Interactive report

Long-term light history modulates the light response kinetics of luminosity (L)-type horizontal cells in the roach retina

A. Jenkins, M.W. Hankins*

Imperial College School of Medicine, Division of Neuroscience and Psychological Medicine, Department of Integrative and Molecular Neuroscience, Fulham Palace Road, London W6 8RF, UK

Accepted 17 October 2000

Abstract

We have examined the effects of prolonged periods of darkness on the responses of luminosity-type horizontal cells (L-HCs) in the freshwater cyprinid, *Rutilus rutilus*. Two groups of retinae were compared, those recorded after 10 min dark adaptation (SA) and those recorded after 3 h dark adaptation (LA). The results suggest that long-term light history does not modify the general responsiveness of the L-HCs in this species. However, there are apparent changes in the receptive field of the cells and modifications to the kinetics of the light-evoked response. The kinetics changes involve both a delay in the onset of light response and a selective effect on the hyperpolarizing light-ON response. Thus the mean time constant ($\tau$) for the SA cells was 32.4±2.39 ms ($n=62$), whilst that for the LA cells was 53.4±3.03 ms ($n=61$). These effects occur in the absence of changes in the relative spectral sensitivity or threshold sensitivity of the HCs. The results suggest that in some vertebrate retinae, prolonged darkness (light-history) may regulate long-term plasticity in the kinetics of the cone–HC pathway. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Sensory systems

Topic: Retina and photoreceptors

Keywords: Retina; Horizontal cell; Adaptation; Plasticity

1. Introduction

There is growing interest in the mechanisms that subserve long-term plasticity of the retinal network. Numerous studies in the vertebrate retina suggest that prolonged dark-adaptation has a profound effect on the responsiveness of cone pathways. Following prolonged dark adaptation, it was first shown that there are anatomical changes in the cone pedicles, which were proposed to be correlated with the loss of colour opponency and cone function at the ganglion cell level of the goldfish retina [16]. Subsequently it was demonstrated in the goldfish that prolonged darkness was associated with suppression of responsiveness of the L-type horizontal cells [32]. Furthermore, it was shown that such dark-suppression was associated with a reduction in the horizontal cell (HC) receptive field [13,14]. By analogy, this effect was interpreted in relation to changes in the activity of the dopaminergic interplexiform neurones, which have been shown to regulate HC coupling [3,20]. However, recent studies have established that dopamine release is not enhanced by periods of prolonged darkness in the daytime [27].

In a detailed study of the white perch retina (*Morone americana*), a similar phenomenon was described [21,29,30], although the extent of dark-suppression was significantly more pronounced than in carp. This suggests that the expression of dark-suppression may have a species-dependent component. It has also been established in the white perch that there are significant changes in the kinetics of the light response after prolonged darkness [12]. However, these changes were associated with profound changes in the threshold sensitivity and chromatic sensitivity, suggesting they are mediated through rod–cone interaction.

Preliminary experiments on the L-HCs in the roach...
retina (*Rutilus rutilus*) recorded through a 24-h diurnal cycle, reported no significant modulation of the HC light response amplitude, but noted significant changes in the light response kinetics [9]. L-HCs in the roach, like most cyprinids receive a dominant LWS (red) cone input, with a weak MWS (green) cone input [2]. This raises the question as to whether long-term light history or circadian/diurnal regulation might drive the changes in cone-driven L-HC kinetics. Interestingly, long-term light-history appears to affect the temporal response of the human photopic (cone) electroretinogram [7]. Thus it was shown that the b-wave implicit time of the photopic cone ERG exhibits a pronounced sinusoidal oscillation, the period of oscillation being ~24 h with peak temporal response in the middle of the day. This raises the possibility that the temporal response of cone pathways is modulated according to long-term light-history.

We have therefore examined the effect of long-term dark adaptation on the light responses of L-HCs cells in the roach retina. Some of this work has been reported in abstract form [5].

2. Methods

Experiments were performed on the isolated freshwater cyprinid *Rutilus rutilus* (roach) retina. The fish were maintained in outdoor holding tanks under natural day–night ambient lighting. The experiments were performed during the middle 3 h of subjective day. The fish were dark-adapted according to one of two regimes. In the case of short dark adaptation (SA) the fish were placed in absolute darkness for 10 min, in long dark adaptation (LA) they were placed in darkness for 3 h. After this period they were killed (T=0) and the retinae isolated under IR illumination. After the preadaptation both groups of retinae experienced the same conditions throughout the recording period. The retinae were placed photoreceptor side upward in a moist oxygenated plexiglass recording chamber. The chamber was semi-enclosed to allow electrode penetration from above. The experimental recording period then lasted from $T=5$ min to $T=20$ min. Recordings were made from luminosity-type horizontal cells (L-HCs) using KCl-filled glass micropipettes (80–100 MΩ), and conventional recording methods. Cell recordings were digitized and recorded (Axon Instruments) for detailed post-experimental analysis. Additional experiments (Fig. 6) utilized the superfused roach retina preparation [8], where the retina was clamped photoreceptor side up in a plexiglass chamber and the retina perfused from the photoreceptor surface with cyprinid ringer.

The light stimuli were generated using a twin-channel Maxwellian optical system. This provided a range of spectral (450, 486, 531, 570, 620 and 650 nm) and spatial stimuli (spot: 0.56–4.1 mm o.d. and annulus 0.9 mm i.d., 4.1 mm o.d.) that were projected from below the preparation. Cells were impaled with no photic stimuli and initially characterized using a single paired red and green flash of moderate intensity (650 nm, 531 nm, $I_{\text{max}}=6.7$ μW cm$^{-2}$, 450–500 ms). These responses provided the data for the principal amplitude and kinetic analysis with a minimal light exposure (SA, n=69; LA, n=79). The responses of the cells were then additionally characterized using a range of spectral or spatial stimuli. These responses provided information confirming the relative spectral sensitivity, and allowed us to assess the extent of the HC receptive fields of individual cells. After the experiments, detailed measurements of the individual light evoked responses (S-potential) were performed in accordance to those outlined in Fig. 1. This involved simple amplitude measurement of the principal components, together with a range of kinetic measurements on the rate of hyperpolarization and depolarization. An assessment of receptive field size was made using two methods. Firstly, the amplitude of the annulus response for each cell was extrapolated to an equivalent amplitude response spot area (Fig. 1c). Secondly, the ratio of the amplitude of the annulus and spot (annulus/spot) was calculated for each cell to give a measure of the relative strength of surround response.

3. Results

3.1. The amplitude of the light response and the receptive field properties

We examined the general light responsiveness of the L-HCs cells using a number of approaches. First we examined the HC response amplitudes to the initial single red stimulus (650 nm, 4.1 mm o.d., 6.7 μW cm$^{-2}$). We found no significant difference in the amplitudes in the two groups; thus the response of the SA cells was 19.6±4.1 mV and that for the LA cells was 20.2±4.1 mV (mean±1 S.E.M.). For a population of cells, we examined the $V/\log I$ relation and found no significant differences in amplitude at any of the intensities (Fig. 2a). This was also confirmed by measuring the 1/2$V_{\text{max}}$ stimulus intensities for the individual cells, and the mean intensity for SA retinae ($\sim 1.2\pm0.2$ log units) was not significantly different from the LA retinae ($\sim 1.3\pm0.27$ log units). We also examined the dark resting membrane potential ($E_m$) for the two groups, and again found no significant difference in the distribution or mean level (SA, $-36.7\pm1.5$ mV; LA, $-38.1\pm1.5$ mV). The relative spectral sensitivity of the cells was assessed at a range of stimulus wavelengths, the results showed no significant difference between the SA and LA cells (Fig. 2b). In the case of six cells, the spectral sensitivity was assessed using the photopic stimuli presented upon a rod saturating (500 nm) background and this also failed to reveal differences between the two conditions (not illustrated). In addition, we examined the $V/\log I$ and spectral sensitivity curves for L-HCs obtained from a
Fig. 1. Measurement of HC light response components. (a) Schematic of the light response waveform of an L-HC to a ~500-ms stimulus. Amplitude measurements were made relative to dark resting membrane potential of the steady state light response at 450 ms. ON-relaxation amplitude was measured from the light peak to the steady state level. The OFF-transient peak was measured relative to $E_m$. Hyperpolarizing and depolarizing rates of change were measured within the pseudo-linear range (25–75%) of the light response (S). (b) Time to reach 2.5, 5, 10, 50 and 66% of the steady light response was also measured for each cell. (c) Method used to determine the equivalent area of the annulus response. The amplitude of the annulus light response of each cell (A) was translated to an equivalent area response (EA) by extrapolation from its response/spot size curve.

group of retinas obtained under infra-red illumination in the middle of the night. The data from the night-HCs were not significantly different from the SA and LA retinae (Fig. 2a,b).

Analysis of the cell responses to a range of spot and annulus stimuli were used to assess the general extent of HC receptive field. The results show there are highly significant ($P<0.001$) differences in the mean extent and distribution of receptive fields according to their long-term preadaptation history (Fig. 3). The annulus/spot ratios were significantly larger in the LA retinae, consistent with an increased sensitivity to the peripheral stimulus. In addition, when we examined the equivalent isoluminant area (see methods) for the annulus stimulus (0.9 mm i.d., 4.1 mm o.d.) we found this value to be 1.95±0.35 mm$^2$ in the SA cells and 5.68±0.59 mm$^2$ in the LA cells ($n=31$, a significant difference at $P<0.001$, $t$-test).

3.2. Changes in the L-HC light response (S-potential) waveform

Whilst we saw no difference in the amplitude of the light response, examination of the waveform suggested that the kinetics were modified following prolonged dark adaptation. To examine this change we took the cells first response to a single red-flash stimulus (650 nm), normalized the response to the steady-state light response level for each cell and averaged these waveforms. The results are shown in Fig. 4b, and establish that there are significant changes in the waveform, most clearly apparent in the speed of hyperpolarization at light-ON. SA cells were significantly faster than those recorded in LA retinae. Cells from the LA retinae were characterized by a slower rate of hyperpolarization, reflected by a long delay in reaching maximal amplitude. Furthermore, these differences in ON-kinetics were also apparent when cells were recorded in the presence of a rod-saturating (500 nm) background. Typical single light responses for SA and LA cells are given in Fig. 4a.

Using the waveform parameters outlined in Fig. 1, we performed a detailed analysis of the light responses recorded in the two groups of retinae. The latencies were examined at a number of %-response increments. The data revealed that the onset of hyperpolarization was significantly delayed in the LA cells compared to that of the SA group (Fig. 5a). The latency (threshold 2.5%) for the SA cells was 13.62±0.41 ms, whilst in the LA group it was 21.48±0.33 ms (mean±1 S.E.M.). We also examined the rate of hyperpolarization for the two groups of cells, by measuring the slope in the pseudo-linear portion of the

Fig. 2. (a) $V/log I$ response curves. Mean steady-state HC light response amplitude (mV) is plotted as a function of stimulus intensity. SA (□) and LA (■) are plotted together with the standard errors of means (±1 S.E.M.). Data are also shown for L-HCs recorded from retinæ obtained in the middle of the night under infra-red (⋯⋯). The data were statistically tested (t-test and Bonferroni correction) and none of the differences between SA and LA were found to be significant at the $P<0.05$ level ($n=19$). Stimulus 650 nm, spot 4.1 mm diameter (o.d.), Log 0=2.19×$10^{12}$ quanta cm$^{-2}$ s$^{-1}$. (b) Relative spectral sensitivity. The amplitude of the light response (mV) is plotted as a function of stimulus wavelength (λ, nm). Data are shown for the spectral sensitivity for the SA (□) and LA (■). Data are also shown for L-HCs recorded from retinæ obtained in the middle of the night under IR (⋯⋯). We found no significant differences in the SA and LA spectral response at any of the wavelengths tested (t-test, Bonferroni correction). Stimulus spot: 4.1 mm, nSA=37, nLA=25 ($I_{\text{max}}$ 6.7 μW cm$^{-2}$).

The light responses of individual HCs can include additional waveform components, including a light-ON relaxation (roll-back) before the response settles to the steady state (450 ms), and/or a light-OFF transient (depolarizing overshoot). Where present, we measured the amplitudes of these components in accordance with the definition outlined in Fig. 1. When we examined the mean amplitudes of both these components in the two groups of preadaptation conditions, we found no significant differences in their relative expression (Table 1). Furthermore the percentage of cells that expressed either of these components did not vary according to the light-history.

3.3. Horizontal cell responses in the superfused retinal preparations

We performed a number of experiments utilizing SA and LA retinae recorded in superfused retinal preparations ($n=25$). We found that in general the responses recorded from L-HCs in superfused retinae were slower. However, the relative difference between SA and LA retinae was maintained, with SA cells displaying characteristically faster responses at light-ON (Fig. 6a). We also examined the effect of exogenous dopamine (DA) in these prepara-
Fig. 4. HC light response waveform. (a) The S-potential light response for a typical cell from the SA group (---) and the LA group (-----). The waveforms are normalized to the steady state light level. Stimuli 650 nm, spot 4.1 mm. (b) The averaged normalized waveform range (±1 S.E.M. error spreads) plotted for the total cell populations in the study. Note the clear shift in the kinetics of the light-ON hyperpolarization in the LA group combined with a significant delay to reach steady state (nSA=74, nLA=87).

Fig. 5. Kinetics of the HC light response. (a) Delay in the onset of the light response. In this graph the time taken to reach 2.5, 5, 10, 50 and 66% of the steady-state amplitude are shown (±1 S.E.M.). The differences between the SA and LA cells were all significant at the P<0.001 level. The latency to 2.5% threshold was 13.62±0.41 ms for the SA cells and 21.48±0.33 ms for the LA cells (mean±1 S.E.M.). (b) The rate of hyperpolarization at light-ON. A comparison of the absolute (mV/ms) and normalized (%/ms) rates for the SA and LA cells, these differences were both significant at the P<0.001 level. (c) The rates, absolute and normalized, for the depolarization at light-OFF, showing no significant difference between the SA and LA cell populations.

4. Discussion

In this study we have examined the influence of long-term pre-adaptation on the light responses of luminosity-type horizontal cells. In the case of the SA condition, retinae were dark adapted for 10 min prior to dissection, whilst in the LA group they were dark-adapted for 3 h. The experiments all took place at a very similar point (within 3 h of midday) of the natural diurnal cycle, so as to avoid potential variation in the retinal circadian status. In these studies we primarily recorded from the non-perfused preparation, although broadly similar results were found in our experiments with the SA and LA retinae in superfused preparations (Fig. 6).

When we examined the steady-state amplitudes of the light responses of L-HCs and their V/log I functions, we found no difference in the cells recorded from the two conditions (Fig. 2a). Furthermore, there was no significant change in the relative spectral sensitivity (Fig. 2b), such as that previously shown in the white perch [12]. This suggests that the length of preadaption (light-history) does not significantly affect the immediate responsiveness of the L-type horizontal cells in the roach retina. Similarly, we found no effect of light history on the resting membrane potential. In these studies, we were able to record from L-HCs in retinae that had been in darkness for up to 3 h (LA) and dissected under infra-red. In most cases, the cells were impaled in darkness and light responses were apparent at normal amplitudes at the very first red/green (650 nm/531 nm) photopic stimulus presentation. This suggests...
Table 1
Analysis of the ON-relaxation and OFF-transient components of the HC light response

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Mean amplitude±S.E.M.</th>
<th>n</th>
<th>% Expression of ≥1 mV</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON relaxation</td>
<td>SA</td>
<td>2.17±0.30</td>
<td>74</td>
<td>55.4</td>
<td>-1.01</td>
<td>0.31</td>
</tr>
<tr>
<td>(mV)</td>
<td>LA</td>
<td>1.78±0.25</td>
<td>87</td>
<td>48.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OFF overshoot</td>
<td>SA</td>
<td>2.34±0.21</td>
<td>74</td>
<td>70.3</td>
<td>1.28</td>
<td>0.20</td>
</tr>
<tr>
<td>(mV)</td>
<td>LA</td>
<td>2.80±0.27</td>
<td>87</td>
<td>65.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data show the mean amplitudes of the components, together with the percentage of cells that express the component. We found that long-term light history had no significant effect on the expression of either the ON-relaxation or the OFF-transient component.

that in *Rutilus rutilus* there is little significant expression of the dark-suppression phenomenon seen so clearly in other species. The absence of dark suppression is also not related to the time of day, since the amplitudes and spectral sensitivity of L-HCs was the same when experiments were performed at night (Fig. 2a,b). Whilst it has been shown, for example, that long periods of darkness have a strong influence on the amplitude of light responses in the white perch retina [29], the extent of dark-suppression appears less profound in the carp and goldfish retina. In contrast, studies of the hybrid bass (*Morone* sp.) have suggested that dark-suppression in this species is stronger than in any other species to date [1]. Phylogenetically the white perch and hybrid bass belong to the Moronidae family (perch), whilst the goldfish, carp and roach belong to the distinct Cyprinidae (carp). Collectively, this may suggest that the expression of dark-suppression is partly species dependent, and that in *Rutilus rutilus* we have a retina where this effect is minimal. Recently, we have recorded L-HC responses in the carp (*Cyprinus carpio*) retina under identical conditions to those reported in this study. In the carp, we see a significant dark-suppression of HC responses in the LA condition, consistent with that reported in previous studies [32].

In contrast, with the minimal effect of long-term light history on general HC responsiveness, we did find that both the receptive field and general kinetics of the light response differed between the SA and LA conditions. Analysis of the annulus/spot ratio (Fig. 3) and the equivalent area index revealed a significant difference in the extent of HC effective receptive field. It appears that the long period of darkness in the LA group results in HCs that are more sensitive to the peripheral stimulus. It is likely that the changes in HC receptive field may reflect the strength of HC coupling which is regulated, at least in part, by retinal DA release in most vertebrate retinai [11,20]. Studies of light-dependent dopamine release in the cyprinid retina have provided somewhat contradictory results, and release appears to be partly dependent upon the diurnal status and long-term light history. Thus, in a recent study it was shown that steady light did not affect release, but flickering light increased dopamine release 2-fold. During prolonged darkness, the release of dopamine increased slowly over 2 h, but only when the experiments were performed at night [27].

The most striking difference between the SA and LA retinae came from our analysis of the light-ON response. There was an apparent increase in the latency to light-ON in the LA group (Fig. 5a). The latency (threshold 2.5%) for the SA cells was around 14 ms, whilst in the LA group this was prolonged by around 50% to 21 ms. The latencies we find in the SA condition are consistent with those previously reported in cone-driven HCs in the light-adapted retina [18,28]. Our results suggest that long-term light history has a pronounced influence on the photoreceptor input to the L-HCs. In addition to the effect on synaptic delay, long-term light history also affected the...
rate of HC hyperpolarization (Fig. 5b). Both the absolute rate of hyperpolarization (mV/ms) and the normalized slope (%-response/ms) were significantly slower in cells from the LA retina. This difference in the response is also apparent when the general waveforms of SA and LA cells were compared (Fig. 4). In contrast, the rate of HC repolarization at light-OFF was not different in the SA and LA retinas (Fig. 5b). Our detailed analysis of the HC response waveform therefore suggests that prolonged periods of darkness during the day results in a selective change in the kinetics of the light-ON hyperpolarization. We examined a potential correlation between the synaptic delay (2.5% latency) and the rate of HC hyperpolarization and found no simple linear relationship. This suggests that light history may affect at least two presynaptic components to the L-HCs.

The kinetics of the HC light response could be affected by changes in the passive membrane properties of the HC, or by modification of the strength of HC–HC coupling [15]. However, such an effect should modify both the light-ON and light-OFF kinetics, and this is clearly not consistent with our results. Whilst the time constant for light-ON in the SA group was 32.4±2.39 ms, that for the LA group was 53.4±3.03 ms. In contrast, there was no significant corresponding variation in the light-OFF time course. It is also important, that since we found no change in the dark resting potential, the kinetic changes cannot be explained in terms of voltage-dependent activity in the HC membrane.

The selective effect upon light-ON might be explained by long-term light-history affecting the rate of glutamate reuptake in the retina. More recently it has been suggested that the HC response waveform, whilst dependent upon changes in the rate of glutamate release, is shaped by glutamate reuptake in the synaptic cleft [4,22]. Thus, it has been shown that the application of dihydrokainate (DHK), a selective uptake inhibitor of retinal glutamate uptake, slows the horizontal cell hyperpolarization at light-ON in a dose-dependent manner, without affecting the repolarization at light-OFF [4]. Such an explanation might be consistent with our observations in the LA condition, since long periods in the dark prior to the experiments are likely to increase the loading on the glutamate uptake system, rendering the system less effective.

The kinetics of the HC light response is also regulated by the activity of HC–cone feedback. Thus it has been shown that GABA can affect the temporal properties of the HC light response in a number of species [19,31]. Furthermore, it appears that the GABA feedback from HC to cones is a novel example of positive feedback that acts to slow down the onset of the HC light response [10]. Whilst in this study we have observed no shift in the HC resting membrane potential, we cannot rule out feedback as a candidate in the temporal changes initiated by long-term light history.

It has also been established that long-term dark-adaptation, or diurnal phase, can initiate a number of anatomical changes in the cone–HC synapse. In addition to the initiation of retino-motor movements, there are reported modifications to the density of presynaptic cone ribbons [23–25] and postsynaptic HC spinules [26], both of which can occur within the time scale of 2 h in darkness. Whilst the precise relationship between the anatomical changes and the physiology of the cone–HC synapse remains somewhat obscure, we cannot exclude these factors contributing to the long-term plasticity in HC response kinetics which we have reported here.

Interestingly we have examined the effects of dopamine (DA) upon the kinetics of L-HC light responses from LA retinas in superfused preparations. Previous studies have shown that dopamine has rather inconsistent effects on roach L-HCs in terms of membrane potential and cell coupling [8], and this may explain some of the differences in the properties of the roach retina. However, we have shown that DA application to the LA retina can evoke selective changes in the light-ON response (Fig. 6b). These results do not establish cause and effect, but imply that some of the differences between the SA and LA retinas may involve retinal dopaminergic activity.

We have described in detail the effect of long-term light-history upon the L-HCs. This has shown that prolonged darkness in the daytime does not significantly modulate the amplitude or sensitivity of the light response of L-HCs cells in the roach retina. We suggest that this species shows no evidence of significant dark-suppression. What has been revealed is that prolonged darkness (light-history) has a profound effect on the kinetics of the HC light response. Interestingly, studies of the human electroretinogram have shown that the latency of the photopic (cone) b-wave varies throughout the diurnal cycle [6]. Furthermore, such temporal changes could be partially initiated by prolonged darkness in the day. It has been suggested that hyperpolarizing neurones, including HCs, may play a role in shaping and terminating the b-wave [17]. It is therefore tempting to suggest that the effects of long-term light history on light response kinetics may be a general feature of cone pathways in some species.

Acknowledgements

This work was supported by an MRC studentship award to Aaron Jenkins. The authors would also like to acknowledge the considerable initial encouragement received from the late Professor Keith Ruddock.

References


