Effect of luminance on response properties of retinal ganglion cells

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Abstract

The mammalian visual system operates over an impressive range of brightness levels covering more than ten orders of magnitude. To ensure efficient encoding in such conditions, the visual system 'measures' ambient light intensity and adapts to it. Light adaptation begins at the very first steps of vision – in the retina, and already there it encompasses more than just a shift of operational range of neurons. The retinal circuits undergo complex reconstructions, which include changing the connection strength between neurons and using different pathways to process visual signals. Extent, localization, mechanisms, and functions of light adaptation-induced changes in the retinal circuits are largely unknown.

In my work I present the results of systematic tracking of light level influence on basic response properties of ganglion cells of the mouse retina. In particular, I tested how luminance levels affect contrast adaptation and responses to simple full-field contrast steps. By using multi-electrode array recordings, I was able to record from the same ganglion cells for many hours and to test each cell with the same stimulus at different luminance levels. This approach provides sampling from many ganglion cells and therefore sheds light onto the big picture of luminance effects on retinal function.

This experimental approach, combined with the use of mutant mouse models with only one functional photoreceptor type, allowed me to establish the conditions *in vitro* under which the retina is driven primarily by rods, by a mixture of rod- and cone-signals, and primarily by cones. I show that the latter regime, so-called photopic, is restricted to a very narrow brightness range *in vitro*, and depends on experimental duration: too bright light can reactivate the rods.

After identifying reliable conditions to characterize retinal processing, I show that the light level has a larger impact on contrast adaptation than previously appreciated, in particular on the firing rate and kinetics adaptation. I also found ON ganglion cells with a novel type of adaptation strategy, and show that this strategy is luminance-dependent. Finally, I analyzed the response structure of ON and OFF ganglion cells to full-field contrast steps and show that responses to such a simple stimulus – in particular responses to light increments – are greatly affected by the ambient light level, to the extent when the cell could behave qualitatively different at different brightness. I show that nearly all OFF ganglion cells display ON responses at least at some light levels, and discuss possible implications for the processing of natural stimuli.

Zusammenfassung

Das Sehsystem der Säugetiere funktioniert über einen Helligkeitsbereich, der mehr als zehn Größenordnungen umfasst. Um effektives Arbeiten unter solchen Bedingungen sicherzustellen, „misst“ das Sehsystem die durchschnittliche Helligkeit und adaptiert entsprechend. Lichtadaptation beginnt bei den ersten Schritten des Sehens in der Netzhaut. Selbst dort passiert bereits weit mehr als ein einfaches Verschieben des Arbeitsbereiches der Nervenzellen. Die neuronalen Schaltkreise in der Netzhaut erfahren einen komplexen Umbau, der Änderungen der Verknüpfungsstärke zwischen Neuronen beinhaltet, sowie die Benutzung unterschiedlicher Leitungsbahnen, um die Sehsignale zu verarbeiten. Das Ausmaß, die Lokalisation, die Mechanismen, und die Funktion der durch Lichtadaptation verursachten Änderungen in den Schaltkreisen der Netzhaut sind zu einem großen Teil unbekannt.

In meiner Dissertation zeige ich den Einfluss von systematischen Helligkeitsänderungen auf grundlegende Antworteigenschaften von Ganglienzellen in der Mäusenetzhaut. Insbesondere untersuchte ich, wie Helligkeitsänderungen sich auf Kontrastadaptation auswirken, und wie sich die Antworten zu Ganzfeldstimulation ändern. Mithilfe von Multielektrodenarrays konnte ich über viele Stunden hinweg die Aktivität der gleichen Ganglienzellen ableiten, und deren Lichtantworten unter verschiedenen Helligkeitsbedingungen charakterisieren. Dieser Ansatz ermöglicht es, viele Ganglienzellen gleichzeitig abzuleiten und daher eine Übersicht über allgemeingültige Einflüsse von Helligkeitsunterschieden auf Netzhautantworten zu gewinnen.

Dieser Versuchsansatz – in Kombination mit Mausmutanten, die nur einen funktionellen Photorezeptortyp besitzen – erlaubte es mir, die Versuchsbedingungen zu definieren, bei denen Netzhautantworten hauptsächlich von Stäbchenaktivität getrieben werden, von einer Mischung aus Stäbchen- und Zapfenaktivität, und ausschließlich von Zapfenaktivität. Ich konnte zeigen, dass der letztere Funktionsmodus, das sogenannte photopische Sehen, *in vitro* auf einen sehr engen Helligkeitsbereich beschränkt ist, der außerdem von der Versuchsdauer abhängt. Zu helles Licht kann zu Reaktivierung der Stäbchen führen.

Nachdem ich verlässliche Versuchsbedingungen bestimmt hatte, um die Signalverarbeitung in der Netzhaut charakterisieren zu können, wies ich nach, dass die absolute Helligkeit einen größeren Einfluss auf Kontrastadaptation hat als dies vorher bekannt war. Insbesondere betroffen waren die Adaptation der Gesamtaktivität (Feuerrate) und der Antwortkinetik. Ich beschreibe ON Ganglienzellen, die eine neuartige Adaptationsstrategie ausweisen, die von der Helligkeit abhängig ist. Als letztes untersuchte ich die Antwortstruktur von ON und OFF Ganglienzellen auf Ganzfeld Stimuli

(schrittweise Erhöhung der Helligkeit oder Abdunklung). Die Antworten zu diesem einfachen Stimulus – insbesondere Erhöhung der Helligkeit – sind sehr stark von der Hintergrund-Helligkeit abhängig. Dies führt so weit, dass viele Zellen qualitativ unterschiedliche Antwortmuster bei unterschiedlichen Helligkeiten aufweisen. Ich konnte zeigen, dass nahezu alle OFF Ganglienzellen auch ON Antworten haben, zumindest bei vereinzelten Helligkeitsstufen. Ich erörtere die möglichen Auswirkungen dieser Befunde auf die Verarbeitung von natürlichen Lichtreizen.

Content

1. General Introduction.

The range of possible outputs generated by a neuron is much smaller than the range of natural sensory inputs. In order to provide the most efficient encoding, sensory systems estimate the statistics of the current inputs and adjust the properties of neuronal circuits. This process is called adaptation.

In the visual system, adaptation to a large degree occurs already at the first steps of processing – in the retina. The retina can adapt to a number of different features of the incoming stimulus. Ambient light intensity is one of the simplest features in terms of statistics the retina has to adapt to; at the same time it might be the most challenging in terms of orders of magnitude. The range of intensities the retina can operate at covers about 10^{12} [1].

The main mechanism underlying the ability of the visual system to function over such an enormous range of light intensities is the use of two different photoreceptor classes, namely the rods and the cones. The corresponding broad categories of light intensity are called scotopic (only rods are active, starlight vision), mesopic (both rods and cones are active), and photopic (rods are saturated, and only cones are active, daylight vision). Rods and cones convey their signals through specialized circuits, eventually converging onto retinal ganglion cells, the output neurons of the retina [2]. The intensity levels in which both photoreceptor systems are simultaneously active is of particular interest: rod and cone driven signals possibly interact on many levels in the retinal circuitry and both contribute to the final responses of ganglion cells.

The light responses of rods and cones themselves are not simply scaled versions of each other. Cone integration time is smaller than that of rods, which leads to faster kinetics of responses also of the downstream neurons [3]. The retinal circuits also undergo considerable changes under light adaptation, sometimes in a nonlinear fashion. For example, the conductivity of gap junctions in the horizontal cell and amacrine cell networks is small in darkness and under bright light, but much bigger in the mesopic regime [4]. These changes affect the responses of ganglion cells.

Ganglion cells can be divided into 15 to 20 functional types [5-6], each of them transmitting information to the brain about a certain subset of features of the image stream impinging onto the photoreceptors. The specific response pattern of each ganglion cell type is largely shaped by the connectivity with the presynaptic circuitry and the precise spatio-temporal interactions of all cells involved. Sophisticated response patterns produced by retinal ganglion cells [7] include direction-selective responses [8-10], approach-sensitive responses [11], object-specific motion sensitivity [12], or color specificity [13-14].

For many of these feature-specific response patterns it is not known if the ganglion cells might report on different aspects of the visual stimulus in rod- and cone-driven regimes, or if the response patterns stay consistent across different light levels. For example, direction-selective ganglion cells are directionally selective over a wide range of conditions [15], while color processing is restricted to the cone regime [16]. The circuit of approach-sensitive ganglion cells involves a gap junction through which the information flow reverses in dark- and light-adapted conditions. The particular circuit structure predicts that these cells are approach-sensitive only in the light-adapted case [11]. Furthermore, ganglion cells can change their spatial and temporal receptive field properties during light adaptation: some ganglion cells obtain a receptive field surround [15, 17-22] some are able to respond to higher temporal frequency stimulation under brighter illumination [19, 23].

A full-field step of light has been extensively used in retinal neurophysiology to characterize ganglion cells and study their circuits. Parameters of these responses, such as latency, duration, and relative amplitude of ON and OFF components, are often used as a basis for ganglion cells classification [5, 24]. In most cases, however, only the responses at one or at most a few light levels have been tested. Other studies investigated more light levels, but only characterized few cells (e.g. [25]). In the study of Sagdullaev [25] some ganglion cells (4/8) showed considerable changes of response patterns in response to a full field step of light: from pure OFF response at low light level to ON-OFF response at 1 log unit brighter background light. Farrow [15] described a ganglion cell type, PV1, which increases spiking activity in response to a large spot of light (1000µm) at low light levels and decreases activity at higher brightness (by a factor of 10). The mechanism of this switch-like behavior involves activation of the receptive field surround at cone threshold through wide-field amacrine cells. These examples suggest that the responses of ganglion cells to full-field steps of light may change dramatically at different light levels.

The interaction of different forms of adaptation has also been scarcely studied. For example, the retina can adapt not only to the mean intensity, but also to the fluctuation of the sensory input, or its variance, both in spatial and temporal domains [26-28]. Adaptation to temporal contrast affects regulation of sensitivity, temporal filtering, and average responses of ganglion cells [28-31]. Some amacrine cells and bipolar cells show similar adaptive changes, whereas horizontal cells and photoreceptors do not [30, 32]. These findings put the sites of adaptation within inner retinal circuits (for review, see [31], which are in turn affected by light adaptation. The consequences of light adaptation on contrast adaptation properties have not yet been understood.

In my work, I aimed to characterize basic properties of retinal processing over eight orders of light intensity, ranging from the scotopic to the high photopic range, with the focus on stimuli that are

widely used in retinal research: Gaussian white noise full-field flicker and full-field steps of light. For recordings, I used multi-electrode arrays, which has the advantage that many ganglion cells of an isolated retina can be recorded in one experiment over several hours. I recorded responses to the same set of stimuli repeatedly presented during 20 to 40 minutes at each of the eight light levels, to investigate the temporal evolution of the response patterns on a large time scale.

In the first chapter of this thesis, I describe the responses of ganglion cells to Gaussian white noise flicker. I establish at which light levels the responses are stable and driven by only rods, both rods and cones, and only cones. I find that the classical trichotomy of light intensity ranges (scotopic, mesopic, photopic) does not completely hold true in the *in vitro* situation. I show that rod photoreceptors can escape saturation in photopic light levels and therefore can always contribute to ganglion cell responses in experimental *in vitro* conditions. I also find that the balance of rod- and cone-driven inputs to ganglion cells changes in either direction under bright illumination, in a stimulus-dependent and circuit-specific way. These findings suggest that the functional relationship between rod- and cone-driven pathways and responses is much more complex than previously appreciated.

In the second chapter, I describe the properties of adaptation to temporal variance (contrast adaptation) at different light levels. I analyze firing rate, and parameters of the linear-nonlinear model: kinetics of the linear filter and the gain. I show that the luminance level modulates each of these parameters in a nonlinear fashion; furthermore, this influence is different for ON and OFF cells. I also show cells which have 'non-typical' adaptation features: they decrease their mean firing rate at high contrast and have accelerated kinetics of the linear filter at low contrast.

In the third chapter, I analyze the responses of ganglion cells to full-field steps of light. I show that all ganglion cells have rather uniform and stable responses to light decrements and more diverse responses to light increments. Many OFF ganglion cells (as identified by linear filter polarity) also have low-latency or delayed ON responses to light increments – this last property is strongly modulated by luminance. Despite variability of responses, I find distinct groups of ganglion cells with consistent changes of the response pattern across light levels. I also investigate asymmetries in responses to light increments and decrements as parts of steps with positive and negative contrasts.

2. Methods

Animals

I used several mouse strains in my experiments. As wild type animals, I used PV-Cre x Thy-S-Y mice [11, 15] which have a C57Bl/6J background. As a model of rod-only retina with non-functional cones, I used Cnga3 -/- [33], kindly provided by M. Biel. As a model of cone-only retina with non-functional rods, I used Rho -/- [34], kindly provided by P. Humphries. As a model of the melanopsin lacking retina, I used Opna4 -/- [35], kindly provided by K.W. Yau. The age of wild type animals was 5 weeks to 6 months at the time of the experiments, Cnga3 -/- animals were 2 months, the Opna4 -/- animals were 5 to 6 months, Rho -/- animals, which show progressive retinal degeneration, were between 5 and 7 weeks. Animal use was in accordance with German and European regulations and approved by the Regierungspräsidium Tübingen.

Retina preparation

Mice were kept on a 12/12 hour light/dark cycle, dark-adapted for 4-16h before the experiment, and sacrificed under dim red light by cervical dislocation. The eye cups were removed, put in Ringer solution (in mM: 110 NaCl, 2.5 KCl, 1 $CaCl_2$, 1.6 $MgCl_2$, 10 D-Glucose, and 22 $NaHCO_3$) bubbled with 5% CO2 / 95% O2. The retina was isolated and attached to a nitrocellulose filter (Millipore) with a central 2x2 mm hole, with the optic nerve head centered. Experiments were performed at different circadian times with no noticeable effects on the outcome.

MEA recordings

All recordings were performed with a perforated 60-electrode MEA (60pMEA200/30iR-Ti-gr, Multichannel Systems, Reutlingen). The mounted retina was placed ganglion cell-side down in the recording chamber, and good electrode contact was achieved by negative pressure through the perforated MEA. The tissue was superfused with Ringer solution at 33-35 °C. Data was recorded at 25 kHz with a USB-MEA-system (USB-MEA1060, Multichannel Systems, Reutlingen).

Light Stimulation and Attenuation

Light stimulation was performed from the bottom with a digital light processing (DLP) projector (PGF212X-L, Sharp) and focused onto the photoreceptors through the condenser of the

microscope (*Fig. 2.1*) 1 screen pixel covered 3x3 μm² on the retina. The light path contained a shutter and two motorized filter wheels, each with a set of neutral density (ND) filters (Thorlabs NE10B-A to NE50B-A), having optical densities from 1 ("ND1") to 5 ("ND5"). To achieve light attenuation stronger than 5 log units, I serially combined an ND5-filter in one filter wheel with another ND-filter in the second filter wheel, to achieve optical densities from 6 to 10. I refer to the filter settings and the corresponding ambient brightness levels as ND1 (the brightest setting I used, 10-fold light attenuation) to ND8 (darkest setting used, 10^8-fold light attenuation).

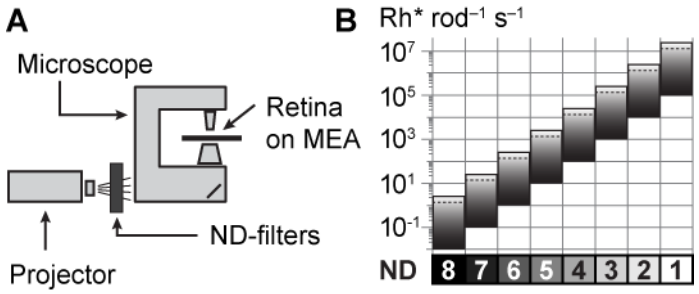

Fig. 2.1. Experimental layout. (A) Schematic of the experimental setup. (B) Light intensities used in the study. Neutral density (ND) filters were inserted into the stimulation light path to set the ambient background light level (indicated by horizontal dashed lines within the boxes). Stimulus intensities varied linearly around that background within the range outlined by the boxes. The maximal contrast of the stimuli at each light level were +1 and –1 (Weber contrast) relative to the ambient intensity.

Experiments usually began at ND8 (i.e. combination of ND5 and ND3 filter). Every 20 to 40 minutes, the ambient stimulation brightness was increased one step by changing the ND filters by 1 unit. Every time when ND filters were changed during the experiment, I closed the shutter to prevent intermittent exposure to bright light. Unless otherwise noted, I presented the same set of visual stimuli at each ND level during an experiment.

The stimulus projector could produce output spanning 3 log units of light intensities (i.e. 1000-fold intensity ratio between black (‘0’) and white (‘255’) pixels). I linearized the projector output, and limited the visual stimuli to the range from ‘0’ to ‘60’, with the background set to ‘30’. As a consequence, the brightest pixels at any given ND-filter setting were 5-fold dimmer than the background illumination at the next brighter ND-setting.

Light stimuli

All stimuli were gray-scale images with pixel values between ‘0’ ("black") and ‘60’ ("white"). The background was kept at ‘30’ ("gray"), and the stimuli were balanced so that the mean intensity over time equaled background. The stimulus set (*Fig. 2.2*) contained:

(1) Full-field steps of light. The duration of each step was 2s, intensity was either ‘50’ (positive step) or ‘10’ (negative step). Given background intensity of ‘30’, the contrast of the steps, calculated as $(I_{st}-I_{bg})/I_{bg}$, was equal to +0.66 and -0.66, correspondingly. Each trial of full-field steps consisted of 5 positive and 5 negative steps, interleaved with 5s interstimulus interval (*Fig. 2.2* A).

(2) Full-field chirp. Stimulus intensity was updated for 8 s according to the formula intensity = $30 + 30 \sin(\pi (t^2 + t/10))$ (frequency-modulated chirp, frequencies range from 1 Hz to 8 Hz, t given in s) and for 8.125 s according to intensity = $30 + 3.81\ t \cos(4 \pi t)$) (amplitude-modulated chirp) (*Fig. 2.2* B).

(3) Full-field Gaussian white noise (GWN) flicker. Screen brightness was updated every frame (60 Hz) and drawn from a Gaussian distribution with mean 30 and standard deviation 1.8 (low contrast) or 9 (high contrast) *(Fig. 2.2* C). The duration of high contrast and low contrast presentations varied in different types of experiments. I used the following GWN stimulus sets:

a) 1 minute trials of high contrast and low contrast, separated by several seconds of background

b) 4 or 5 minute trials where 30s of high and 30s of low contrast were interleaved

c) 1 minute trial where 10s of high and 10s of low contrast were interleaved

The conclusion drawn from different stimulus sets did not differ.

(4) Checkerboard Gaussian white noise flicker, 8 min. The screen was divided into a checkerboard pattern; each checker covered 60x60 μm^2 on the retina. The intensity of each checker was updated independently from the other checkers in the same way as for the high-contrast full-field Gaussian flicker (*Fig. 2.2* D).

(5) Natural movie: gray scale with intensity range from ‘0’ to ‘60’. The movie consisted of snippets of several seconds duration, taken from https://www.youtube.com/watch?v=cG6n3Z7qwVs. Total length was 655 frames shown at 30Hz refresh rate (total duration 21.8s).

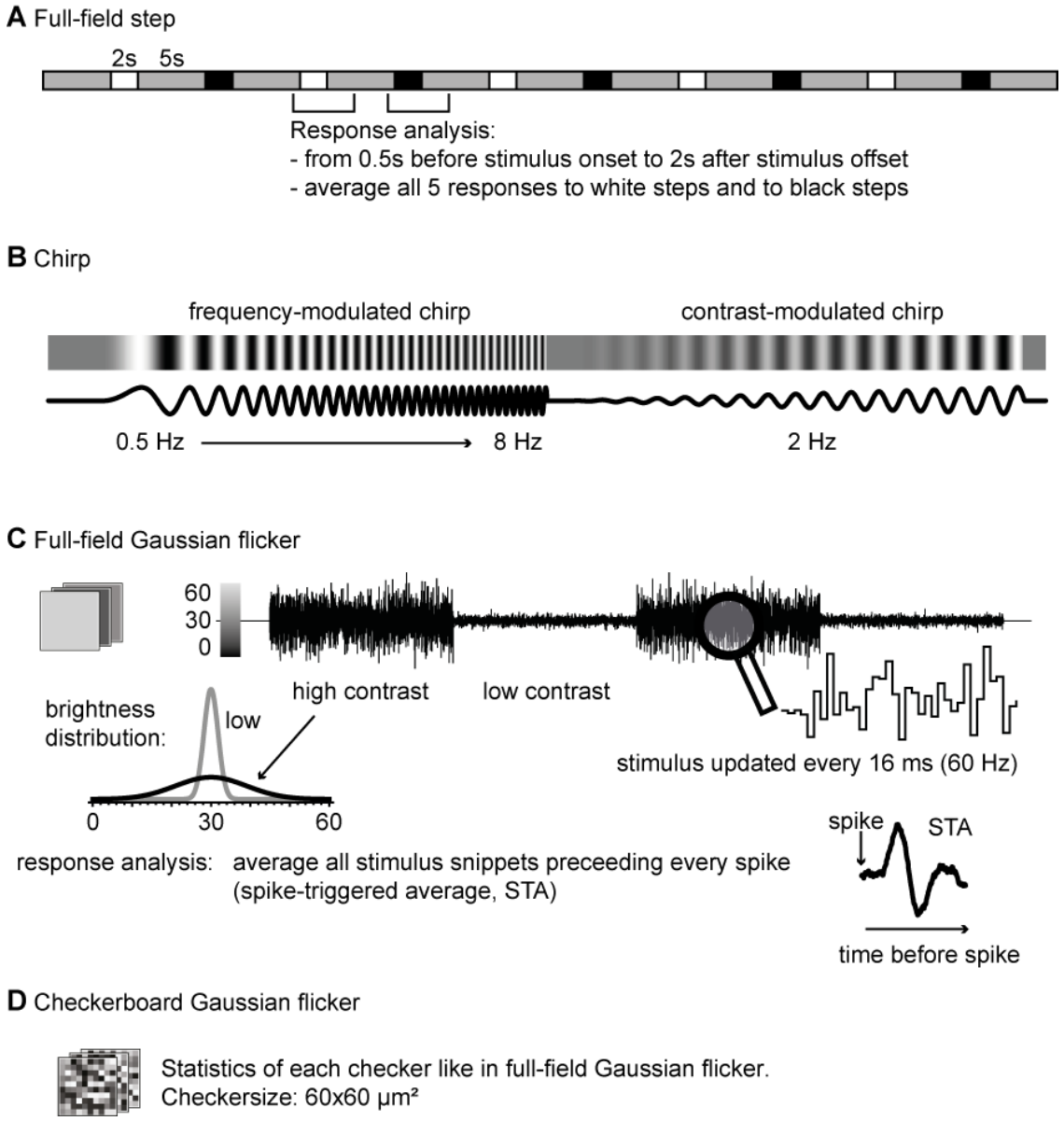

Fig. 2.2. Schematic of used stimuli. A. Full field steps. B. Chirp. C. Full-field Gaussian flicker. D. Checkerborad Gaussian flicker.

I used different combinations/subsets of these stimuli in different experiments, some of them repeated several times at each brightness level. In any combination, the complete experimental stimulus set lasted at least 20 minutes at each light level. Details are given in the results.

Light Intensity Measurements

I measured the spectral intensity profile (in $\mu W \cdot cm^{-2} \cdot nm^{-1}$) of my light stimuli at the position of the retina in the experimental setup, but without the MEA in place, with a calibrated USB2000+ spectrophotometer (Ocean Optics). The stimulus intensity was then transformed into equivalents of

photoisomerizations per rod and second, assuming dark-adapted rods, as described in [11]. Briefly, the spectrum was converted to photons·cm^{-2}·s^{-1}·nm^{-1}, then convolved with the normalized spectrum of rod sensitivity [36], and finally multiplied with the effective collection area of rods (0.5 μm²) [37]. The results range from 2 Rh*·s^{-1} per rod (ND8) to 2·10^{7} Rh*·s^{-1} per rod (ND1), see *Fig. 2.1.* According to the calculations, ND8 to ND6 is expected to be scotopic (ganglion cells are driven only by rods), ND5 – mesopic (both rods and cones drive ganglion cells), and ND4 to ND1 – photopic (only cones drive ganglion cells).

Equivalently, I calculated the isomerization rates for pure M- and UV-cones (collecting area 0.2 μm²) [38]. They were 1/2 (M) and 1/200 (UV) of the rod values, respectively. The weak UV-cone activation was due to a built-in UV-filter in the projector.

Spike sorting

Data was originally acquired with MultiChannel Systems software MCRack. I designed and programmed software (MATlab, MathWorks) for preliminary data analysis. Main steps included:

- high-pass filtering of the raw data (500Hz, 10th-order butterworth filter)
- extraction of spike waveforms and spike times
- spike sorting, and thereby assignment of spikes to individual units (presumably ganglion cells) was performed semi-manually with custom written software (Matlab). Quality of each unit was manually assessed by interspike interval and spike shape variation.

Data analysis was based on the spiking responses (spike times) of individual units.

Local field potentials (LFPs)

Data was low-pass filtered (100Hz, 2$_{nd}$-order butterworth filter) and then downsampled to 1kHz for the analysis of LFP responses.

Data analysis

Calculation of linear filters. I calculated linear filters in response to the Gaussian flicker (full-field or checkerboard) by summing the 500 ms stimulus history before each spike. The stimulus intensity was first normalized to range from -1 to 1 (instead of '0' to '60'). Peak latency and amplitude of the linear filter were determined by latency and amplitude of the first peak. To make those estimates more robust against noise, I fit the first peak of the filter with a Gaussian. The latency was then taken as the position of the Gaussian fit along the time axis; the amplitude was read from the apex of the Gaussian fit. Another latency parameter was taken as the time of the first zero crossing after the first

peak of the filter. To calculate linear filters from the LFP responses, for each time point of the LFP response the 500 ms stimulus history before that point was multiplied with the LFP amplitude at that point, and these entire weighted stimulus vectors were summed. The resulting linear filters were much less noisy than the spike-based linear filters; latencies and amplitudes could thus be read directly from the filter without Gaussian fits.

Calculation of nonlinearity. First, I convolve the stimulus (GWN flicker) with linear filter obtained as described in the previous section. The resulting vector is a linear prediction of the cells response, the so-called 'generator signal' [39]. Then I split the generator signal values into 61 bins, and averaged the actual firing rate of the cell corresponding to generator signal bins. The resulting 61-vector is then fitted by a sigmoidal function:

$$y = x_0 + \frac{m}{1 + \exp(\frac{x_{1/2} - x}{r})},$$

where parameters have clear physiological meanings: x_0 is the basal firing rate, m is the maximal firing rate, $x_{½}$ is the x value at 50% of maximal firing and $1/r$ represents the gain [40].

Firing rate calculation. I estimated the instantaneous firing rate of ganglion cells by convolving the spike train (i.e. time series of 0's and 1's) with a Gaussian with sigma of 40 ms and amplitude of 0.25 $\sqrt{e}$ sigma^{-1} (10.3 Hz for sigma = 40 ms).

Chirp analysis. Estimation of response modulation for chirp stimuli. I first calculated the instantaneous firing rate of individual ganglion cells in response to chirp stimuli. Then, I subtracted the mean firing rate during spontaneous activity (i.e. 3s prior the stimulus). This can result in negative firing rates if the stimulus causes suppression of responses, which also is a form of response modulation (without this correction, only minor details in the outcome are different, and the main conclusions are not influenced). Next, I took the absolute value, and calculated the running modulation strength by taking a moving average with a window of fixed size (500 ms). The choice of the window size introduces minor artifacts in the frequency-modulated chirp (ringing, most clearly visible in *Fig. 3.10* in the rod-only responses), which does not affect the further analysis – the same conclusions about the modulation strength at higher frequencies hold true for smaller and larger windows. I chose 500 ms because this is the stimulus period for the intensity-modulated chirp.

Statistics. I used the Wilcoxon signed-rank test to assess the significance of difference between distributions. Sometimes I also used paired Student t-test; in these cases it will be noted.

Computational model

To estimate the effect of photoreceptor bleaching on our results, we modeled the number of rhodopsin molecules R (i.e. opsin with bound 11-*cis* retinal) in a photoreceptor:

$$\mathrm{d}\,R / \mathrm{d}\,t = \rho\,(R_{\max} - R(t)) - stimulus(t)\,(R(t) / R_{\max})$$

$R_{\max}$ is the total number of opsin molecules; ρ is the regeneration rate, i.e. the rate at which bleached opsin ($R_{\max} - R(t)$) is being reloaded by 11-*cis* retinal. This regeneration of rhodopsin is counteracted by bleaching, the rate of which depends on stimulus intensity. In the model, stimulus intensity is given in R* rod^{-1} s^{-1}, assuming all opsin molecules are loaded with 11-*cis* retinal and are available for the photon response.

We estimated the maximum regeneration rate ρ as it might be expected in the *in vivo* situation, from the data of Pepperberg (their Figure 6) [41]. After 50% bleaching, they found rod responses to recover to maximum sensitivity in about 60 min with "infinite" supply of 11-*cis* retinal. *Fig. 2.3* shows the recovery behavior in our model with $\rho = 10^{-3.3}$, overlaid on the data of Pepperberg. We used this value to estimate photoreceptor responses *in vivo* (*Fig. 3.11* A,C). The *in vitro* value of ρ, valid for our experimental conditions, was estimated from experiments when we returned to dark luminance conditions (protocol as shown in *Fig. 3.11* B). Ganglion cells responded robustly down to ND5, but never at ND6 or darker. This is consistent with earlier reports that rod responses at the scotopic level cannot be recovered *in vitro* after a bleaching stimulus [42]. The model reproduced this behavior when ρ was reduced to 10^{-6}. With this setting of $\rho = 10^{-6}$, the behavior of the model at the ND5/ND6 boundary was robust against changes of the value of $R_{\max}$, the total number of opsin molecules, between 10^7 and 10^9. However, the model behavior at ND3 depended strongly on $R_{\max}$. With $R_{\max} = 10^{7.6}$, the model reproduced the drop below saturation at ND3 with the time course shown in *Fig. 3.11* B. With smaller $R_{\max}$, this effect could already be seen at ND4, with larger $R_{\max}$, only at ND2. For all simulations shown in *Fig. 3.11*, $R_{\max} = 10^{7.6}$ was used.

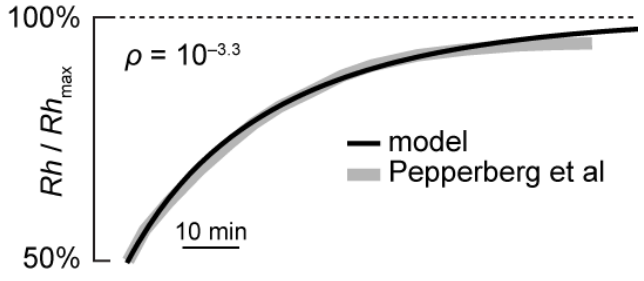

Fig. 2.3. Estimation of the in vivo regeneration rate for the computational model. With a factor $\rho = 10^{-3.3}$, our model matched the rate measured by Pepperberg [41].

3. Effect of luminance on kinetic properties of ganglion cells.

3.1. Introduction

The visual system is sensitive enough to detect single photons. The same system can efficiently handle a photon flux which is 10^{12} fold brighter. To make this possible, light adaptation begins already in the retina. The adjustment of sensitivity predominantly happens in the photoreceptors. With adaptation to increasing ambient light levels, many properties of retinal processing change. One well documented change is that retinal responses become faster, which is initiated in the outer retina and can be shown by using Gaussian white noise (GWN) flicker stimuli and the formalism of the linear-nonlinear model [3, 39]. The theoretical basis of this model is well described in the literature, and this model has become a popular framework to characterize various features of retinal circuits. The linear filter – part of the model – represents the approximation of the impulse response and reflects the temporal properties of the retinal circuit converging on a ganglion cell. It is then expected that the linear filter will accelerate as the light level increases. This has indeed been shown for different cell types (bipolar, amacrine, and ganglion cells, see Introduction); however, a consistent measurement of the linear filter kinetics in the same cells across many light levels is still missing. I therefore chose the GWN stimulus and linear-nonlinear model to probe the influence of luminance on the kinetics of ganglion cell responses.

3.2. Results

I recorded 520 cells in 15 wild type retinas. The stimulus set in each experiment contained 2 to 24 trials of full-field Gaussian white noise flicker (GWN) and was repeated at 8 luminance levels. For each trial, I calculated the spike-triggered average stimulus (linear filter) from the spiking responses of every individual ganglion cell at each of the eight brightness levels (ND8-ND1). Typical linear filters of an ON and an OFF cell at ND7-ND1 are shown in *Fig. 3.1* A,B. In this figure, a linear filter obtained from the first and the last trial (1min after an ND switch, and 25 min later) of high contrast GWN are shown. To characterize the kinetic properties of each filter, I measured its time-to-first-peak (peak latency, marked by arrows in *Fig. 3.1* A-B).

In the experiment shown in *Fig. 3.1*, I presented 1-minute trials of full-field high contrast GWN flicker every 2 minutes, in total 12 trials at each brightness level. This experiment had the best time resolution to observe temporal development the linear filter kinetics. The mean peak latency of the linear filters of 35 cells recorded in this experiment is depicted in *Fig. 3.1* D.

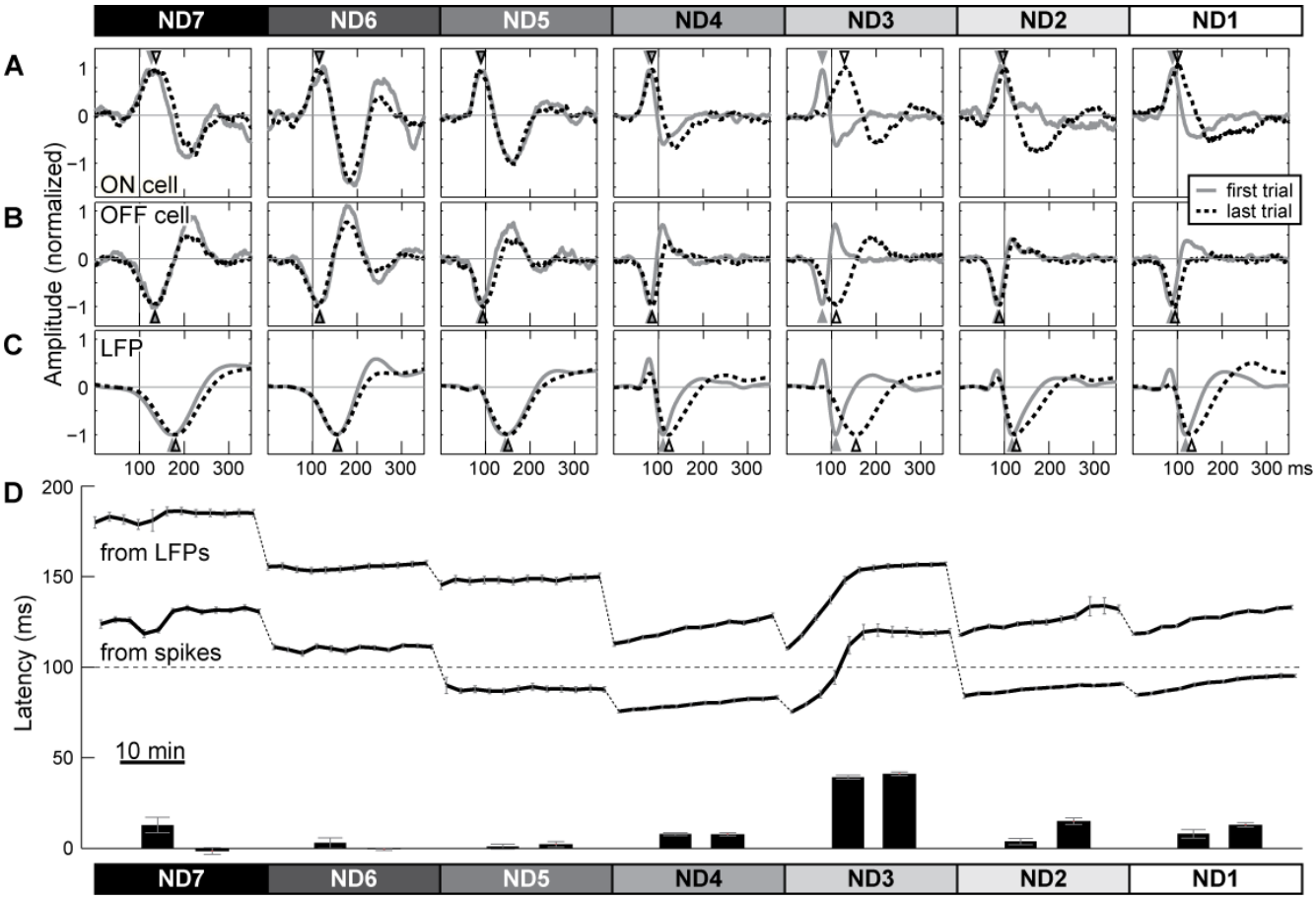

Fig. 3.1. Linear filters and their latencies in wild type retina. Linear filters were calculated by reverse correlation in response to Gaussian full-field flicker stimulation, either from spiking responses of ganglion cells (A: example ON cell, B: example OFF cell), or from the LFP responses (C: average from all 59 electrodes). Flicker stimuli lasted for 1 min and were repeated 12 times every other minute. A-C show the linear filters of the first and last trial at each brightness level. The timing of the first peak is indicated by arrowheads. (D) Latencies of linear filters for one retina (35 cells, mean ± s.e.m.). Bars at the bottom: latency increase between first and last trial (mean ± s.e.m.) for all cells (left bars) or LFPs (right bars) from 6 retinas.

At each brightness level, the latency was immediately reduced during the first minute after the ND-change and was relatively stable throughout the rest of the same brightness level. While responses did tend to slow down by a few ms at all ambient intensities, cells never returned to latency values of

the previous (darker) brightness level, with the striking exception of ND3. This light level roughly corresponds to a standard LCD computer monitor showing a white screen. Initially, as expected, the latency decreased within the first minute at ND3 as well, but then it began to rapidly increase again. Eventually it stabilized at a level that is typical for ND6-ND5 (i.e. high scotopic to mesopic range, see Methods). At the next brighter light level, ND2, the latency returned to the previous, fast ND4 values, already in the first minute, and remained relatively stable throughout the measurement.

This behavior was consistent for all recorded ganglion cells (n=520 from 15 retinas), independent of their response polarity (ON cells (*Fig. 3.1* A), OFF cells (*Fig. 3.1* B), ON-OFF cells), response kinetics (transient cells, sustained cells), and spatial location (ventral or dorsal retina). It also did not depend on the stimulus sets: each experiment where GWN was presented early and late after the ND switch showed latency increase at ND3, independent of other stimuli shown in between. This suggests a mechanism underlying the observed brightness-latency profile which is common to all ganglion cells. Another observation suggested that the mechanism is probably located presynaptic to ganglion cells: on the MEA electrodes, I recorded not only spiking responses of ganglion cells, but also local field potentials (LFPs) that originate mainly from extracellular (synaptic) currents located in the tissue column above the recording electrode, representing activity presynaptic to the ganglion cells. When I reverse-correlated the LFP signal with the stimulus, I obtained linear filters (*Fig. 3.1* C) that behave similar to the ganglion cell spike-triggered average. Specifically, although they were generally slower, LFP-based filters showed the same brightness-latency profile as the spiking-based linear filters (*Fig. 3.1* D), placing the mechanism at least as early in the pathway as the bipolar cells.

3.2.1. Possible mechanism for the latency increase at ND3

Speeding up of the filters with increasing brightness is consistent with smaller integration time of cones compared to rods. A similar behavior has been shown for other cell types, e.g. horizontal cells [3]. Slowing down of the linear filter at ND3, however, lacks explanation. Possible reasons of such behavior include:

- experimental timing (tissue being in the recording chamber for a long time)

- activation of melanopsin in intrinsically photosensitive ganglion cells (ipRGCs), which may potentially modulate RGC timing through some metabolic pathways

- interaction of the center and the periphery of the receptive field of the ganglion cell at high brightness

The next sections describe control experiments which I performed to check the above listed mechanisms. These experiments led to the conclusion that none of these potential mechanisms is involved.

3.2.1.1. Timing

Long recordings, i.e. long time in the dish, might affect the physiology of the retina. My experiments usually started at ND8 and the stimulus set took 25-40min at every brightness level. Therefore, time to reach ND3 was between 100 and 160 min in different experiments, which already is an hour difference. However, the phenomenon of slowing down always occurred at ND3 in all experiments, independent of the length of the stimulus set. I performed additional control experiments, where presentation began at ND4 instead of ND8 (n=2 retinas), so that time to reach ND3 was only 25min. The results are shown in *Fig. 3.2* and *Fig. 3.4* A. The results were not qualitatively different from standard experiment starting at ND8: stable short latency at ND4, slow increase of latency at ND3, and faster and stable filters at ND2.

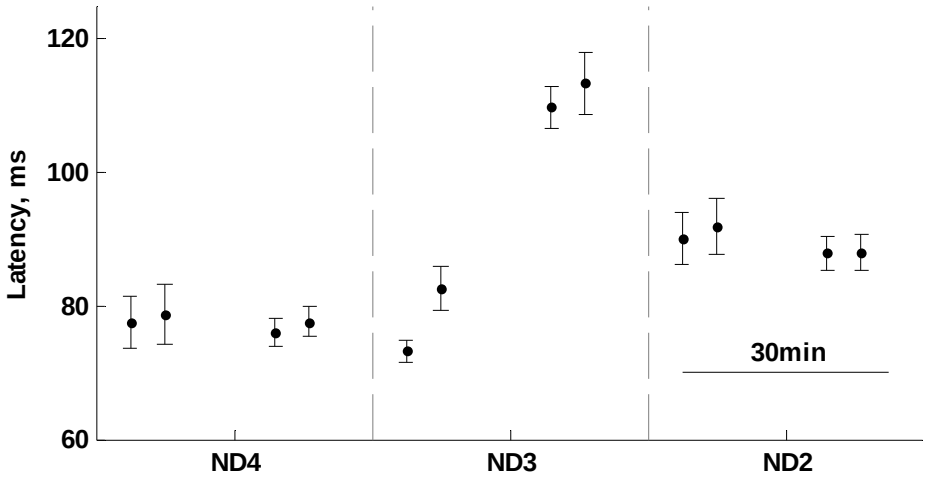

Fig. 3.2. Latency of the linear filter also increases at ND3 when the experiment began at ND4. Latencies averaged across 28 cells recorded in 2 experiments (mean ± s.e.m.).

Skipping ND3, i.e. switching from ND4 directly to ND2, did not cause slowing down at ND2 (*Fig. 3.3*, n=2 retinas). I conclude that the latency shift does not depend on the time the retina stayed in the dish, but is specific to actual brightness.

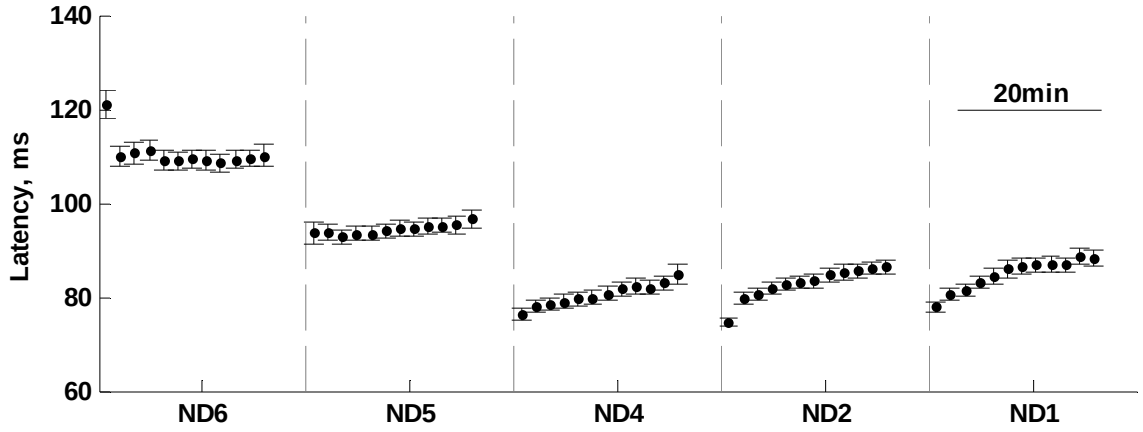

Fig. 3.3. Latency of the linear filter does not increase at ND2 if ND3 was skipped. Latencies averaged across 56 cells recorded in 2 experiments (mean ± s.e.m.).

3.2.1.2. Melanopsin activation

Photoreceptors are not the only photosensitive cells in the retina. About a decade ago intrinsically photosensitive retinal ganglion cells (ipRGCs) were described [35, 43]. These cells contain a special chromophore, melanopsin, which is activated by sufficiently bright light and depolarizes the ganglion cell. Mouse retina contains at least 5 distinct types of ipRGCs [44]. A tight connection between melanopsin containing cells and circadian rhythms has been shown; however, functions and pathways of ipRGCs in the retina are not fully understood yet, and it is possible that they perform a number of tasks during light adaptation process [45-46].

Photoactivation threshold of melanopsin is higher than that of rods and cones [47]. As described in Methods, the projector I used in my experiments had a UV-filter and stimulated S cones more weakly. Spectral sensitivity of melanopsin peaks at 484nm [43], which is between S and M cones optima (360 and 508nm correspondingly, e.g. [42]). Therefore melanopsin could be sufficiently stimulated in our setup, although at higher brightness than M-cones, which makes it a good candidate to affect retinal processing at ND3.

To test the involvement of ipRGCs in the slowing down of ganglion cell responses at ND3, I used a mouse strain in which the OPN4 gene was knocked out (OPN4-/-, [35]), which lacks melanopsin. However, these retinas demonstrated the same properties of linear filter adaptation as wild type (n = 4 retinas, *Fig. 3.4* B,C). Therefore melanopsin does not seem to initiate the latency increase at ND3.

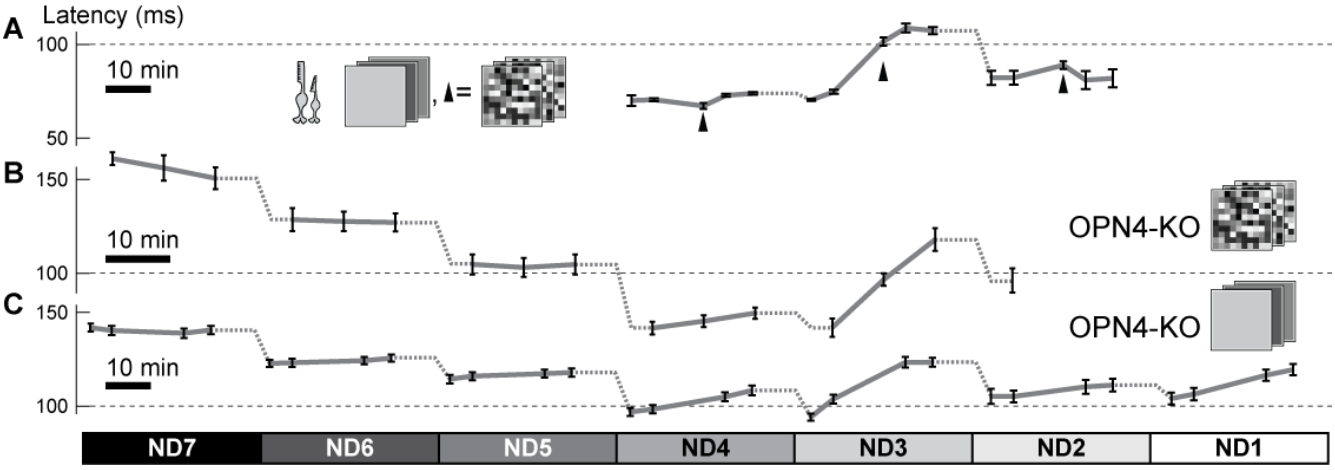

Fig. 3.4. Latency shift is robust against time the retina is in the experiment, spatial stimuli, and lack of melanopsin. (A) Latency of linear filter in wild type retina; mean ± s.e.m. of 28 cells (full field) and 36 cells (checkerboard) of 2 retinas. Experiment was started at ND4 and contained an 8-min checkerboard flicker stimulus midway through each luminance level. (B) Latency of linear filter in an OPN4-KO retina; mean ± s.e.m. of 10 cells of 1 retina. The experimental paradigm consisted of three 8-min checkerboard flicker stimuli. (C) Latency of linear filter in OPN4-KO retina; mean ± s.e.m. of 38 cells of 3 retinas.

3.2.1.3. Interactions between receptive field center and periphery

The receptive field of a ganglion cell is traditionally viewed as consisting of two concentric areas: center and periphery, with antagonistic reactions to light steps [48-49]. Stimulation of the center with a spot of light of increasing diameter will evoke responses with increasing magnitude. However, when the spot diameter exceeds certain values, the responses will diminish. The size of the spot evoking maximal response determines the size of the receptive field center.

The periphery of the receptive field becomes stronger in light-adapted conditions [15, 17]. Since I used full-field stimulation, both periphery and center of the receptive field were activated. The kinetics and relative amplitude of the inputs driven by center and surround stimulation may therefore depend on the light-adaptation state [15, 21, 25]. I checked whether simultaneous activation of the whole receptive field is responsible for the latency shift at ND3.

To probe the influence of spatial processing on the described phenomenon, I included a checkerboard white noise flicker into the stimulus set to activate ganglion cell receptive fields center and surround independently (n = 36 cells from 2 wild type retinas, *Fig. 3.4* A, and 10 cells from 1 OPN4-/- retina, *Fig. 3.4* B). The checkerboard covered the whole retina, but each checker (60x60 μm²) independently changed its brightness with the same statistical properties as in the high contrast

Gaussian full-field flicker stimulus. The linear filters, obtained from the checkers stimulating only the receptive field centers, had certain features distinct from the filters obtained by full-field stimulation, but their temporal properties showed the same overall adaptive changes at each ND-level. The observed brightness-latency profile was therefore not a result of center-surround interactions in the receptive field.

3.2.1.4. Animals with only one functional photoreceptor type

Background light at ND4 corresponds to 10^4 photoisomerizations per rod per s. Rods are supposed to be completely saturated at this light level [42] and never recover responses in vitro [50]. However, the latencies of linear filters at ND3 (both spike-based and LFP-based) fall within the range typical for values obtained under scotopic or low mesopic conditions (i.e. up to about ND6). I therefore tested if the increasing latency at ND3 might reflect a reactivation of rods at photopic brightness levels.

I recorded from retinas of transgenic mice in which the cones are non-functional due to a mutation in the cone-specific cyclic nucleotide gated channel subunit Cnga3, to which I will refer to as "rod-only retinas". In these recordings (n = 53 cells from 2 retinas, *Fig. 3.5* A), the linear filters were robust and had high signal to noise ratio. Most cells had highest amplitude of the linear filter at ND6. I normalized all linear filters of each cell to the amplitude at ND6 to be able to directly compare the relative amplitude changes across brightness levels. As expected for rod-only retinas, at ND7 and ND6 (scotopic light levels) linear filters were robust and had high-amplitude. The amplitude dropped by half at ND5 (indicating beginning saturation), and vanished completely at ND4. This is in line with my initial calculation, estimating that rods would be saturated at ND4, making this the lowest photopic light level (see Methods).

At ND3, however, the linear filters gradually gained amplitude again, so that it became comparable to the values at ND6, the ‘optimal rod luminance’. The time course at ND3 of this amplitude increase in rod-only retinas (*Fig. 3.5* A) matched the time course of the latency increase in wild-type retina when compared to the experiments with the same stimulus set (*Fig. 3.1* D; inset in *Fig. 3.5* A). At ND2, the linear filters disappeared completely for most cells and decreased strongly in amplitude for others; at ND1, linear filters were flat for all cells. Taken together, these results suggest that the ganglion cells can be driven by rod activity even at photopic light levels. It also suggests that the shift of the latencies at ND3 might be explained by return to mesopic vision.

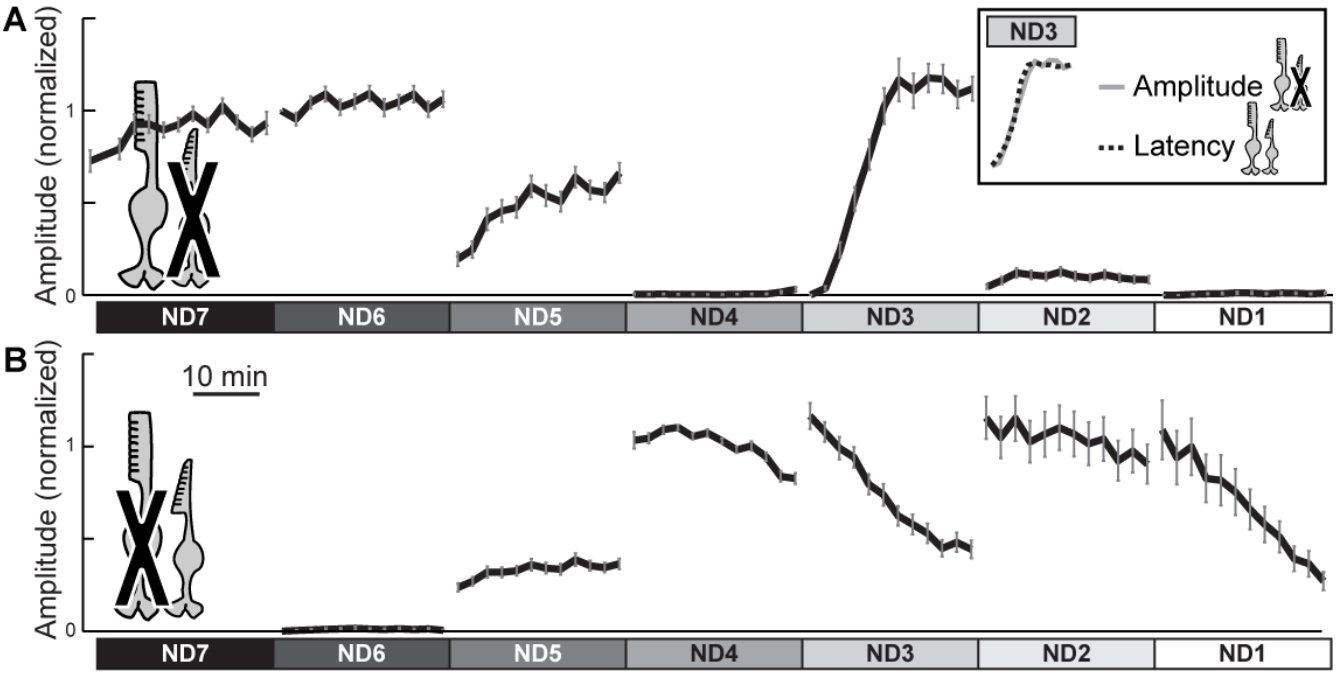

Fig. 3.5. Amplitudes of linear filters in the mutant retinas. (A) Cnga3-KO (rod-only retina), mean ± s.e.m. of 53 cells in 2 retinas. Amplitudes are normalized to the mean amplitude at ND6. Inset: Comparison of time course at ND3 of amplitude change in rod-only mice and latency change in wild type mice. (B) Rho-KO (cone-only retina), mean ± s.e.m. of 121 cells in 6 retinas. Amplitudes are normalized to the mean amplitude at ND4.

To test how well cones drive activity of ganglion cells, I repeated the experiments on 6 retinas of another transgenic mouse strain, Rho-/-. These mice have a mutation in the rhodopsin gene *Rho*, which makes their rods non-functional. I will refer to these retinas as "cone-only".

Light-evoked activity of cone-only retinas was notably worse than that of rod-only or wild type mice. Signal-to-noise ratio was on average lower. The reason of that is probably related to disturbed cone-function in absence of rods (rods comprise 97% of photoreceptors in mice [42] and cones have been shown to degenerate soon after rod degeneration in any rod-degeneration model [51]. Cones in the Rho-/- retinas start to degenerate at 6 weeks of postnatal age. I used for experiments younger animals (4 to 5 weeks postnatal): before the onset of degeneration but after the retina has fully developed. The linear filters of almost half of the recorded cells had low signal-to-noise ratio. Disregarding those, I took for the analysis only the 121 cell with reliable filters.

As expected, at light intensities darker than ND6, I could not record light-driven activity in ganglion cells from cone-only retinas, firmly placing the light levels ND8 and ND7 into the scotopic range. At ND6, the linear filters were distinguishable from noise only in very few ganglion cells, and they had very small amplitude. At ND5, most cells already had reliably filters. The maximal amplitude

and most stable linear filters were obtained at ND4. I normalized the amplitude of linear filters for each cell to the amplitude at ND4 to compare relative changes directly.

Responses generally were robust also at higher photopic light levels than ND4; however, in the course of ND3, linear filter amplitudes remarkably decreased. At ND2, the amplitude was more variable, but on average as high as at ND4 and also quite stable in the whole duration of this brightness level. At ND1, the responses gradually decreased during half an hour.

Observations made on both rod-only and cone-only retinas are consistent with the hypothesis that slowing of the linear filter kinetics observed in wild type retinas is caused by shifting the photoreceptor balance towards the more sluggish rods.

Thus, specific changes at ND3 occurred not only in the wild-type, but also in rod- and cone-only retinas with corresponding time courses. It is therefore likely that the latency shift at ND3 in the wild type is caused by switching the photoreceptor balance back to the mesopic range. It seems that this process occurs independently of either photoreceptor system alone and is not likely due to a winner-takes-it-all competition between them. It should be noted, however, that the mutant mouse models I used differ in the level of photoreceptors malfunction. The cone-only retina does not have functional rhodopsin, i.e. the phototransduction cascade is not even initiated inside the rods. It means that the rods do not contribute to the cone activity at all; however, the cones are not completely healthy and the conclusions should be drawn with caution. The rod-only Cnga3 -/- retina, on the other hand, has vigorous light responses at expected light levels and appears healthy; but the mutation in cones affects relatively late stages of cone functioning. Indeed, the initial stages of the phototransduction cascade in cones are intact, but non-functional cGMP-gated channels prevent translation into membrane voltage. Although the cones in such retinas are morphologically misshaped and do not maintain normal metabolism [52], I can not completely rule out the possibility that some intermediate (metabolic) product of phototransduction in cones is necessary for the latency shift at ND3.

3.2.1.5. Triggerness

As experiments with rod-only retinas showed, rods indeed reactivate in the middle of the photopic brightness range (ND3). It is a slow process taking about 10 min to complete. The mechanism of this phenomenon is not clear, and one of the questions is whether this process is only triggered by a certain light level, or if this light level is constantly required until the process has been completed. I checked this by presenting a full stimulus set at each brightness level from ND8 to ND4. Then, at ND3

I presented only a single 1-minute trial of high contrast full field flicker, and then switched back to ND4 and repeated the complete stimulus sequence. If the effect of rod reactivation (slowing down of linear filter kinetics) is already triggered by brief exposure to the ND3 brightness level, one should observe these processes developing during the second presentation at ND4.

I applied this experimental paradigm to one CNGa3-/- (rod-only) and one wild type animal. For the rod-only retina, I used 9-trials set of stimuli, all high contrast, at ND8-ND4 (every ND took 10 minutes). Then, I switched to ND3, presented 1 minute of high contrast GWN, and immediately switched back to ND4. There, I presented 24 trials, 12 of high contrast and 12 of low contrast, interleaved. As described above for rod-only retinas, I measured the amplitude of obtained filters for every trial, and then normalized them to the amplitude at ND6 for each cell. The results are plotted in *Fig. 3.6*. At ND8-ND4, the amplitudes look typical for rod-only animals: high-amplitudes at ND7-ND6, weaker at ND5, and vanishing at ND4. The single trial at ND3 yielded no filters whatsoever (as expected). However, further stimulation at ND4 led to robust amplitude recovery, albeit it took longer than at ND3 in the other experiments with standard ND sequence.

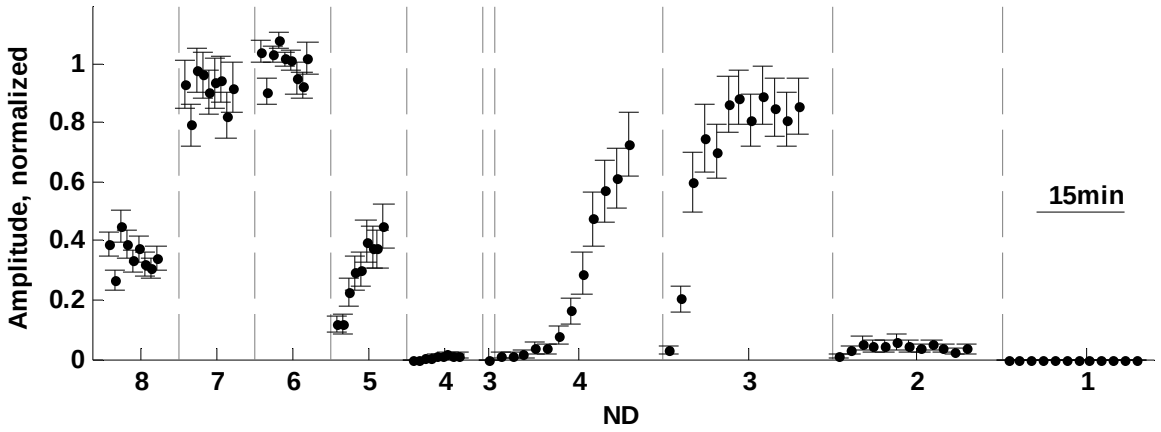

Fig. 3.6. Rod reactivation at ND4 could be triggered by 1 minute stimulation at ND3 in the Cnga3-KO (rod-only retina). Mean amplitudes of the linear filters, normalized to the amplitudes at ND6 for each cell. Mean ± s.e.m. of 25 cells of 1 retina.

For the wild type retina, I used the standard stimulus set of 12 trials of high contrast and 12 trials of low contrast (interleaved) at ND8-ND4. At ND3, I used 1 minute trial of high contrast flicker. Then I switched to ND4 and repeated the 24-trials set up to ND1. I measured the latency of the first peak of the linear filter as described above. The results are shown in *Fig. 3.7*. Like in the rod-only retina, I observed the changes typical for ND3 at the second presentation of ND4, and it also took longer then

in the experiments with standard ND-sequence. At the second repetition of ND3, linear filters initially sped up and then slowed down again; the slowing down had a faster rate constant than at ND4

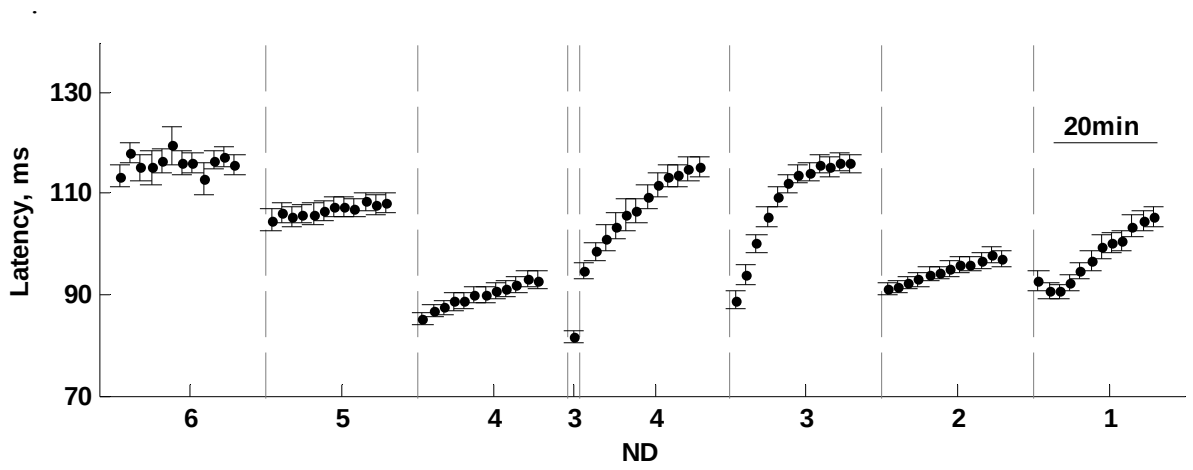

Fig. 3.7. Latency increase at ND4 may be triggered by 1 minute stimulation at ND3 in the wild-type retina. Mean ± s.e.m. of 27 cells of 1 retina.

Since the speed of the rod reactivation depends on the brightness level, I checked whether it can be triggered by longer duration of ND4. For that, I performed 2 experiments with wild type retinas in which I stayed at ND4 and repeated the stimulus sequence 4 times before switching to ND3 (total time at ND4: 100 min). The results are plotted in *Fig. 3.8*.

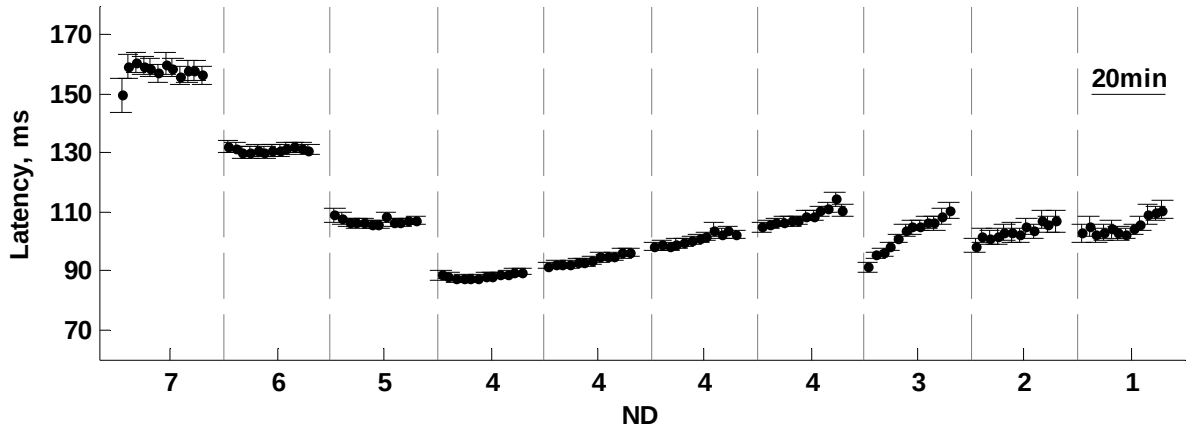

Fig. 3.8. Latency of the linear filter gradually increases when the retina is kept under ND4 for 100min and reaches values typical for ND5. During ND3, latency increases to the same level in 25 minutes. Mean ± s.e.m. of 53 cells of 2 retinas.

The latency of linear filters had typical behavior at ND8-ND5. At first presentation of ND4, it moderately increased (as in other experiments). Further, it continued to slowly increase throughout all

ND4 repetitions. At ND3, latency first decreased, and then rapidly grew. Interestingly, the values at the end of ND3 were roughly equal to the values at the end of the 4th presentation at ND4.

The results of the described experiments suggest that the onset of rod reactivation, and the speed at which it occurs, depend on the actual amount of light the retina receives; in other words, it quantitatively depends on the luminance. Reaching a certain light level is enough to trigger rod reactivation process, but its speed is then further influenced by the ambient light intensity. On the other hand, prolonged exposure to a dimmer light will evoke similar changes, but on a longer time scale.

3.2.2. Rod vision in the photopic range

Obvious rod reactivation in the photopic regime was an unexpected observation. All ganglion cells recorded in rod-only retinas had flat filters at ND4 (n = 53/53, *Fig. 3.5* A) when the standard, one-directional ND sequence was used. However, the experiment with the 1-minute trial at ND3 and return to ND4 showed that after a while cells start responding at ND4 as well. It is then an open question whether rods themselves are truly silent at high light levels, or if reorganization of downstream circuits prevents rod signals from being conveyed to ganglion cells at low photopic levels (ND4), while allowing them to be transmitted again at higher photopic levels (ND3).

The stimulus set for experiments with rod-only retina also contained full field steps of light and the chirp stimulus (see Methods, *Fig. 2.2* A,B), presented starting from the 25th minute after the ND switch (i.e. the retina did have time to adapt to the new background level). Consistent with the vanishing linear filters at ND4, some ganglion cells in rod-only retinas (n=9 or 17% of recorded cells, *Fig. 3.9* A) did also not respond to the steps of light and chirp. However, most ganglion cells did have responses at ND4 to these stimuli (83% of cells, *Fig. 3.9* B), including ON-responses to 2-s light increments. Moreover, some of these cells responded with larger amplitude at ND4 than at any other brightness level (including ON responses of some ON cells). Rods therefore cannot be fully saturated at ND4, but the properties either of the rods themselves, or of the retinal circuitry, change so that white noise full-field flicker does not lead to robust stimulus-locked responses in ganglion cells.

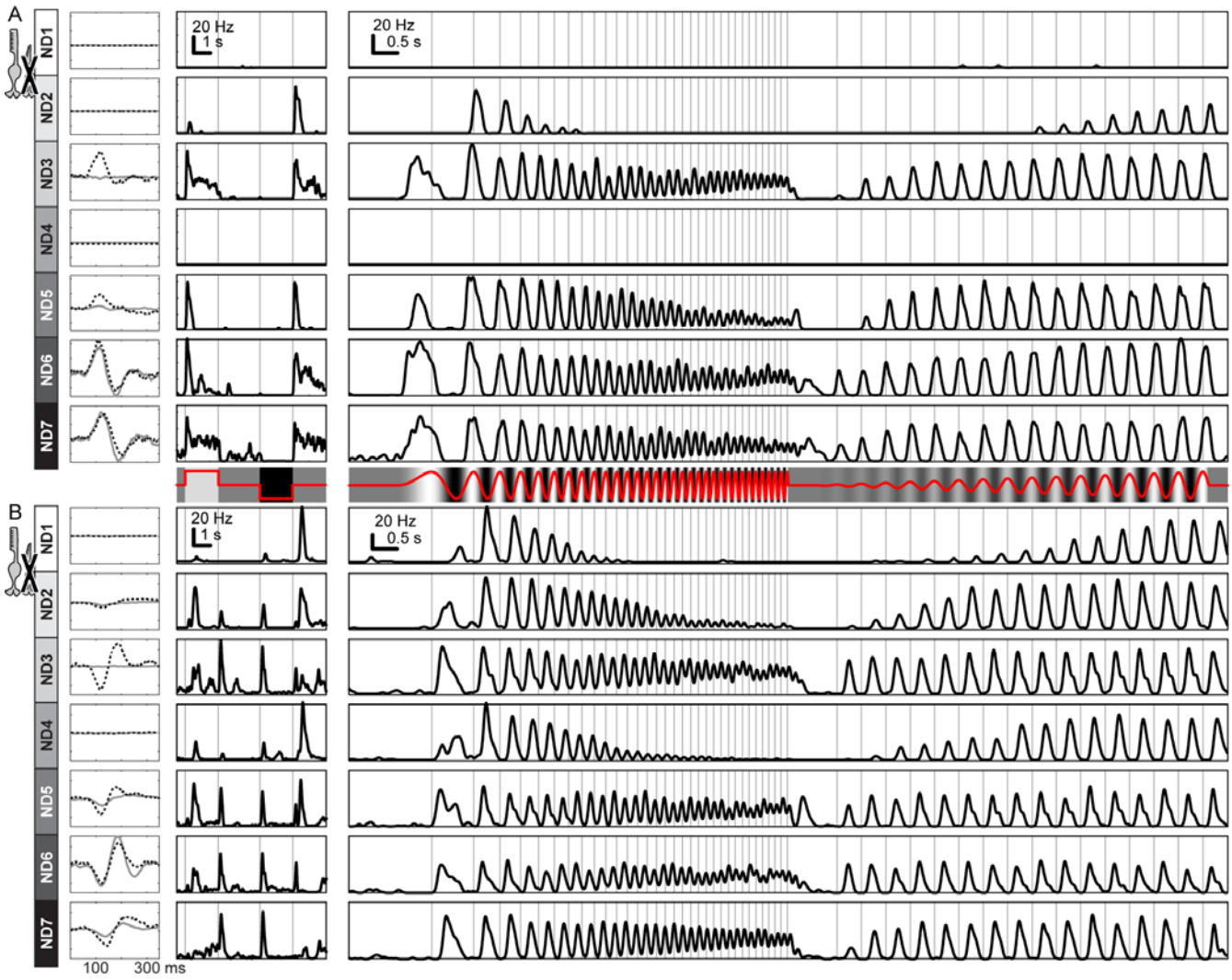

Fig. 3.9. Examples for individual ganglion cell responses in rod-only retina. Left: Linear filters presented as in Fig. 3.1 A. Middle: responses to step stimuli (mean of 5 responses). Right: responses to chirp stimuli (mean of 5 responses). (A) Example cell whose responses completely disappear at ND4. (B) Example cell from the same retina which still responds vigorously to step and chirp stimuli, despite vanishing filters at ND4 and ND1.

In these rod-only retinas, ganglion cell responses to the chirp stimulus had distinct characteristics at ND4 in comparison to lower brightness levels. For those ganglion cells that did have light responses at ND4, low frequency and high contrast parts of the chirp evoked robust and high amplitude responses, not distinguishable between luminance levels. Responses to high frequency and low contrast parts of the chirp, on the other hand, were much weaker at ND4 than at ND7-5 (*Fig. 3.9*). In fact there was a tight correlation between the response properties to the chirp stimulus and the amplitude of the linear filters at different brightness levels (*Fig. 3.10*). Namely, robust linear filters in response to Gaussian full-field flicker (right column in *Fig. 3.10*) were associated with strong response

modulation by the higher frequencies of the chirp (> 4 Hz) (black circles in *Fig. 3.10*). Medium amplitude linear filters corresponded to medium response modulation at higher frequencies (dashed circles), and missing or very small linear filters corresponded to weak response modulation (gray dashed circles).

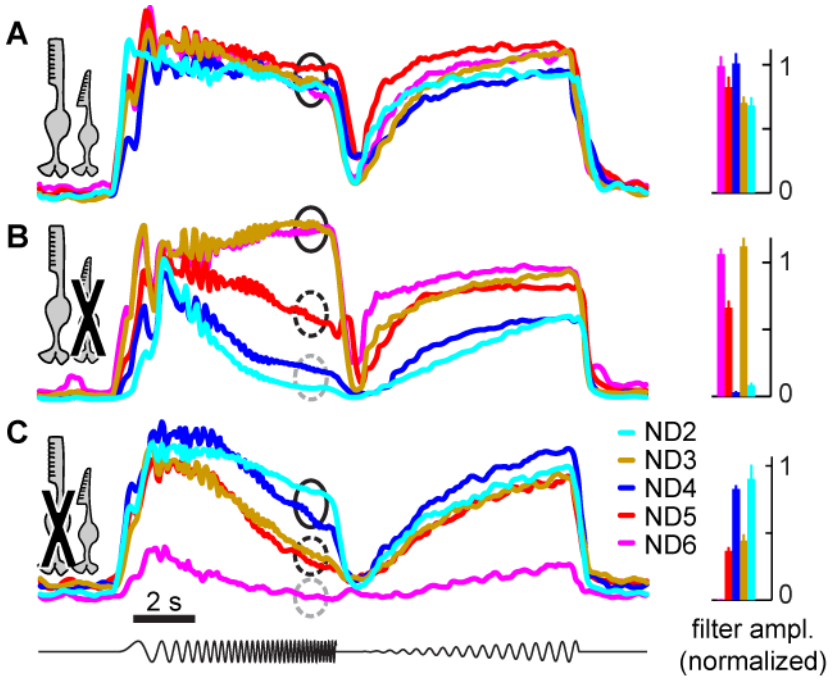

Fig. 3.10. Modulation strength of ganglion cell responses to chirp stimulation. Mean modulation strength of responses from wild type retina (A, 118 cells), rod-only retina (B, 53 cells), cone-only retina (C, 121 cells), in response to full field chirp stimuli (bottom) at different NDs. Black circles: NDs at which ganglion cells have large amplitude linear filters (right) in response to Gaussian full-field flicker. Dashed circles: NDs with medium amplitude linear filters. Gray dashed circles: NDs with low amplitude or absent linear filters. The linear filter amplitude range is given as mean ± s.e.m. of the last presented flicker trial for each ND, i.e. closest in time to the presented chirp stimulus.

Therefore, it appears that the strength (or lack) of linear filters can be explained by the (in-)ability of the rod- or cone-system, including the associated circuitry, to follow high-frequency stimulation. In other words, the linear filters, calculated in response to GWN full-field flicker, only captures the response properties to a specific subset of stimulus features, namely low contrast and high frequency. Notably, both rods and cones appear to be active at brightness levels ND6 and above, and both rods and cones are inherently able to respond to high frequencies between 4 and 8 Hz. However, the transmission of these fast signals by rod- or cone-driven pathways seems to be dynamically regulated as a function of luminance. It thus appears that dynamic regulation of circuit properties is sufficient to explain the brightness-latency profile of linear filters in the wild-type retina (*Fig. 3.1*). The

latency of linear filters seems to reflect the balance at which high frequency signals are carried by rod-driven pathways (longer latencies) and by cone-driven pathways (shorter latencies).

3.2.3. Mechanism of rod reactivation: bleaching

The experiments described so far show that rods can contribute to retinal responses at all luminance levels, and are not, as would have been expected, saturated at high luminance. Non-saturating behavior is traditionally considered a cone feature. Behavioral experiments with mice suggested that rods are indeed saturated at high brightness [53]. In humans, the existence of rod saturation is psychophysically supported by rod monochromats; in these patients, vision fades at high light intensities [54-56]. The rod-only mouse is a model for rod monochromacy. However, the vigorous light responses of the rod-only retina (*Fig. 3.5*, *Fig. 3.9*) seem to contradict the current view of the abilities of rods to support vision at high light levels.

For cones, an established mechanism to support vision at very bright light levels is bleaching adaptation [57]. With increasing light intensity, the concentration of rhodopsin (i.e. opsin with bound 11-*cis* retinal) in photoreceptors drops. Consequently, compared to the situation when the photoreceptor is fully loaded with unbleached rhodopsin, both the steady-state and stimulus-induced photoisomerization rates are substantially lower at higher brightness levels. This allows cones to escape saturation.

The signal amplification in the rods at the different steps of phototransduction is much higher than in the cones, which is the basis for their higher sensitivity. However, it also leads to a proportionally lower saturation threshold, and forms the basis for the different operational ranges of these two photoreceptors types.

The process of bleaching is counteracted by the process of pigment restoration into its initial form (which is one aspect of dark adaptation). In vivo, the balance of the bleaching and regeneration rates leads to a certain steady state of functional rhodopsin in the photoreceptor, which depends mostly on the ambient light level. However, in our experiments, when the retina is isolated from the retinal pigment epithelium (which is the main sources of the chromophore 11-*cis*-retinal, a necessary component of pigment restoration), the rate of regeneration is likely reduced [41]. Bleaching might thus have a more pronounced effect on photoreceptor responses.

We estimated the effect of bleaching in my experiments in comparison to the *in vivo* situation using a computational model. The model calculates the rhodopsin concentration, and the resulting isomerization rate, depending on two counteracting processes: (1) rhodopsin concentration drops due to

bleaching as a function of stimulus intensity and (2) it rises due to pigment regeneration. The estimation of the in vivo regeneration rate ρ was based on published data ([41], see Methods, *Fig. 2.3*). *Fig. 3.11* A shows the expected activity of rods *in vivo* in response to the experimental paradigm with switching between ND filters. The saturation threshold drawn in the figure is chosen such that the rods become saturated at photopic light levels at ND4 and above (*Fig. 3.11* A). This is in line with the traditional picture of rod function, but clearly not what my in vitro experiments have shown (*Fig. 3.9*). The corresponding value of the rhodopsin regeneration parameter ρ in vitro must be substantially lower. *Fig. 3.11* B shows the outcome of the model with $\rho=10^{-6}$. Assuming the saturation threshold to be equal to that in the in vivo situation, due to prevailing bleaching rods would escape saturation in vitro few minutes after the switch to ND3 and drive responses again.

Overall, the model suggests that bleaching adaptation is, in principle, a possible mechanism of rod reactivation at high luminance levels. It should be noted, however, that the model only looks at the isomerization rate in the rods, and does not take into account additional reactions and equilibria inside the rods that could influence whether or not there is an actual light response. In particular, no adaptation mechanisms of the phototransduction cascade are considered. Furthermore, there are some features of the experimental results which are not captured in the model:

1) In the model, rods are saturated at the ND4 light level which is consistent with the lack of linear filters in my experiments with rod-only retinas. However, most ganglion cells in the rod-only retina do respond to steps of light and chirp stimuli at ND4. Some cells among those are ON cells and they produce large-amplitude responses to light increments (comparable to the responses at ND6, in the preferable rod-range). This evidence suggests that the rods are not (fully) saturated at ND4.

2) At ND3, R* per rod per s drops almost to the ND7-level according to the model. This should be below cone sensitivity. However, cones in the cone-only mouse model could still drive ganglion cells at ND3, although less effectively than at ND4.

3) At ND2, rods are still below saturation threshold in the model, and should be as effective in driving ganglion cells as in the end of ND3. However, ganglion cells had robust linear filters of high amplitude at ND3, as well as responses to chirp and step stimuli, in the rod-only mouse, but almost vanishing responses at ND2. At ND1, they were completely absent.

Changing the value of ρ or the saturation threshold in the model could not overcome these discrepancies. It suggests that although bleaching likely plays a role in rod reactivation observed in my experiments, taken alone it cannot fully account for the observed behavior of ganglion cells.

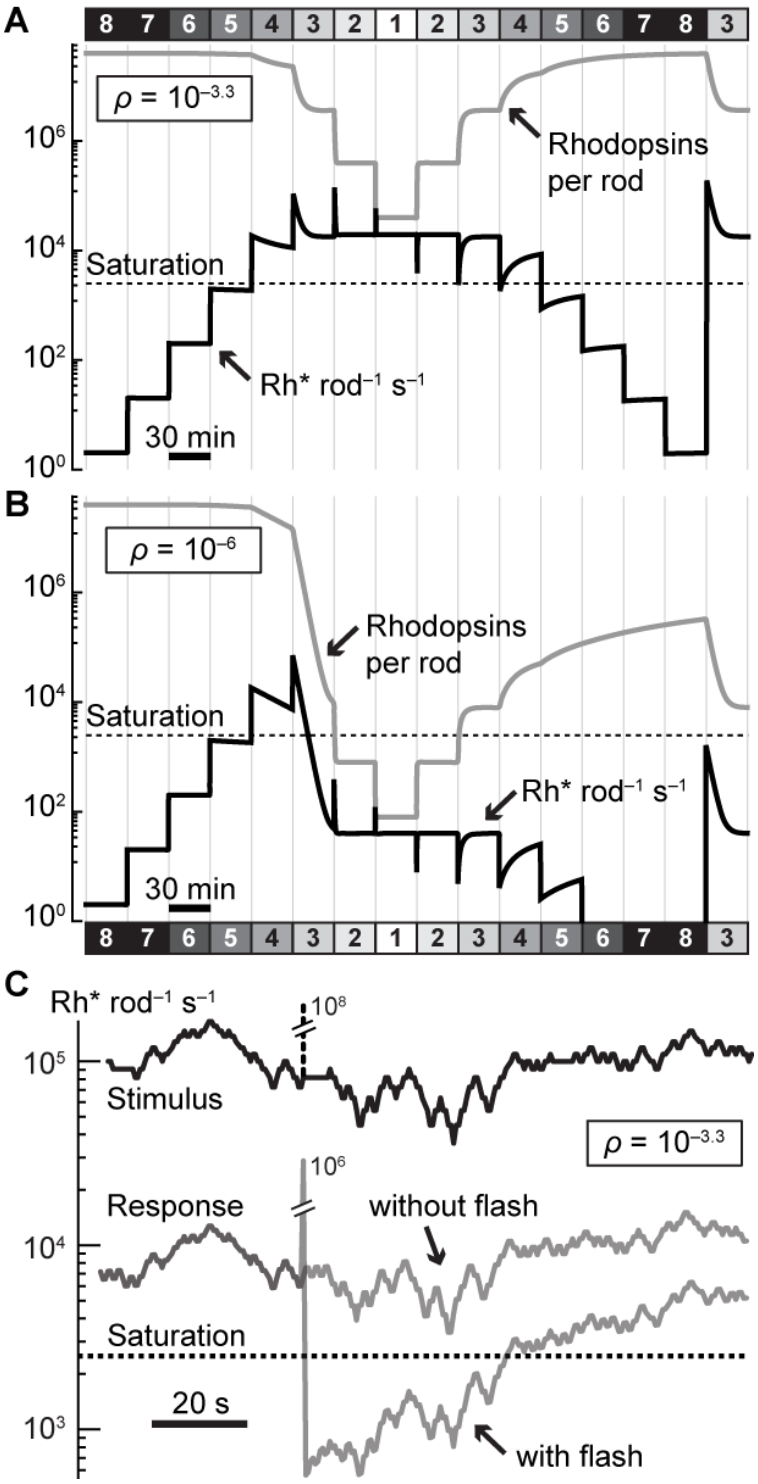

Fig. 3.11. Computational model of bleaching effects. (A, B) Rhodopsin content and isomerization rate in rods during our experimental paradigm with in-vivo (A) and in-vitro (B) regeneration rates. The saturation level is drawn at 2500 Rh* rod^{-1} s^{-1}. (C) A "random walk"-type intensity modulated stimulus with average intensity corresponding to ND3, with or without a brief flash of high intensity. After brief exposure to bright light, rods can escape saturation even with in-vivo regeneration rates.

3.3. Discussion

The experiments described above compromise the commonly accepted light adaptation model – at least in the *in vitro* situation. Once the ambient light level reaches a certain values (above the rod saturation threshold), a process of rod reactivation is triggered. The duration of this process depends on the actual brightness and is likely caused, at least in part, by bleaching adaptation in the rod photoreceptors. The ability of bleaching adaptation to regulate the photoreceptor's contribution to light responses of ganglion cells appears to be strongly underestimated.

These results raise two important questions. First, is bleaching adaptation in rods also relevant for retinal information processing in the behaving animal, when the regeneration rate is much higher? The retina of an actively foraging animal is never exposed to stable luminance levels. Different retinal patches can be briefly exposed to very high brightness, for example when an animal walks under dense canopy (~ ND3) and briefly looks at sunlit objects (ND1) or direct sunlight (~ ND0). The model suggests that short (less than one second) exposure to ND0 bleaches enough rhodopsin to relieve rods from saturation for several tens of seconds after the exposure (*Fig. 3.11* C). At the same time, cones would be brought down close to their sensitivity threshold. Functionally, the exposed retinal patch will work in the "mesopic mode", even though the brightness there (in absolute terms) has not changed compared to the level before the brief exposure to bright light, i.e. absolute brightness is still "photopic". The photoreceptor's return to its photopic operational regime would require sufficient regeneration of rhodopsin (which is traditionally thought of as part of dark adaptation). In rods, this is resulting in saturation. The model thus suggests that bleaching adaptation in rods may contribute to dynamically alternating conditions in which either only cones, or both rods and cones, drive retinal responses *in vivo*.

The second question concerns *in vitro* studies of light adaptation. In fact, many studies use a retinal preparation without the pigment epithelium – whole mounts or retinal slices. In these conditions, it is difficult to maintain the retina in the photopic regime (i.e. in a state in which retinal responses are driven exclusively by cones). Despite very bright adapting light (or maybe in fact because of it), the responses might still be mediated by rods or by mixture of rods and cones, whereas the researcher would assume it is well above the rod saturation range. Duration of the experiment plays a role as well since 'return' to the mesopic regime may take relatively long time (depending on the intensity of the light level, up to tens of minutes). Last but not least, the origin of the ganglion cells inputs may depend on the stimulus characteristics (also not related to the actual stimulus intensity - e.g. frequency) **and** light level.

In my experimental paradigm, ND8 and ND7 may be considered as truly scotopic, meaning that only rods drive retinal activity. ND6 may be considered as high scotopic, meaning that the activity is driven predominantly by rods, and the cone input is weak or absent for the stimuli I use. ND5 represent truly mesopic light level, because rods and cones appear to be equally active (amplitude of the responses in rod-only and cone-only retinas is about half of the maximal). ND4 may be considered as 'mostly' photopic, meaning predominant input from cones at least during the first 30 minutes. It should be noted, however, that whereas the responses to the full field flicker most likely are exclusively driven by cones, in other stimuli certain proportion of the rod input cannot be completely ruled out.

ND3 is a special light level, because in the beginning the responses are mostly mediated by cones, and later in time by rods. A hallmark of this process is that it happens relatively slowly, whereas every switch to a new ND, i.e. 10-fold increase of brightness, evokes immediate changes in the photoreceptor balance (as indicated by abrupt changes and subsequent stability of light response properties at most light levels, see *Fig. 3.1*). ND2 appears to be mostly cone-driven (based on the linear filter kinetics and experiments with rod-only and cone-only retinas). However, the activity of ganglion cells is somewhat lower than at ND4, which may be explained by bleaching of a substantial part of the rhodopsin pool. It is also possible that S-opsin is sufficiently activated at this light level to support light responses in the retina. At ND1, all responses are absent in the rod-only retinas, gradually decline in the cone-only retinas and are rather unstable in the wild type retinas, which suggests that it is too bright for normal functioning of the retina (at least in experimental conditions without pigment epithelium). According to my light intensity measurements (see Methods) ND0 roughly corresponds to a brightness level when one would look directly into the sun. ND1 is only 10 times dimmer and may exceed physiological limitations of the retina.

In the following chapters, I will analyze contrast adaptation and basic properties of ganglion cells (such as response polarity and transiency) at different light levels. For completeness and for reference, I will show the responses at all eight light levels used in the experiments, but I will concentrate my analysis on ND6 (predominantly scotopic), ND5 (mesopic), and ND4 (predominantly photopic) light levels, which I will refer to as scotopic, mesopic, and photopic for simplicity.

4. Effect of luminance on adaptation to temporal contrast.

4.1. Introduction

The retina is able to adapt not only to the mean luminance, but also to stimulus variance, which is called contrast adaptation. Adaptation to temporal contrast adjusts both gain and kinetics of ganglion cells responses, as has been established using linear-nonlinear model. In particular, the zero crossing of linear filters, obtained from responses to a white noise flicker, has bigger latency for stimuli of smaller contrast, and the slope of the nonlinearity becomes steeper (higher gain) [28, 58]. Several sites of contrast adaptation have been identified (see [29, 31, 59]). In a recent study, Ozuysal and Baccus use a linear-nonlinear-kinetic model, and are able to accurately reproduce the responses to the white noise flicker of different contrast, by adjusting a single (kinetic) block of the model [60]. The authors note that this block corresponds to the properties of synaptic vesicle release in bipolar cells terminals. In the resting state, all three vesicle pools – a readily-releasable, a recycling, and a much larger reserve pool – are fully filled with the neurotransmitter [61]. During the GWN stimulation, first the readily-releasable pool, and then the recycling pool, will be depleted. The rate constant of the depletion depends on the contrast. Refilling from the reserve pool is slower than from the recycling pool. Vesicle depletion during high contrast will be faster and refilling will be slower, which leads to reduction of gain. Kinetic properties of the filter, according to this work, depend on combination of signal transduction and membrane and synaptic properties.

Stimulus variance also affects the firing rate of ganglion cells [26-27, 40]. Most studies report an initial and instantaneous increase of firing rate after the switch to high contrast flicker which then decreases in the process of contrast adaptation. Switch to low contrast stimulus leads to abrupt decrease of firing rate. Interestingly, a subset of cells then further decreases spiking activity (“adapting cells”) and another subset sensitizes, partially recovering firing [40]. It has been shown that the speed of recovery/decline depends on the trial duration: shorter times of high/low contrast presentation are associated with faster stabilizing of the firing rate after the switch [62].

Contrast adaptation properties depend on the cell type as well. OFF cells change gain and kinetics stronger than ON cells [28, 63]. Little is known about adaptation properties of ON-OFF cells.

How does contrast adaptation interact with luminance adaptation? Contrast adaptation is likely to occur in the inner retina. A considerable fraction of light adaptation can be accounted for by changes in the outer retina: rods have sluggish responses compared to cones, which leads to slower kinetics of

the linear filters in the scotopic regime (*Fig. 3.1*). Mante et al. showed with recordings of LGN neurons in the cat that the gain control during luminance adaptation and contrast adaptation occurs independently[64]. However, they compared only two mean light levels, differing roughly by one log unit. Dunn has shown that adaptation in cone vision at low light intensities happens in cone bipolar cells, and in cones at higher light intensities. Rod signals generally pass through more cells before reaching the ganglion cells layer (rod bipolar cells are connected with ganglion cells not directly but via amacrine cells) [65]. Beaudoin studied contribution of different gain control sites under bright light and in scotopic conditions [63]. This work shows that cone bipolar-driven pathways display gain control already in synaptic currents into the ganglion cell, whereas under rod bipolar-driven conditions, gain control of the ganglion cell was accounted for mostly by intrinsic properties of ganglion cells. All these evidences suggest that adaptation to temporal contrast in scotopic conditions may therefore have different properties than under the bright light.

In this chapter, I describe the results I obtained using trials of high and low contrast white noise flicker, drawn from Gaussian distribution with sigma of 9 and 1.8 correspondingly, at several brightness levels. I recorded 331 cells in 10 experiments. In the 10 experiments, I used different sets of stimuli with different high/low contrast sequences and durations (details see in Methods). The results obtained from different sets were identical, unless otherwise noted.

4.2. Results

Contrast adaptation may affect at least three characteristics of ganglion cells responses: statistical parameters of the firing rate (mean and variance), speed of the linear filter, and gain of the nonlinearity. In the following sections I will compare these features at high and low contrasts at different light levels.

4.2.1. Firing rate

Spikes produced by a ganglion cell are discrete events. They can be represented as a sequence of zeros and ones. The mean spike count (firing rate) over a certain time window and the distribution of interspike intervals (firing rate variance) are parameters of neural activity frequently used to study the neural code [66-67]. I calculated these parameters for every cell at high and low contrast GWN trials at different brightness levels.

First, I convolved the spike events with a Gaussian kernel (sigma 40ms, see Methods). I was interested in the steady-state firing rate during GWN flicker, and omitted the first second after the

stimulus onset (contrast switch) to reduce the influence of fast adaptational processes on the mean firing rate. As a reference baseline, the spontaneous firing rate was calculated in the 2s interval prior the stimulus onset, when constant background was shown (intensity equaled the mean intensity of the flicker stimulus of either contrast). If there were several trials of GWN flicker at one ND, I took the average of the 2s intervals before each trial.

Fig. 4.1 summarizes the mean and the variance of firing rates during spontaneous activity, high and low contrast flickers averaged for ON and OFF cells at eight light levels. At most light levels, the majority of OFF cells had lower spontaneous firing rate than ON cells, consistent with [25, 58, 63, 68]. Moreover, the spontaneous firing rate of OFF cells appears stable across NDs, whereas ON cells depended on the ambient light level: the lowest firing was registered at ND5 and at the brightest light levels, ND2-ND1. The latter may be explained by overall reduction of activity due to strong bleaching of the photoreceptors. ND5 is a mesopic light level, and decrease of the firing rate may reflect competition/mutual inhibition of rod- and cone-signals. Interestingly, neither this potential mechanism, nor the decrease of ON cell activity as such had any noticeable effect on the spontaneous OFF cells spiking. During GWN stimulus presentation, however, the reduction of firing rate at ND5 is observed for both ON and OFF cells.

Firing rate during stimulation by GWN showed different tendency than the baseline activity: it was higher in OFF cells than in ON cells starting from ND6 (at high contrast) and ND5 (at low contrast). The variance of the firing rate was also higher in OFF cells.

Fig. 4.2 shows the same data as the upper right panels of *Fig. 4.1*, but grouped differently to emphasize the change of the firing rate at high and low contrast. The left panel shows the mean firing rate of ON cells to high and low contrast, the right panel shows OFF cells. In OFF cells, luminance strongly modulated the firing rate at high contrast (compare ND7-ND5 dynamics), while firing rate at low contrast changed little. ON cells, in their population average, revealed no difference of mean firing rate to contrast up to ND4, and more equal impact of the luminance level on the firing rate dynamics at high and low contrast.

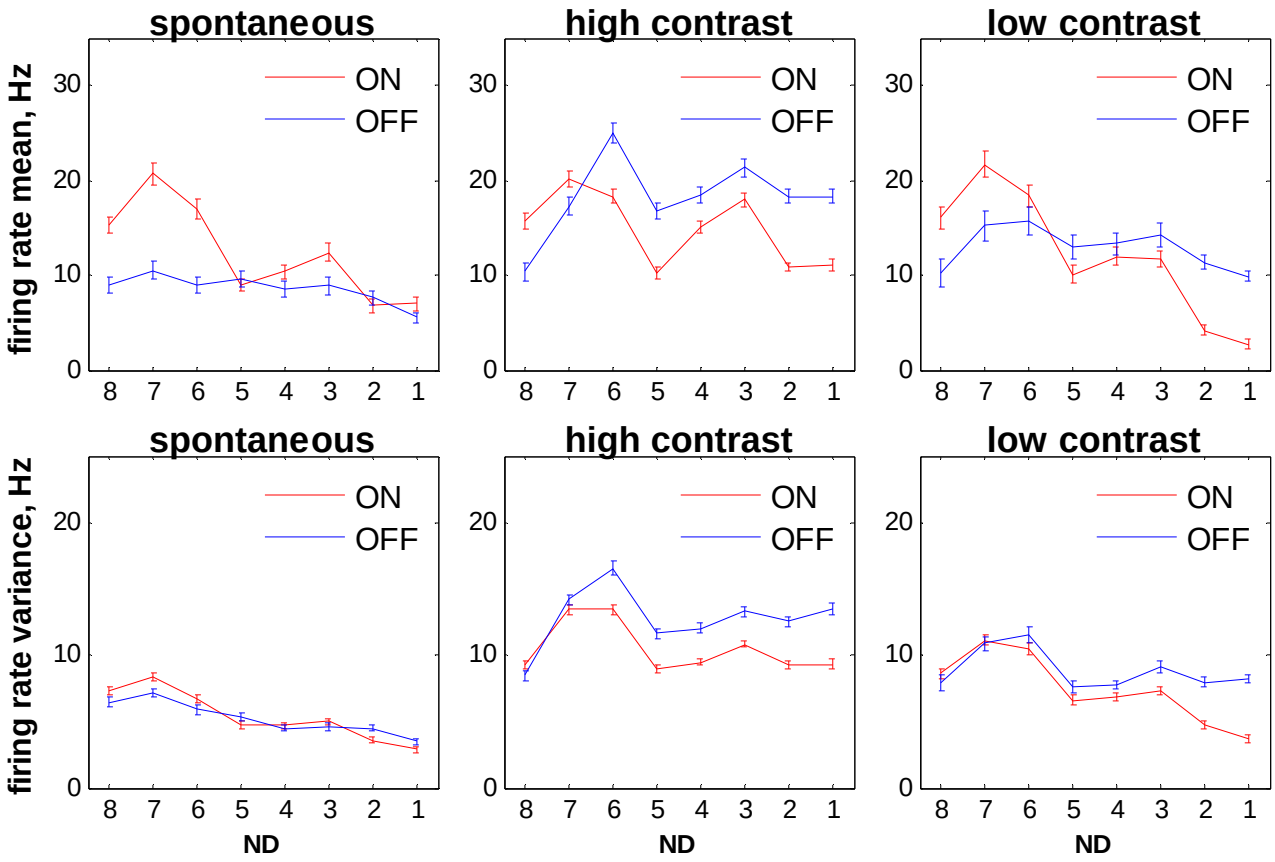

Fig. 4.1. Firing rate of ganglion cells during different stimuli. Mean (upper panels) and variance (lower panels) of the firing rate of ON (red) and OFF (blue) cells during spontaneous (left panels) activity, high contrast flicker (central panels) and low contrast flicker (right panels).

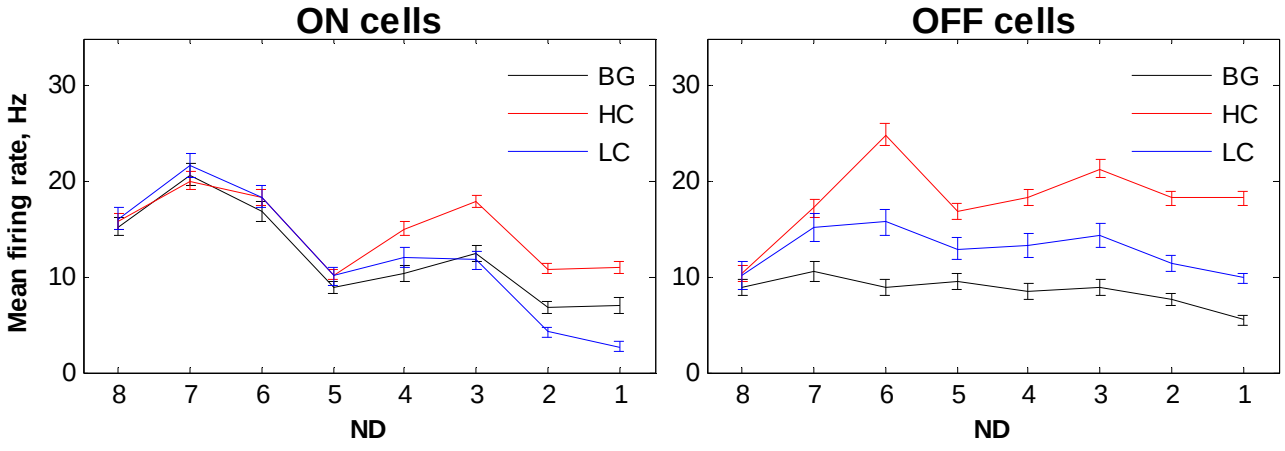

Fig. 4.2. Comparison of firing rate during spontaneous activity (BG), high (HC), and low contrast (LC) stimuli of ON (left panel) and OFF (right panel) cells at different light levels.

When a transition from high contrast to low contrast occurs, cells adjust their gain and consequently firing rate. In an ideal scenario, a cell would scale its output equally to the scaling of the stimulus. Bigger change of the firing rate parameters at different contrasts would reflect therefore weak adaptation. Small changes of the firing rate would mean ideal contrast adaptation or, alternatively, weak responsiveness to the stimulus of either contrast. ON cells, when averaged across the population, have similar mean firing rates at high and low contrast at ND8-ND5 (*Fig. 4.2*). This may be an indication of good adaptation; on the other hand, there may be cells with opposing adaptation patterns (increasing and decreasing firing rate), masked by the population average. To check this, I calculated the difference of the firing rate at high and low contrast and plotted it against the baseline firing rate for ON cells (red) and OFF cells (blue) (*Fig. 4.3*). Negative y-values indicate that the mean firing rate during the high contrast stimulus is smaller than during the low contrast stimulus, and zero values would indicate no difference between firing rates (good adaptation or weak responsiveness).

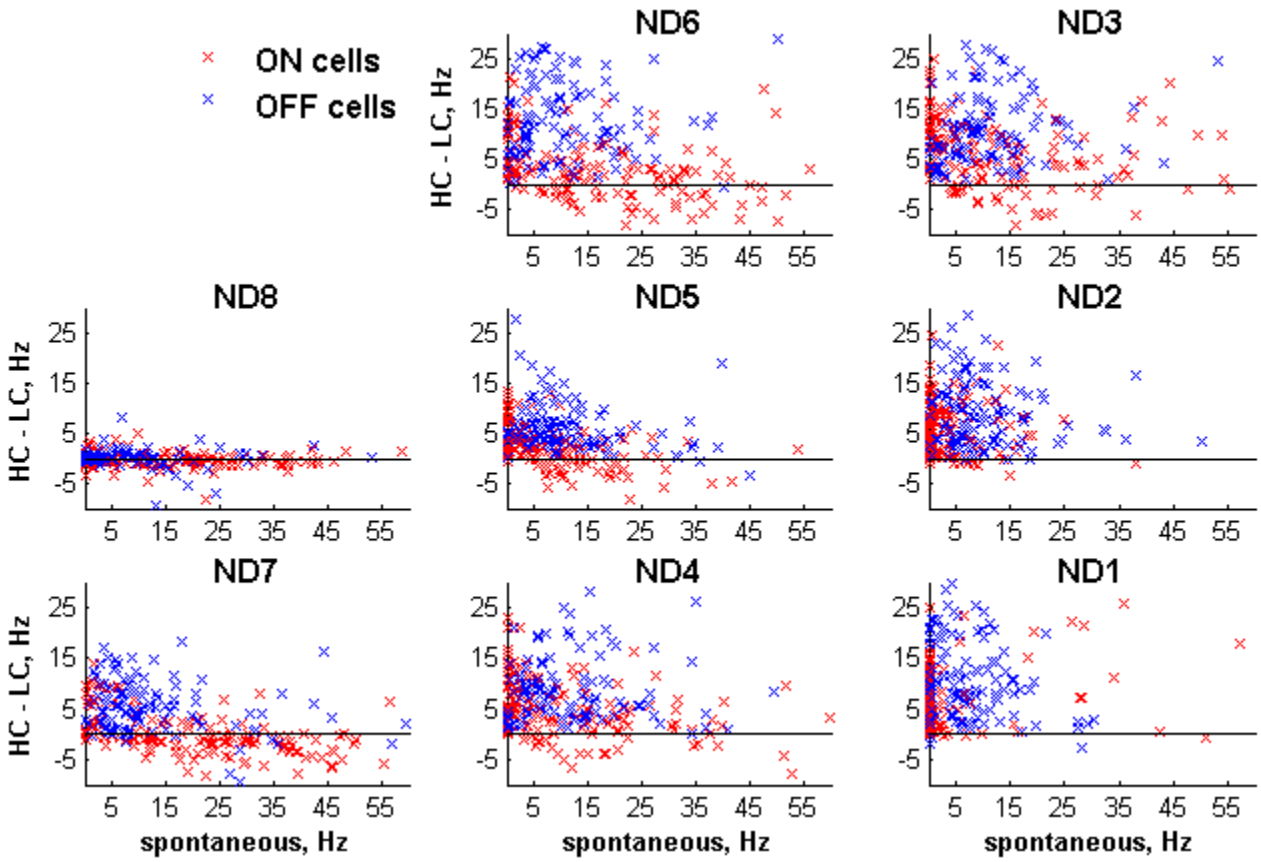

Fig. 4.3. Firing rate change at high contrast. Most cells increase their firing rate at high contrast compared to low contrast GWN stimulus (y-axis, positive values). Some ON cells, however, decrease it (negative y values). This property of ON cells is not necessarily linked to the strength of the background firing rate (x-axis).

As *Fig. 4.3* shows, the only light level where most ON cells were close to zero was ND8 – the dimmest setting, so that in this case such distribution is likely to reflect weak responsiveness. At other light levels, ON cells are distributed both above and below zero line. This means that some of them increase their firing rate at high contrast compared to low contrast, whereas others do the opposite. The observed similarity of high and low contrast firing rates when averaged across population (*Fig. 4.2*) does therefore not represent such an outstanding adaptation among ON cells, but rather diversity of adaptation strategies. In comparison, OFF cells are mostly situated above the zero line (indicating higher firing rate at high contrast) at every brightness level, as also visible in the population average (*Fig. 4.2*).

A decrease of the firing rate at high contrast compared to low contrast is surprising. Somewhat related effects have been described by Smirnakis [26], regarding adaptation to spatial contrast. In this work the authors used either full-field flicker or checkerboard patterns with various sizes of checkers to stimulate the retina. ON cells had higher steady-state firing rate to checkerboard stimulation than to full-field flicker, and this effect was negatively correlated with the checker size. Most OFF ganglion cells, however, showed a nonlinear relationship between steady-state firing rate and checker size. For smaller sizes, OFF cells decreased their firing rate, and for larger sizes it increased, compared to the firing rate to full-field flicker. The authors suggest center-surround antagonism to explain this behavior, and propose a model consisting of two blocks, one with narrow antagonistic receptive fields and the other with a wide uniform receptive field, converging onto the OFF cells. In my experiments, I varied temporal contrast and used a full-field stimulus, therefore such a spatially heterogeneous model can not explain the decrease of firing rate at high contrast. Furthermore, in my experiments, I observe the 'unexpected' behavior mostly in ON cells, not OFF cells. For the further discussion, I call cells that respond more rigorously at high contrast 'typical', and cells that respond more weakly 'non-typical' ON cells.

What could be the mechanism of firing rate suppression by high contrast stimuli in 'non-typical' ON cells? It has been shown that ON cells are less rectifying than OFF cells [58, 69]. Less rectification means that the cell not only increases its activity to ON stimuli, but also decreases its activity to OFF stimuli. This suggests that the cell is being actively suppressed by the antipreferred stimulus (light decrements in case of ON cells). Since the GWN flicker stimulus equally contains events where the stimulus is dimming (activating OFF pathways) and brightening (activating ON pathways), more efficient presynaptic inhibitory OFF pathways (compared to excitatory ON pathways) would decrease the mean firing rate. This simple mechanism – stronger inhibitory OFF inputs relative to excitatory ON inputs - could explain the unexpected behavior of 'non-typical' ON cells.

The idea of strong inhibitory OFF input in the 'non-typical' ON cell population also is supported by their responses to other stimuli. *Fig. 4.4* shows the responses to full field step stimuli averaged across 'typical' and 'non-typical' ON ganglion cells. The 'non-typical' cells (in red) indeed have smaller amplitude of the ON response to light increments compared to the group of 'typical' cells (shown in blue), and at the same time much stronger suppression at light decrements.

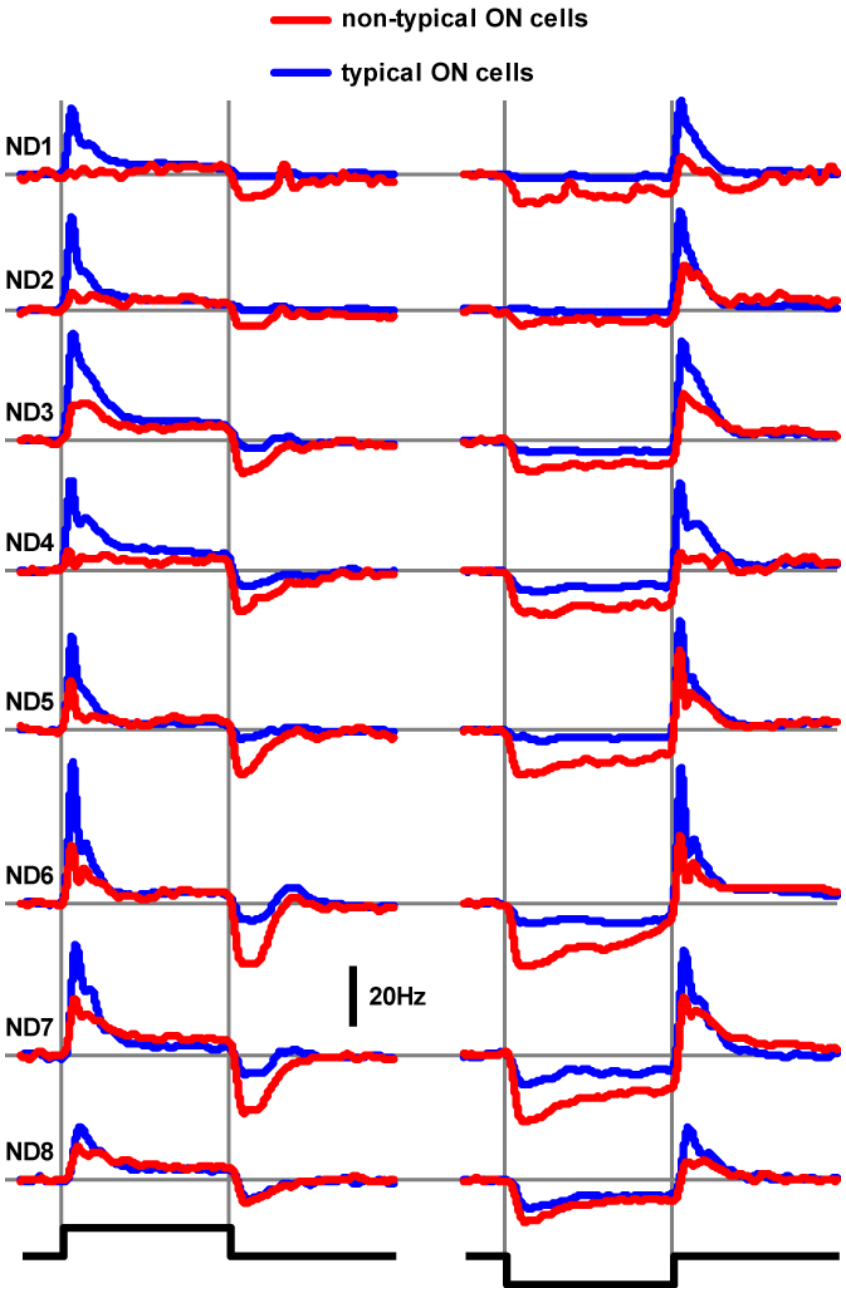

Fig. 4.4. Averaged responses of 'typical' (blue) and 'non-typical' (red) ON ganglion cells to white and black full-field steps at different light levels. Mean spontaneous firing rate was subtracted from each cell at each light level. 'Non-typical' cells have smaller responses to light increment and bigger suppression of spiking to light decrements.

Spiking responses of ON cells do not allow measuring the input currents of ganglion cells directly, therefore it is not possible to test the relative strength of ON excitatory and OFF inhibitory inputs in my experiments. However, one can approximate it by calculating the area of the firing rate fluctuation in response to white and black steps. This procedure is likely to underestimate the inhibitory inputs (when spikes are totally suppressed); sometimes it can also underestimate the ON excitatory input (if it was combined with ON inhibition, pulling the firing rate down). The interpretation of the results may therefore only show the tendency and should be taken with caution. For my analysis, I compared 'ON excitation' and 'OFF inhibition' by calculating the difference between the area of the ON response during first second of the white step and the area of the OFF response during the first second of the black step. Then, I averaged these differences across 'typical' and 'non-typical' ON cells. The results are plotted in *Fig. 4.5*. When 'OFF inhibition' exceeds 'ON excitation', this analysis yields negative values. As expected, this was indeed the case for 'non-typical' ON cells, at least for ND8 to ND4. 'Typical' ON cells, on the other hand, yielded the opposite result. The brightest light levels ND3-ND1 did not differ between the two groups of ON cells. The likely reason for this is the underestimation of the OFF inhibitory input: most ON cells have very low spontaneous activity at high brightness and can not be further suppressed by the black step.

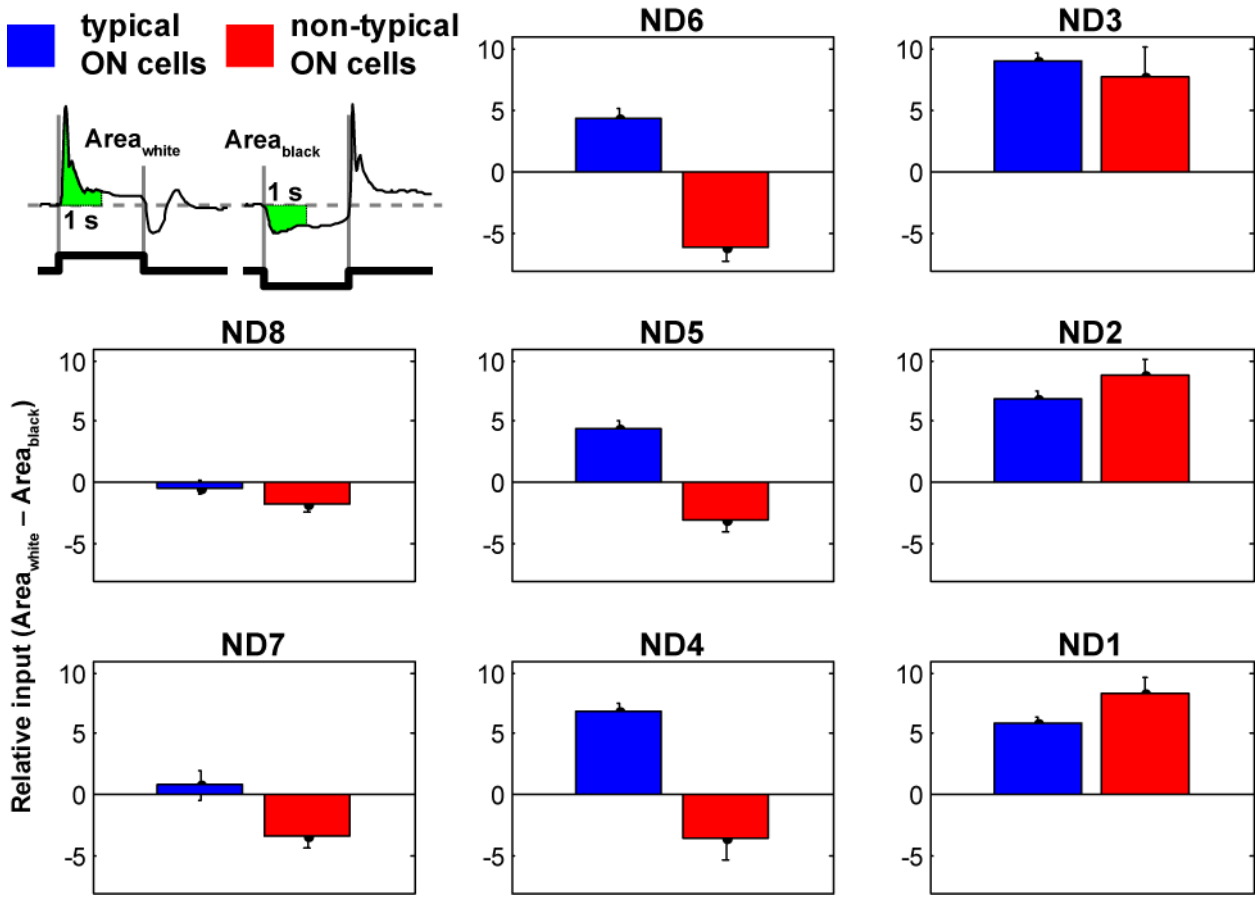

Fig. 4.5. The response area to the white step is larger than the response area to the black step for typical cells but not for 'non-typical' ON cells at ND7-ND4. It likely reflects stronger OFF inhibition in 'non-typical' cells compared to ON excitation.

Responses to chirp stimuli also support the prevailing OFF inhibition in 'non-typical' ON cells (*Fig. 4.6*). These cells (in red) were modulated by the chirp mostly below their baseline activity (baseline activity was subtracted prior the averaging), whereas 'typical' cells (in blue) were modulated mostly above their spontaneous activity. For comparison, I plotted the responses of the OFF cells to the chirp stimulus (*Fig. 4.7*). They resemble the responses of 'typical' ON cells with the corresponding phase shift.

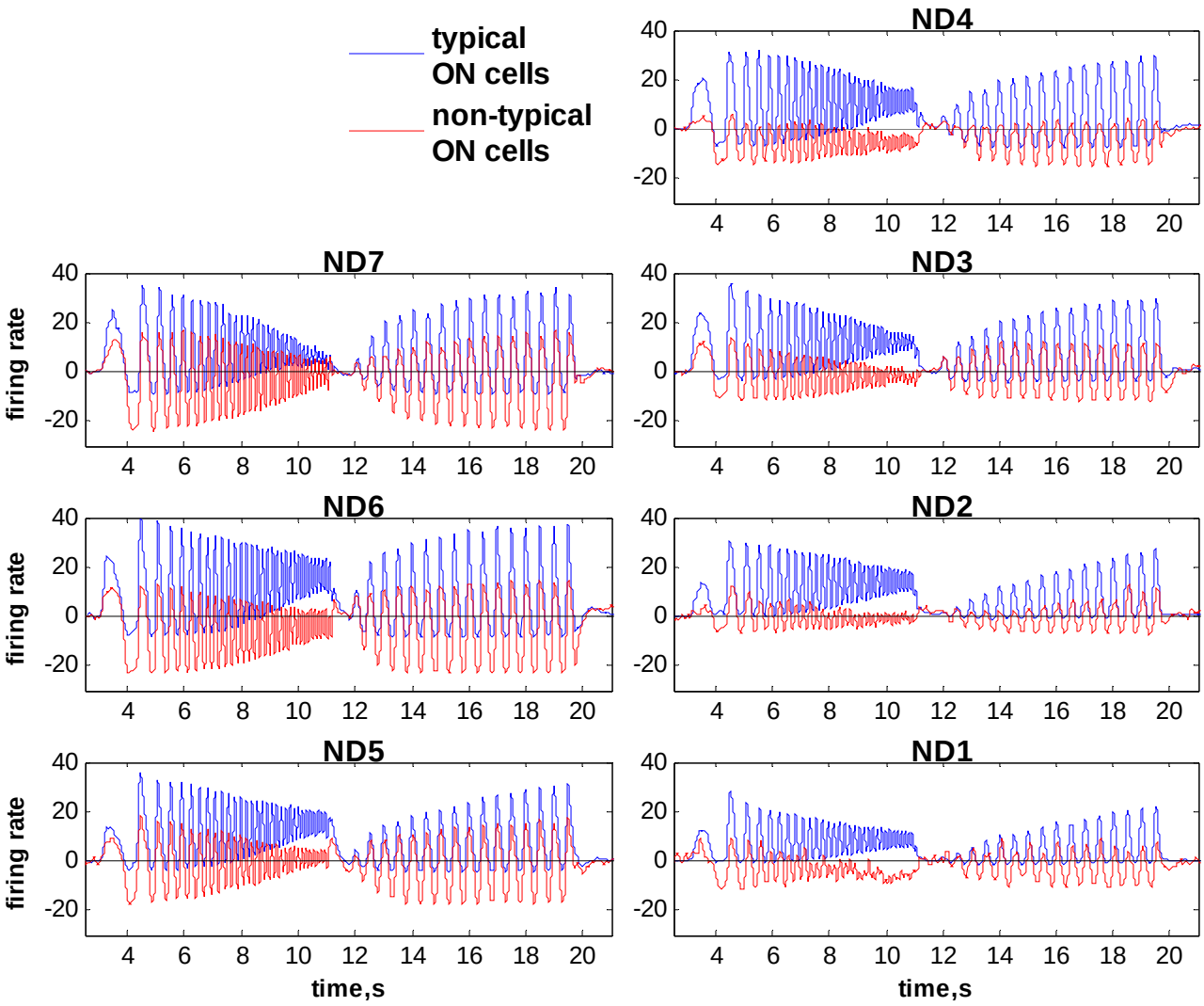

Fig. 4.6. Averaged responses of 'typical' (blue) and 'non-typical' (red) ON ganglion cells to the chirp stimulus. Mean spontaneous firing rate was subtracted from each cell at each light level. 'Non-typical' cells are much less rectifying.

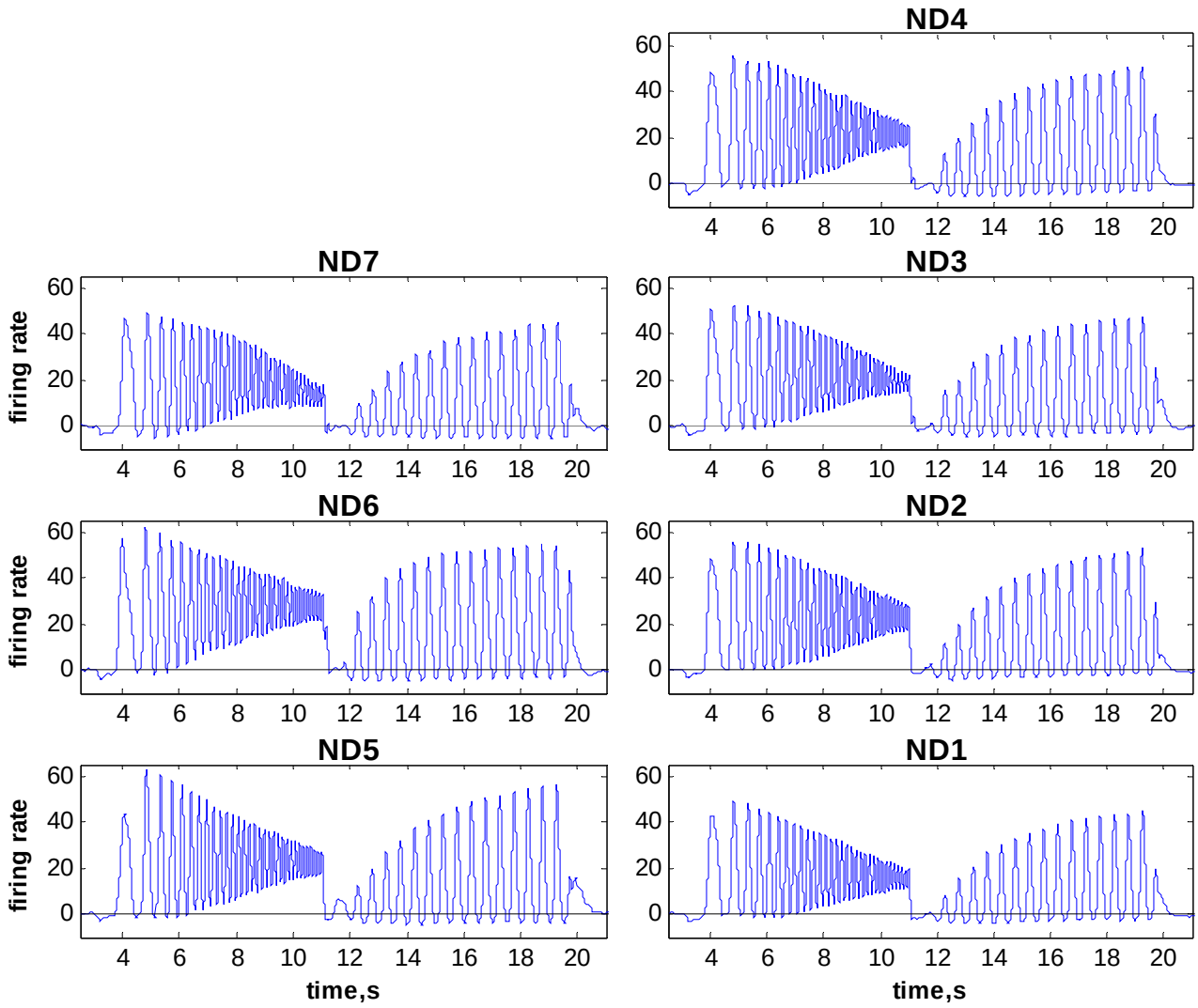

Fig. 4.7. Averaged responses of OFF ganglion cells to the chirp stimulus. Mean spontaneous firing rate was subtracted from each cell at each light level. OFF cells strongly rectify and are similar to 'typical' ON cells in this respect (see Fig. 4.6).

I counted the fraction of 'non-typical' ON cells at each brightness level. Most numerously these cells were represented at ND7 (>60%, *Fig. 4.8*). At higher light levels they were fewer: about one fifth of all recorded ON cells at ND4-ND3 and <10% at ND2-ND1. Note that 'non-typical' OFF cells (i.e. decreasing their firing rate at high contrast compared to low contrast, analogously to 'non-typical' ON cells), shown for comparison, hardly exist: less than 10% of cells at ND7 and less than 5% at higher light levels.

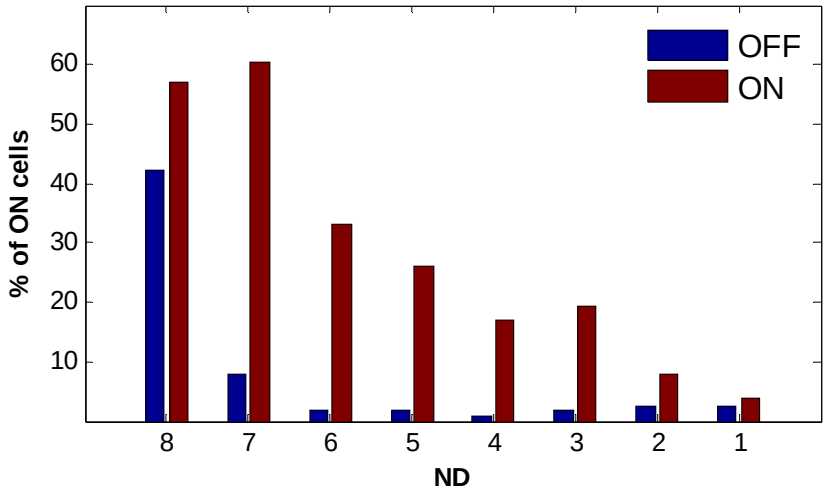

Fig. 4.8. Percentage of 'non-typical' cells in OFF (blue) and ON (red) cell populations (in 15 experiments).

To test how firing rate adaptation to temporal contrast is modulated by mean luminance, I calculated the 'firing rate adaptation index' for each cell: the ratio of the mean firing rates at low and high contrasts. Then I checked whether the distribution of these indices is significantly different between ND7-ND4. Among ON cells, there was no significant difference ($p>0.05$) only between ND5 and ND4, and among OFF cells, only between ND7 and ND5 and between ND5 and ND4. All other NDs differed from each other. This test further confirms the luminance effect on firing rate adaptation.

4.2.2. Speed of linear filter

Numerous studies have shown that contrast adaptation reveals itself in tuning to lower temporal frequencies at low contrast compared to high contrast [28-29, 31]. I took the time-to-first-peak and the time-to-first-zero-crossing-after-the-first-peak (in biphasic filters) as parameters to represent linear filter kinetics. I found that different cells show diverse behaviors: some cells slow down at low contrast, while others remain stable or even accelerate. *Fig. 4.9* compares the linear filters of three individual ON cells obtained to high (blue) and low (red) contrast GWN flicker stimuli at 6 different brightness levels, from ND7 to ND2. The amplitudes of the linear filters were normalized by the first peak better compare the kinetic properties. The first cell has slower linear filter at low contrast (left column), and therefore shows the 'expected' behavior. The second cell has kinetically very similar filters (middle column), and the third cell even has a faster linear filter at low contrast (right column). Remarkably, every cell consistently behaves in the described way at most brightness levels, except the dimmest and the brightest ones shown (ND7 and ND2).

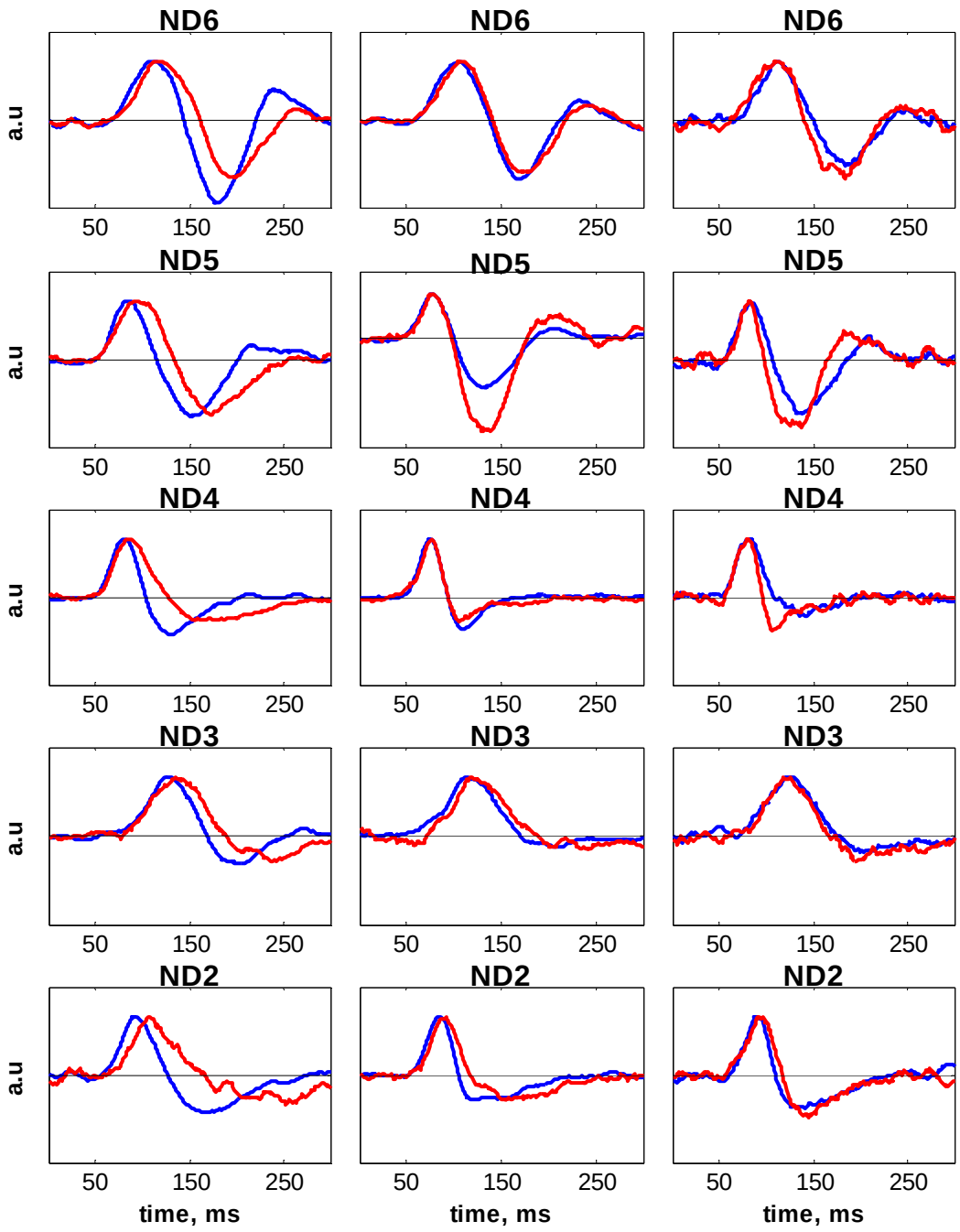

Fig. 4.9. Linear filters of three ganglion cells obtained in response to high (blue) and low (red) contrast trials. Amplitude normalized to the positive peak. Left column. Cell speeding up at high contrast. Middle column. Cell not changing at high contrast. Right column. Cell slowing down at high contrast.

Fig. 4.9 also illustrates that kinetics changes of the linear filters are not constant at every time point of the filter. For example, the zero-crossing latency differs more than the peak latency for cells in

left and right columns. To avoid bias towards a specific time point in the linear filter, I used a more general method to estimate the change of linear filter kinetics: I calculated the cross-correlation between high and low contrast filters, and used the lag corresponding to the peak in the cross-correlogram as the parameter representing adaptation of the linear filter kinetics. Cells with unstable filters at low contrast resulted in low maximal cross-correlation coefficient (< 0.6), and were excluded from further analysis. *Fig. 4.10* shows the distribution of these lags, separately for ON and OFF cells at every brightness level. Negative lags represent the "expected" behavior, namely faster kinetics during high contrast stimuli. Positive lags mean that the filter accelerates at low contrast: some fraction of ON cells does so at ND7-ND5. The fraction of OFF cells with this kind of adaptation is much smaller. At ND7-ND3, ON cells adapt significantly less than OFF cells ($p<0.001$), i.e. the distribution of ON cells is closer to 0 lag.

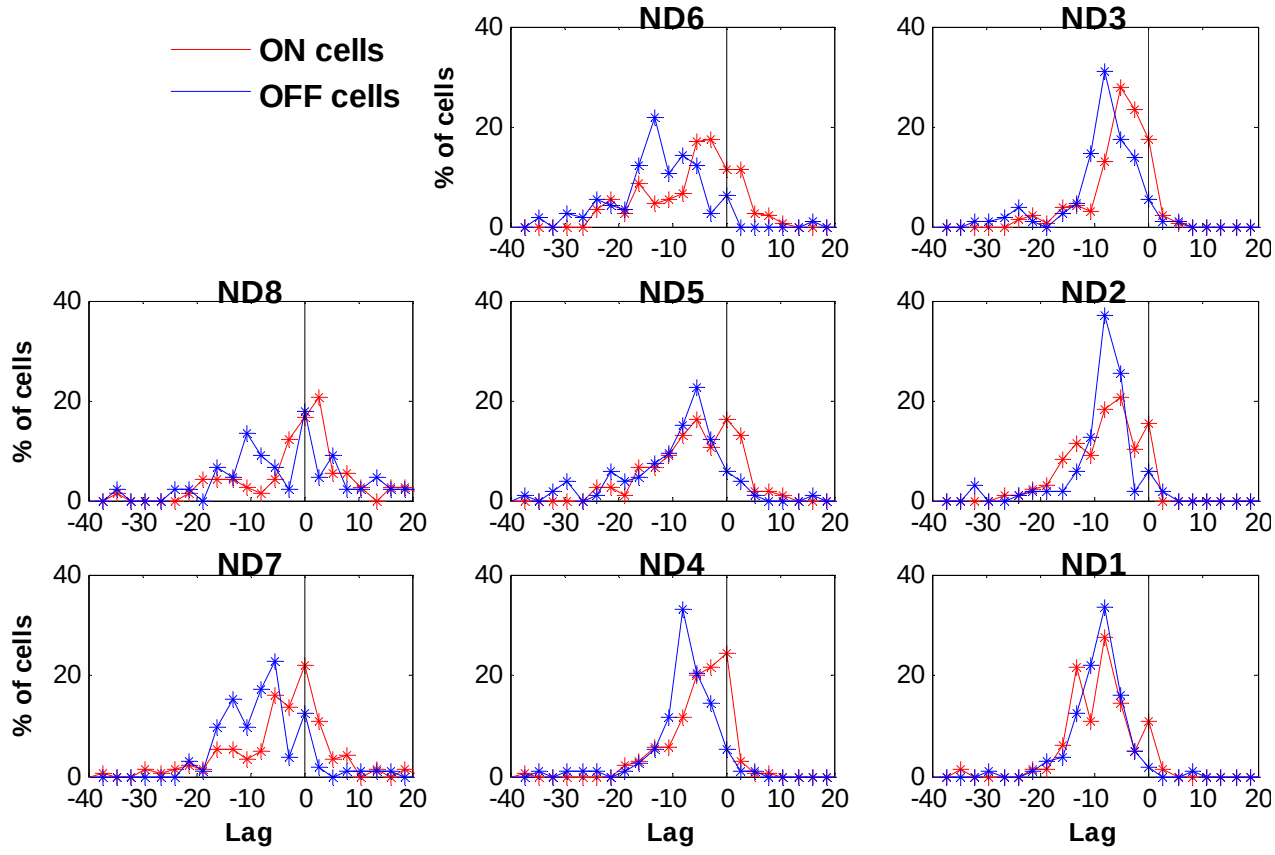

Fig. 4.10. Distribution of lags of the maximal cross-correlation coefficients between linear filters at high and low contrast at different light levels of ON cells (red) and OFF cells (blue). Negative values correspond to speeding up of the linear filter at high contrast.

Does the distribution of these lags change across different brightness levels? I.e., does mean luminance effect the way in which cells adapt the relative kinetic of their responses to high and low contrast? The distribution of the kinetic changes was indeed influenced by luminance. OFF cells had statistically different mean of the lag distributions at ND6 compared to all other ND (*Table 4.1*). Among ON cells, distributions at ND7 and two brightest NDs (ND2 and ND1) were the most distinct, while the distributions at ND6 to 3 were not significantly different. Interestingly, the effect of luminance on contrast adaptation was statistically significant mostly between low light levels and the brightest, most bleached, conditions (*Table 4.2*).

Table 4.1. Light levels with significantly different (*: $p<0.05$ and **: $p<0.01$) distributions of lag of the maximal cross-correlation coefficients between linear filters to high and low contrast in OFF cells population (see also Fig. 4.10)

ND	7	6	5	4	3	2	1
8	**	**	**	**	**	**	**
7		**	-	-	-	-	-
6			**	**	**	**	**
5				-	-	-	-
4					-	-	**
3						-	*
2							-

Table 4.2. Light levels with significantly different (*: $p<0.05$ and **: $p<0.01$) distributions of lag of the maximal cross-correlation coefficients between linear filters to high and low contrast in ON cells population (see also Fig. 4.10)

ND	7	6	5	4	3	2	1
8	**	**	**	**	**	**	**
7		*	-	*	**	**	**
6			-	-	-	*	**
5				-	-	**	**
4					-	**	**
3						**	**
2							-

4.2.3. Gain control

Decrease of stimulus variance (low contrast) generally leads to increased responses to weak stimuli. This process is referred to as gain control and is one of the most prominent and well-studied features of contrast adaptation. In work of Mante, it has been shown that gain adjustment observed in LGN neurons of the cat is controlled independently by luminance and contrast adaptation [64]. I tested this observation over my large range of light levels, using the linear-nonlinear model formalism.

I calculated the nonlinearity as described in Methods, and then fitted it with a sigmoid function. The fit contains a single parameter characterizing the gain of the nonlinearity. The gain increase at low contrast was calculated as ratio of slope parameter at high contrast and low contrast. For the analysis, I only took gain ratio not exceeding 20, as such high values are likely caused by fits to noise data. *Fig. 4.11* summarizes the gain ratio averaged for all ON cells and OFF cells at each brightness level.

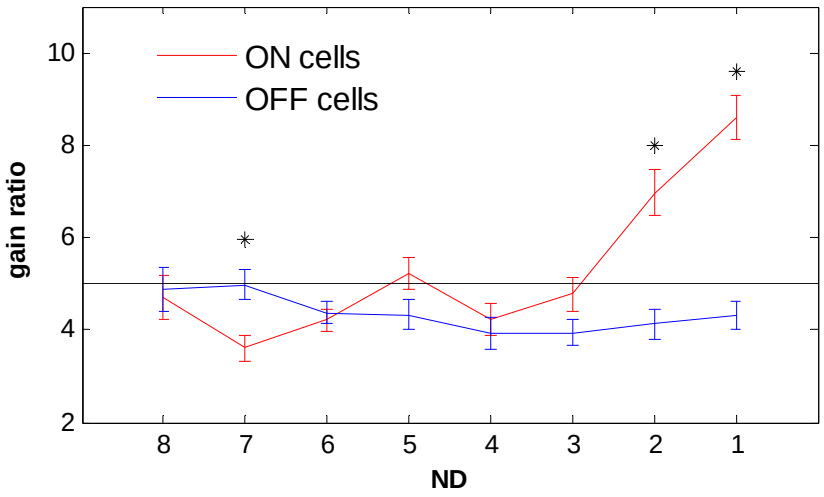

Fig. 4.11. Ratio of gain at low and high contrast at different light levels in ON and OFF cell populations. Mean ± s.e.m. * mark significant ($p<0.05$) difference between gain ratio of ON and OFF cells.

An 'ideal adapter' would have a gain ratio approximating the contrast ratio (5 in my experiments; marked as a black horizontal line in *Fig. 4.11*). This means that a 5-times weaker stimulus would produce, on average, the same responses. However, on average OFF cells only 'ideally' adapt at ND8 and ND7; at brighter light levels, they 'underadapt' (gain ratio<contrast ratio). This means it takes a stronger stimulus than 5-times-weaker to achieve the same response. ON cells behave differently: they underadapt at most light levels, getting close to the 'ideal' gain control only at ND5 and ND3, and

strongly overadapt at ND2-ND1. The difference between ON and OFF cells distribution of gain ratios is statistically valid ($p<0.05$) at ND7, ND2, and ND1.

To estimate the influence of luminance on gain control in more detail, I compared not only the mean of the gain ratios, but also the distributions of gain ratios at different light levels for both OFF cells and ON cells (Fig. 4.12). The results of the Wilcoxon test are given inTable 4.3 (OFF cells) and Table 4.4 (ON cells).

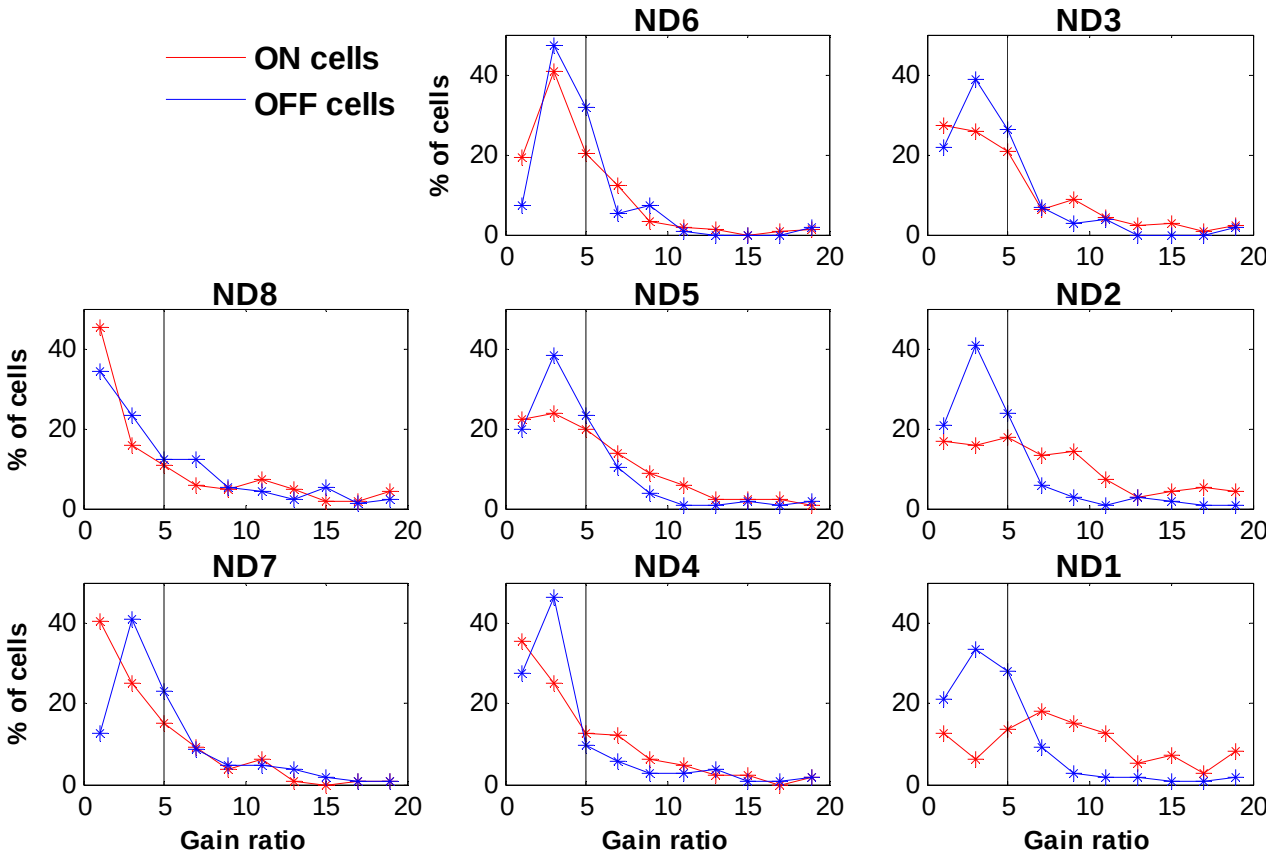

Fig. 4.12. Distribution of gain ratio at low and high contrast in ON and OFF cells populations at different light levels. Black vertical line marks 5, ratio of stimulus contrasts.

Table 4.3. Light levels with significantly different (*: $p<0.05$ and **: $p<0.01$) distributions of gain ratio of low and high contrast in OFF cells population (see also Fig. 4.12).

ND	7	6	5	4	3	2	1
8	-	-	-	-	-	-	-
7		-	-	**	*	*	-
6			-	**	-	-	-
5				*	-	-	-
4					-	-	*
3						-	-
2							-

Table 4.4. Light levels with significantly different (*: $p<0.05$ and **: $p<0.01$) distributions of gain ratio of low and high contrast in ON cells population (see also Fig. 4.12).

ND	7	6	5	4	3	2	1
8	-	-	*	-	-	**	**
7		**	**	-	*	**	**
6			-	-	-	**	**
5				*	-	**	**
4					-	**	**
3						**	**
2							**

Table 3 suggests that gain control in OFF cells caused by contrast adaptation indeed almost does not depend on the luminance. Gain increase at ND4 is statistically lower than at most other NDs, and at ND7 it is statistically higher than at ND4-ND2. This contrasts with the ON cells group (Table 4), where gain control at ND7 was significantly lower than at any other light level (except ND4). ON cells also showed overadaptation at ND2 and ND1; it was significantly different from all other light levels.

4.3. Summary and Discussion

There is evidence of significant effects of the ambient light level on all three investigated features of contrast adaptation: firing rate, temporal filtering, and gain control. Interestingly, the luminance level often has distinct, sometimes even opposite, impact on the contrast adaptation properties of ON and OFF cells.

A large proportion of ON cells was found to decrease the firing rate in response to high contrast stimuli. The activity of these 'non-typical' ON cells is strongly suppressed in response to their 'antipreferred' stimulus, i.e. in response to light decrements. A corresponding behavior is very rare among OFF cells (1-3% of OFF cells). This is consistent with previous reports showing larger rectification in OFF cells compared to ON cells [58, 68-69].

Remarkably, many 'non-typical' ON cells not only are strongly suppressed by OFF stimuli, but this suppression is even stronger than the activation by ON stimuli (judging by the difference of response areas to light increments and decrements, *Fig. 4.5*). In a circuit, such cells report light decrements better than light increments, if the target neuron cares about the firing rate changes regardless of the sign (see also chirp responses in *Fig. 4.6*). In a sense, 'non-typical' ON cells could be thought of as 'secondary' OFF cells, reporting the same stimulus via the opposite code. Indeed, the responses of OFF cells and 'non-typical' ON cells are highly negatively correlated. The correlation between responses of OFF cells and 'typical' ON cells is also negative, but much weaker because the responses of both cell groups are strongly rectified (*Fig. 4.13*, correlation is calculated between chirp responses averaged across corresponding groups of cells, see also *Fig. 4.6* and *Fig. 4.7*). The intragroup correlation between OFF cells is of course strongly positive.

What advantage could there be to having two opposing ways of coding for OFF stimuli? Due to the high spatial and temporal correlations of natural stimuli, the ganglion cells in a given retinal patch receive correlated input, and share correlated noise. It has been shown that in presence of correlated noise, neurons with negatively correlated responses carry more information about the stimulus than positively correlated or uncorrelated neurons [for review, see [70]. 'Non-typical' ON cells, together with OFF cells, may then increase the information transmitted by ganglion cells on the population level, and decrease sensitivity to noise. Furthermore, it has been shown that negative contrasts are more abundant in natural scenes than positive contrasts [71]. This might explain why such a double coding strategy exists for OFF stimuli (i.e., 'non-typical' ON cells are abundant), but not for ON stimuli ('non-typical' OFF cells are hardly found).

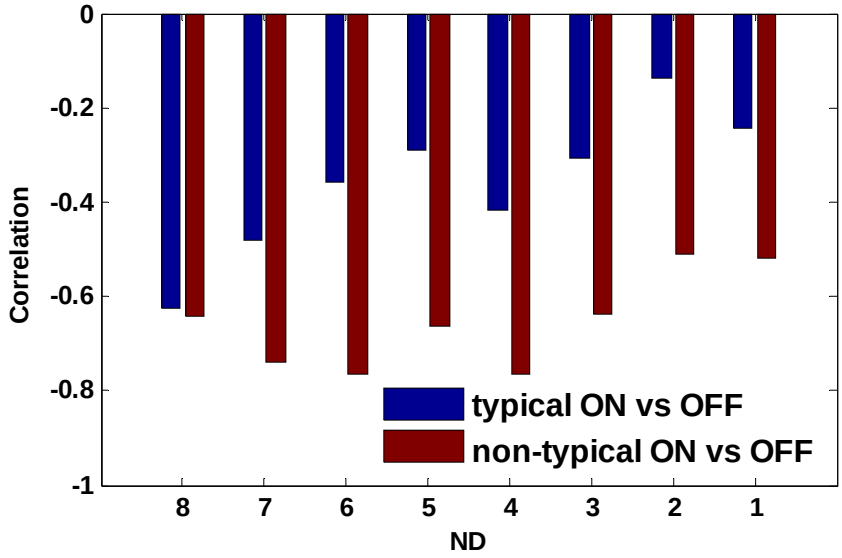

Fig. 4.13. Correlation coefficient between average responses to the chirp stimulus of OFF cells and 'typical' or 'non-typical' ON cells.

Interestingly, the fraction of 'non-typical' ON cells diminishes as the light level increases (*Fig. 4.8*), which suggests differential light level-dependent regulation of their synaptic inputs (ON excitation vs OFF inhibition). In particular, the relative strength of ON excitation grows, which leads to stronger responses to light increments and shifts the cell's behavior toward 'typical' ON cells. This evidence fits very well with the natural images statistics (negative contrasts are more frequent in the natural environment [71]) and the fact that light decrements are harder to detect in low luminance [72]. Since 'non-typical' ON cells 'support' OFF cells (see previous paragraph), functionally it would be more beneficial to have more such cells in dimmer conditions.

ON cells also have more variable strategies of kinetics adaptation than OFF cells. Some ON cells do not speed up at high contrast, but even slow down (inverse kinetic adaptation). The proportion of such cells was also luminance-dependent (*Fig. 4.10*). *Fig. 4.14* shows the latency difference of the linear filter at low and high contrast (negative values on x-axis: latency at high contrast shortens, while positive values correspond to inverse kinetic adaptation) versus firing rate difference at high and low contrast (negative values on y-axis, 'non-typical' cells, positive values, 'typical' and OFF cells). Most cells with inverse kinetic adaptation (slowing down at high contrast) lie below the zero line on y-axis and therefore belong to 'non-typical' ON cells.

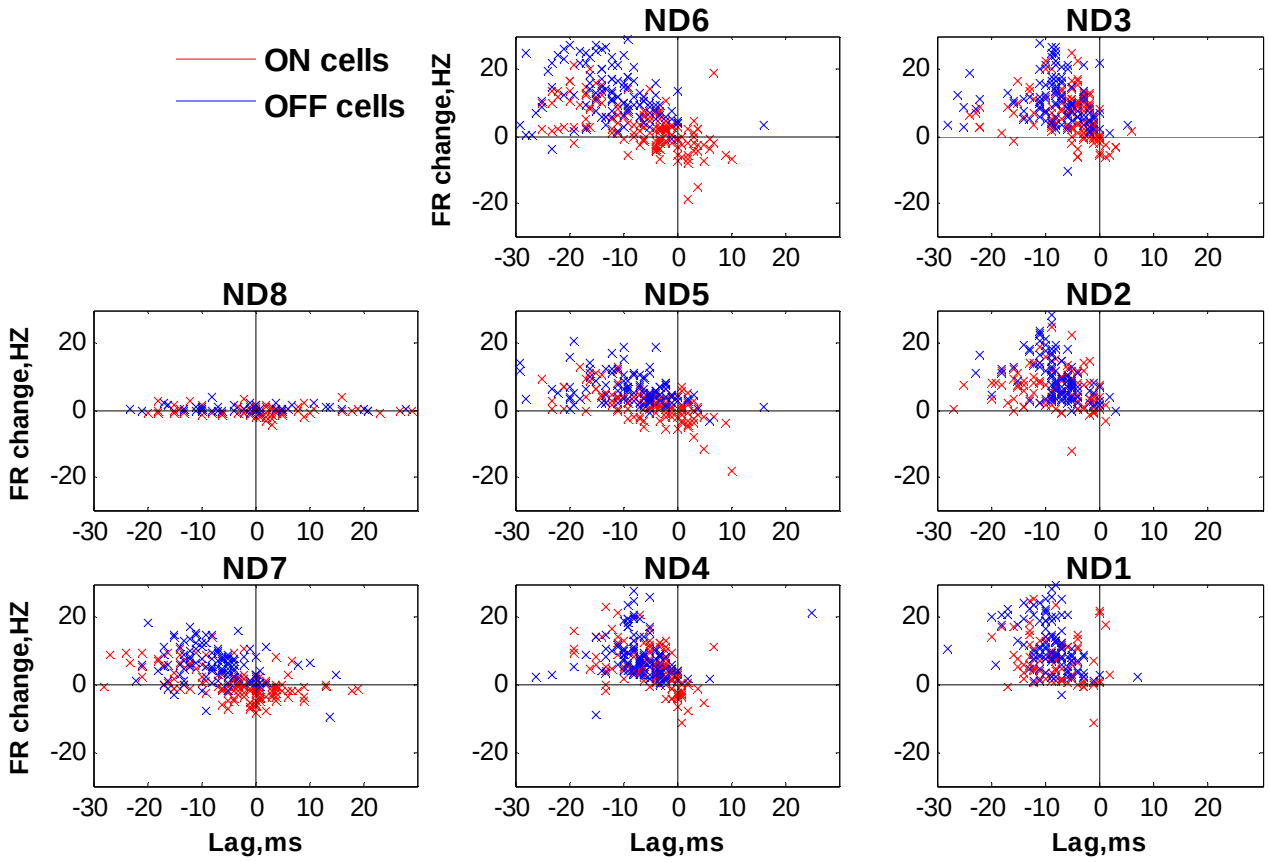

Fig. 4.14. Relation between firing rate adaptation and linear filter kinetics adaptation to GWN contrast in ON and OFF cell populations at different light levels. X-axis, lag of the maximal cross-correlation coefficient between linear filters obtained at high and low contrast. Y-axis, difference of mean firing rate at high contrast and low contrast. 'Non-typical' ON cells are below horizontal zero line and may have both positive and negative lags (i.e. accelerate or slow down at low contrast).

Interestingly, 'non-typical' ON cells in general have rather weak kinetic adaptation (note the small horizontal spread of marks below the horizontal zero line in *Fig. 4.14*). I calculated the mean delay of linear filters across groups of 'non-typical' ON, 'typical' ON, and OFF cells (*Fig. 4.15*). For this analysis, cells which had moderate (less than 2 Hz) differences between firing rate to high and low contrast were left out to account for jitter. 'Typical' ON cells and OFF cells, as expected, sped up at high contrast, on average by 8 ms. The distribution of delays was significantly different from zero at ND7 to ND1 (t-test, $p<0.001$). The mean delay of 'non-typical' ON cells, in contrast, was close to zero at all NDs (t-test, $p>0.2$ at ND7 to ND1).

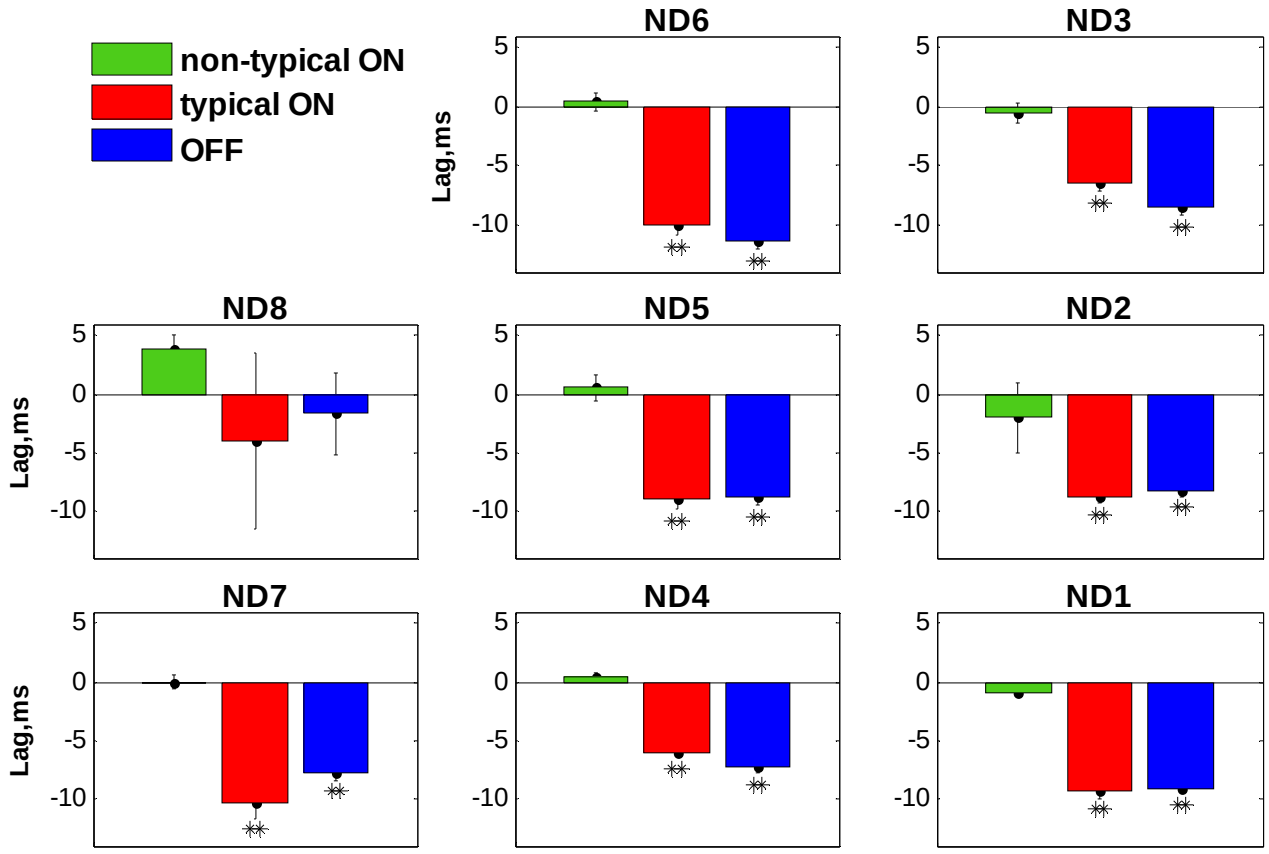

Fig. 4.15. Lag of maximal cross-correlation coefficient between linear filters obtained at high and low contrast in 'non-typical' ON, 'typical' ON, and OFF cell populations. Mean ± s.e.m., ** mark p<0.001 (t-test). 'Typical' ON and OFF cells slow down at high contrast, whereas 'non-typical' cells adapt on average very weakly (not significantly different from 0 at all light levels).

This analysis suggests that kinetic adaptation in ON cells is tightly connected with their input composition, in particular, with the ratio of ON excitation and OFF inhibition, since it is the basis of the distinction of these two groups of cells. If one of the pathways (e.g. ON excitation) has adaptation properties similar to that in 'typical' ON cells, the other pathway (OFF inhibition) might have opposite properties. The resulting kinetic changes will then be moderate - slight slowing down or accelerating of the responses at high contrast. However, the exact mechanism of kinetic adaptation has not been established yet, and current models of contrast adaptation do not account for such 'inverse' kinetic changes [31, 60].

Gain control on average was found to be the least affected by luminance. OFF cells tended to underadapt at most brightness levels (*Fig. 4.11*). ON cells, in general, were more variable, changing from underadaptation to overadaptation at different light levels (*Fig. 4.11*). A remarkable feature of ON cells was strong overadaptation at ND2 and ND1. The reason might be related to the strongly decreased firing rate of ON cells at low contrast at these NDs (*Fig. 4.1*, top right). In fact, at these NDs it is even

smaller than the mean spontaneous firing rate. As discussed above, the decrease of firing rate in response to GWN stimulus is likely to be caused by stronger OFF inhibition than ON excitation (in ON cells). Since ON cells behave as 'non-typical' at low contrast and as 'typical' at high contrast at ND2 and ND1, the ratio of ON excitation and OFF inhibition appears to depend on contrast. This implies different sensitivity of the two pathways to contrast at very bright light: more sensitive OFF inhibition, providing a strong input into the cell at low contrast and saturating at high contrast, and less sensitive ON excitation, which needs stronger stimulus to activate the cell. Since the two inputs signal different features of the stimulus (light increments and decrements), the resulting gain increase is even higher than the contrast ratio of the stimulus.

Taking together, these results suggest that the influence of luminance on contrast adaptation has been underestimated. Firing rate and kinetics adaptation, as well as gain control, may be significantly modulated by the ambient light level. My experiments indicate at least one mechanism of this modulation, namely the differential regulation of ON excitatory and OFF inhibitory inputs into so-called 'non-typical' ganglion cells. To the best of my knowledge, contrast adaptation of inhibitory circuits has not been studied/modeled in terms of the linear-nonlinear system, and my results may serve as a basis to implement and generalize in the LNL model. Another novel aspect of my work is the suggested light-dependent role of 'non-typical' ON cells, which may serve to 'support' OFF cells and reduce the influence of correlated noise on information processing.

5. Effect of luminance on steps responses

5.1. Introduction.

Retinal ganglion cells are not homogeneous in their properties and functions. Instead they form several 'information channels' processing different aspects of the incoming visual signal in parallel. The physiological basis of this separation begins at the photoreceptor-bipolar cell synapse where signal flow broadly separates into ON and OFF channels, determined by the nature of the glutamate receptor expressed by bipolar cell dendrites. Downstream circuits are specialized and extract very complex features of the visual input [8-12]. Ganglion cells may combine inputs from both ON and OFF pathways through sign-reversing or sign-conserving synapses. Complete and detailed physiological classification of ganglion cells is one of the major goals and challenges in retinal research which has not yet been solved [73].

An additional difficulty in ganglion cell classification is posed by the variety of species used in research. The most popular models – tiger salamander, mouse, rat, rabbit, cat, primates – occupy very different ecological niches, and the correspondence of established cell classes between species remains unclear [74]. Furthermore, usage of different experimental conditions (dark- or light-adapted retinas) and stimuli makes direct comparison difficult even within one species, as it is likely that cells of a given type will adapt (change) their properties depending on the current conditions.

One of the most basic characteristic properties of ganglion cells is their response polarity – their response type to light increments and decrements [75-76]. Cells which increase their firing rate in response to a brighter (than the background) spot of light in the center of their receptive field are called ON cells. They may decrease spiking activity in response to a dark spot. OFF cells react in the opposite way: increase activity to dark spots, and (potentially) suppress activity to bright spots. Cells which respond with increased activity to both darkening and brightening of their receptive field center are called ON-OFF.

ON and OFF cells are sometimes considered as mirroring each other. However, a number of publications have shown asymmetries between them [25, 58]. These asymmetries are luminance-dependent: they are more pronounced in dark adapted conditions [19, 23, 58, 72]. Some studies show that some cells can even change their polarity under specific luminance and stimulation conditions

[77], or obtain ON-OFF responses [25]. The latter work also shows that polarity of cells may depend on the stimulus size (full field stimulation vs only receptive field center).

Therefore, classification of cells by even such a basic property as polarity may depend on the luminance level and the specific stimulus it is probed under. There has been no attempt to routinely characterize ganglion cell response properties to a simple light increment or decrement over several orders of light magnitude. In this chapter, I analyze ganglion cells responses to full-field positive (white) and negative (black) steps of light over 8 orders of magnitude of luminance (details see in Methods). For the discussion below, I classify cells as ON and OFF based on the polarity of the linear filter, as described in Section 3. This property was found to be stable, in contrast to responses to full-field steps of light. Then, I describe groups of cells which not only have similar responses at one brightness, but also change the response properties in the same way at least at three light levels: ND6 (predominantly scotopic), ND5 (mesopic), and ND4 (predominantly photopic).

5.2. Results.

5.2.1. Motivation: Cell responses across luminance conditions are highly variable

520 cells were recorded in 15 experiments. I calculated the firing rate of every cell in the 4.5s interval from 500 ms prior to the 2-second full-field step to 2s after the step offset (see Methods). To reduce noise, I then averaged the responses to the 5 steps of each contrast presented in one trial, yielding a response pattern to white and black steps at every brightness level. If several trials were presented at one brightness level, I averaged the responses across trials as well.

Before systematic description of my analysis of ganglion cell responses to this step stimulus across different brightness levels, I show two individual examples which demonstrate the stability and variability of ganglion cell responses.

The first example (*Fig. 5.1*) depicts an ON cell: it increased its firing rate in response to light increments, and it decreased its firing rate to light decrements at all mean light intensities. However, the responses at different brightness obviously change. In summary, for this cell, these changes include:

1) transient response to the white step onset (light increment) at all levels except ND4, where the response is sustained;

2) transient response suppression to the black step onset (light decrement) at all levels except ND4, where it is sustained;

3) qualitatively similar reaction at all NDs to the white step offset (also light decrement): transient inhibition followed by a short rebound. The amplitude of the rebound varies;

4) peak amplitude of the ON response to a white step at different NDs varies across brightness levels and reaches its maximum at ND3. At ND2 and ND1, it is greatly suppressed, whereas the ON response to the black step offset has as high an amplitude as at lower light intensities.

There are more differences in the responses across brightness levels. Nevertheless, this cell may be consistently characterized as 'ON' at any brightness level.

The second example, shown in *Fig. 5.2*, depicts another cell. This cell has transient responses at light decrement at all NDs, for both white and black steps – a typical response of an OFF-cell. However, at ND5 the cell also responds to light increments, with rather large latency (~500ms). From here on, I will refer to this type of response as "delayed ON response". At ND4, the cell has responses with short latency to both light increment and decrement, which is normally a signature of an ON-OFF cell. At higher light intensities, the cell returns to a pure OFF-like behavior. If only a single light level had been tested, this cell could have fallen into the 'pure OFF', 'pure ON-OFF', or 'OFF with delayed ON' category.

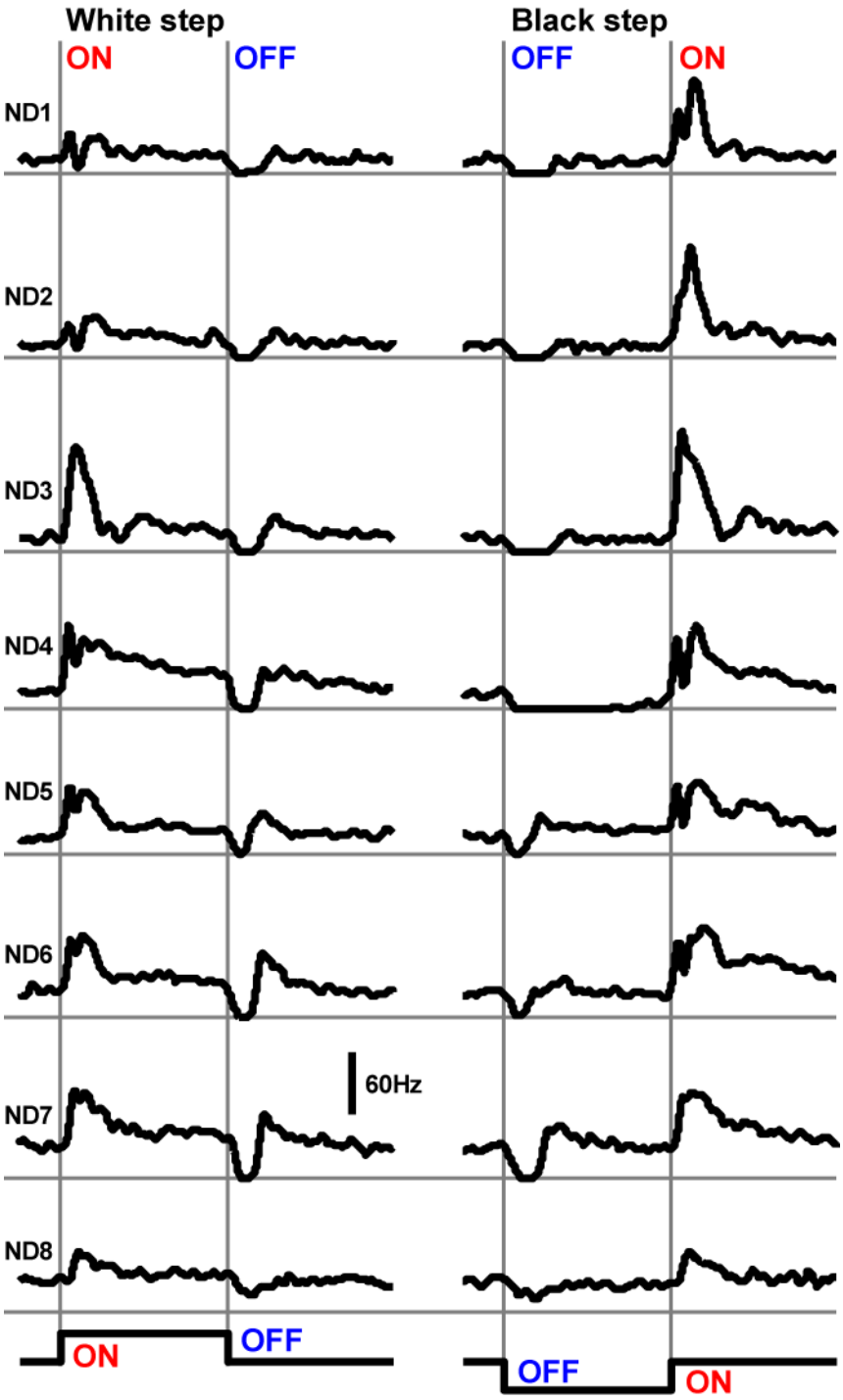

Fig. 5.1. Example responses of a ganglion cell to full-field positive and negative steps at different mean luminance. Overall, the cell has large responses at light increments and activity suppression at light decrements, therefore this cell can be classified as an 'ON' cell. The horizontal line in each panel indicates 0 Hz firing rate.

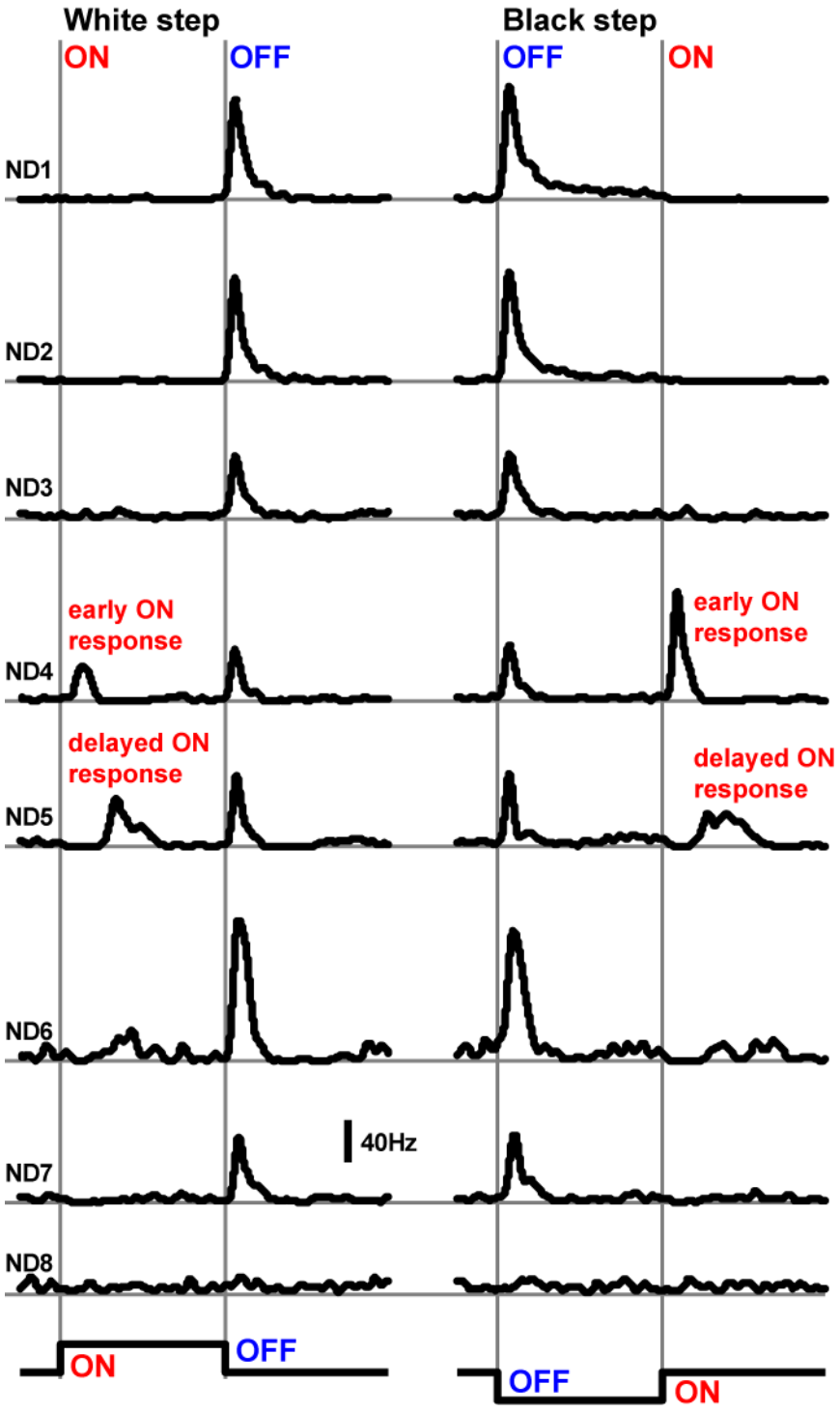

Fig. 5.2. Example responses of another ganglion cell to full-field positive and negative steps at different mean luminance. The cell has large responses at light decrements at all light levels but ND8, whereas responses to light increments vary greatly. The cell can not be therefore unambiguously classified as either 'OFF' or 'ON-OFF' based on responses to full-field steps.

These two examples illustrate how much the responses of ganglion cells depend on the luminance, even when a simple full-field step of light is used as stimulus. Even classification of the cell as ON, OFF, or ON-OFF may be greatly influenced by the ambient light level.

Instead of using an apparently unreliable (because luminance-depended) classification of a cell as ON, OFF, or ON-OFF based on the responses to full-field step stimuli, I used the polarity of the cells' linear filters. Gaussian white noise full-field flicker (see Methods and Section 3) was part of the stimulus set in every experiment. 200 out of 520 cells had OFF-like filters, 249 had ON-like filters. 71 cells with flat or unstable filters were not included in this analysis. It turned out that ON-OFF ganglion cells, when classified in this way, fall into the group with OFF-type linear filters. In the next sections, I will analyze and discuss cells with ON- and OFF-type linear filters separately. The goal is to characterize how the ambient light level influences the response properties of these ganglion cell types.

5.2.2. Cells with OFF-like linear filters (OFF cells)

5.2.2.1. Mean population response.

I first analyzed the mean response of all OFF cells to positive and negative steps of light at 8 brightness levels (*Fig. 5.3*). The main observations are:

- OFF responses consist of a single sharply declining peak at light decrements (for both white and black steps) that differ mainly quantitatively at different brightness levels;
- The two OFF responses to light decrements during black and after white steps have similar amplitude, but differ in transiency;
- ON responses have several distinct components (early and delayed ON response); these components asynchronously appear and disappear at different light levels;
- The two ON responses during white and after black steps are qualitatively different at most brightness levels.

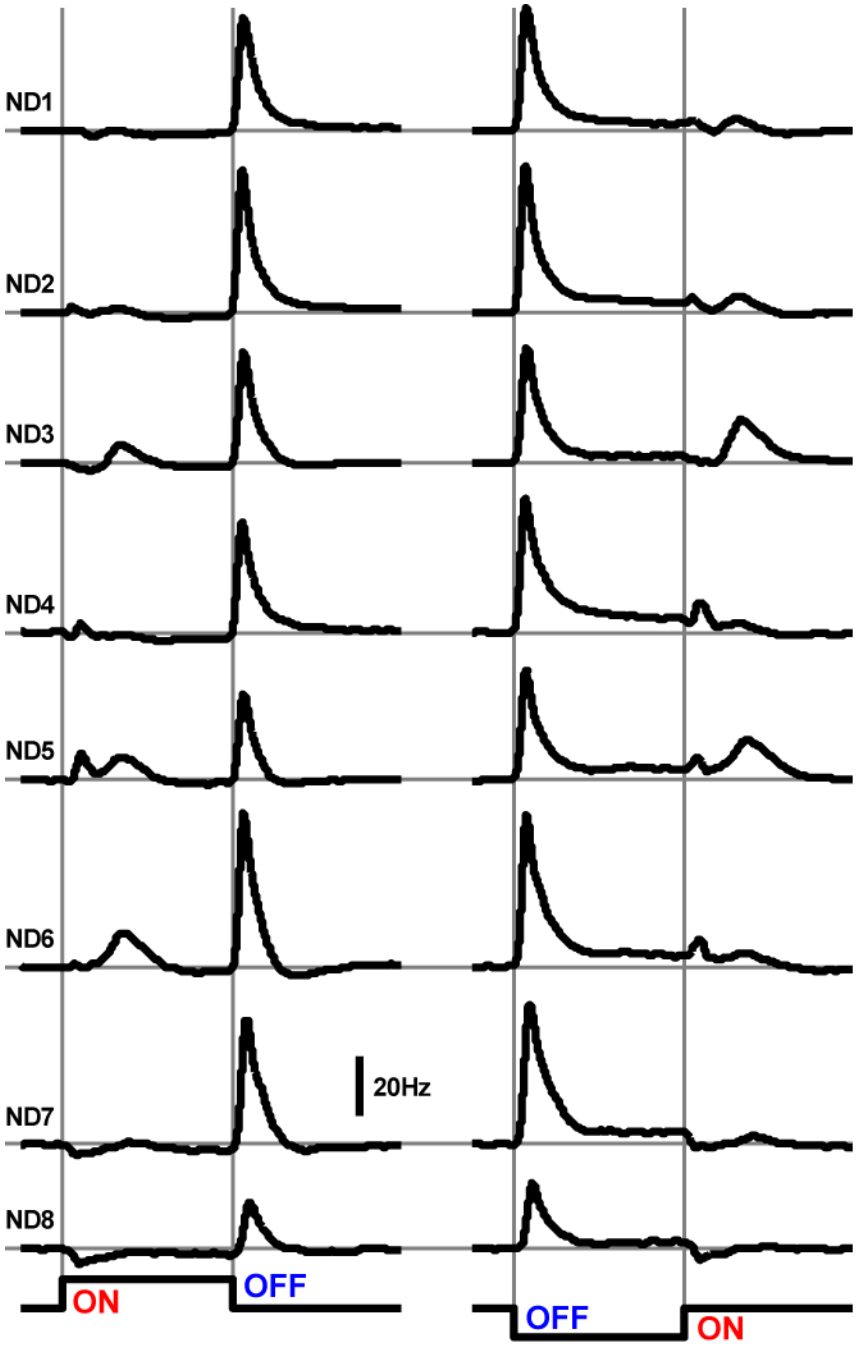

Fig. 5.3. Average responses to full-field white and black steps in the OFF cell population (n=200, from 15 experiments) at different light levels. Spontaneous firing rate was subtracted for each cell individually at each light level.

These observations are made on averaged population responses. Individual OFF cells may maintain the same response across brightness level. However, it is obvious that even if some cells do so, other cells must change their responses. Two observations are of special interest: the pronounced change of the ON response across brightness levels, and the asymmetry of responses to corresponding parts (i.e. light increments or decrements) during white and black steps. First suggests light-level

dependent regulation of ON inputs to at least some OFF cells, which may affect their function (e.g. when a pure OFF cell becomes an ON-OFF cell) and mislead classification attempts. Second suggest nonlinearities in ganglion cells' circuit. These two observations are dissected in more detail below.

5.2.2.2. ON response of OFF cells: variability and dynamics across NDs.

The responses of individual OFF cells to light increments can be strongly modulated at different brightness levels, as the example in *Fig. 5.2* and the population responses (*Fig. 5.3*) have already demonstrated. To characterize OFF cells more rigorously, I first classified different types of responses at three brightness levels: ND6, ND5, and ND4. This is the minimal set of brightness levels which includes all three basic regimes of retinal processing: high scotopic, mesopic, and photopic (see Discussion in Section 3). *Fig. 5.4* shows the standard deviation of the firing rate in response to the black step across the OFF cell population. It suggests that the biggest variability between cells exists during the immediate OFF response and during two distinct phases of the ON responses: one with latency of about 120-200ms (early ON response) and another with the latency of about 600-700ms (delayed ON response).

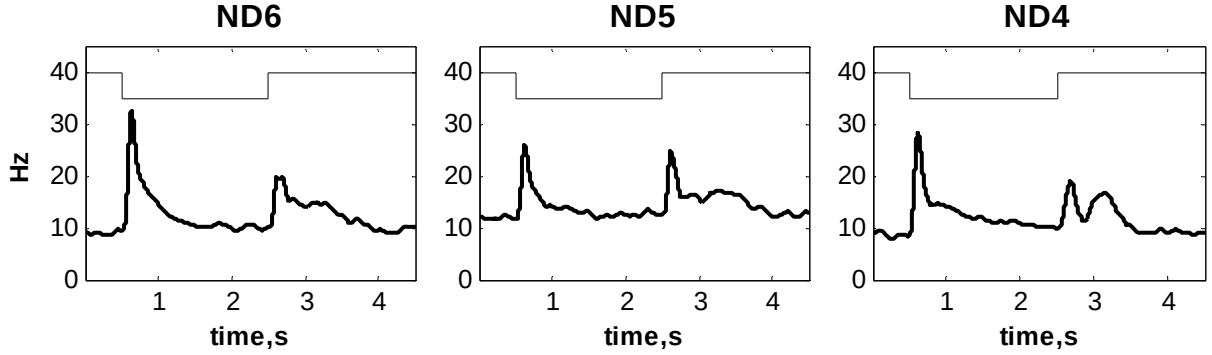

Fig. 5.4. Standard deviation of responses of OFF cells to black steps at ND6-ND4. Left: ND6 left, middle: ND5, right: ND4. It is maximal soon after the onset and offset of the stimulus. At stimulus offset, it has two distinct peaks.

It was surprising that OFF cells display such a rich diversity in their responses to light increments. In fact, the ON responses of the OFF cells are affected more by luminance changes than the OFF responses. I thus characterized the two distinct ON responses, "early" and "delayed", to investigate the effect of luminance on OFF ganglion cells. Indeed, different cells may have no ON response (*Fig. 5.5* left panel), only early (*Fig. 5.5* middle panel), only delayed (*Fig. 5.5* right panel), or

even both of them, which allows for qualitative classification. The luminance effect on OFF responses of OFF ganglion cells will be analyzed later.

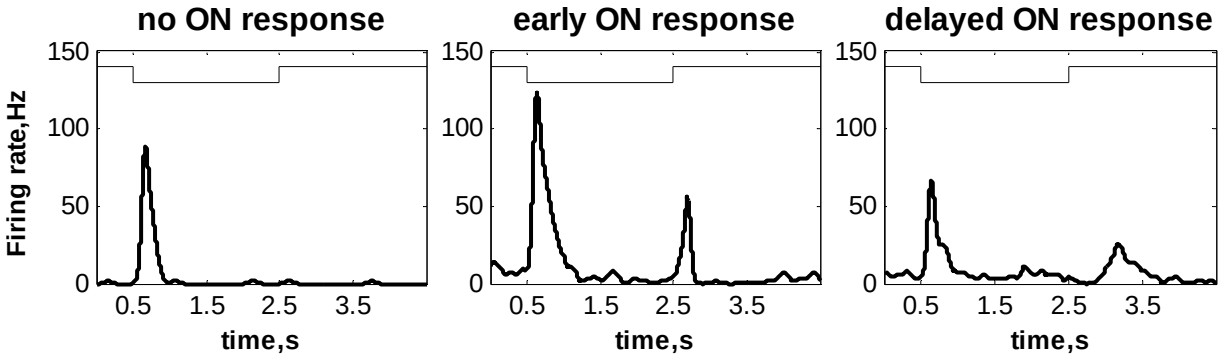

Fig. 5.5. Variability of ON responses of OFF ganglion cells. Responses of three individual OFF cells to a black step at ND6 are shown. Left, cell display no ON response. Middle, cell has an early ON response. Right, cell has a delayed ON response.

Thus, every OFF cell was first categorized according to the presence or absence of early and delayed ON responses (regardless of the amplitude) in response to the light increment after a black step at each of the three brightness levels ND6, 5, and 4. For the moment, I ignore responses to the white step; response asymmetries to black and white steps will be discussed later (section 5.2.2.5). Cells were then placed into groups based on consistent patterns in their ON response structure across light levels.

Fig. 5.6 shows the responses of the 6 groups containing most cells (62% of OFF cells). Each panel shows the averaged responses of all group members (baseline subtracted). The largest group (20% of recorded OFF cells) had no ON responses at ND6-ND4. They behaved as pure OFF cells, and their response pattern to the step stimulus was modulated by light adaptation only quantitatively. The second group (8% of cells) also maintained the same response pattern under all brightness levels. They consistently had early ON responses, and thus behaved as typical ON-OFF cells. Groups 3 and 4 resemble groups 1 and 2, with the addition that at ND5 (mesopic), they also demonstrate delayed ON responses, i.e. they qualitatively change their response characteristics in the mesopic regime. Group 5 represents cells with delayed ON response in scotopic and mesopic conditions; it disappears in photopic conditions. The most interesting case is represented by the cells in group 6: they have no ON responses in the scotopic regime, delayed ON response in the mesopic regime, and early ON response in the photopic regime. The example cell shown earlier in *Fig. 5.2* belongs to this group. Other cells formed different groups, but each group contained less than 5% of recorded OFF cells, and I omit them here.

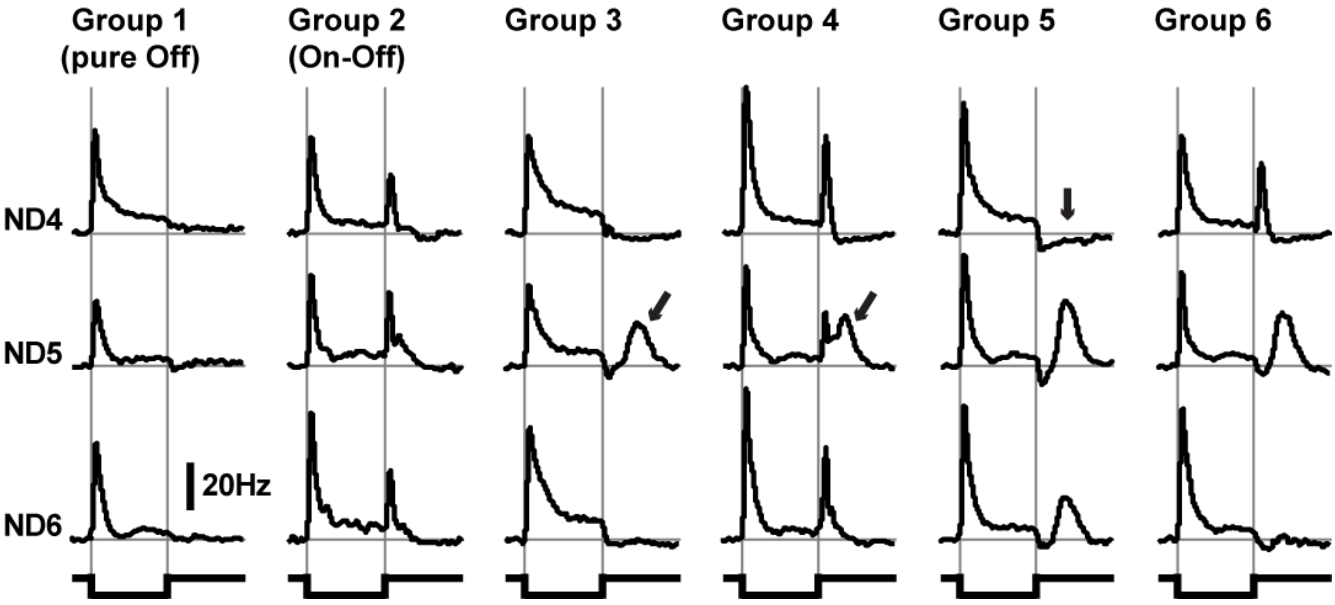

Fig. 5.6. Average response to black steps of 6 groups of OFF cells with consistent ON responses patterns at ND6, ND5, and ND4.

In summary, only in 28% of recorded OFF cells (pure OFF and pure ON-OFF cells), the response pattern to light increments (ON responses) was modulated only quantitatively by light adaptation. ON Responses of more than 70% of OFF cells, however, qualitatively depended on the light level (34% belong to groups 3 to 6 shown in *Fig. 5.6*; 36% belonged to less populated groups not shown).

Despite the diverse effects of the ambient light level on the responses of OFF cells, there are certain tendencies. Early ON responses were encountered in about 35% of cells at each brightness level (*Fig. 5.7*, left group). The overall fraction of OFF cells with early ON response was therefore weakly affected by luminance. More than half of those cells (19% of total) consistently had it at all three brightness levels. Together with cells which had no early ON response at any light level ND6-ND4 (therefore also not modulated by luminance), they make up 65% of all OFF cells (*Fig. 5.8*). Delayed ON responses (*Fig. 5.7* right group), on the contrary, were very light-level dependent: present in 29% of cells at ND6, 58% of cells at ND5 (2-fold increase), and only in 15% of cells at ND4 (almost 4-fold decline). Only ~3% of OFF cells had consistent delayed ON responses at all three light levels, and in 30% it was consistently absent (i.e. together 33% of cells in which delayed ON response was not modulated by luminance between ND6 and ND4, see *Fig. 5.8*). More than two thirds of OFF cells therefore showed some sort of luminance dependent modulation of the delayed ON response.

Taken together, early and delayed ON responses appear to be regulated by luminance independently. This observation, together with examples of cells with various combinations of early

and delayed ON responses (e.g. *Fig. 5.15*), suggests that the responses are generated by independent mechanisms.

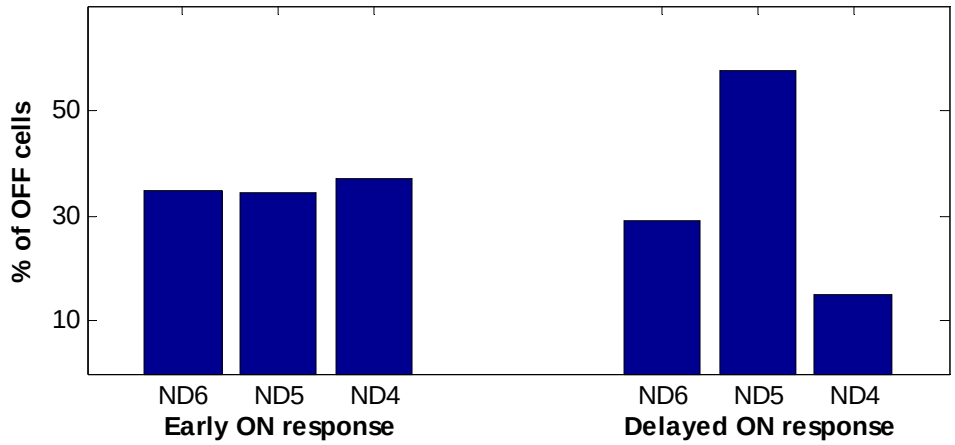

Fig. 5.7. Percentage of OFF cells with early and delayed ON response (independently of each other) at ND6, ND5, and ND4. Early ON responses are encountered in ~35% of OFF cells at each light level, whereas occurrence of delayed ON responses is more luminance-dependent.

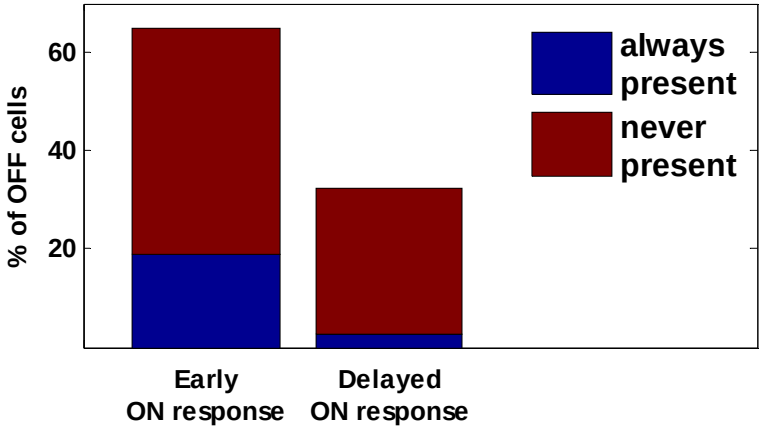

Fig. 5.8. Stability of early and delayed ON responses in OFF cells at ND6, ND5, and ND4. Early ON response is more stable: 65% of OFF cells either have early ON response at all three light levels or do not have it at any of them, versus 33% for delayed ON response.

5.2.2.3. OFF response of OFF cells.

OFF responses of OFF cells showed less qualitative diversity on the population level (*Fig. 5.3*). I checked whether differences are more pronounced among individual cells. *Fig. 5.9* shows the peak of the firing rate after the onset of the black step versus its latency (spontaneous firing rate was subtracted prior the calculation). Amplitudes were distributed rather continuously at every light level, suggesting only quantitative differences. Latencies were also densely grouped at every light level. *Fig. 5.10* shows

the mean peak amplitude and latency of OFF responses across OFF cells at different light level. It suggests that the luminance effect on these parameters of the OFF responses is rather weak.

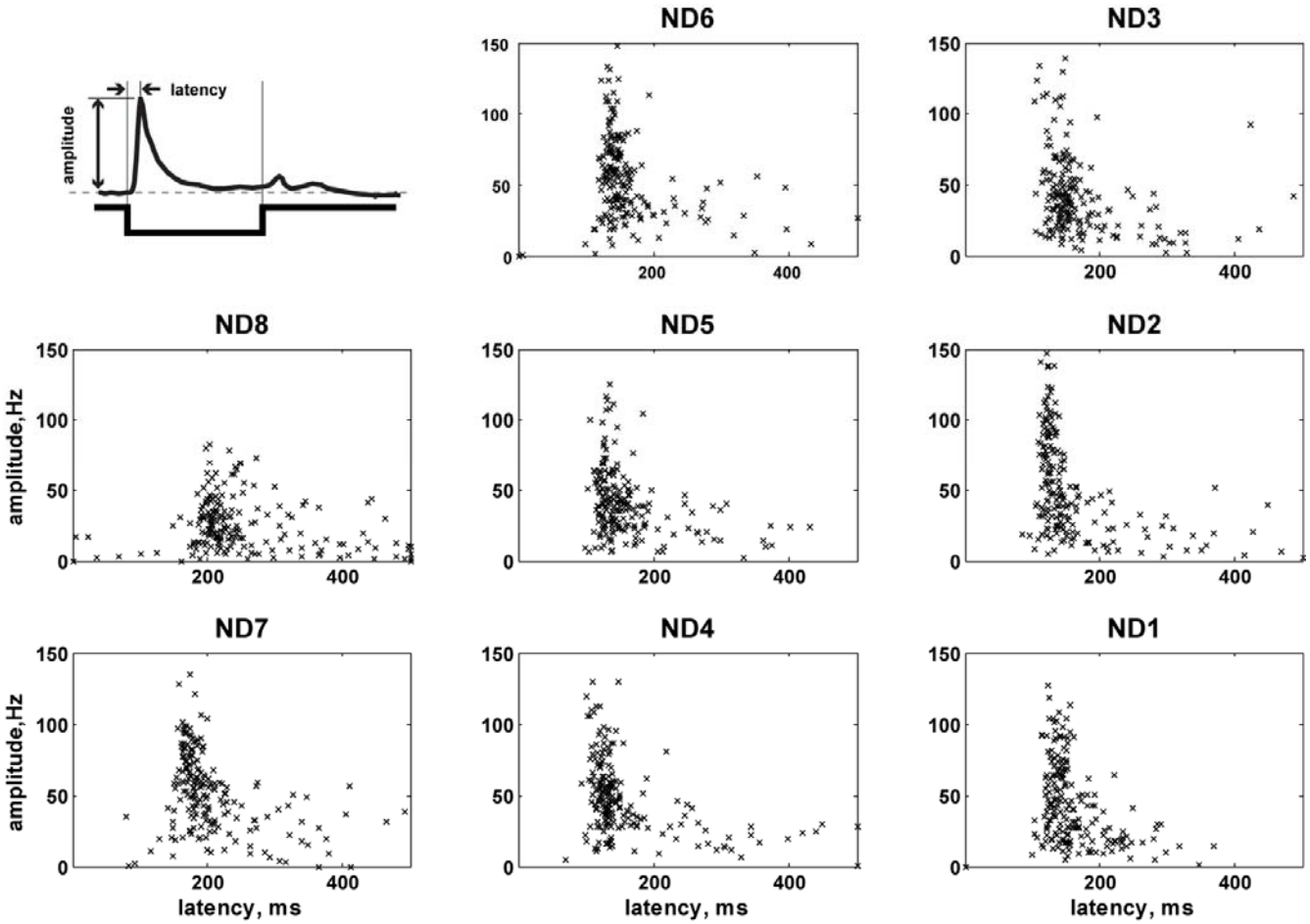

Fig. 5.9. Amplitude versus latency of the peak OFF response of OFF ganglion cells at different light levels. Mean background firing rate was subtracted prior the calculations.

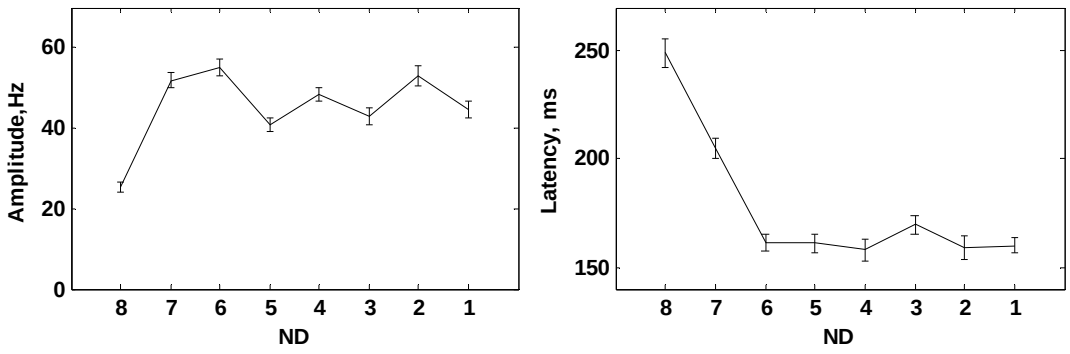

Fig. 5.10. Amplitude and latency of the peak OFF response of OFF ganglion cells to the black step at different light levels. Mean ± s.e.m.

Another parameter often used to characterize ganglion cell responses is transiency. There is no commonly accepted approach to determine transiency (e.g. it may be calculated as the time point when certain fraction of all spikes during the stimulus have occurred or as the time point when the firing rate declines by 1/e after the peak). Manual inspection of the responses suggested that they smoothly decline after the peak. I thus defined the transiency index of OFF responses as the time it took the cell to return to the mean baseline activity level plus one standard deviation (i.e. to "almost" return to baseline). The distribution of transiency indices across NDs is shown in *Fig. 5.11*. At each light level, there are two groups of cells: relatively transient (returning to the baseline in about 500-700ms) and sustained (not returning to the baseline during 2s). Interestingly, the fraction of sustained cells was the largest at ND4, ND2, and ND1 (about 40%, vs less than 30% at ND7-ND5). These light levels are predominantly photopic, and it is surprising that they favor sustained responses, for several reasons. First, rods themselves have bigger integration time and more sluggish responses than cones. Furthermore, RGCs are tuned to higher frequencies in the photopic regime [19, 23], so that I would have expected that responses tend to be more transient. Second, I used a full-field stimulus, which activates both periphery and center of the receptive field. Periphery has antagonistic response to the center, but is more sluggish, so that full-field stimulation diminishes both amplitude and duration of the late response component [22, 25, 78]. However, my findings contradict this expectation.

To further quantify the effect of light adaptation on the transiency of OFF responses, I tested how many cells stably remain sustained from ND6 to ND4 (i.e., don't return to baseline during the 2-second black step, which is a rather strict criterion for stustainedness), and how many cells stably remain transient. The stably sustained cells comprised 13.5% of all OFF cells, and 48% were always transient. Less than 40% switched between the two categories. These numbers suggest that this property of cells is weakly modulated by luminance.

Overall, this analysis confirmed the impression from population average: OFF responses of OFF cells are less diverse and more stable than ON responses.

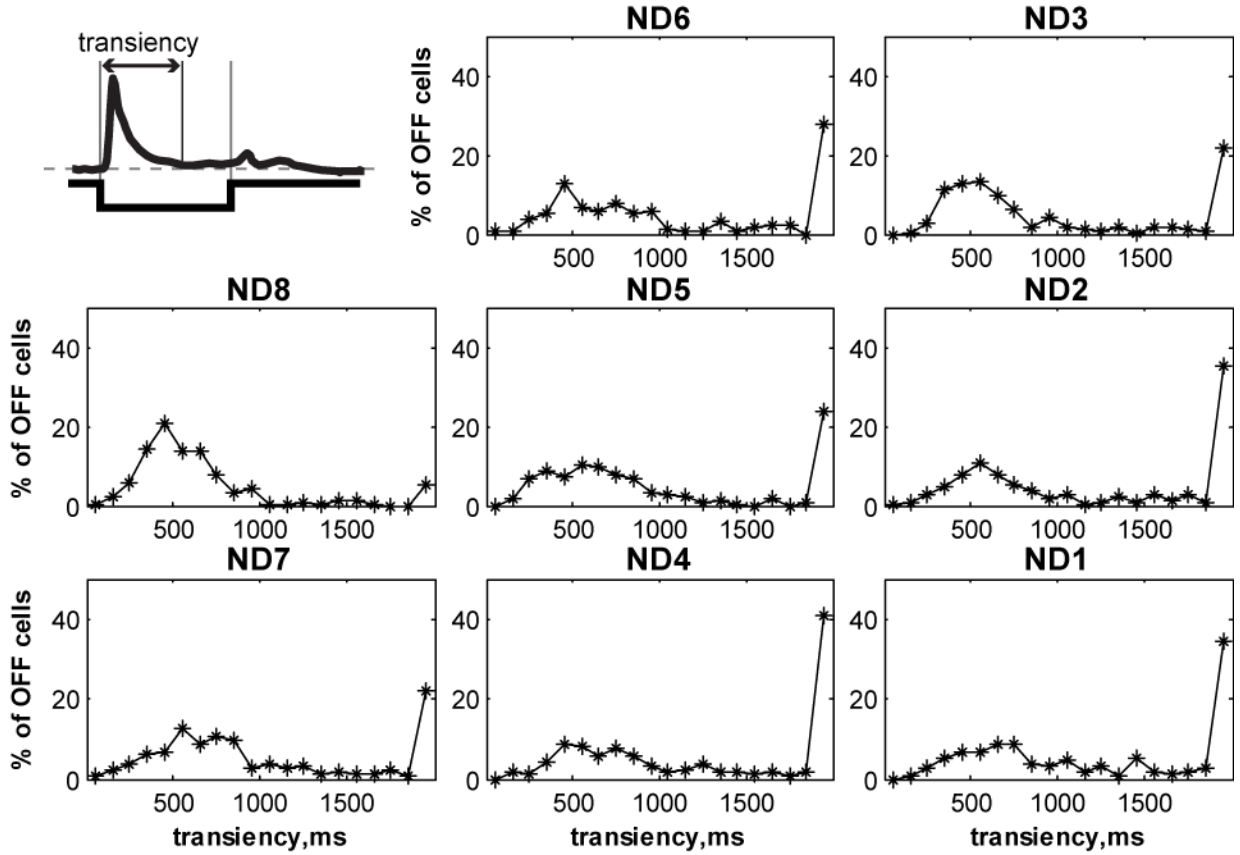

Fig. 5.11. Distribution of transiency index of OFF response of OFF ganglion cells to the black step. At every light level, a large fraction of OFF cells has index of 2000ms (sustained cells). Note that the largest fraction of sustained cells is at ND4 (photopic range).

5.2.2.4. Asymmetry of responses to black and white steps: OFF responses.

The previous section only considered responses to the black step stimulus. White and black steps used in my experiments had equal absolute contrast (+0.66 and -0.66, see Methods) and differed only in sign. Therefore, the offset of the white step was equivalent to the onset of the black step in terms of luminance change, and vice versa. However, the responses to these corresponding parts of the black and white stimuli were not symmetrical.

As described above, luminance modifies OFF responses mostly quantitatively, whereas ON responses are modified qualitatively. The asymmetries between OFF responses to black and white steps were as well rather quantitative than qualitative. To quantify this, I calculated the correlation between the corresponding responses. *Fig. 5.12* shows the distribution of correlation coefficients between OFF responses to black and white steps (blue), and ON responses to these stimuli (red). In the vast majority

of OFF cells, OFF responses are well correlated, whereas ON responses are sometimes even negatively correlated, especially at ND5 and ND4.

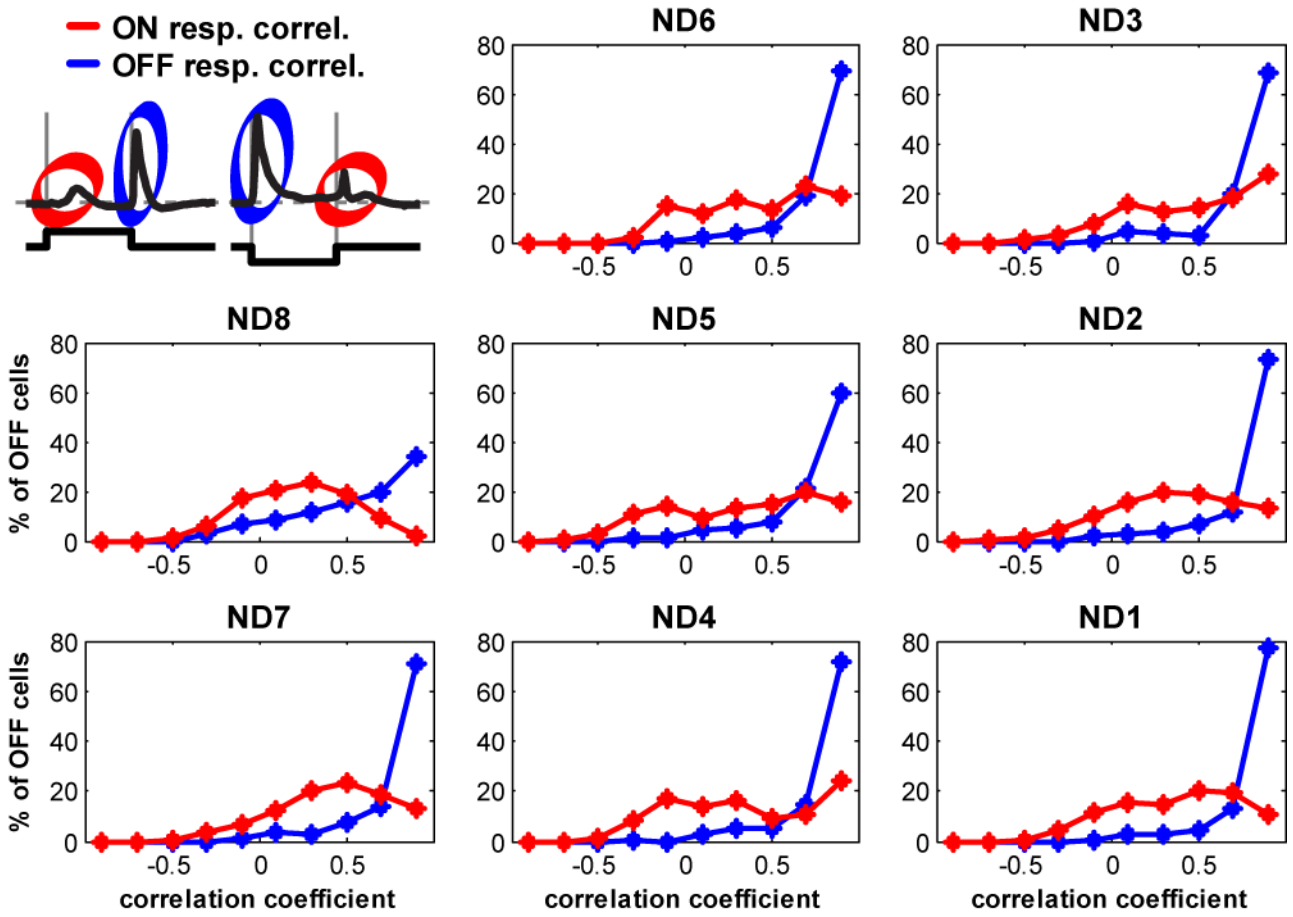

Fig. 5.12. Distribution of correlation coefficients between responses of OFF ganglion cells to light decrements in black and white steps (blue) and to light increments in black and white steps (red) at different light levels. Responses to light increments (ON responses) are notably less correlated and some of them are even negatively correlated.

In the analysis of OFF response asymmetries I concentrated on their quantitative parameters (amplitude, latency, and transiency), as described in the previous section.

To compare the amplitude of the OFF response to black and white steps, I calculated their ratio for every cell (peak firing rate, white step/black step). Values more than 1 would mean that the response is larger at the end of the white step, less than 1 that the response is larger during the black step. The distribution of the amplitude ratio across cells was normal at every ND (Kolmogorov-Smirnov test, 5% significance level), and the mean values at each ND are shown in *Fig. 5.13* A. Asterisks mark light levels where the distribution was significantly different from unity (t-test, $p<0.05$). Interestingly, at ND6 the mean ratio was significantly more than 1 (white step offset evoked bigger

responses), and at ND5 it was significantly less than 1 (black step onset evoked bigger responses). At ND7 and ND4 the cells on average responded equally well to both stimuli.

To compare the latency of the responses to black and white steps, I took the difference of time-to-peak of the responses, $Latency_{white\ step} - Latency_{black\ step}$. Negative values indicate that the OFF response is faster after white steps. The distribution of latency differences was also normal at each ND (Kolmogorov-Smirnov test, 5% significance level), and the mean (shown in *Fig. 5.13* B) at each ND was significantly less than zero (t-test, $p<0.05$). It indicates that cells reached the peak amplitude after the white step offset earlier than after the black step onset.

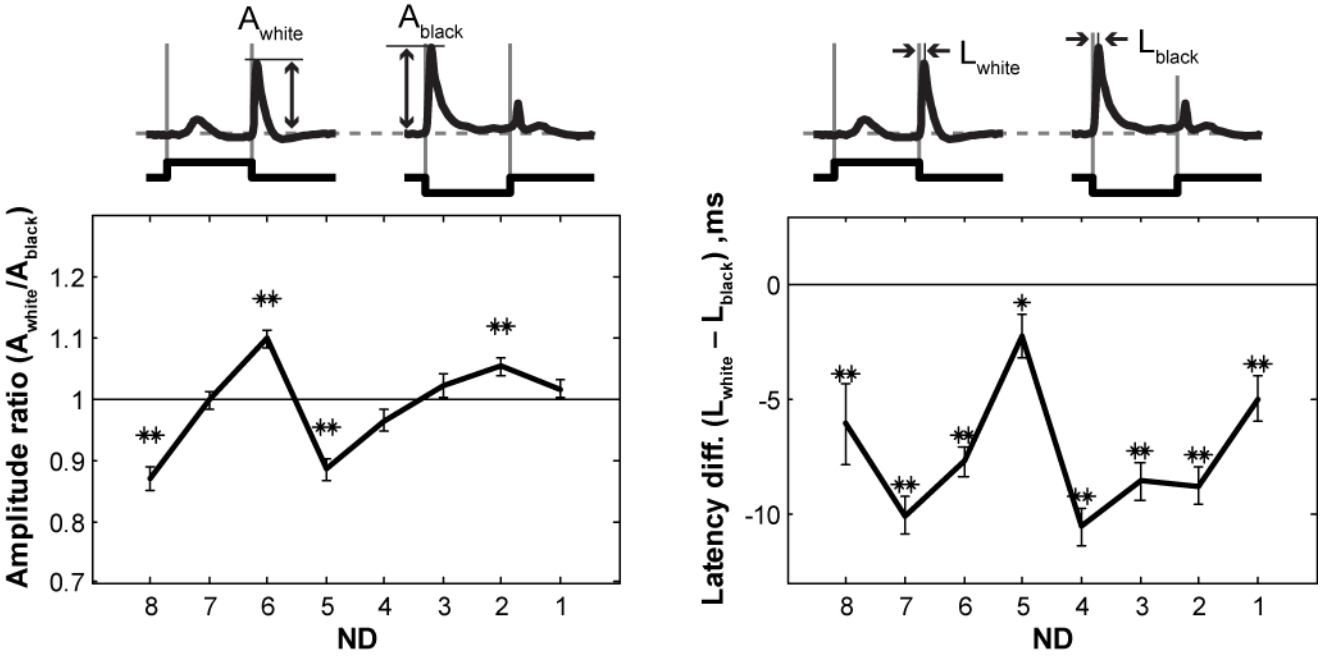

Fig. 5.13. Amplitude ratio and latency difference of OFF responses to black and white steps of OFF cells at different light levels. Mean ± s.e.m. Asterisk marks values statistically different from '1' (amplitude) or '0' (latency), *: $p<0.05$; **: $p<0.001$ (t-test).

These results suggest that the luminance does have some effect on relative amplitudes of OFF responses to black and white steps. ND6 (high scotopic level), favors white steps offset, whereas ND5 (mesopic level) favors black step onset. On the other hand, the influence of luminance on the asymmetry of latency is only quantitative: at every light level, the OFF response reaches its peak amplitude faster after a white step. This difference is smallest at ND5; however, it does not appear to be related to amplitude ratio change towards black steps at ND5: the correlation coefficient between latency difference and amplitude ratio was 0.17 at ND5 and less (in absolute values) at all other NDs except ND8 (not shown).

Transiency of OFF responses was calculated as described in the previous section (the time it took the cell to return to the mean baseline activity level plus one standard deviation). *Fig. 5.3* suggests more sustained OFF responses during the black step. Indeed, many cells did not return at all to their baseline firing rate during the 2s of the black step (*Fig. 5.14* x-axis, see also *Fig. 5.11*). After the end of the white step, it took most cells only about 500ms to return to the firing rate they had before the beginning of the white step at most brightness levels (*Fig. 5.14* y-axis). This asymmetry is revealed in the scatterplot as most values lie below the unity line (diagonal black lines): the vast majority of the cells had more sustained OFF responses during the black step than after the white step ($p<0.001$ at all light levels).

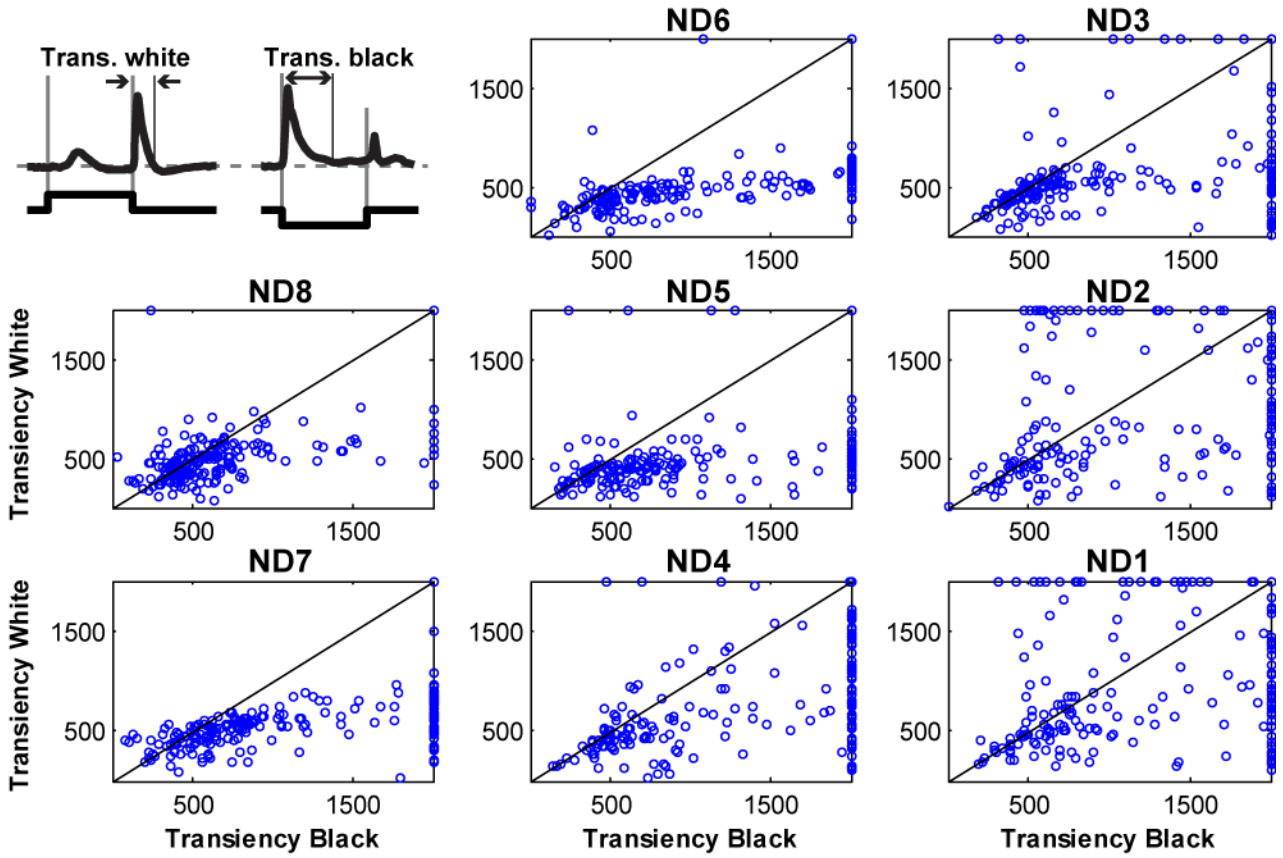

Fig. 5.14. Transiency index of OFF responses of OFF ganglion cells to light decrements in black (x-axis) and white (y-axis) steps. Transiency was calculated as difference between the time point of light decrement and time of the firing rate return to the baseline activity level after the response peak. Black steps lead to more sustained responses in OFF cells: the indices are distributed below the diagonal unity line.

5.2.2.5. Asymmetry in responses to black and white steps: ON responses.

Fig. 5.15 shows an example of an OFF cell with highly asymmetrical ON responses from ND6 to ND3. Notably, the structure of asymmetry is not constant: early and delayed ON responses change places, appear and disappear at different brightness levels, whereas OFF responses remain similar at all brightness levels.

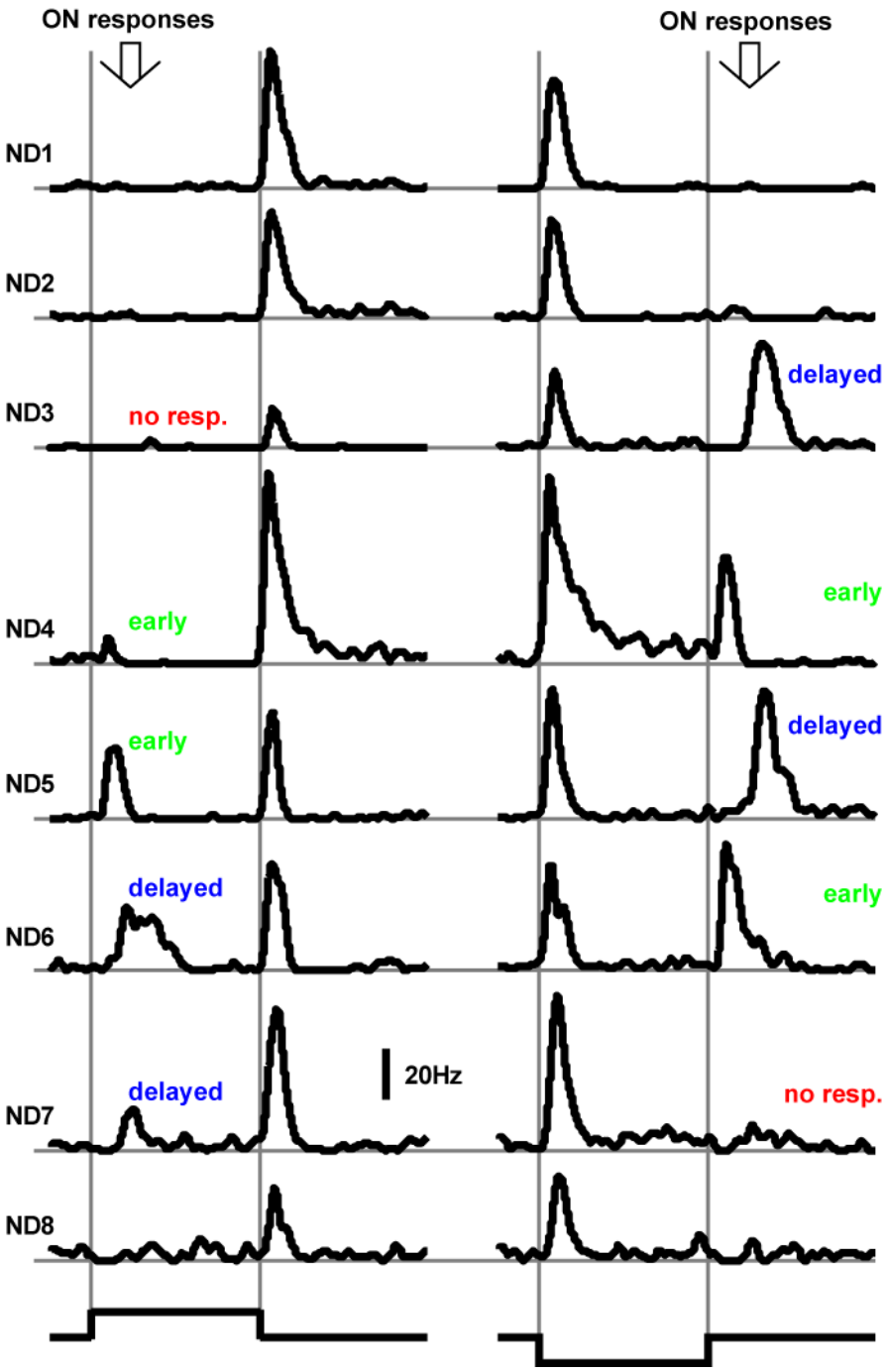

Fig. 5.15. Example of a cell with asymmetrical ON responses to light increments in black and white steps at ND6-ND3. Note that OFF responses to the two stimuli are very similar at every light level.

I categorized the ON responses to the white step onset the same way as described for the black step offset (early and delayed ON responses).

The amount of cells with at least one ON response type to white step onset is shown on *Fig. 5.16* (data for black steps, taken from *Fig. 5.7*, are shown for comparison). The dynamics of occurrences is very different for both response types. At ND5, both black and white steps evoke early ON responses

in ~35% of OFF cells (i.e., cells respond as ON-OFF). ND6 and ND4, however, ON-OFF responses were encountered in ~35% of cells to black steps, but only in ~20% to white steps. Delayed ON responses at ND6 to black steps were underrepresented compared to the white steps, whereas at ND5 and ND4, their occurrence in population was similar for both black and white steps.

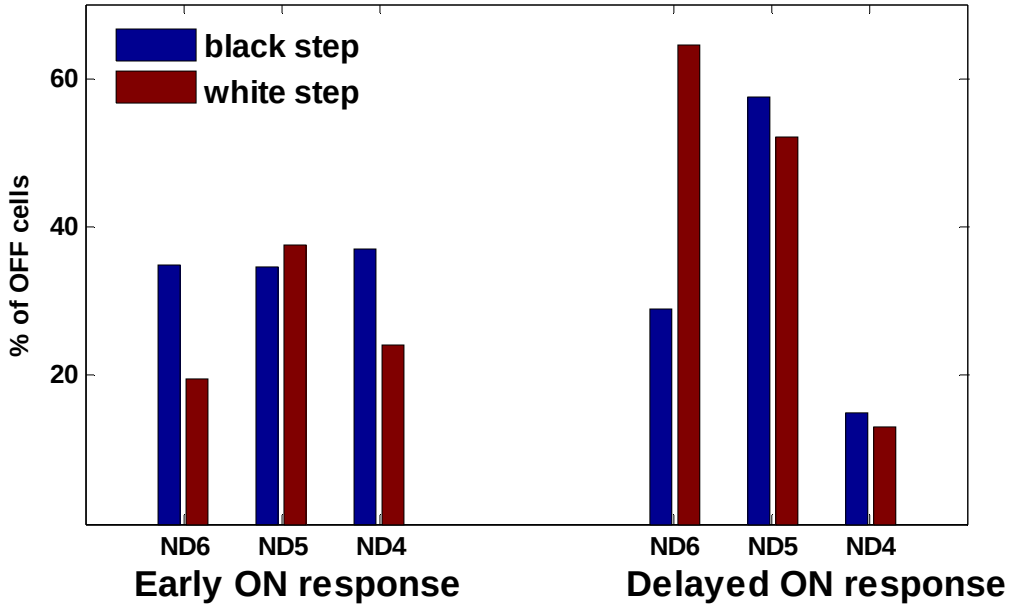

Fig. 5.16. Percentage of OFF cells with early (left group) and delayed (right group) ON responses to white (red bars) and black (blue bars) steps at ND6, ND5, and ND4. Note that luminance has distinct effect on generation of ON responses to black and white steps.

In some cells, ON responses to white steps at ND6-ND4 were stable (always present or always absent), whereas in others they depended on luminance. *Fig. 5.17* shows the fraction of cells with stable early and delayed ON responses to the white step, and to the back step for comparison (taken from *Fig. 5.8*). These results suggest asymmetrical regulation of ON responses to black and white steps by luminance. On average, in OFF ganglion cells, ON responses to white steps are more light-dependent than ON responses after black steps.

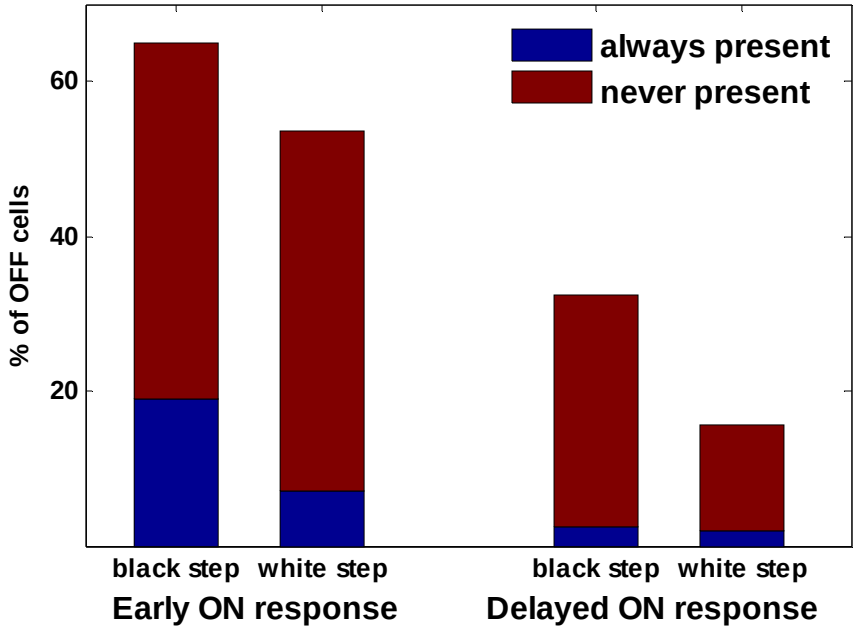

Fig. 5.17. Stability of early and delayed ON responses to black step and white step in OFF cells at ND6, ND5, and ND4. Overall, ON responses of each type to white steps are less stable.

As described above, most OFF ganglion cells changed their ON response pattern in a luminance-dependent manner. Quite often, I also encountered inconsistencies in the ON responses during white and after black steps. I next tested how many cells had consistent ON response patterns to black and white steps at least at ND6 to ND4, regardless of luminance-induced changes of the pattern. An example could be a cell with early ON responses to both black and white steps at ND6 to ND4, and no delayed ON response (a consistently stable cell). Another example is the cell shown in *Fig. 5.18*. It has delayed ON responses to both black and white steps at ND6 and ND5, and no ON responses at ND4. Note that this cell also has consistent ON responses at ND8, ND7, and ND1, but not at ND2. ND3 is ambiguous.

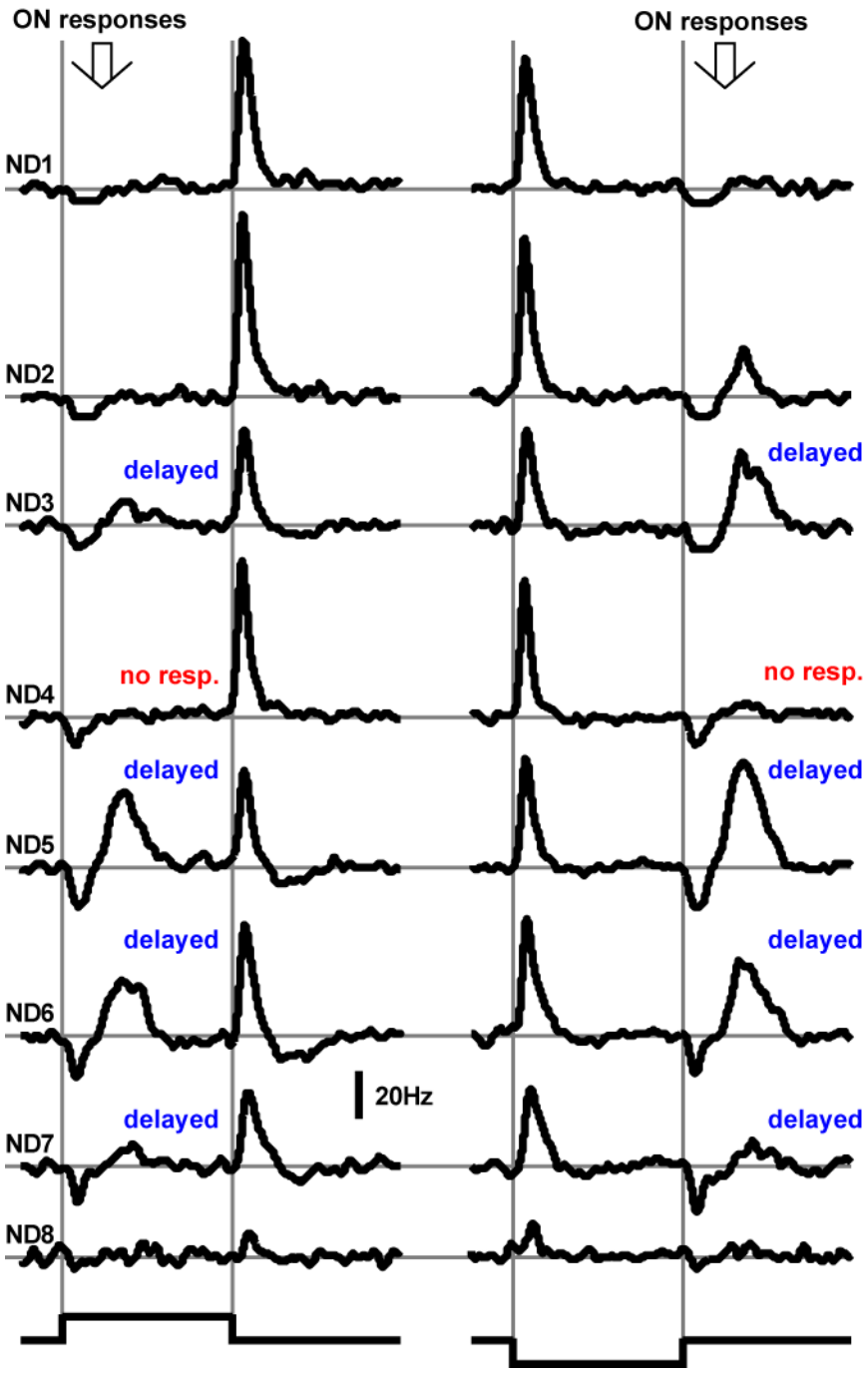

Fig. 5.18. An example cell with consistent changes of ON response pattern to black and white steps at ND7 to ND4. Note that OFF responses to black and white steps are similar at each light level.

Surprisingly, only 16.5% of OFF ganglion cells had consistent ON response patterns to black and white steps even when only three light levels - ND6 to ND4 – were considered. 83.5% of cells had qualitative asymmetries of ON responses in at least one of the three considered light levels. This also means that these 83.5% of cells had an ON response at least at one of the three light levels. Further

investigation confirmed that only 5 OFF cells out of 200 (2.5%) were found to consistently have no ON response at ND6-ND4. *Fig. 5.19* shows an example of such cell. Note, however, that after the offset of the black step at ND5 the cell has a small but apparent increase of the firing rate after the initial inhibition. This increase was not automatically detected because of its small amplitude and relatively high noise of the cell, but manually, it can be classified as a delayed ON response (judging by its latency). The same held true for the other 4 cells in which no ON response was detected.

In summary, ON responses were observed in every single OFF ganglion cell in response to full field steps, even if it was present at only one light level, only at the beginning of a white step or at the end of a black step, and only of the “early” or “delayed” type.

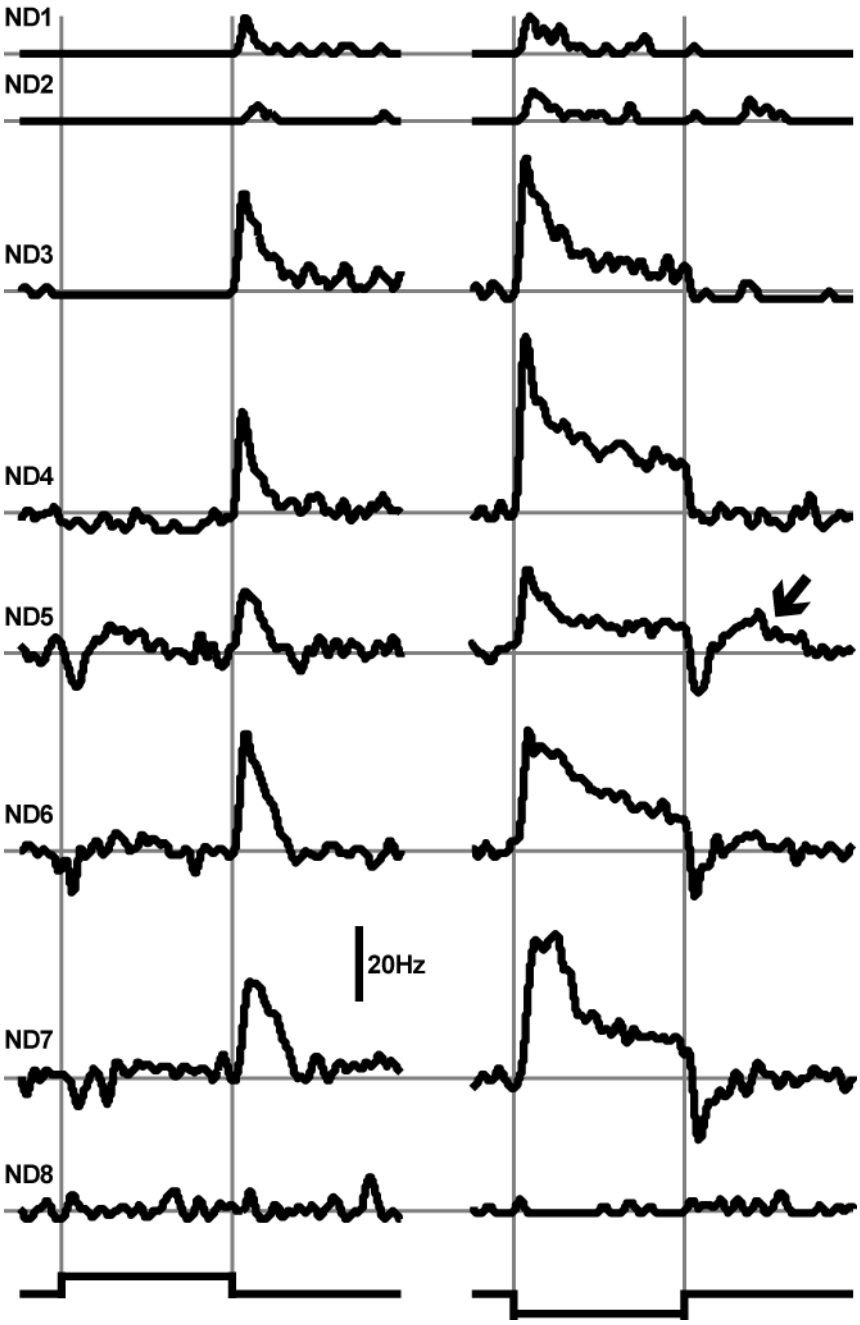

Fig. 5.19. An OFF cell which had no automatically identified ON response at ND6 to ND4. However, the black step offset at ND5 evokes an inhibition which is followed by relatively small but obvious increase of the firing rate with latency typical for delayed ON responses.

5.2.3. Cells with ON-like linear filters.

5.2.3.1. Mean population response.

Like for the OFF cells, I first analyzed the mean response of all ON cells to black and white steps of light (*Fig. 5.20*).

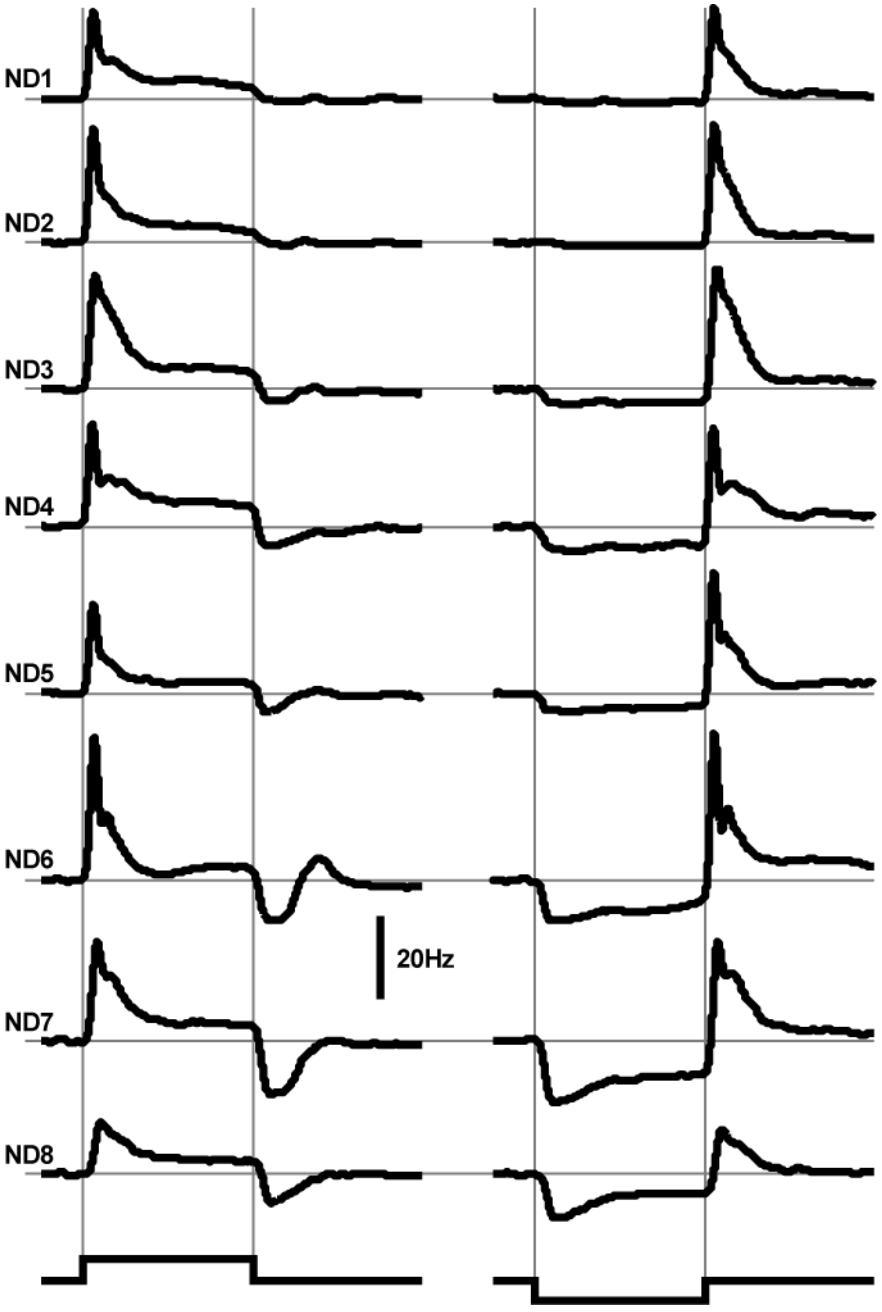

Fig. 5.20. Average responses to full-field white and black steps in ON cell population (n=249, from 15 experiments) at different light levels. Spontaneous firing rate was subtracted for each cell individually at each light level.

ON cells, like OFF cells, demonstrate on average stable, sustained, high amplitude responses to the preferred stimulus (light increment). However, there are at least two very distinct features compared to OFF cells:

- ON responses do not return to the baseline activity level during 2s after both black and white steps.

- there is no positive OFF response to light decrements. Instead, cells are strongly and sustainedly inhibited. The only exception is a delayed OFF response to the white step at ND6.

In other words, there is less variability across brightness levels (as compared to the OFF cells), and also less qualitative asymmetry between response patterns to white and black steps. To estimate which parts of the response are most variable, I calculated the standard deviation of the responses to white steps at ND6, ND5, and ND4. The results are plotted in *Fig. 5.21*. Unlike OFF cells, ON cells have the smallest response variance across population right after the offset of the preferred stimulus (end of the white step), representing consistent suppression. On the other hand, the variance of the responses to the preferred stimulus is not as smooth as among OFF cells (compare to *Fig. 5.4*).

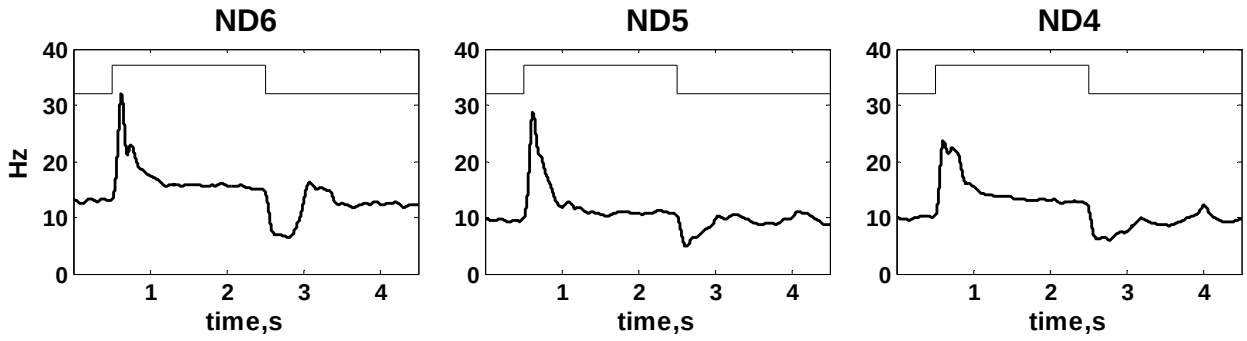

Fig. 5.21. Standard deviation of responses of ON cells to white steps at ND6 (left), ND5 (middle), and ND4 (right). It is maximal soon after the onset of the stimulus, and minimal at the offset (compare with Fig. 5.4 depicting the same parameter for OFF cells).

Such variance indicates that the ON response is diverse between subtypes of ON cells. Manual inspection of individual cell responses showed that there are three major types of ON responses among ON cells, represented by the 3 example cells shown in *Fig. 5.22*. The cell plotted in the left panel has a classical transient response; the cell plotted in the middle panel is classically sustained. The cell plotted in the right panel also has a sustained response, but it has a noticeable gap between the initial rise of the

firing rate and the sustained part. I will refer to these three response types as ‘transient’, ‘sustained’, and ‘gap’ response.

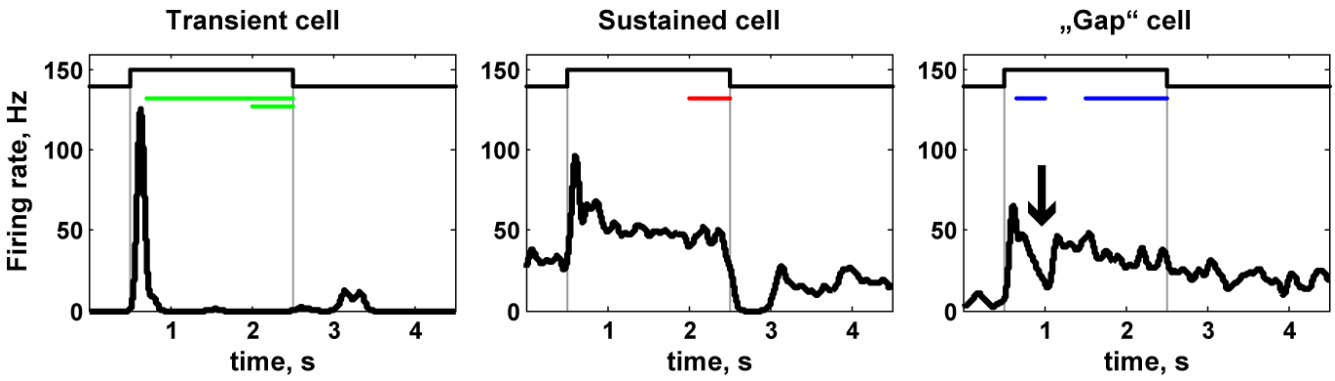

Fig. 5.22. Example responses of three individual ON cells to the white step at ND6. Note that the cell in the left panel is pure transient, the cells in the middle panel is pure sustained, and cell in the right panel decreases its firing rate after initial response, but then returns to the sustained type of response. The response strengths during the time periods indicated by colored bars were used for automatic classification of the response type.

I tested if these three response types are a consistent property of ON cells or if they are affected by luminance. Like for OFF cells in the previous sections, I begin by considering only the responses to the preferred stimulus, i.e. to the white step. Asymmetries to black and white steps will be discussed later (section 5.2.3.3). To classify cells at every brightness level, I first subtracted the mean spontaneous firing rate and then used the following algorithm (all timings are given relative to the onset of the white step, see also *Fig. 5.22*):

1) transient cells: both mean firing rate in 200-2000ms and in 1500-2000ms did not exceed 1 Hz (the second check excluded cells with a deep gap and further recovery of the response).

2) gap cells: the mean firing rate in 150-500ms was less then the mean firing rate in 1000-2000ms, and the cell did not belong to the transient type.

3) sustained cells: mean firing rate in 1500-2000ms was higher than one standard deviation of the spontaneous firing rate and higher than 1Hz, and the cell did not belong to the gap type.

These criteria were manually chosen. Some variations of the cut-off timings led to different classification of few cells, but did not change the overall results described below. Some cells (12 to 20% at ND6 to ND1), however, were not captured by these criteria. For these cells, I calculated the correlation coefficient between their responses and the mean response of the transient, sustained, and gap groups of cells. If correlation with some response type was high enough (>0.7), the cell was marked as belonging to this type. This method allowed classifying about 95% of all ON cells at ND6-

ND1. The mean responses of all cells falling into the 3 groups are shown in *Fig. 5.23*. The percentage of cells of each group at different light levels is shown in *Fig. 5.24*.

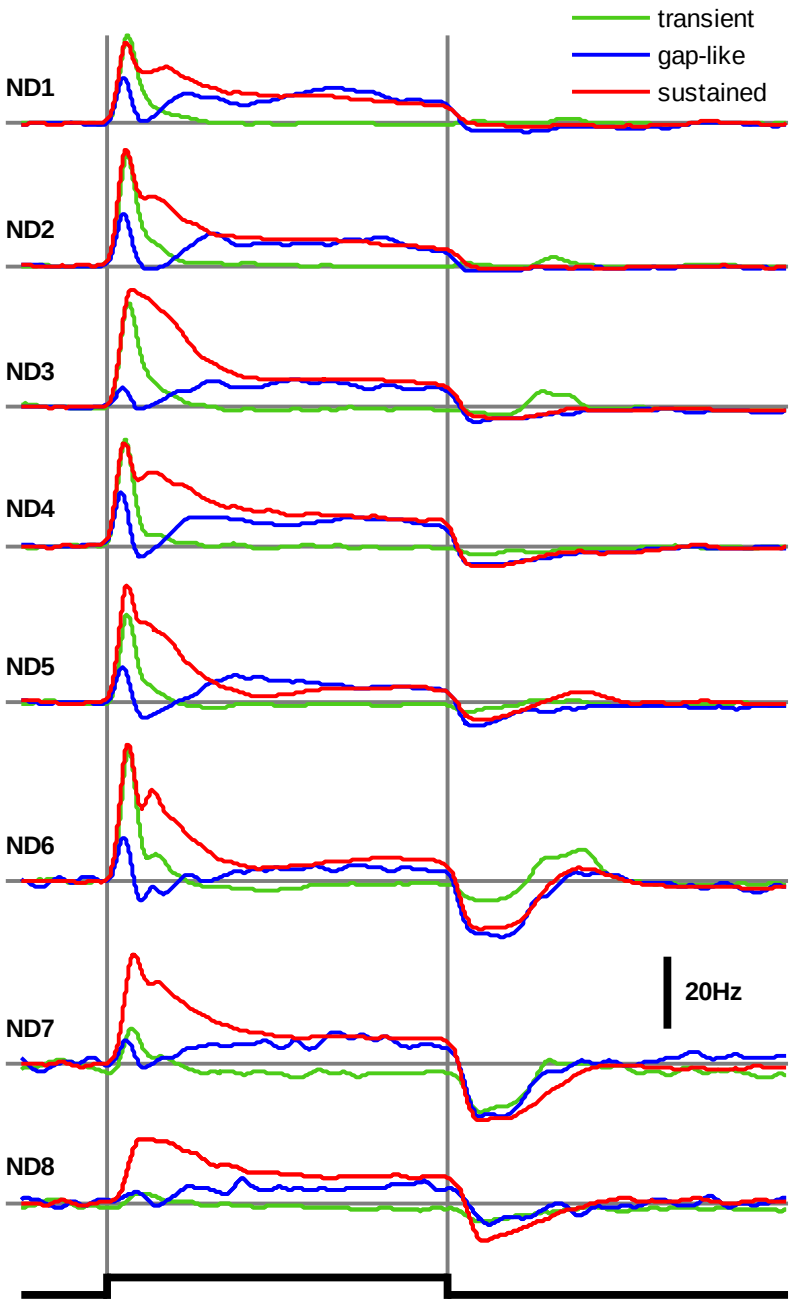

Fig. 5.23. Averaged responses of ON cells with transient, gap-like, and sustained responses to white steps at different light levels. Gap-like and sustained cell have very similar late phases of ON responses. OFF responses to the offset of the stimulus are similar in all cell groups, except ND6 and ND3, where transient cells have an overshoot after the initial activity suppression.

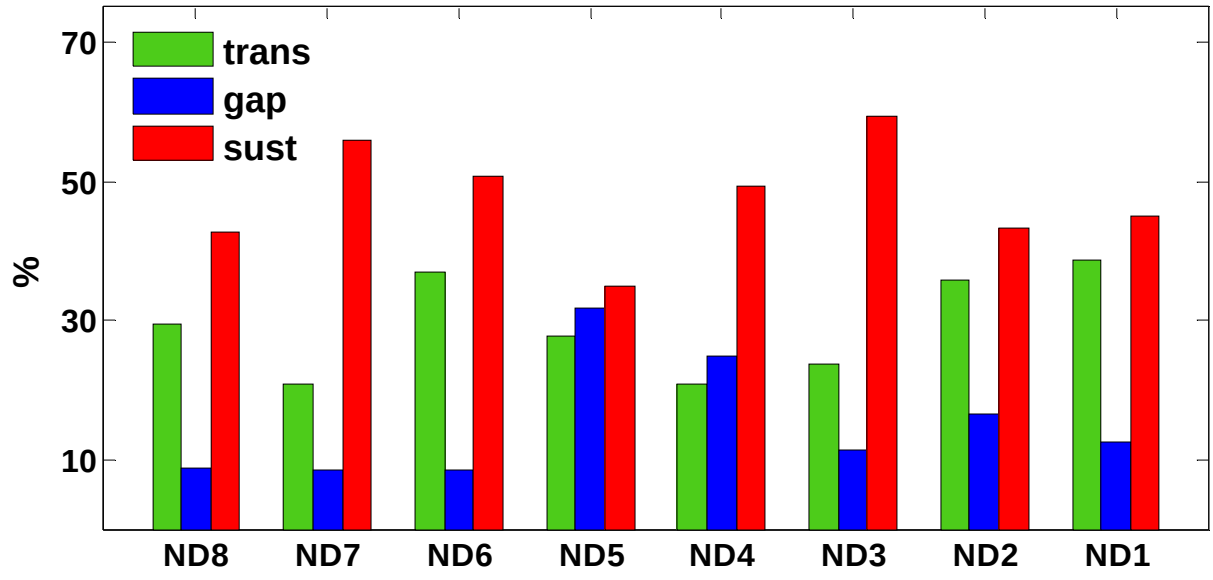

Fig. 5.24. Percentage of ON cells with transient, gap-like, and sustained responses to white steps at different light levels. At ND7 and ND6, most cells are sustained, whereas at ND5, all three cell types are similarly represented. At ND4, sustained cells strongly dominate again.

These two figures allow making interesting observations. First, three cell groups comprise different fractions of ON cells at different light levels, suggesting luminance-dependent regulation. Second, sustained and gap cells are very similar during the late phases of the responses (1000-2000ms after the white step onset). In other words, "gap cells" look like sustained cells with additional short (but strong) inhibition soon after the response peak. Moreover, gap and sustained cells had similar mean spontaneous firing rate ($p>0.05$ for all NDs except ND5, see *Fig. 5.25*), whereas transient cells had significantly lower spontaneous firing rate ($p<0.05$). Third, despite very different dynamics of ON responses in the three groups of cells, the OFF responses differ mainly in amplitude at all brightness levels except ND6 and ND3, where transient cells had a delayed OFF response.

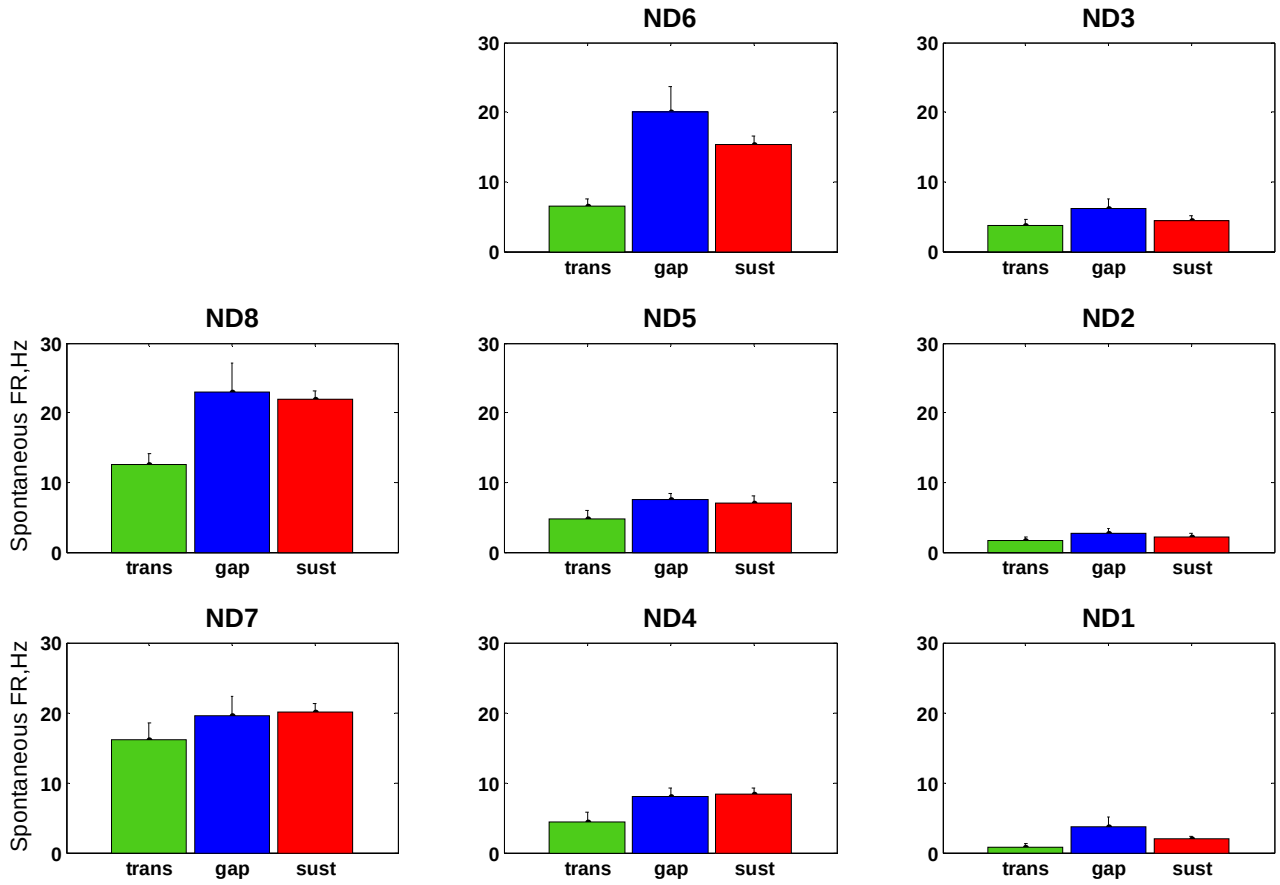

Fig. 5.25. Mean + s.e.m.of spontaneous firing rate by ON response type. Difference is not statistically significant between sustained and gap cells at all NDs except ND5 (p=0.03), whereas it is significant between transient and sustained cells at all NDs and transient and gap cells at all light levels but ND7 (p=0.07).

Indeed, in the scotopic range – up to ND6 – transient and sustained cells are predominant, and there hardly is any "gap" behavior. In contrast, all three response types are roughly equally represented in the mesopic range (ND5). In the photopic range, half of the cells have sustained response, whereas gap-like and transient responses are found in about a quarter of cells each.

In general, increase of luminance favors prolonged responses. If one would only consider the late phase of the response, gap cells would be categorized as sustained. Then sustained cells would amount to 59% at ND6, 67% at ND5, and 74% at ND4. Decrease of transient cell number and increase of sustained cell number in photopic conditions (ND4) resembles the tendency I observed in the OFF cells (*Fig. 5.11*). My observations of ON cells also contradict the expectation of more transient responses under bright light.

I counted cells which maintained the same response type at ND6, ND5, and ND4 (*Fig. 5.26*). Almost one fifth – 19% - of all ON cells had consistent sustained responses at all three NDs. About

10% had consistent transient responses, and only 2.5% had consistent gap-like responses. Most cells therefore (68%) should have migrated between response types at least once. I found 26 distinct cell groups according to their changing response pattern across ND6-ND4. However, in most of these groups (altogether 47% of all ON cells) the migration was between gap-like and sustained responses. Together with ~21% of always sustained or always gap-like cells, those cells make 68% - and represent cells which have consistent elevated late response at all 3 light levels. This observation suggests that the inhibitory activity causing the gap is more dependent on ambient luminance than the sustained excitatory input.

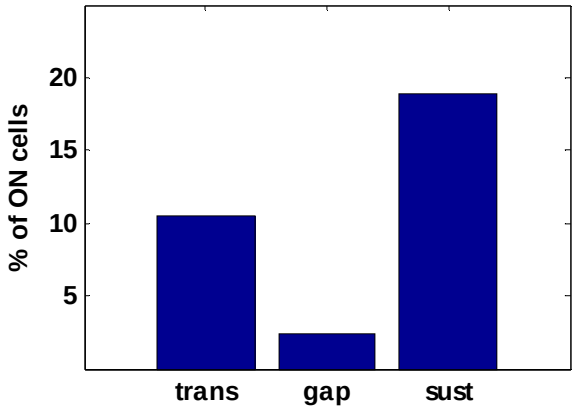

Fig. 5.26. Percentage of ON cells maintaining transient (left), gap-like (middle), or sustained (right) response to white steps at all three light levels ND6 to ND4.

The complex shape of the ON responses of gap cells is particularly interesting, because it sheds light on dynamical interaction of excitatory and inhibitory inputs in ON cells. Both sustained and gap cells receive excitatory input leading to similar initial and late responses at many brightness levels. In sustained cells, inhibitory input is weak or absent. In gap cells, this input is obviously stronger. It pulls the response down, in most cases before it reaches the full amplitude. The relative strength of inhibitory input varies from cell to cell and across NDs (see *Fig. 5.27*), maybe optimizing the amount of cells with certain characteristics to actual visual tasks at certain luminance level.

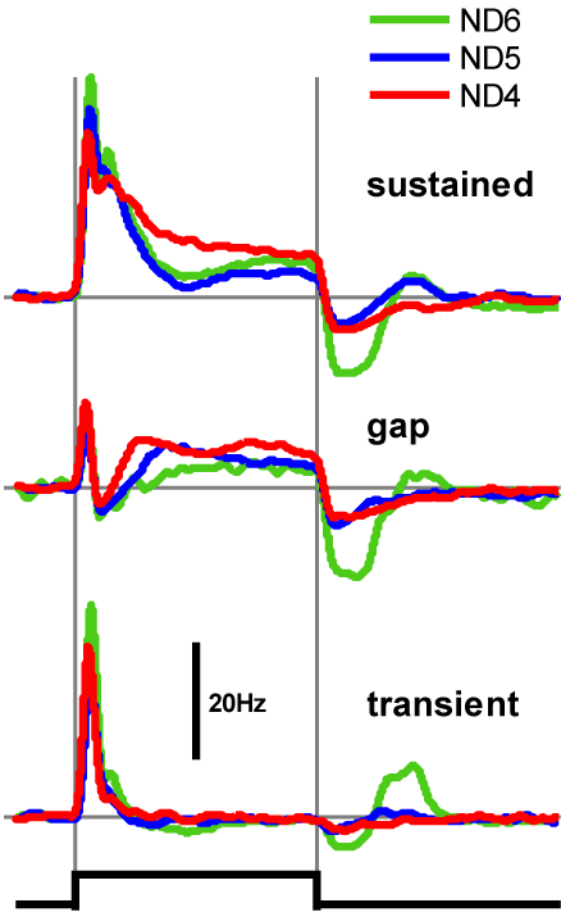

Fig. 5.27. Overlay of averaged responses of transient, gap, and sustained ON cells to white steps at ND6, ND5, and ND4.

5.2.3.2. OFF responses of ON cells.

Fig. 5.23 and *Fig. 5.27* also illustrate OFF responses of transient, gap, and sustained cells. As noted above, OFF responses appear very similar in all cells at most light levels, and, unlike ON responses in OFF cells, mostly reveal themselves in nearly total suppression of spiking. At ND6 the suppression is often followed by a delayed spiking increase. Interestingly, suppression was not necessary in order for such delayed OFF responses to occur: some transient ON cells with no spontaneous activity (i.e. they could not be suppressed after the white step offset) also had a burst of spikes in 500-700ms after the step offset. In some cells, the delayed OFF response was even larger than the ON response. Only 2 cells out of 249 had short-latency OFF response, therefore the vast majority of the 'true' ON-OFF cells were identified as OFF cells by linear filter polarity. This suggests that the 'true' ON-OFF cells in the mouse retina predominantly receive OFF input.

5.2.3.3. Asymmetry of responses to white and black steps: ON responses.

Overall, the ON responses of ON cells were less correlated than the OFF responses of OFF cells (*Fig. 5.28*, compare with *Fig. 5.12*). The fraction of highly correlated responses to the preferred stimulus (here: light increments) was notably smaller, and to the opposite stimulus (here: light decrements) rarely exceeded 0.9. For ON responses, it may be explained by high variability of the relative strength of the ON inhibitory and ON excitatory inputs. Responses to light decrements were more uniform (strong suppression of spiking activity). However, the duration of suppression was much longer for black steps than after the offset of white steps. This led to rather low correlation coefficients.

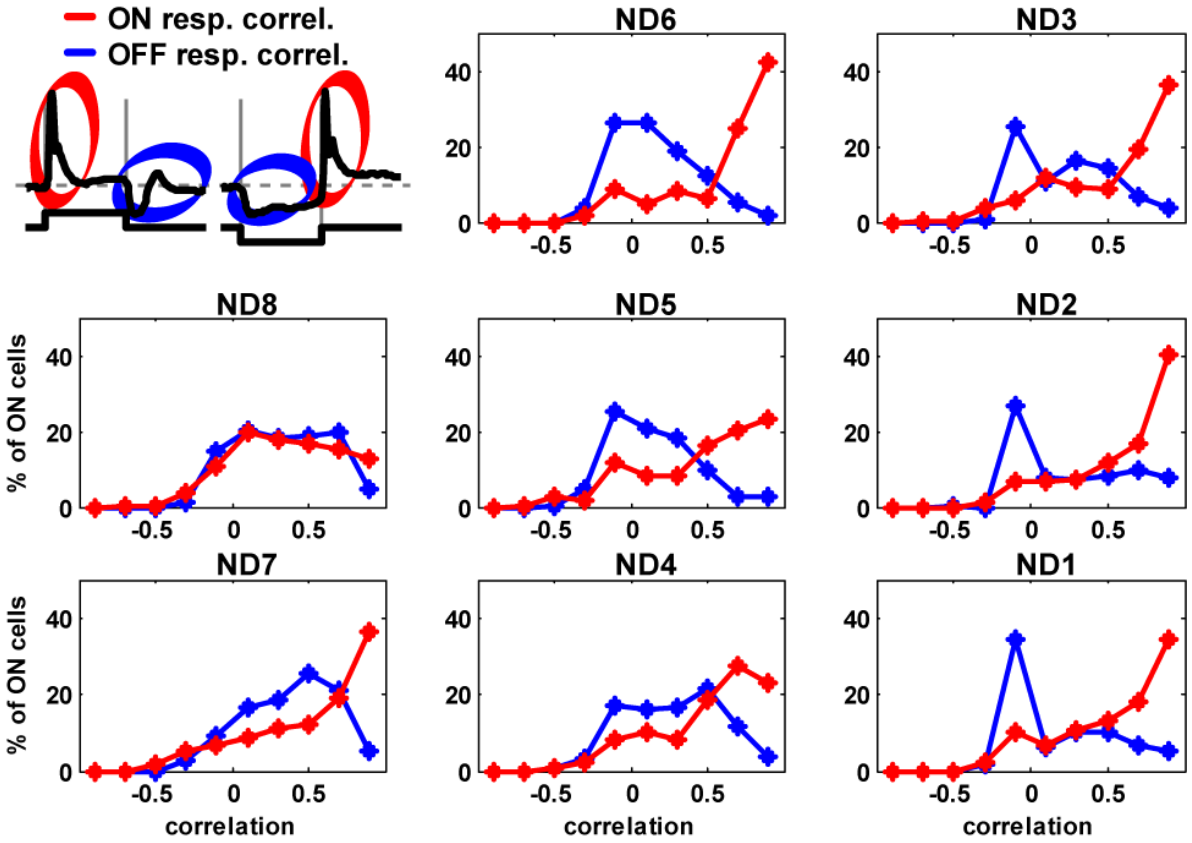

Fig. 5.28. Distribution of correlation coefficients between responses of ON ganglion cells to light decrements in black and white steps (blue) and to light increments in black and white steps (red) at different light levels.

Since ON cells had three major categories of ON responses to white steps, I conducted the same analysis with the response to the light increment after the black step. The results are summarized in *Fig. 5.29*. In general, I found the same three distinct response types (transient, sustained, and gap-

like), which accumulated about 90-98% of all ON cells. The distribution of the types across brightness levels was similar to the distribution seen during the white step, with exception of ND5 and ND4 (*Fig. 5.30*, compare to *Fig. 5.24*). At ND5, gap-like responses had only 10% of cells to the black step offset versus 35% of cells to the white step onset. At ND4, all three response types to the black step offset were similarly represented, whereas the white step onset evoked predominantly sustained responses.

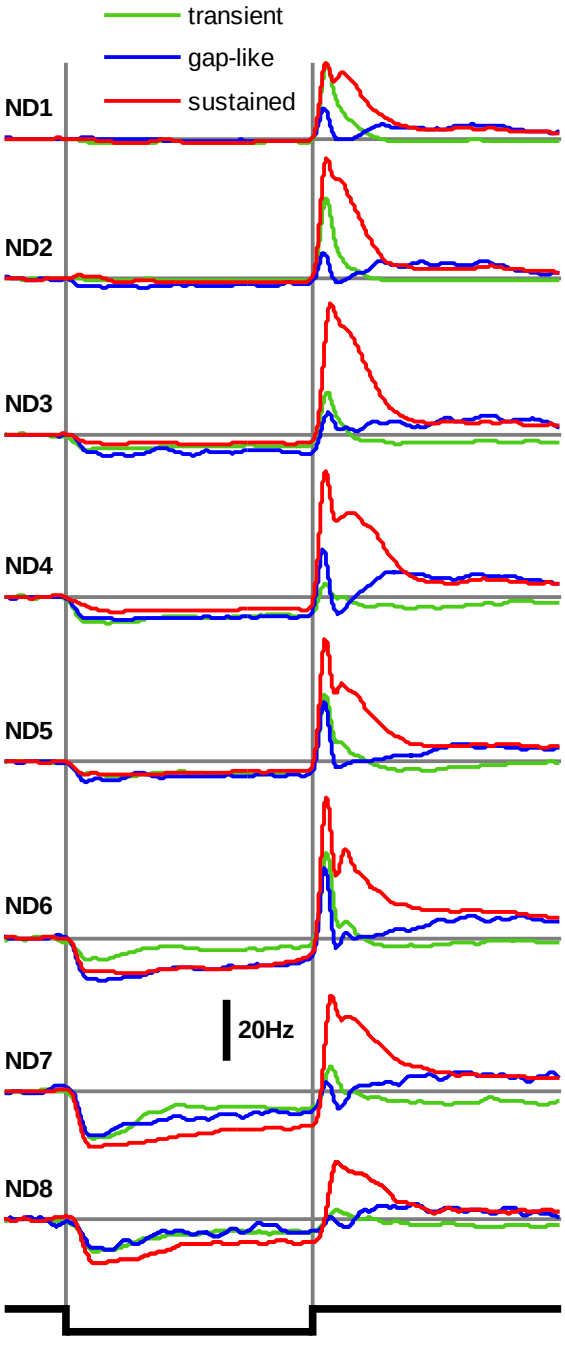

Fig. 5.29. Averaged responses of ON cells with transient, gap-like, and sustained responses to black steps at different light levels.

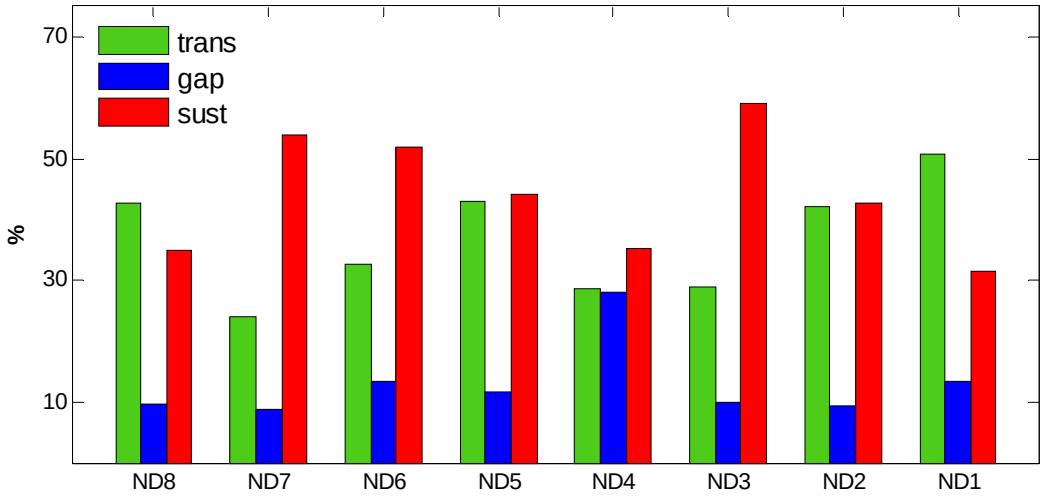

Fig. 5.30. Percentage of ON cells with transient, gap-like, and sustained responses to the black step offset at different light levels.

Then I compared the amplitude ratios and latency differences of ON responses to black and white steps for ON cells like described above for OFF cells (taking light increments instead of light decrements in the stimulus), regardless the response type. *Fig. 5.31* A shows mean amplitude ratio of ON responses of ON cells at eight light levels, and *Fig. 5.31* B shows mean latency difference of ON responses of ON cells (corresponding parameters of OFF responses of OFF cells, taken from *Fig. 5.13*, are shown for comparison).

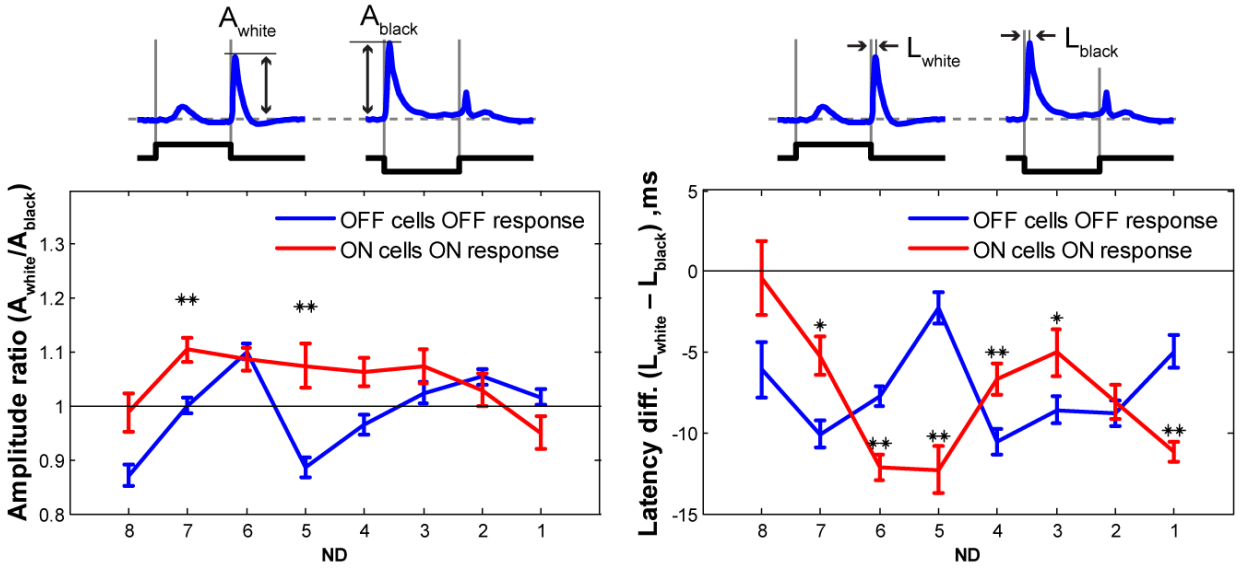

Fig. 5.31. Amplitude ratio and latency difference of responses to the preferred contrast between black and white steps, averaged across ON cells (red) and OFF cells (blue). Mean ± s.e.m. * marks statistically different values between ON and OFF cells, $p<0.05$; ** $p<0.001$.

Note that ON cells behave very differently than OFF cells: amplitude ratio of ON cells almost does not change with luminance. Latency difference is modulated in both groups of cells, but in an opposite manner, although it remains negative: both cell groups are faster to reach the peak amplitude of the response to white step compared to black step.

Fig. 5.32 shows transiency of ON responses of ON cells to black (x-axis) and white (y-axis) steps (analogous to *Fig. 5.14* for OFF cells). Transiency was calculated like described for OFF cells: time for each cell to return to the baseline activity plus one standard deviation. In this analysis, gap-like cells tend to behave like transient cells. The indices of ON cells are distributed much more symmetrically then the indices of OFF cells (the responses of OFF cells to the preferred stimulus were more sustained almost without exception, see *Fig. 5.14*).

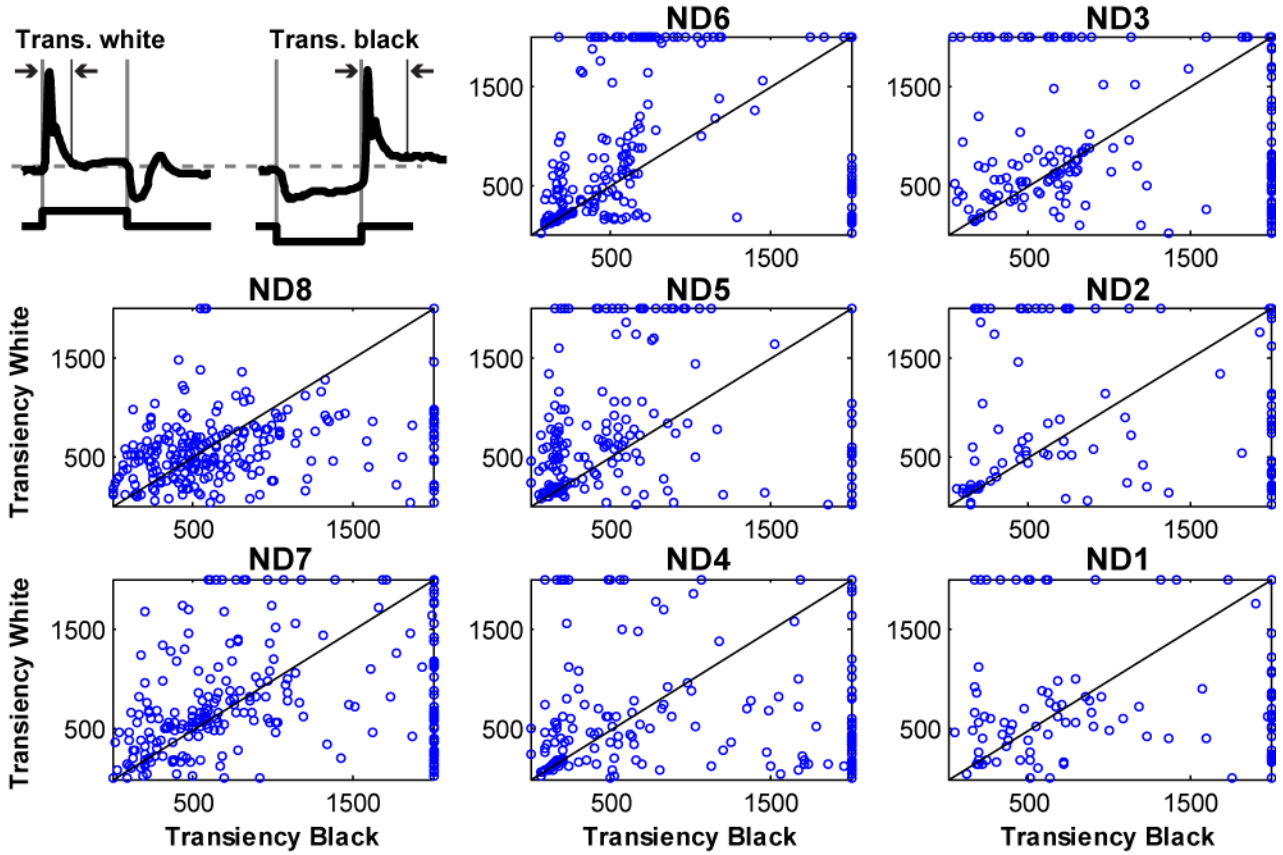

Fig. 5.32. Transiency index of ON responses of ON ganglion cells to light increments in black (x-axis) and white (y-axis) steps. Transiency was calculated as difference between the time point of light increment and time of firing rate return to the baseline activity level plus one standard deviation after the response peak.

5.2.3.4. Asymmetry of responses to white and black steps: OFF responses.

Responses of ON cells to light decrements, as seen in *Fig. 5.20*, are usually represented by a suppression of the response below the baseline firing rate. This suppression had very different duration for black and white steps. I calculated the transiency of the firing rate suppression using similar algorithm as for firing rate elevation: time the cell needed to recover the firing rate to the level of the baseline activity minus one standard deviation. *Fig. 5.33* shows the scatter plot of the results for black step (x-axis) and white step (y-axis). Many cells are suppressed throughout the entire duration of the black step, whereas offset of the white step suppresses the cells not longer than 500-600ms. Thus, the suppression of ON cells and the activation of OFF cells after light decrements have similar time courses (compare *Fig. 5.33* and *Fig. 5.14*).

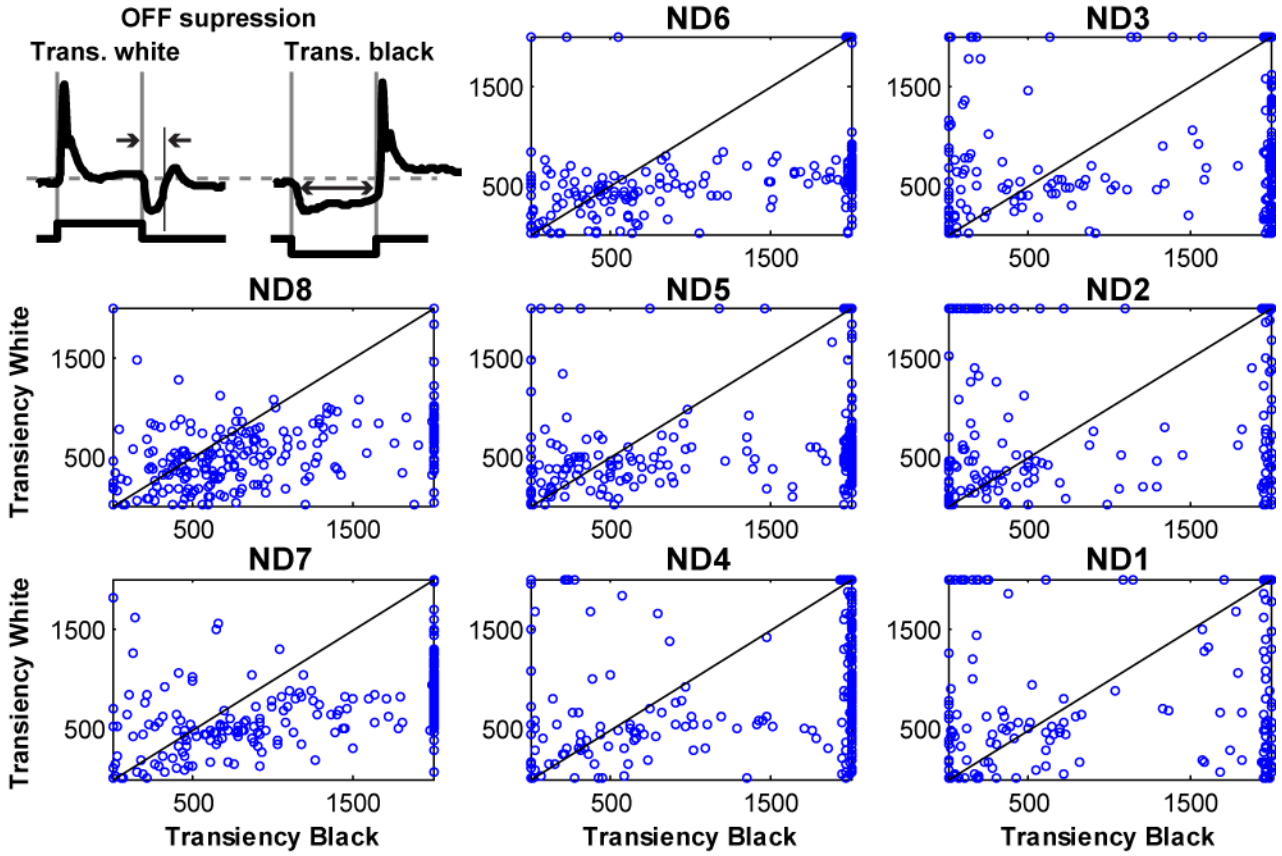

Fig. 5.33. Transiency index of OFF suppression of ON ganglion cells to light decrements in black (x-axis) and white (y-axis) steps. Transiency was calculated as time between the time point of light decrement and time of firing rate return to the baseline activity level minus one standard deviation after the maximal suppression.

5.2.4. Temporal development of responses to the full-field steps at different light levels.

In 12 experiments I presented trials of full-field steps several times at each light level, distributed over time. To estimate the stability of the population response at each light level I averaged the responses obtained during the first trial (within 5 min after the ND switch) and during the last trial (20-30min after the ND switch). The results are plotted in *Fig. 5.34*, separately for OFF cells (left) and ON cells (right).

Overall, the responses were stable at most light levels, or changed quantitatively (decrease of amplitude, more transient responses). This was the case for ON cells at all light levels. OFF cells, however, demonstrated qualitatively changing ON responses at two light levels: ND5 and ND3 (marked by arrows in *Fig. 5.34*). In particular, the delayed ON response to the white step was very small or absent at first trials at both NDs, and reached relatively high amplitude at the last trials. At ND3, the same also happened with the ON responses to the black step offset.

Interestingly, these changes occur at the light levels where the photoreceptor balance is likely changing (see Section 3): ND5 is the mesopic level where rods are not fully responsive, and at ND3 the slow rod reactivation is happening (*Fig. 3.5*). At ND5, the linear filters of cells from the rod-only retina initially had rather small amplitude (20% of that at ND6), which was then slowly increasing up to 50% of the amplitude at ND6. This observation suggests that the rods might gradually increase their impact on ganglion cell responses at ND5, similar to ND3 (but starting from higher values and ending with lower values than at ND3). These observations on the response properties to Gaussian white noise flicker in rod-only retinas correlate remarkably well with the observations from the OFF cell responses to contrast steps: at ND6 (rods are active) the delayed ON response is robust and has high amplitude; at ND5 it is smaller in the beginning and increases with time (rods become more active); at ND4 it is barely present (cones are predominantly active); finally, at ND3 it is completely absent in the beginning but strongly increases towards the end. Such dynamic changes may indicate a connection between the delayed ON response in OFF cells and the rod-driven circuitry. It should also be noted, however, that in low scotopic conditions ND8 and ND7 the delayed ON responses are hardly observed, although rods are active there as well. A possible explanation could be insufficient activation of rods at these light levels (e.g. origin of the response in less sensitive rod-pathways). Alternatively, the delayed ON response arises from some form of interaction between rod- and cone driven circuitry.

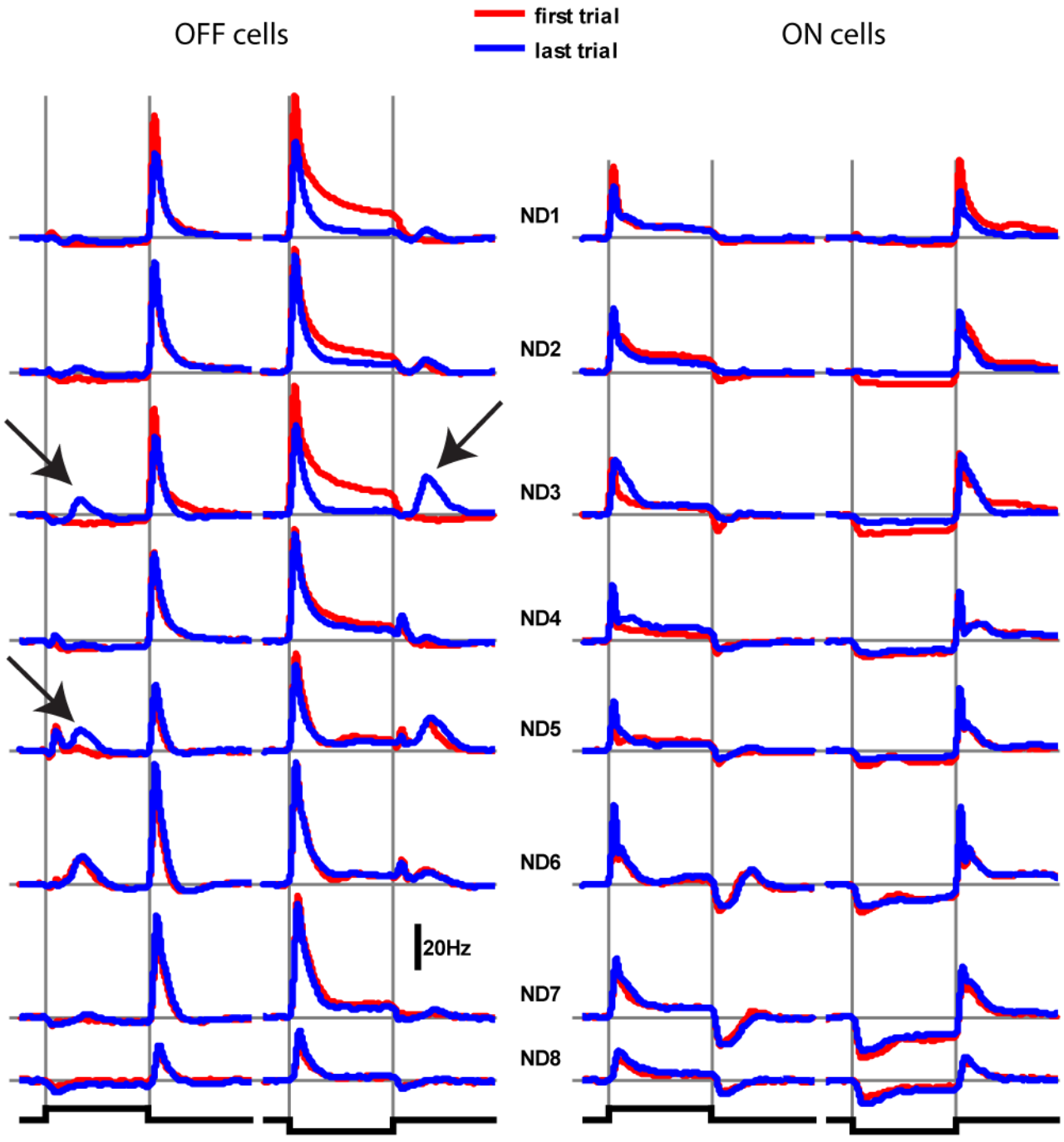

Fig. 5.34. Temporal development of responses to contrast steps of OFF (left) and ON (right) cells at different light levels. Red curves show the average responses to the first trial after the light level increase (<5min), blue curves represent the last trial (20+ min). Overall, the responses are remarkably similar within one light level. However, there are qualitative changes of delayed ON responses in OFF cells at ND5 and ND3 (arrows).

5.3. Summary and Discussion

I have analyzed in detail ganglion cell responses to positive and negative full-field steps over several log units of ambient light intensity. Here is a collection of the most interesting observations:

- OFF responses of OFF cells can be transient (15-20% of cells) or sustained (~75%). This property is moderately modulated by luminance.

- ON responses of OFF cells can have short latency (early responses) or be delayed. All OFF cells have excitatory ON responses at least at one light level from ND6 to ND4. The type of ON response can change under light adaptation.

- ON responses of OFF cells to light increments in white and black steps are often asymmetrical. This asymmetry can change with luminance.

- The vast majority of ON-OFF cells (as defined by their responses to the full-field steps of light) have OFF-like linear filters.

- ON cells display at least three types of ON response to light increments: transient, gap-like, and sustained. The response type may change at different brightness levels.

- OFF responses of ON cells are rather uniform and reveal themselves as strong suppression of spikes. OFF inhibition to black steps last much longer than after the offset of the white step.

From these observations, I draw the following conclusions:

(1) the responses to a stimulus as simple as a full-field step of light are highly dependent on the ambient light level;

(2) if there is no shift in the photoreceptor balance, changes in the response structure of ganglion cells occur within the first minutes after the luminance increase and remain stable afterwards;

(3) responses to light increments are modulated by luminance *qualitatively* in both ON and OFF cells, whereas responses to light decrements are modulated only *quantitatively*.

Since I did not use any pharmacological agents in my experiments, the origin of the diverse responses to light increments can not be derived from my data directly. The early ON response is likely to be caused by an excitatory ON input from ON bipolar cells. However, many cells behave like ON-OFF at some light levels and as OFF cells at others. Even more intriguing, the early ON response to light increments in white steps and black steps is highly asymmetrical in some cells. It is quite common to observe a robust early ON response to the black step offset and very weak or absolutely no early ON

response to the white step onset (*Fig. 5.16* and *Fig. 5.17*). This evidence makes the origin of this response from ON bipolar cell output arguable.

Few previous studies have also shown long-latency ON responses in OFF cells [24, 79]. However, there were several important differences between my data and data of Renteria et al. [79]: there, abnormal, normally masked, delayed ON responses were studied. They were not observed in control conditions, but only in OFF cells of mGluR6 knockout mice (i.e. with no transmission from photoreceptors to ON bipolar cells) and in OFF cells of wild type mouse if APB was applied (i.e. photoreceptor → ON bipolar cells transmission was blocked). Renteria et al. suggested that the responses originate directly in OFF bipolar cells. However, the latency of these responses was larger than that of the delayed ON responses in my experiments (beginning at 700ms or later, whereas in my experiments delayed ON responses already peaked at 650-700ms). This and the conditions of their manifestation suggest that these responses have different nature than the delayed ON responses described in this thesis.

In my experiments, delayed ON responses in OFF cells were often preceded by a drop of firing rate, which suggests it may be a rebound reaction when the ganglion cell's membrane voltage rises after a strong inhibitory input. However, the rebound mechanism can not account for the delayed ON response in the cells which have both early and delayed ON responses (like cells in the Group 4 in *Fig. 5.6*). Moreover, in some cases the delayed ON response reaches very high amplitude. An example of such behavior is shown in *Fig. 5.15*: in response to the black step at ND5, the cell produced a high-amplitude OFF response and then an equally high-amplitude delayed ON response; at ND3, the delayed ON response was even stronger than the OFF response. The duration of these delayed ON responses was longer than that of the OFF responses at corresponding NDs. Finally, in some cells the firing rate remains at the background level in the period of time between the black step offset and the onset of the delayed ON response. Taken together, these data suggest that at least in some cells the delayed ON responses are likely initiated by an independent excitatory input and not by a rebound after inhibition due to intrinsic ganglion cell properties.

Taken together, the input to OFF ganglion cells evoked by a light increment may consist of three independent components with different temporal properties: early excitation, early inhibition, and delayed excitation. The early excitation seems to be universal for rod- and cone-driven circuitries, whereas the delayed excitation appears to be connected with the rod-driven circuitry. Various vertical interactions between ON and OFF pathways have been described in the retina [80-81]. The concrete circuit of each of the three inputs may differ for different ganglion cell types (e.g. the 6 groups shown in *Fig. 5.6*), but the responses corresponding to each input have very similar latencies and durations at

each ambient light level across all ganglion cells. Generally speaking, the response pattern of a cell to the light increment is then determined by the relative strength of the three inputs (counting no input of a certain type as '0'). Independent modulation of the inputs by ambient light intensity would then cause the overall response pattern to change.

ON cells, as noted above, also had variable responses to light increments: transient, sustained, and gap-like. The transiency of the response may be a simple consequence of the temporal characteristics of the bipolar cell outputs: it has been shown that the excitatory current into ganglion cells may be transient or sustained [82-87]. 'Gap-like' behavior in ON cells is not a new finding either: it was mentioned (but not analyzed) by Renteria [79]. The 'gap' between the initial response peak and the late sustained phase may be mediated by a (delayed) ON inhibitory input. The variety of observed responses may be explained by variation of the relative strength of the ON inhibition and ON excitation across cells and light levels. Independent modulation of their amplitudes by luminance can make a cell migrate from one group to another (based on my criteria of type identification, see section 5.2.3.1). Note that the migration from the sustained to the transient group was rather rare (<10% of cells), much more common was a modulation of the 'gap'-effect.

Responses to light decrements were much more uniform across cells and light levels. Remarkably, the timing of the responses of ON and OFF cells to light decrements, averaged across their populations, perfectly matched (see *Fig. 5.35* – overlay of *Fig. 5.3* and *Fig. 5.20*). For example, the responses at the onset of the black step were prolonged, while they were notably shorter at the offset of the white step. Furthermore, ND6 was the only light level where ON cells had a noticeable overshoot after typical inhibition to the white step offset (marked by an arrow in the figure). This was also the only light level where OFF cells had a noticeable drop of firing rate below the baseline after the initial excitatory OFF response. Again, the timing of these complementary responses was well matched. Both ON and OFF cells also took longer to return to the baseline activity after the white step offset at ND4 (arrow). Overall, the responses to light decrements of ON and OFF cells appear to perfectly mirror each other. It may partially be caused by disinhibition in the OFF pathways: at least two studies suggest non-reciprocal ON→OFF circuit inhibition [68-69], likely via ON amacrine cells. In the model of Zaghloul [68], OFF ganglion cells receive inhibitory input from ON amacrine cells (see their Fig.7). Negative contrast suppresses basal glutamate release from the ON bipolar cell, which, on one hand, reduces spiking in ON ganglion cells. At the same time, the inhibitory ON amacrine cells also becomes less active, resulting in disinhibion of the OFF cell. This might explain the tight temporal link between the suppression of ON cells and activation of OFF cells. However, this model was proposed for OFF

brisk-transient (Y) cell circuitry and the extension of the model to the majority of OFF cells is arguable; more experiments should be conducted to establish the circuits for different types of cells.

High basal release from ON bipolar cells might also partially explain why responses to light decrements are stronger and last longer during the black step than after the white step (see *Fig. 5.35*), even though the contrast was identical. The rate R_0 of basal release itself is likely a parameter that is adjusted by luminance adaptation, either by mechanisms inside the bipolar cells themselves, or by regulating the synaptic input strength from photoreceptors. During a black step, ON bipolar cells might then be hyperpolarized in a sustained fashion, and basal release drops and stays below R_0. This would lead not only to a sustained suppression of ON ganglion cell spiking, but also to a sustained facilitation of OFF ganglion cells through the disinhibitory pathway described in the previous paragraph. The situation would be different after a white step. There, the light level drops back to the 'ambient' condition, so that there is only a transient undershoot and the release rate R_0 is quickly re-established. If this hypothetical scenario were true, we could make the following prediction: a longer white step (longer than 2 seconds) would trigger luminance adaptation, bringing the ON bipolar cell to a new R_0, so that the response at the offset of the white step would become longer. In other words, the longer the white step, the more the offset responses should resemble the observed responses to the black step. A corollary conclusion would be that the light adaptation mechanisms that set the operating range of ON bipolar cells take more than 2 seconds to be completed, but they take less than 1 minute, which is the earliest time point when I measured full field step responses after ND changes (note, however, that an ND change is a stronger contrast than the white step stimulus).

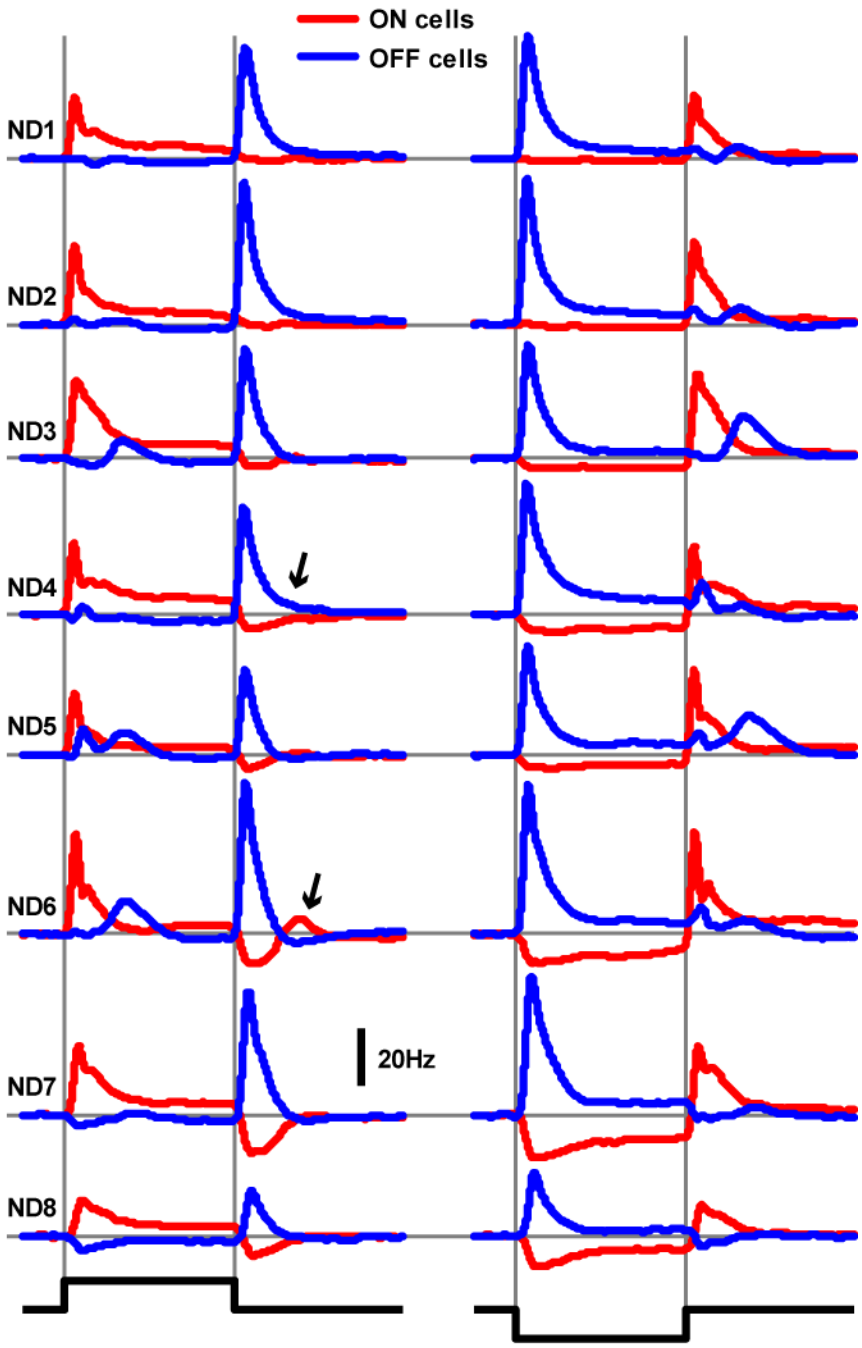

Fig. 5.35. Overlay of averaged responses of ON and OFF cell populations to white and black steps at different light levels. Note that responses to light decrements of ON and OFF cells are antisymmetric, whereas responses to light increments are not. Arrows highlight features discussed in the text.

The surprising diversity of luminance effect on basic properties of ganglion cells responses raises important questions about the "retinal code", i.e. the information about the visual environment that a particular ganglion cell transmits to the brain. It has been shown that ganglion cells are tuned to higher frequencies in day-light conditions compared to the scotopic regime, but that is not likely to

qualitatively influence the features a ganglion cell extracts from the visual input. However, if a cell acquires drastically different properties – e.g. when a pure OFF cell becomes an ON-OFF – it is likely that such cell will then detect qualitatively different events. The functional meaning of the delayed ON response in OFF cells is less clear: would it affect processing of natural stimuli, or is it an artifact of a spatially homogeneous stimulus like the full-field contrast step and would be masked in the 'normal' viewing conditions?

In fact, I found that the feature selectivity of a ganglion cell to a natural movie can change, similar to the changing response patterns to full-field steps. *Fig. 5.36* shows the responses of an example OFF cell to full-field steps and to repeated presentations of a natural movie at ND6, ND5, and ND4. The cell had strong transient OFF responses to the black step at all brightness levels. Responses to the white step varied: delayed ON response and small OFF response at ND6, same at ND5 (but amplitude ratio is very different from ND6), and no ON response at ND4 (but OFF response has higher amplitude). This cell had distinct response patterns to a 25s natural movie at different light levels (raster plots in *Fig. 5.36*, first 16s of the responses are shown). The movie was repeated 18 times at each brightness level (6 times in the beginning, 6 times after 10min of adaptation, and 6 times after 20min of adaptation), and the responses appeared to be stable at each ND. At all three light levels, the cell had strong transient responses at the time points highlighted green in the raster plots. However, some features in the movie evoked robust responses only at certain light levels (highlighted pink in the raster plot), while there was no response at other light levels (open pink boxes). These results demonstrate that if basic response properties as seen from the full-field step of light stimulus are affected by luminance, the feature selection – and therefore information extraction – from naturalistic stimuli may also change.

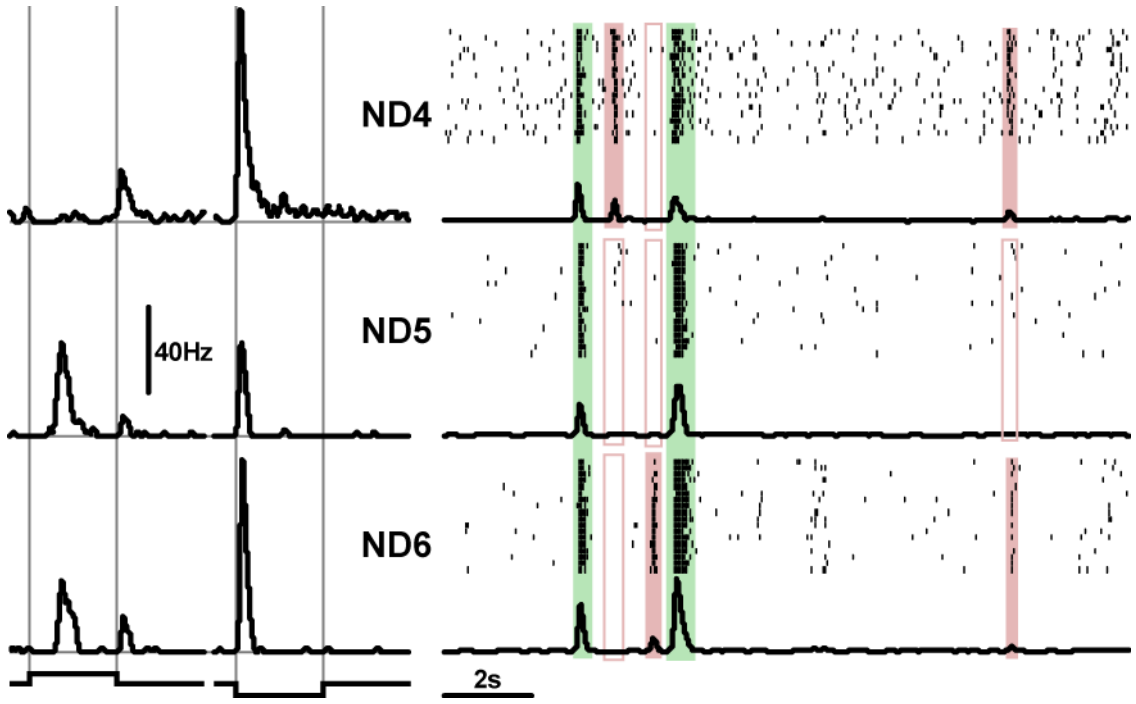

Fig. 5.36. Example of luminance effect on ganglion cell responses to contrast steps and naturalistic movie. Left panels: responses to the full-field steps (white and black) at ND4, ND5, and ND6 correspondingly. Right panels: raster plots of spikes in response to a natural movie (18 trials per brightness level) and mean firing rate of all 18 trials at ND6, ND5, and ND6 correspondingly. All plots show the same cell. "Green" highlights events that consistently activate the cell at all brightness levels. "Pink" events are only reported at some brightness levels.

In summary, I aimed to characterize the responses of ganglion cells on a phenomenological level. The mechanisms responsible for the observed behavior need to be tracked down by additional methods: using pharmacological agents, histology, patch clamp, and others. Many of these methods are not applicable for long experiments at many light levels. My work provides a basis for further research and indicates specific conditions and stimuli to test the cells. I also show that cell classification based on classical parameters, such as response polarity, amplitude, and latency to full-field steps, probed at a single luminance, has high chances to be incomplete/inaccurate and cell types might be mixed or confused. Besides, I show that these luminance-induced changes may lead to different feature selectivity in naturalistic movies and therefore be relevant for processing of natural scenes. Further investigation is required to better understand the complex relationship between basic cell properties, its functions in natural environment, and luminance effects on both.

6. General Discussion.

Light adaptation in the retina is a complex phenomenon, which serves not only to shift the operational range of neurons according to the new mean luminance, but also to tune the retinal circuits to process the most relevant aspects of the visual input at this luminance. The tasks of visual processing in relative darkness and under bright light are different; it follows from both the ecological needs (different behavior patterns in the night and day) and from limitations of the image statistics [19, 23, 71-72]. The variety and extent of changes in the retina under light adaptation are only beginning to be appreciated, even though it might have a significant impact on both our fundamental understanding of the nervous system principles and practical applications, such as retinal prosthetics.

I attempted to characterize the effects of luminance adaptation on basic properties of ganglion cells, such as kinetic properties of the impulse response (linear filter), adaptation to temporal contrast (including firing rate, kinetics, and gain control), and responses to full-field steps of light. In my experiments, I consistently used a framework where the retina is tested under a broad range of brightness levels, spanning 8 log units, and where I repeated the same set of stimuli with constant contrast relative to the mean luminance at each light level. This approach allowed tracking and characterizing the changing response patterns of the same cells under light adaptation.

6.1. Rod reactivation under bright light

One of the most important outcomes of my experiments was the finding that bright light may cause rod reactivation at least under *in vitro* conditions. In particular, I have shown that at a mean light intensity equivalent to about 10^5 of photoisomerizations per rod per s, it will take only 10 to 15 min until rods begin to drive ganglion cells activity (*Fig. 3.1*). At 10^4 photoisomerizations per rod per s, this process will take about 1.5 hours (*Fig. 3.8*). Cones, on the other hand, will at the same time diminish their activity (*Fig. 3.5*), and the responses of ganglion cells will resemble that in mesopic or even scotopic conditions.

I have proposed a possible mechanism of rod reactivation. Without the pigment epithelium, the photopigment restoration (loading of opsin with retinal) happens at much lower rates than *in vivo*. Bleaching is therefore much more prominent *in vitro*, and eventually the fraction of unbleached rhodopsin becomes small enough for the photoisomerization rate to drop to relatively low levels – the rods then become able to translate the fluctuations of photoisomerizations into voltage changes (*Fig. 3.11*). This process is commonly known as bleaching adaptation and has been known to be an important

mechanism of the cones; it prevents them from saturating at high brightness levels [57]. My work suggests that it may also play a role in rods, and most ironically, its effect becomes drastic under bright light.

The phenomenon of rod reactivation has two important consequences. First, from the point of view of retinal research, it compromises the interpretation of those mouse retina studies conducted *in vitro* without the pigment epithelium (which is by far the most common setup to study the mouse retina. Besides, this phenomenon may also be relevant for other species). Bright adapting light does not guarantee that the retina operates in the photopic regime, i.e. that only cones drive the ganglion cell light responses. Since rod reactivation is a relatively slow process happening on a time scale of tens of minutes, the absolute light level and the duration of the experiment are crucial: the ganglion cells may be as responsive after an hour of the recording as in the beginning, but the balance of the rod and cone signaling may be reversed (*Fig. 3.1*, *Fig. 3.7*, *Fig. 3.8*). Moreover, ganglion cell responses to a certain stimulus may be driven predominantly by rods and to another stimulus predominantly by cones, even when the intensity properties (contrast range) of these stimuli are identical but temporal properties (frequency) differ (*Fig. 3.9*, *Fig. 3.10*).

Second, it is possible that the described phenomenon of bleaching-mediated rod reactivation might be relevant in the *in vivo* situation. According to the computational model (*Fig. 3.11* C), the relationship between rod- and cone signaling may be much more complex than previously appreciated, and the retina may be frequently shifting between photopic to mesopic operational ranges even under bright illumination. This process may have been missed so far because it only reveals itself under certain conditions, which are not used in standard experimental settings, and is ignored in every-day experience due to constancy of perception (as human observers do not notice the switch from mesopic to photopic conditions as the dawn progresses). My results can be used to identify the conditions under which the rod-cone relationship may be tested *in vivo*.

6.2. Influence of luminance on ganglion cell properties

I have investigated how light adaptation influences the properties of ganglion cells in steady state conditions (i.e., the retina had time to adapt to the new light level) in light ranges which were predominantly rod-, rod- and cone-, and cone-driven. Here, I concentrated on two stimuli: (1) Gaussian white noise flicker (to probe contrast adaptation properties, Section 4), and (2) simple full-field steps of positive and negative contrast (to probe basic cell properties such as ON and OFF response characteristics, Section 5).

Adaptation to mean light level and to its variance (contrast) is believed to have independent mechanisms for mid to high brightness at least as measured by responses of LGN neurons of the cat [64]. I used broader intensity ranges to test this observation in ganglion cells of the mouse retina, and found that luminance indeed has rather weak impact on gain control during contrast adaptation (*Fig. 4.11*), but it does have some impact on changes in kinetics of the linear filters (*Fig. 4.10*) and significant effect on firing rate adjustment (*Fig. 4.2*, *Fig. 4.8*).

Weak modulation of gain control by luminance may have a good practical reason. The retina computes the stimulus variance and adjusts the gain so that the cell encodes the maximal range of the stimulus. This range depends on its variance, not on its mean. Temporal filtering, however, may have different implications when the light level increases (tuning to higher frequencies).

Light adaptation had more pronounced effects on ON cells than on OFF cells. I have described ON cells which adapt to contrast in unexpected ways: some decrease their firing rate at high contrast ('non-typical' ON cells, *Fig. 4.3*), some accelerate at low contrast. The second group is a subset of the first group: most cells accelerating at low contrast reduce their firing rate at high contrast (*Fig. 4.14*) Kinetics adaptation in 'non-typical' ON cells seems to be weak in general (*Fig. 4.15*). One possible reason of this may be two converging inputs with opposite kinetic adaptation patterns: OFF inhibition and ON excitation. Such two pathways may have different gain control, which would lead to different signal amplification at low contrast compared to high contrast, and therefore to dynamic regulation of these inputs ratio. Distinct extent of kinetic adaptation in these circuits, or different temporal tuning, may then reduce the acceleration of the linear filter at high contrast, or even reverse it. According to responses of 'non-typical' ON cells to full-field steps of light and chirp stimuli, they indeed may receive stronger OFF inhibition than ON excitation (*Fig. 4.5*). Such circuit would also easily explain the properties of firing rate adaptation.

'Non-typical' ON cells may serve as 'secondary' OFF cells. Whereas in general these cells behave as typical ON cells - activate at light increments and suppress spiking at light decrements – their ability to signal light decrements is strongly increased. I propose at least two specific functions of these cells: supporting regular OFF cells in light decrement detection (possibly gaining information about negative contrasts) and reduction of correlated noise (decreasing information loss). These hypotheses may be directly tested in future research.

Finally, I showed that the response properties of ganglion cells to full-field contrast steps highly depend on luminance, and that this dependence is much more pronounced for responses to light increments than decrements in both ON and OFF cells (*Fig. 5.3*, *Fig. 5.20*). ON responses of OFF cells may have short or long latency (*Fig. 5.5*). The mechanisms generating ON responses are yet unclear,

although the short- and long-latency responses seem to be independent. Intriguingly, the vast majority of OFF cells, maybe all of them, had ON responses at least at some brightness level (*Fig. 5.19* and Section 5.2.2.5.). To my knowledge, this has not been reported so far. One of the reasons might be that the ganglion cells are usually not tested in such a broad range of brightness in one preparation. At any given light level, many OFF cells without early ON response (i.e. cells that would be considered 'OFF', and not 'ON-OFF' in the classical sense) do not display any ON response (especially in photopic conditions, see *Fig. 5.7* and *Fig. 5.16*). This may explain why these frequently observed ON responses in OFF cells have been missed so far. It also emphasizes the importance of testing the cells in a broad range of luminance for classification purposes.

The variability of responses to light increments was not an artifact of the experiment: although the response pattern may change drastically as the light level increases (e.g. an OFF cell becomes an ON-OFF cell), they remained very stable throughout the measurement at one light level (except ND5 and ND3, where the photoreceptor balance is unstable, *Fig. 5.34*). Using the distributions of response types which I obtained in my experiments, one could identify the underlying circuits by using different techniques such as patch-clamp, that do not allow for such extended recording times but have a better cellular and synaptic resolution. The functional meaning of response variability is intriguing: there are indications of relevance for naturalistic stimuli processing (*Fig. 5.36*). This evidence may eventually compromise the concept of 'labeled lines' for the retinal cells: the retinal 'code' may change with luminance; at a new light level, some new features will be extracted and others will be ignored. This finding is yet another direction for further research, and it yet again emphasizes the role of light adaptation in the retinal processing.

7. References

1. Shapley R., E.-C.C., *Visual Adaptation and Retinal Gain Controls.* Progress in Retinal Research, 1984. **3**: p. 263-346.
2. Wassle, H., *Parallel processing in the mammalian retina.* Nat Rev Neurosci, 2004. **5**(10): p. 747-57.
3. Naka, K.I., R.Y. Chan, and S. Yasui, *Adaptation in catfish retina.* J Neurophysiol, 1979. **42**(2): p. 441-54.
4. Bloomfield, S.A. and B. Volgyi, *The diverse functional roles and regulation of neuronal gap junctions in the retina.* Nat Rev Neurosci, 2009. **10**(7): p. 495-506.
5. Farrow, K. and R.H. Masland, *Physiological clustering of visual channels in the mouse retina.* J Neurophysiol, 2011. **105**(4): p. 1516-30.
6. Field, G.D. and E.J. Chichilnisky, *Information processing in the primate retina: circuitry and coding.* Annu Rev Neurosci, 2007. **30**: p. 1-30.
7. Gollisch, T. and M. Meister, *Eye smarter than scientists believed: neural computations in circuits of the retina.* Neuron, 2010. **65**(2): p. 150-64.
8. Barlow, H.B. and W.R. Levick, *The mechanism of directionally selective units in rabbit's retina.* J Physiol, 1965. **178**(3): p. 477-504.
9. Fried, S.I., T.A. Munch, and F.S. Werblin, *Directional selectivity is formed at multiple levels by laterally offset inhibition in the rabbit retina.* Neuron, 2005. **46**(1): p. 117-27.
10. Fried, S.I., T.A. Munch, and F.S. Werblin, *Mechanisms and circuitry underlying directional selectivity in the retina.* Nature, 2002. **420**(6914): p. 411-4.
11. Munch, T.A., et al., *Approach sensitivity in the retina processed by a multifunctional neural circuit.* Nat Neurosci, 2009. **12**(10): p. 1308-16.
12. Olveczky, B.P., S.A. Baccus, and M. Meister, *Segregation of object and background motion in the retina.* Nature, 2003. **423**(6938): p. 401-8.
13. Breuninger, T., et al., *Chromatic bipolar cell pathways in the mouse retina.* J Neurosci, 2011. **31**(17): p. 6504-17.
14. Dacey, D.M. and O.S. Packer, *Colour coding in the primate retina: diverse cell types and cone-specific circuitry.* Curr Opin Neurobiol, 2003. **13**(4): p. 421-7.
15. Farrow, K., et al., *Ambient illumination toggles a neuronal circuit switch in the retina and visual perception at cone threshold.* Neuron, 2013. **78**(2): p. 325-38.

16. Reitner, A., L.T. Sharpe, and E. Zrenner, *Is colour vision possible with only rods and blue-sensitive cones?* Nature, 1991. **352**(6338): p. 798-800.
17. Barlow, H.B., R. Fitzhugh, and S.W. Kuffler, *Change of organization in the receptive fields of the cat's retina during dark adaptation.* J Physiol, 1957. **137**(3): p. 338-54.
18. Bisti, S., et al., *Spatial frequency and orientation tuning curves of visual neurones in the cat: effects of mean luminance.* Exp Brain Res, 1977. **27**(3-4): p. 335-45.
19. Dedek, K., et al., *Ganglion cell adaptability: does the coupling of horizontal cells play a role?* PLoS One, 2008. **3**(3): p. e1714.
20. Enroth-Cugell, C. and J.G. Robson, *The contrast sensitivity of retinal ganglion cells of the cat.* J Physiol, 1966. **187**(3): p. 517-52.
21. Muller, J.F. and R.F. Dacheux, *Alpha ganglion cells of the rabbit retina lose antagonistic surround responses under dark adaptation.* Vis Neurosci, 1997. **14**(2): p. 395-401.
22. Rodieck, R.W. and J. Stone, *Analysis of receptive fields of cat retinal ganglion cells.* J Neurophysiol, 1965. **28**(5): p. 832-49.
23. Bai, X., et al., *Mechanisms that limit the light stimulus frequency following through the DL-2-amino-4-phosphonobutyric acid sensitive and insensitive rod Off-pathways.* Neuroscience, 2009. **162**(1): p. 184-94.
24. Carcieri, S.M., A.L. Jacobs, and S. Nirenberg, *Classification of retinal ganglion cells: a statistical approach.* J Neurophysiol, 2003. **90**(3): p. 1704-13.
25. Sagdullaev, B.T. and M.A. McCall, *Stimulus size and intensity alter fundamental receptive-field properties of mouse retinal ganglion cells in vivo.* Vis Neurosci, 2005. **22**(5): p. 649-59.
26. Smirnakis, S.M., et al., *Adaptation of retinal processing to image contrast and spatial scale.* Nature, 1997. **386**(6620): p. 69-73.
27. Brown, S.P. and R.H. Masland, *Spatial scale and cellular substrate of contrast adaptation by retinal ganglion cells.* Nat Neurosci, 2001. **4**(1): p. 44-51.
28. Chander, D. and E.J. Chichilnisky, *Adaptation to temporal contrast in primate and salamander retina.* J Neurosci, 2001. **21**(24): p. 9904-16.
29. Baccus, S.A. and M. Meister, *Fast and slow contrast adaptation in retinal circuitry.* Neuron, 2002. **36**(5): p. 909-19.
30. Rieke, F., *Temporal contrast adaptation in salamander bipolar cells.* J Neurosci, 2001. **21**(23): p. 9445-54.
31. Demb, J.B., *Functional circuitry of visual adaptation in the retina.* J Physiol, 2008. **586**(Pt 18): p. 4377-84.

32. Jarsky, T., et al., *A synaptic mechanism for retinal adaptation to luminance and contrast.* J Neurosci, 2011. **31**(30): p. 11003-15.
33. Biel, M., et al., *Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3.* Proc Natl Acad Sci U S A, 1999. **96**(13): p. 7553-7.
34. Humphries, M.M., et al., *Retinopathy induced in mice by targeted disruption of the rhodopsin gene.* Nat Genet, 1997. **15**(2): p. 216-9.
35. Hattar, S., et al., *Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity.* Science, 2002. **295**(5557): p. 1065-70.
36. Umino, Y., E. Solessio, and R.B. Barlow, *Speed, spatial, and temporal tuning of rod and cone vision in mouse.* J Neurosci, 2008. **28**(1): p. 189-98.
37. Nikonov, S.S., et al., *Photoreceptors of Nrl -/- mice coexpress functional S- and M-cone opsins having distinct inactivation mechanisms.* J Gen Physiol, 2005. **125**(3): p. 287-304.
38. Nikonov, S.S., et al., *Physiological features of the S- and M-cone photoreceptors of wild-type mice from single-cell recordings.* J Gen Physiol, 2006. **127**(4): p. 359-74.
39. Chichilnisky, E.J., *A simple white noise analysis of neuronal light responses.* Network, 2001. **12**(2): p. 199-213.
40. Kastner, D.B. and S.A. Baccus, *Coordinated dynamic encoding in the retina using opposing forms of plasticity.* Nat Neurosci, 2011. **14**(10): p. 1317-22.
41. Pepperberg, D.R., et al., *Visual pigment and photoreceptor sensitivity in the isolated skate retina.* J Gen Physiol, 1978. **71**(4): p. 369-96.
42. Wang, Y.V., M. Weick, and J.B. Demb, *Spectral and temporal sensitivity of cone-mediated responses in mouse retinal ganglion cells.* J Neurosci, 2011. **31**(21): p. 7670-81.
43. Berson, D.M., F.A. Dunn, and M. Takao, *Phototransduction by retinal ganglion cells that set the circadian clock.* Science, 2002. **295**(5557): p. 1070-3.
44. Hu, C., D.D. Hill, and K.Y. Wong, *Intrinsic physiological properties of the five types of mouse ganglion-cell photoreceptors.* J Neurophysiol, 2013. **109**(7): p. 1876-89.
45. Munch, M. and A. Kawasaki, *Intrinsically photosensitive retinal ganglion cells: classification, function and clinical implications.* Curr Opin Neurol, 2013. **26**(1): p. 45-51.
46. Schmidt, T.M., S.K. Chen, and S. Hattar, *Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions.* Trends Neurosci, 2011. **34**(11): p. 572-80.
47. Rollag, M.D., D.M. Berson, and I. Provencio, *Melanopsin, ganglion-cell photoreceptors, and mammalian photoentrainment.* J Biol Rhythms, 2003. **18**(3): p. 227-34.

48. Kuffler, S.W., *Discharge patterns and functional organization of mammalian retina.* J Neurophysiol, 1953. **16**(1): p. 37-68.
49. Wiesel, T.N., *Receptive fields of ganglion cells in the cat's retina.* J Physiol, 1960. **153**: p. 583-94.
50. Wang, J.S. and V.J. Kefalov, *An alternative pathway mediates the mouse and human cone visual cycle.* Curr Biol, 2009. **19**(19): p. 1665-9.
51. Bovolenta, P. and E. Cisneros, *Retinitis pigmentosa: cone photoreceptors starving to death.* Nat Neurosci, 2009. **12**(1): p. 5-6.
52. Michalakis, S., et al., *Impaired opsin targeting and cone photoreceptor migration in the retina of mice lacking the cyclic nucleotide-gated channel CNGA3.* Invest Ophthalmol Vis Sci, 2005. **46**(4): p. 1516-24.
53. Naarendorp, F., et al., *Dark light, rod saturation, and the absolute and incremental sensitivity of mouse cone vision.* J Neurosci, 2010. **30**(37): p. 12495-507.
54. Hess, R.F. and K. Nordby, *Spatial and temporal properties of human rod vision in the achromat.* J Physiol, 1986. **371**: p. 387-406.
55. Hess, R.F. and K. Nordby, *Spatial and temporal limits of vision in the achromat.* J Physiol, 1986. **371**: p. 365-85.
56. Michaelides, M., D.M. Hunt, and A.T. Moore, *The cone dysfunction syndromes.* Br J Ophthalmol, 2004. **88**(2): p. 291-7.
57. Burkhardt, D.A., *Light adaptation and photopigment bleaching in cone photoreceptors in situ in the retina of the turtle.* J Neurosci, 1994. **14**(3 Pt 1): p. 1091-105.
58. Chichilnisky, E.J. and R.S. Kalmar, *Functional asymmetries in ON and OFF ganglion cells of primate retina.* J Neurosci, 2002. **22**(7): p. 2737-47.
59. Demb, J.B., *Multiple mechanisms for contrast adaptation in the retina.* Neuron, 2002. **36**(5): p. 781-3.
60. Ozuysal, Y. and S.A. Baccus, *Linking the computational structure of variance adaptation to biophysical mechanisms.* Neuron, 2012. **73**(5): p. 1002-15.
61. Rizzoli, S.O. and W.J. Betz, *Synaptic vesicle pools.* Nat Rev Neurosci, 2005. **6**(1): p. 57-69.
62. Wark, B., A. Fairhall, and F. Rieke, *Timescales of inference in visual adaptation.* Neuron, 2009. **61**(5): p. 750-61.
63. Beaudoin, D.L., M.B. Manookin, and J.B. Demb, *Distinct expressions of contrast gain control in parallel synaptic pathways converging on a retinal ganglion cell.* J Physiol, 2008. **586**(Pt 22): p. 5487-502.

64. Mante, V., et al., *Independence of luminance and contrast in natural scenes and in the early visual system.* Nat Neurosci, 2005. **8**(12): p. 1690-7.
65. Dunn, F.A., M.J. Lankheet, and F. Rieke, *Light adaptation in cone vision involves switching between receptor and post-receptor sites.* Nature, 2007. **449**(7162): p. 603-6.
66. Bialek, W., et al., *Reading a neural code.* Science, 1991. **252**(5014): p. 1854-7.
67. Stanley, G.B., *Reading and writing the neural code.* Nat Neurosci, 2013. **16**(3): p. 259-63.
68. Zaghloul, K.A., K. Boahen, and J.B. Demb, *Different circuits for ON and OFF retinal ganglion cells cause different contrast sensitivities.* J Neurosci, 2003. **23**(7): p. 2645-54.
69. Liang, Z. and M.A. Freed, *The ON pathway rectifies the OFF pathway of the mammalian retina.* J Neurosci, 2010. **30**(16): p. 5533-43.
70. Averbeck, B.B., P.E. Latham, and A. Pouget, *Neural correlations, population coding and computation.* Nat Rev Neurosci, 2006. **7**(5): p. 358-66.
71. Koch, K., et al., *Efficiency of information transmission by retinal ganglion cells.* Curr Biol, 2004. **14**(17): p. 1523-30.
72. Pandarinath, C., J.D. Victor, and S. Nirenberg, *Symmetry breakdown in the ON and OFF pathways of the retina at night: functional implications.* J Neurosci, 2010. **30**(30): p. 10006-14.
73. Sterling, P., *How retinal Circuits Optimize the Transfer of Visual Information.* in The Visual Neuroscience, 2004: p. 234-259.
74. Rockhill, R.L., et al., *The diversity of ganglion cells in a mammalian retina.* J Neurosci, 2002. **22**(9): p. 3831-43.
75. Kuffler, S.W., R. Fitzhugh, and H.B. Barlow, *Maintained activity in the cat's retina in light and darkness.* J Gen Physiol, 1957. **40**(5): p. 683-702.
76. Calkins, D.J. and P. Sterling, *Evidence that circuits for spatial and color vision segregate at the first retinal synapse.* Neuron, 1999. **24**(2): p. 313-21.
77. Geffen, M.N., S.E. de Vries, and M. Meister, *Retinal ganglion cells can rapidly change polarity from Off to On.* PLoS Biol, 2007. **5**(3): p. e65.
78. Kaplan, E. and E. Benardete, *The dynamics of primate retinal ganglion cells.* Prog Brain Res, 2001. **134**: p. 17-34.
79. Renteria, R.C., et al., *Intrinsic ON responses of the retinal OFF pathway are suppressed by the ON pathway.* J Neurosci, 2006. **26**(46): p. 11857-69.
80. Roska, B. and F. Werblin, *Vertical interactions across ten parallel, stacked representations in the mammalian retina.* Nature, 2001. **410**(6828): p. 583-7.

81. Werblin, F., B. Roska, and D. Balya, *Parallel processing in the mammalian retina: lateral and vertical interactions across stacked representations.* Prog Brain Res, 2001. **131**: p. 229-38.
82. Awatramani, G.B. and M.M. Slaughter, *Origin of transient and sustained responses in ganglion cells of the retina.* J Neurosci, 2000. **20**(18): p. 7087-95.
83. DeVries, S.H. and E.A. Schwartz, *Kainate receptors mediate synaptic transmission between cones and 'Off' bipolar cells in a mammalian retina.* Nature, 1999. **397**(6715): p. 157-60.
84. DeVries, S.H., *Bipolar cells use kainate and AMPA receptors to filter visual information into separate channels.* Neuron, 2000. **28**(3): p. 847-56.
85. Euler, T. and R.H. Masland, *Light-evoked responses of bipolar cells in a mammalian retina.* J Neurophysiol, 2000. **83**(4): p. 1817-29.
86. Ichinose, T., C.R. Shields, and P.D. Lukasiewicz, *Sodium channels in transient retinal bipolar cells enhance visual responses in ganglion cells.* J Neurosci, 2005. **25**(7): p. 1856-65.
87. DeVries, S.H., W. Li, and S. Saszik, *Parallel processing in two transmitter microenvironments at the cone photoreceptor synapse.* Neuron, 2006. **50**(5): p. 735-48.

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