Luminance adaptation increased the contrast sensitivity of retinal ganglion cells

[VISION, RETINA]

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Abstract

In the present study, the activity changes of chicken retinal ganglion cells in response to light stimuli with defined contrast were investigated, in the presence of various levels of sustained background illumination. Following a step increase of light illumination, the firing rate of most retinal ganglion cells increased abruptly, and then decreased to a steady-state level with a much lower firing rate during the sustained application of light. However, when a test flash was applied, which superimposed the prolonged background illumination, an increased firing rate was observed. Moreover, the neuron firing rate was increased to a greater extent when the intensity of the background illumination was higher. This may suggest that the neuron sensitivity can be modified by the background illumination level, although the neuron firing rate was reduced during sustained illumination.

INTRODUCTION

In the early stages of the visual pathway, two distinct types of adaptation were observed, which were termed light adaptation [3,10] and contrast adaptation [1,4,11,12]. The biological significance for retinal neurons to have adaptation is that individual neurons have

limited signaling ranges but must encode stimulus intensities that vary over much wider ranges. The property of adaptation allows the visual system to adjust its sensitivity and activity in facing the varying environment and thus to deal with it better [2]. Without such adaptive adjustment, small signals will drown in neuronal noise, and large signals will saturate the system.

Adaptation to the mean illumination intensity, which is termed ‘light adaptation’, regulates the gain and dynamics of retinal responses to environmental illumination [2]. After an abrupt change in environmental illuminance level, there is evidence of a fast response mechanism (acting over the first tens of milliseconds) with the increased firing of retinal ganglion cells, and a slower adaptation mechanism (taking several seconds to completion) with decreased firing activities of the retinal ganglion cells [5,8].

A second type of adaptation – called ‘contrast adaptation’ – responds not to the mean level of the visual stimulus but to its ‘contrast’, which is the relative fluctuation around the mean illumination [2,15]. Similarly, following a switching from a low-contrast environment to one of high contrast, the firing rate of retinal ganglion cells increased abruptly (<0.1 s), and then decreased exponentially to a much lower level (~10 s) [1]. By matching their sensitivity to the fluctuations in the inputs, visual neurons can efficiently encode signals with widely varying temporal and spatial structure [11,12,15].

Both light adaptation and contrast adaptation revealed phenomena analogous to each other: an increase of activity at the transient switching from weak stimulus to intense stimulus, and a slow reduction in firing rate during adaptation to sustained stimulation. On the other hand, the biological significance of adaptation is to adjust the response activity and sensitivity of neurons. The purpose of this study is therefore to investigate the possible relationship between the sustained illumination and the neuron's contrast sensitivities to the fast change in stimulus.

In the present study, the retinal ganglion cells that were examined revealed typical phenomena of light adaptation and contrast adaptation, similar to the observation made in other species [1,4,15]. After 30 s of sustained unitary illumination, the neurons were exposed to white flashes with defined contrast. It was observed that neuron firing activities in response to the test flashes were dependent on the background illumination level. Given a flash with defined contrast, more spikes could be elicited when the sustained illumination was brighter. These results suggest that neuron sensitivities can be

modified by the background illumination level, although neuron firing rate was reduced on exposure to sustained illumination.

METHODS

Experimental procedure:

Extracellular recordings were made in isolated chicken retina using a multi-electrode array (MEA60, Multi Channel Systems MCS GmbH, Reutlingen, Germany) that consisted of a 5×5 cm transparent quartz glass plate with 60 substrate integrated and insulated golden connection lanes running from connector pads at the edge of the plate to the electrode matrix at the center. The tips of these conductors serve as the electrodes (10 µm in diameter) and are arranged in an 8×8 matrix with 100 µm tip-to-tip distances [6,7]. The patterned area of microelectrodes is enclosed in a glass ring forming a tissue perfusion chamber.

Eyes were obtained from newly hatched chickens (about 2–4 days old). After decapitation and enucleation of the eye under dim red illumination, the eyeball was hemisected with fine scissors or a razor blade. The cornea and lens were separated from the posterior half. The vitreous that adhered to the retinal surface was removed with tweezers. The isolated retina was dissected into 4×4 mm squares. For electrical recording, a small piece of retinal segment was attached with the ganglion cell side to the surface of the MEA. For improving adhesion, the MEA was initially covered with 3 µl dissolved cellulose nitrate solution (1.0 cm^2 of Sartorius cellulose nitrate filter in 10.0 ml methanol) and dried in air. The preparation was kept in standard perfusate containing (in mM) 100.0 NaCl, 5.0 KCl, 3.0 $MgCl_2$, 1.8 $CaCl_2$, 25.0 $NaHCO_3$ and 25.0 glucose, and bubbled with a mixed gas of 95% O_2 and 5% CO_2 with a pH value of 7.5±0.2. The perfusion was delivered at a rate of 0.8 ml/min and the tissue was kept at 37°C using a temperature control unit (Thermostat HC-X, Multi Channel Systems MCS GmbH) [13]. An Ag/AgCl pellet, which was dipped into the bath solution, acted as the reference electrode.

Multi-unit photoresponses were simultaneously recorded from all 60 electrodes of the MEA and were amplified with a 60-channel amplifier (single-ended amplifier, bandwidth 10 Hz to 3.4 kHz, amplification 1200×, amplifier input impedance >10^{10} [OMEGA], output impedance 330 [OMEGA]) [6]. The selected channels of recording along with one stimulus signal were digitized with a commercial multiplexed data acquisition system (MCRack) and stored in a Pentium-based

computer. The data were sampled at a rate of 20 kHz, plotted on screen instantaneously, and then stored on the hard disk for off-line analyses.

Spike events recorded from each electrode were then classified into neuronal activities on the basis of principal component analysis (PCA), as described in previous reports [7,9].

Stimuli:

Light stimulus was generated using a computer monitor, and was focused to form a 0.7×0.7 mm full-field white light spot on the isolated retina via a lens system. Three stimulation protocols were applied.

Protocol A consisted of a sustained full-field spot switching between light and dark every 32 s (Fig. 1). This protocol was used to examine the properties of retinal ganglion cells in response to sustained light illumination.

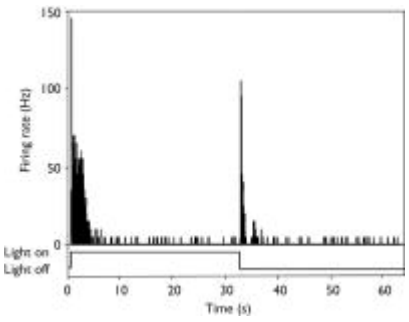

Fig. 1. Firing activities of an ON–OFF cell in response to spatially uniform light stimulation. Firing rate was computed for one ON–OFF cell, in 50-ms time bins averaged over four trials. The lower trace illustrates the time course of the light stimulation between dark (0 mW/m^2) and light (10 mW/m^2).

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Protocol B was a full-field flickering stimulation sequence, the intensity of which was renewed every 50 ms following a pseudorandom m-sequence with a given mean level and defined contrast of light illumination (Fig. 2b) [14].

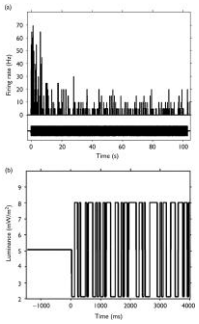

Fig. 2. Ganglion cell activity changes in adaptation to temporal contrast. (**a**) Responses of a ganglion cell to full-field pseudorandom flashes (temporal contrast). The lower trace indicates the contrast (0.5) of the flashing stimuli. The mean level of luminance intensity is 5.9 mW/m^2. The firing response was calculated using 50-ms time bins. (**b**) Temporal pattern of intensity changes of the stimulus in (a) shown in an expanded time scale.

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Protocol C consisted of a 30-s sustained illumination at requested levels, followed by a series of test flashes. The test flashes had a light-ON duration of 1000 ms and light-OFF intervals of 9000 ms, with the mean intensity equal to the leading conditioning light and contrast defined as requested (Fig. 3).

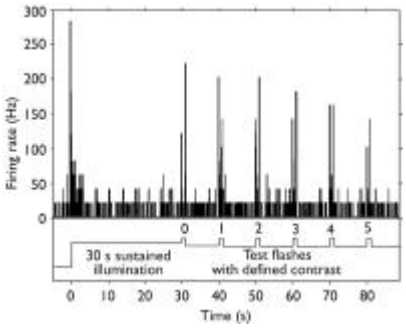

Fig. 3. Response activity of an ON-retinal ganglion cell to test flashes superimposed on the leading sustained illumination. The firing activity of an ON-ganglion cell in response to stimuli (lower trace) consisting of a 30-s sustained illumination (3.9 mW/m^2) followed by six test flashes (marked 0, 1, 2, 3, 4 and 5 in the figure) with defined contrast (0.167 in this example). The light-ON duration for each test flash is 1000 ms and the light-OFF intervals are 9000 ms, with the mean intensity level equal to the leading sustained illumination.

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The light intensity of the monitor's output during each stimulus was measured using a light detection system manuf. (IL1400,

Newburyport, MA, USA) at the retina level when the light was delivered through the optical system. The contrast of stimuli is defined as $(L_{max}-L_{min})/(L_{max}+L_{min})$, where L_{max} and L_{min} are the maximum and minimum luminance in each flash, respectively.

RESULTS

For the recordings made in this study, most retinal ganglion cells showed increased activities at light-ON and/or light-OFF transients.

Figure 1 is an example of the activity changes of an ON–OFF retinal ganglion cell in response to light-ON and light-OFF transients. Following a step increase in light intensity, the firing rate of the cell increased abruptly, with a peak level reaching 150 Hz, and then decreased to a much lower level of less than 5 Hz. Similarly, firing activity had a swift increase after the incident of light offset, which decreased to a very low level afterwards.

The retinal ganglion cells also adapt to the range of fluctuations around the mean intensity, which is termed ‘contrast adaptation’ [12,15]. In our experiment, those cells with increased activities at the light-ON transient also had obvious contrast adaptation as shown in Fig. 2a. Such ‘contrast adaptation’ was observed from all the ON, OFF and ON–OFF transient cells investigated, but the phenomenon was more significant in the ON-cells or during the ON-response of the ON–OFF cells. Therefore, we chose to use the cell's ON-response for statistical analyses.

It was previously reported that the presence of background illumination would result in some changes in the retinal neurons' sensitivity to light intensity changes. The ganglion cell's sensitivity to contrast stimulus was relatively low immediately after the onset of ambient illumination, and a slow increase in sensitivity could be observed during the sustained application of light [8]. To test the relationship between the intensity of sustained ambient illumination and the neurons' sensitivity to contrast discrimination, light stimuli consisting of a 30-s sustained illumination followed by six test flashes with defined contrast were applied in this study. One example of the experimental results is plotted in Fig. 3, where a sustained full-field white light (3.9 mW/m^2) was applied for 30 s prior to the application of test flashes. It is shown that the ganglion cell's firing activity increased to a peak level of 280 Hz at the light-ON transient; this was followed by a gradual decrease to a lower level of around 20 Hz during the sustained application of unitary illumination. However, when

superimposed white test flashes (with contrast being 0.167) were applied, increased spikes were detected again.

Neuron activities were then measured following the time schedule illustrated in Fig. 3, with test flashes given at various contrast levels (ranging from 0.1 to 0.45), in the presence of sustained (30 s) background illumination (3.9 mW/m^2). Generally, the firing rate of retinal ganglion cells increased when the contrast of the testing flash was increased. Similar results were observed from 12 retinas under investigation. An example in Fig. 4 gives the statistical results of 11 ON–OFF ganglion cells recorded from one retina. In this plot, the activity of each neuron was measured by looking into the cell's responses to five white flashes (flashes 1, 2, 3, 4 and 5 as marked in Fig. 3) with defined contrasts. For each cell, the spikes fired during the light-ON period were counted and averaged for the cell's response to the five flashes, for each level of contrast. The relative firing index during response to various contrast tests was then calculated for each cell by normalizing the cell's response activity against its maximal level among these events, as plotted by closed symbols in Fig. 4a. The relative activity across the neurons for each contrast level is presented by the curve in the figure (mean±SD). It is shown that more spikes could be elicited by a light flash with a higher contrast.

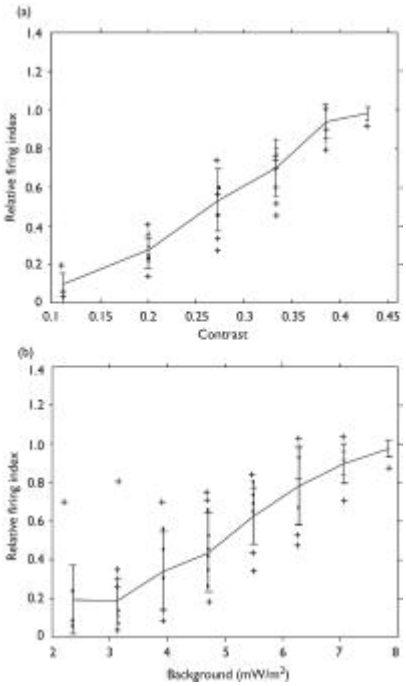

Fig. 4. Relative firing activities calculated during various stimulation conditions. (**a**) Firing activity (closed symbols) detected when the retina was exposed to test flashes with various contrast levels (ranging from 0.1 to 4.5) after application of a 30-s sustained background illumination (3.9 mW/m^2). (**b**) Firing activity (closed symbols) elicited by flashes with a defined contrast (0.167) superimposed on a 30-s sustained background illumination (ranging from 2.3 to 7.8 mW/m^2). Data were collected from 11 ganglion cells in one retina.

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Then, keeping the test contrast at a level of 0.167, the sustained illumination level was changed within a range from 2.3 to 7.8 mW/m^2. Experimental results were observed from 11 ganglion cells, and the statistics are plotted in Fig. 4b. The averaged activity was calculated for each cell across various stimulation levels and normalized against the maximum, as represented using closed symbols. The average across neurons is illustrated by the curve (mean±SD). It is shown that when the test flashes were applied with a certain contrast level, the response activity of neurons was closely related to the intensity of the continuously applied background illumination. More spikes were elicited when the intensity of the background illumination was stronger.

Because the contrast is defined as $(L_{max}-L_{min})/(L_{max}+L_{min})$, it might be argued that for a certain level of contrast, the difference between the high and low levels of illumination should be greater when the mean illumination is brighter, so the increased difference might be responsible for the increased firing activities when various levels of sustained illumination were applied. To avoid such ambiguity, another set of control experiments was therefore performed, in which the difference between the high and low levels of the test flashes was kept constant, while the conditioning background illumination was applied at various levels. Again, more spikes could be observed when the intensity of the sustained illumination was increased. Figure 5 shows the firing activities of 11 neurons (from one retina) in response to the flashes with $L_{max}-L_{min}=1.96$ mW/m^2, which superimposed on the continuously applied (30 s) background illumination. Similar observations were made in a total number of 12 chicken retinas.

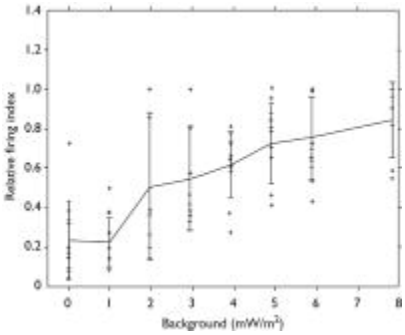

Fig. 5. Relative firing activities to flashes with fixed difference versus various levels of background illumination. The firing activities (closed symbols) in response to light flashes with fixed intensity (1.96 mW/m^2) in the presence of the background illumination. Data were collected from 11 ganglion cells of one retina.

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DISCUSSIONS

The chicken retinal ganglion cells examined in this study showed typical light adaptation (Fig. 1) and contrast adaptation (Fig. 2), which is similar to that observed from retinal ganglion cells in other species [1,4,15]. Both luminance adaptation and contrast adaptation revealed two phenomena when retinal neurons were exposed to stimulus changes: a fast response (<0.1 s) and a slow adaptation (~10 s). The prolonged presence of contrast or luminance stimuli reduced the firing rate of retinal ganglion cells during the adaptation phase [8,15]. However, our results suggest that the information might be stored in the retinal neural network in spite of the reduction in firing rate during slow adaptation.

This phenomenon was observed from retinal ganglion cells, including ON, OFF and ON–OFF subtypes, investigated in our experiment. However, because the phenomenon was more significant in ON-cells or during the ON-response of the ON–OFF cells, we chose to use the cell's ON-response for statistical analyses.

The basic finding in this study was that after the completion of luminance adaptation, a significant increase in neuron firing rate could be observed when the retina was exposed to light flashes superimposed on the continuously applied background illumination. Moreover, sensitivities to the contrast stimuli were dependent on the level of background illumination (Fig. 4). More spikes were elicited by the flashes when the sustained illumination became stronger. The results suggest that neuron sensitivities can be modified by the presence of background illumination, although the neuron firing rate was reduced during sustained illumination.

It has been previously reported that the increase in ambient illumination could result in a rapid increase in the ganglion cell's firing rate, and in the meantime reduce the cell's sensitivity to the superimposed light stimulus [9]. Similarly, a recent study demonstrated that fast contrast adaptation was marked by increased firings and decreased sensitivity [15]. During the sustained illumination applied in this study, the neuron firing rate was reduced; however, the sensitivity was increased. Furthermore, when the intensity of background illumination was increased, the ability for contrast discrimination was increased accordingly.

These results suggest that information on ambient illumination might be stored in the retinal neural network, which results in some increase in the ganglion cells' sensitivity of contrast discrimination. A broadband adaptive mechanism could, therefore, help explain why the contrast discrimination increased when the background illumination was increased. However, the detailed mechanism is not clear, and needs further investigation.

CONCLUSION

1. Slow luminance adaptation reduced the firing rate but increased the contrast discrimination of retinal ganglion cells.
2. Information on ambient illumination might be stored in the retinal neural network, which results in some increase in the ganglion cell's sensitivity to contrast discrimination.

Acknowledgements

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