Pflügers Arch (1994) 427:373–377

Pflügers Archiv
European Journal of Physiology

Spectral sensitivity and mechanism of interaction between inhibitory and excitatory responses of photosensory pineal neurons

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Received August 2, 1993/Received after revision December 6, 1993/Accepted December 28, 1993

Abstract. The characteristics and distribution of chromatic-type neurons in the photosensory pineal organ of the river lamprey, *Lampetra japonica*, were investigated electrophysiologically. Neuronal activity was inhibited by light of short wavelengths and excited by middle to long wavelengths. The maximum sensitivities of the inhibitory and excitatory responses were at about 380 nm and 540 nm respectively. The spike activity of the neurons during steady illumination for a 10-min period was measured. Although a flash of short-wavelength light caused a strong inhibition in the neuron, this effect was not sustained during 10 min of photic stimuli. It was found that the inhibitory effect continued when excitatory (middle-wavelength) light was delivered together with inhibitory (short-wavelength) light. The result supports the hypothesis of photoregeneration in the pineal photoreceptor, which occurs when photoreceptors having high sensitivity to short wavelengths receive middle-wavelength light. Contrary to the inhibitory response, the excitatory one caused by middle wavelengths continued during stimulation. Spike frequency of the neuron was determined by the spectral composition of the light. Since environmental light contains both inhibitory and excitatory components, the neuron would keep both sensitivities during the daytime and could measure the variation in the spectral composition. Judging from the recording sites, the chromatic-type neurons are distributed in the peripheral part of the pineal organ.

Key words: Pineal organ – Chromatic-type neuron – Circadian rhythm – UV receptor – Photoregeneration – Lamprey

Introduction

The pineal organ plays an important role in the circadian system. The organ is identified as a major circadian pacemaker in lower vertebrates [22], and is necessary to entrain the locomotor activity rhythm to the light/dark cycle [13]. The pineal organ of lower vertebrates contains photoreceptor cells [2] and photic information obtained by the photoreceptors is transmitted to the central nervous system via second-order neurons in the pineal organ. Two types of neurons have been identified, namely, luminosity- (achromatic) and chromatic-type neurons [3, 4].

The luminosity-type neuron is spontaneously active in darkness and is inhibited by light of all wavelengths tested. The maintained discharge rate of the neuron decreases in proportion to the luminance of the background [7–9, 21]. The neuron probably acts as a dosimeter for solar radiation and informs the brain of the ambient light level [5].

The chromatic-type neuron indicates the presence of an opposing color mechanism showing inhibition for short-wavelengths and excitation for medium and longer wavelengths. However, little is known about the function of these neurons. Experiments on the pineal neurons so far have examined the responses to short flashes of light. It is important to examine their neural activity when stimulated for a long time in order to determine their function in the circadian system.

The present study was focused on the chromatic-type neuron of the pineal organ in the river lamprey, *Lampetra japonica*. First, spectral sensitivities of the excitatory and inhibitory components were measured. Then, steady illumination for 10 min was applied and the change in spike frequency was observed. In addition, the distribution of the chromatic-type neurons in the organ was surveyed.

Materials and methods

The experiments were performed on the pineal organs of the adult river lamprey, *Lampetra japonica*. Lampreys were collected from the Ishikari River in Hokkaido, Japan. Before the experiment, they were stored in aquaria with aerated and filtered water at a temperature of 7–10°C. The experimental animals were quickly decapi-

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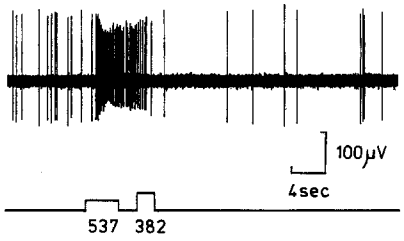

Fig. 1. Responses to light of a chromatic-type neuron. Action potentials were recorded from a pineal organ. Light stimulation is indicated by upward deflection of a lower beam. The numbers under the beam are the wavelengths used as stimulation. Log I (intensity) of each stimulation = −2.0 for 537 nm and −3.3 for 382 nm

tated and their pineal organs were isolated with a small piece of the adjacent brain tissue. The tissue was placed dorsal side upward onto a filter-paper in a small chamber. Before recording, the chamber was filled with oxygenated lamprey Ringer's solution for 30 min in the dark. The ionic composition of the Ringer solution was (in mM): 122.4 NaCl, 2.7 KCl, 1.8 $CaCl_2$, 16 glucose and 4 HEPES, and the pH was adjusted to 7.5 with NaOH solution. During the recording, the level of the solution in the chamber was just enough to expose the top of the pineal organ.

Electrophysiological recordings were made with an insulated tungsten microelectrode. The tip diameter of the electrode was about 5 μm. The position was adjusted until spike responses of a single unit were observed. The potentials were amplified and displayed on an oscilloscope (Tektronix, 5103N). A microcomputer with a window discriminator (ATAC-450, Nihon-Koden) was used for counting the spike frequency. Data were directly recorded on a chart recorder and/or stored on magnetic tape.

Light was projected onto the pineal organ from a photostimulator consisting of two optically equivalent pathways. The light source of the photostimulator was a 500-W xenon arc lamp. The light energy was attenuated by neutral-density filters and the wavelength of the light stimulus was controlled by interference filters. The intensity of the light at neutral density = 0 was determined as 1.2×10^{14} photons cm^{-2} s^{-1}. Duration was controlled by electromagnetic shutters driven by square-wave pulse generators.

Results

Spectral sensitivities of the chromatic-type neurons

The activity of chromatic-type neurons in the photosensory pineal organ was inhibited by light of short wavelengths and excited by middle to long wavelengths (Fig. 1). The intensity of the inhibitory light in Fig. 1 was about 1/20 that of the excitatory light. However, the inhibitory effect was strong and continued for over 10 s after the light was turned off.

Spectral sensitivities of the neurons are shown in Fig. 2. The inhibitory curves were determined by measuring the relative energy that decreases the spike frequency to half the rate in darkness or in a steady background for 10 s. The excitatory curve was acquired by measuring the energy necessary to increase the spike frequency to double the rate in the dark for 10 s.

Maximum sensitivities of the inhibitory and excitatory responses were obtained at 380 nm and at 540 nm respectively. There was a shoulder at about 480 nm in the inhibitory curve. When the wavelength of the test

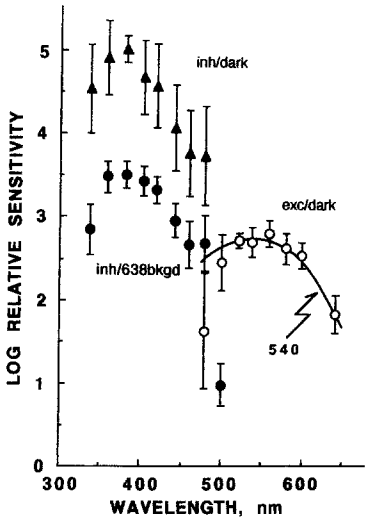

Fig. 2. Spectral sensitivity of chromatic-type neurons. Test light was illuminated in the dark (*inh/dark* and *exc/dark*) or upon a 638-nm background (*inh/638bkgd*) where log I = −0.9. ●, ▲, Mean values from eight (*inh/dark*) or ten (*inh/638bkgd*) neurons in inhibitory components; ○, mean values from eight (*exc/dark*) neurons in excitatory components. *Vertical bars* represent SD. The *solid curve* plots the light absorption curve of a vitamin-A_2-based photopigment peaking at 540 nm [15]

light was longer than 521 nm, the excitatory curve closely followed the absorption curve of a vitamin-A_2-based pigment nomogram, peaking at 540 nm. Comparing the logarithm of the sensitivity of the inhibitory response at 380 nm in the dark with that of the excitatory response at 540 nm, the former was about 2 units higher than the latter to cause the criteria response.

Neuronal activity of the chromatic-type neurons during steady illumination

To examine the neuronal activity during steady illumination, pineal organs were illuminated for 10 min by excitatory (537 nm) or inhibitory (382 nm) light (Fig. 3A, B). In the excitatory responses, the peak spike frequency appeared early in the illumination. Then, the initial maximum frequency decreased and thereafter persisted at about a steady frequency. The neurons stayed in the excitatory state during the stimulation. There was no tendency for the activity of the neurons to adapt.

Contrary to the excitatory responses, the inhibitory responses were not maintained. Although the neurons were inhibited almost completely at first, the spike discharges appeared gradually and the inhibitory effect of the light became remarkably weak. Figure 3B shows that the inhibitory light almost suppressed the spike activity for the first 1 min, but the effect became gradually weaker. Interestingly, when the inhibitory light was superimposed on the excitatory light, the inhibitory effect on the excitatory response was maintained. Figure 3C shows that the excitatory responses were conspicuous before and after the inhibitory light was superimposed, but the effect of the excitatory light was continuously suppressed during illumination with the inhibi-

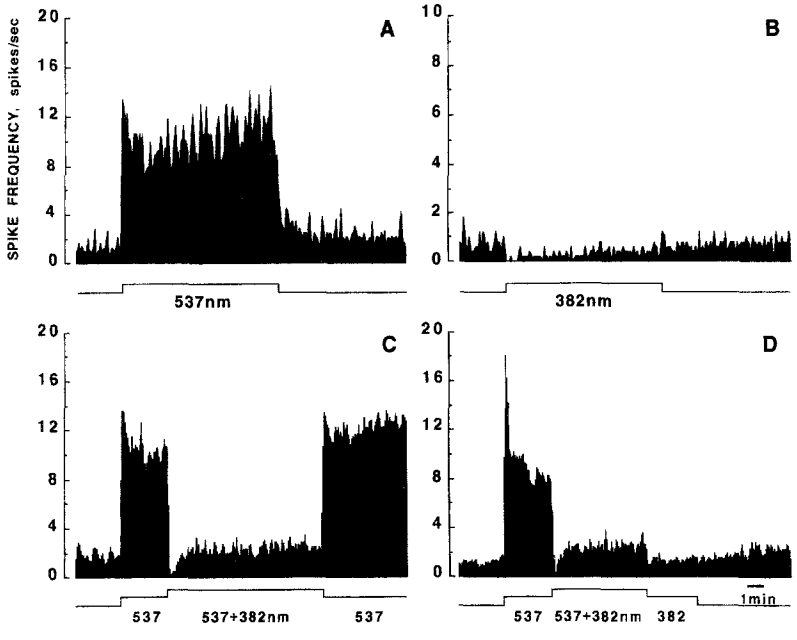

Fig. 3A–D. Rate-meter recordings from a chromatic-type neuron showing responses to background illumination. **A** Excitatory light (537 nm, log $I = -2.0$) illumination for 10 min. **B** Inhibitory light (382 nm, log $I = -3.0$) illumination for 10 min. **C** After illumination of the excitatory light for 3 min, the inhibitory light was superimposed for 10 min. **D** After illumination of the excitatory light for 3 min, the inhibitory light was superimposed for 6 min. Then the excitatory light was put out and only the inhibitory light was on for 3 min. **D** The inhibitory light after cessation of the excitatory light almost ceased to suppress the spikes if compared with the dark spontaneous level before and after the light stimuli. **A** and **B** as well as **C** and **D** were recorded from the same cells

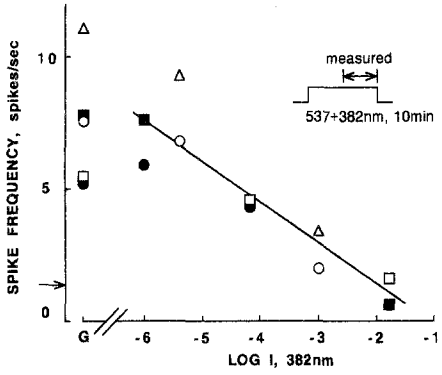

Fig. 4. Matching of excitatory (537 nm) and inhibitory (382 nm) wavelengths. Two beams of light were focused on the pineal organ and mean spike frequency during the latter half of the stimulation was measured. Test light of 382 nm was added to a constant intensity (log $I = -2.2$) of 537 nm for 10 min. Different symbols represent different animals. The *line* is the calculated regression line. *G*, only excitatory light was on. *Arrow*, the mean spike frequency in the dark

tory light. Even if the inhibitory light was unable to sustain its effect on spontaneous spike activity, it had a good effect in cancelling the influence of the excitatory light. Figure 3D shows that the excitatory response was conspicuous before the inhibitory light was on. However, the effect of the excitatory light was almost suppressed by superimposition of the inhibitory light, although the inhibitory light could not suppress spontaneous spike activity after cessation of the excitatory light.

In order to understand the relationship between the spike frequency and the mixing ratio of inhibitory and excitatory light, two beams of light were applied simultaneously for 10 min and mean spike frequency during the latter half of the stimulation was measured. The log intensity of the excitatory light was fixed at -2.2 and the intensity of the inhibitory light was varied (Fig. 4). Once the effect of the inhibitory light appeared, the spike frequency decreased in proportion to the log intensity of the inhibitory light.

Distribution of the chromatic-type neurons

With flashing excitatory light, the pineal organ was sampled by recording at points forming approximately a square grid at 20- to 50-μm intervals (Fig. 5). When the response elicited by the light was excitatory, it was labeled as a chromatic-type response. If the response was inhibitory with off-discharges, it was labeled as a luminosity-type response. Recordings of 605 points from five pineal organs showed that chromatic responses predominated in the peripheral part of the organs. It was especially easy to elicit a chromatic response in the rostral portion. On the other hand, luminosity responses were recorded from every part of the pineal organs. Judging from the recording sites, the chromatic-type neurons are distributed in the peripheral part of the organ.

Discussion

The experimental results presented here show the response properties of chromatic-type neurons in lamprey pineal organs. The peak sensitivity of the excitatory response was 540 nm and that of the inhibitory response was 380 nm. Electrophysiologically, intracellular recording has revealed two types of photoreceptors in the lamprey pineal organ. One is a photoreceptor with a peak sensitivity at 522–525 nm [11, 16], and the other is a UV receptor peaking at 380 nm [20]. The peak sensitivity of the inhibitory response of the chromatic-type neuron and that of the UV receptor were almost the same. These neurons were situated in the peripheral part

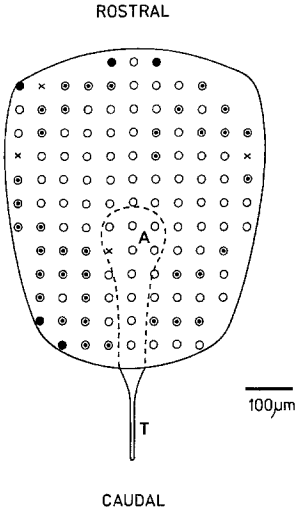

Fig. 5. Recorded points of chromatic and luminosity responses in a pineal organ. ●, Chromatic response; ○, luminosity response; ⊙, both responses were recorded simultaneously; ×, no response. *A*, atrium; *T*, pineal tract

of the pineal organ, coinciding with the distribution of the UV receptors. Therefore, we infer that the chromatic-type neuron makes synapses with the UV receptors and, in turn, the receptors are concerned with the inhibitory response. Since the UV receptor is hyperpolarized by light stimulation [20], the synaptic mechanism from the UV receptor to the chromatic-type neuron is probably a type of disfacilitation, which is the same as that of the synaptic type of pineal photoreceptors to a luminosity-type neuron [21].

The sensitivity of the inhibitory response of the neuron abruptly decreased at 500 nm. This may be due to interaction between the inhibitory and excitatory components. However, the sensitivity to wavelengths longer than 500 nm of the UV receptor itself also sharply decreases [20]. Thus, the influence of the inhibitory component on the excitatory one would be low at wavelengths longer than 520 nm, and the measured spectral sensitivity at such wavelengths should reflect only the excitatory component. When the sensitivity curve of the photoreceptor with a peak at 522−525 nm was compared with that of the excitatory response, they were very different from each other. We could not fit the curve of the photoreceptors to that of the chromatic response, even at wavelengths longer than 520 nm, where the chromatic response is almost free from the influence of the inhibitory response. Interaction of the photoreceptor having a peak at 522−525 nm and the neuron would be slight. The sensitivity curve of the excitatory response fits the absorption curve of a vitamin-A_2-based photopigment peaking at 540 nm, especially in the longer wavelength range. Therefore, a third type of photoreceptor, with an approximate λ_{max} of 540 nm, should exist in the pineal organ of *L. japonica*. This photoreceptor would relate to the excitatory response of a chromatic-type neuron.

The responses of the wavelengths shorter than 520 nm deviate from the absorption curve. This is due to the influence of the inhibitory process on the excitatory response. Since light between 500 nm and 520 nm has a partial inhibitory effect on the neuron, stronger light is needed to induce an excitatory response.

The peak of the excitatory response of the European river lamprey, *Petromyzon fluviatilis*, was reported as 535 nm [10]. This result almost coincides with ours. Further, luminosity-type neurons of the European river lamprey had a peak sensitivity at 525 nm [10], which is the same as that of *L. japonica* [21]. It is interesting that two different species of lampreys have almost the same sensitivities in the pineal organ.

Immunocytochemical studies revealed that at least four types of pineal photoreceptors exist in frogs [23] and five types in lampreys [6, 18, 19]. Which type of photoreceptors has properties revealed by electrophysiological techniques should be investigated. Further studies will provide more insight into the neural networks of pineal photoreception.

When the duration of stimulation is within a few seconds, the light sensitivity of the inhibitory response in the frontal organs of frogs is much higher than that of the excitatory response [4, 5]. In the present study, the same pattern was observed in the result of the spectral sensitivity of both responses. It has not been elucidated why the sensitivity of the inhibitory response is so much higher than that of the excitatory response. However, it should be noted that inhibitory light gradually lost its effect during steady illumination. The inhibitory effect was retained if the system was illuminated simultaneously with excitatory light.

Tamotsu and Morita [17] showed that irradiation of orange light following *cis*-to-*trans* isomerization by blue light caused *trans*-to-*cis* isomerization in the lamprey pineal organ, by using high performance liquid chromatography (HPLC). Their result suggested that photoregeneration occurred on the blue-sensitive visual pigment. Our finding of the effect of excitatory light on the inhibitory response could be applied to support the theory of pineal photoregeneration.

Though the UV receptor loses its light sensitivity by absorption of short-wavelength light quanta, long-wavelength illumination recovers the sensitivity of the receptor by the mechanism of photoregeneration. Hence, if the test light is applied together with long-wavelength light, the UV receptor can keep its sensitivity to short-wavelength light, and since the inhibitory response of a chromatic-type neuron is caused by the UV receptors, the neuron can keep the inhibitory response.

What is the biological meaning of the chromatic-type neuron? Daylight shows cyclic variations in light intensity and spectral composition, and it contains both excitatory and inhibitory light for a chromatic-type neuron. Our present data show that the spike frequency of a chromatic-type neuron is determined by the spectral composition of the stimulus. Thus, the neuron could monitor the variation in the spectral distribution of environmental light. The change in the spectral distribution is most prominent during twilight [14]. It can be assumed that the chromatic-type neuron acts as a sensor of the differential *Zeitgeber* [5, 12] as described by As-

choff [1]. Furthermore, the light intensity and spectral composition are very different in summer and winter. This seasonal variation must also affect the pineal function through the chromatic-type neuron.

Acknowledgements. This work was supported in part by a Grant-in-Aid for Scientific Research (no. 61570040) and for International Joint Research on Pineal Photoreception (no. 63044059) from the Ministry of Education, Science and Culture of Japan to Y. M.

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