we were able to confirm that at least some of the light-responsive cells were indeed melanopsin positive (e.g., Figures 6C and 6D). The combined technique was not sufficiently robust to provide an accurate assessment of the proportion of light-sensitive cells that express melanopsin. However, since we estimate that 2.7% of the neuronal population in the GCL responds to light, it is very unlikely that all of these neurons express this putative photopigment, as it is present in only 1% of the ganglion cells [8]. It remains to be seen if the nonmelanopsin intrinsically photosensitive cells are directly light sensitive (i.e., not coupled) or express another class of photopigment.

Using calcium imaging in whole-mount preparations, we have for the first time been able to define a complete network of light-sensitive inner-retinal neurons that function in the absence of rod and cone inputs. We have established that an increase in intracellular Ca^{2+} plays

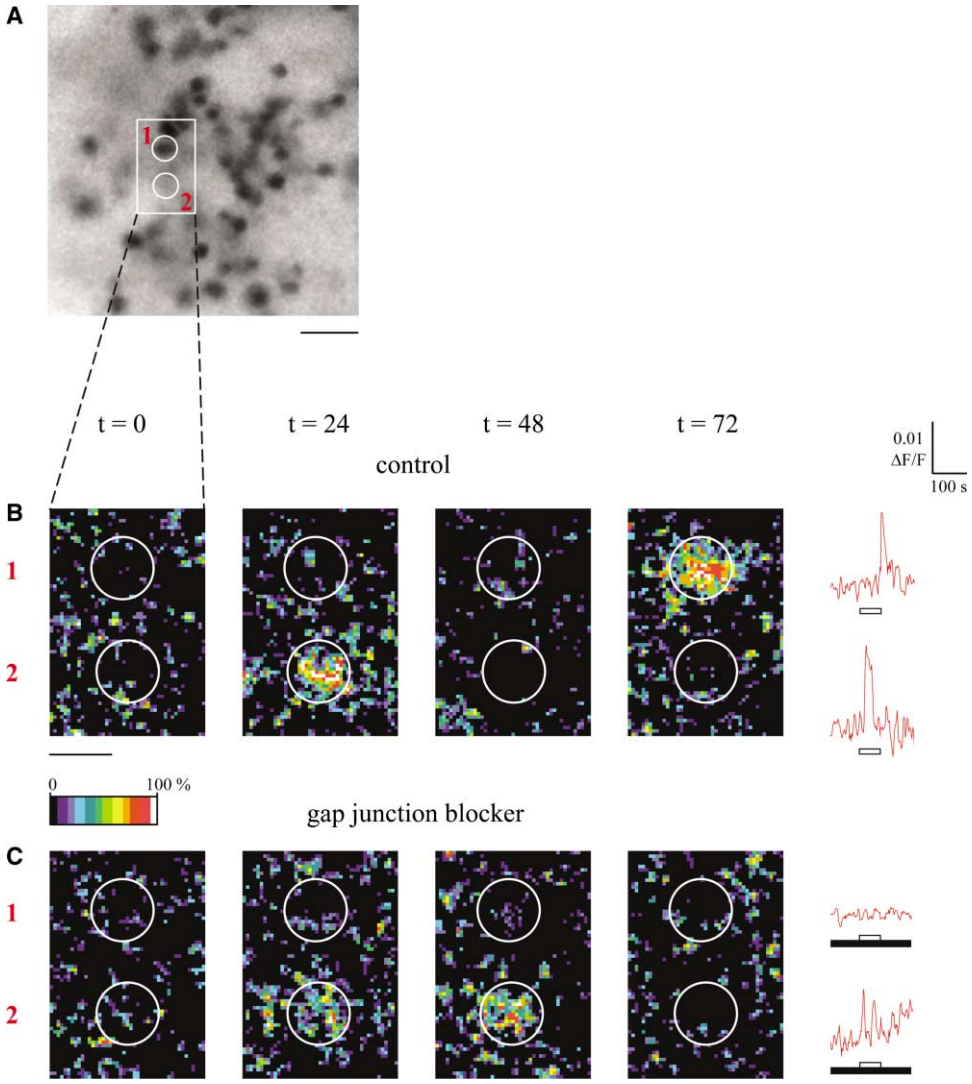

Figure 5. Coupling of Light-Responsive Cells in the GCL

(A) A typical fluorescence ratio image of an *rd/rd cl* retina. The scale bar represents 25 μm.

(B and C) A temporal sequence of the change in fluorescence (ΔF/ΔT) in the two cells indicated during application of the 470 nm light stimulus (at t = 0; 6.7×10^{14} photons/cm^2/s) is displayed in the (B) absence and (C) presence of the gap junction blocker carbenoxolone. Images were processed by using rolling averaging and subtraction as described in the Experimental Procedures. The normalized fluorescence intensity traces for each cell is shown on the right (ΔF/F). In the control condition, light stimulation (open bar) induced transient increases in fluorescence in both cells. The response in cell 1 was delayed by several seconds. In the presence of the gap junction blocker (closed bar), cell 2 continued to respond to light stimulation, whereas no change in fluorescence was detected in cell 1. The scale bar represents 10 μm.

a role in the light sensitivity of neurons of the GCL in the *rd/rd cl* retina (Figure 1). Phototransduction in several invertebrate species is associated with an influx of Ca^{2+} through light-gated cation channels [16, 17], and, indeed, melanopsin may share common features with the invertebrate-like opsins [15]. Currently, there is a paucity of information regarding the signaling cascades linked to novel photopigments. Our results suggest that Ca^{2+} influx is a component of the cell's physiological response to light; however, this does not preclude the involvement of other ions.

It was striking that of the thousands of GCL neurons sampled, some 2.7% showed light-induced Ca^{2+} fluxes, greater than the number of ganglion cells that form the RHT. The present study provides the first comprehensive sample of light sensitivity of all the cells in the GCL of the mouse retina, including any displaced amacrine cells, which are known to form a significant proportion (~50%) of the GCL neurons [18]. Thus, the extent of photosensitivity observed within the inner retina may reflect responses from multiple retinal cell types.

Consistent with a heterogeneous population of light-sensitive inner-retinal neurons, we observed a diversity of light response phenotypes (Figure 2). In addition to multiple cell types, the Ca^{2+} response subtypes may reflect retinal projections to different brain regions [4, 19–21] or, alternatively, innervation of the same regions, but conveying different photic information [22–24]. In-

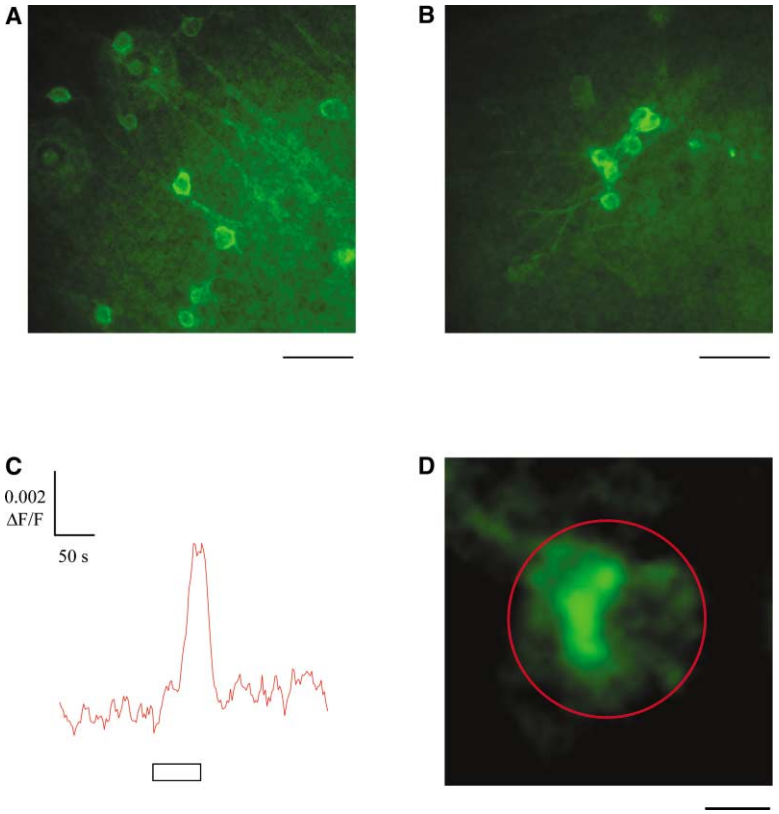

Figure 6. Melanopsin Immunocytochemistry in the *rd/rd cl* Retina

(A) Several melanopsin-expressing neurons are shown in the GCL of the *rd/rd cl* retina. Axonal projections were observed extending toward the optic nerve. The scale bar represents 50 μm.

(B) Clusters of melanopsin-positive neurons were observed in several preparations. The scale bar represents 50 μm.

(C and D) To determine if light-responsive neurons also expressed this protein, FURA-2 imaging was combined with melanopsin immunocytochemistry. A proportion of the light-responding cells expressed melanopsin. (C) For example, a strong increase in the normalized fluorescence (ΔF/F) following light stimulation was observed in the melanopsin-positive cell shown in (D). The scale bar represents 5 μm.

deed, our approach to examining intrinsically light-sensitive cells assumes nothing about the potential retinorecipient target sites that could include the SCN, IGL, VLPO, and OPN. It is unclear if a single or multiple population of ganglion cells innervates each of these targets, either directly or through axon collaterals [19, 25]. Furthermore, recent studies in the human retina suggest that a novel photopigment plays a role in the regulation of local retinal physiology [26]. Thus, the multiplicity of Ca^{2+} response phenotypes could also represent the activity of local circuit neurons responsible for entraining a retinal clock [27].

We have shown that communication between neurons in the retinal GCL accounts for a proportion of the light-sensitive cells. This finding suggests the presence of both intrinsically light-sensitive and extrinsically light-driven cells. Conventional synaptic interactions are unlikely since ganglion cells are not known to communicate centrifugally. Furthermore, if synaptic interactions are present, they were not inhibited by glutamate receptor blockers (Figure 4A). Application of a gap junction antagonist significantly reduced light sensitivity in over 50% of the cells (Figures 4B and 5). Coupling between ganglion cells has been identified in mammalian retinas [28–31]. Intercellular gap junctional communication may provide an important secondary population of light-driven inner-retinal neurons whose role remains to be established.

Conclusions

The data suggest that in the *rd/rd cl* retina, an extensive, heterogeneous coupled network of inner-retinal photoreceptors exists that may mediate a range of NIF responses to light. In the wild-type retina, interactions between rod/cone pathways and the novel photoreceptors may introduce an additional level of complexity. Indeed, the reciprocal coupling of the intrinsically light-sensitive cells in the normal retina may be an important factor in the integration and processing of signals from image-forming and NIF pathways.

Experimental Procedures

Isolated Retinae

C3H/He *rd/rd cl* mice (as previously described [3]) were maintained under a 12:12 light:dark cycle. Food and water were available ad libitum. Animals greater than 90 days of age were killed by cervical dislocation in accordance with UK Home Office guidelines. One eye was removed and placed in ice-cold Ringer solution containing (in mM) 119 NaCl, 2.5 KCl, 1 KH_2PO_4, 1.3 $MgCl_2$, 2.5 $CaCl_2$, 26.2 $NaHCO_3$, and 11 D-glucose, equilibrated with 95% O_2/5% CO_2 (pH 7.4). The eye was hemisected, and the lens and vitreous were removed. The retina was isolated and transferred ganglion cell side up to the recording chamber. The tissue was incubated in Ringer solution containing 10 μM FURA-2AM (Molecular Probes) for 1 hr while bubbling with 95% O_2/5% CO_2. Following dye loading, the retina was washed for 30 min with fresh oxygenated Ringer solution and was continuously superfused during experimentation. The preparation was maintained at a temperature of 28 ± 1°C. All procedures were carried out under dim red light (>610 nm).

Fluorescent Imaging

The retina was viewed with a 40× water immersion objective (0.8 NA) of a Zeiss Axioskop FS2 microscope. The dual wavelength Ca^{2+}-sensitive indicator was excited alternately at 340 ± 15 nm (5×10^{12} photons/cm^2/s) and 380 ± 15 nm (1×10^{14} photons/cm^2/s) via a monochromator (Cairn Research) with exposure times of 400 and 250 ms, respectively. At the specified intensities, the UV excitation light would be too weak to excite the photopigment [12, 7]. Emitted

fluorescence of 510 ± 15 nm was captured with an intensified CCD camera (IPentamax, Princeton Instruments) every 2 s. Ratio images were generated with Metafluor software (Universal Imaging).

Data Analysis

Each cell in the field of view was manually identified, and the averaged fluorescence within each region of interest was plotted over time. The drift in the baseline fluorescence was normalized to the prestimulus values ($\Delta F/F$) [32, 33] by curve fitting with a single asymptotic exponential function ($y = a - bc^x$). To measure the relative Ca^{2+} changes, integrated fluorescence levels were calculated (F/s^2). In order to spatially map the light-sensitive cells in a field of view, captured images were processed by using rolling averaging and subtraction. A five-frame average was applied. To generate subtracted images, the image obtained 20 s previously was subtracted from each frame ($\Delta F/\Delta T$). Where applicable, data are expressed as mean ± standard error of the mean (SEM).

Immunocytochemistry

Immunolabeling with melanopsin antibodies was performed in the recording chamber following fluorescent imaging. The retina was washed with Tris-buffered saline (TBS) and then fixed in 70% cold ethanol in TBS for 10 min. The retina was incubated with melanopsin antibody at 1:500 in TBS/0.3% Triton X-100/0.015% normal goat serum for 1 hr at 37°C. The preparation was subsequently washed for 15 min with TBS before incubation with the secondary antibody, Alexafluor 488 (goat anti-rabbit IgG; Molecular Probes) at 1:200 in TBS, for 1 hr at room temperature.

Pharmacological Treatments

All chemicals were made up in Ringer solution at the concentrations specified: L-amino-4-phosphonobutyrate (L-AP4; 100 μM; Tocris), cadmium (Cd^{2+}; 1 mM; Sigma), carbenoxolone (100 μM; Sigma), cis-2,3-piperidinedicarboxylic acid (PDA; 1 mM; Sigma), kynurenic acid (KYN; 1 mM; Tocris).

Supplemental Data

Supplemental Data including a movie of the effect of light stimulation (470 nm) on the change in FURA-2 fluorescence in neurons of the GCL of the *rd/rd cl* mouse retina revealing a synchronous network of light-sensitive cells are available at http://www.current-biology.com/cgi/content/full/13/15/1290/DC1/.

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