Light and Dark Adaptation of Tectal Neurones in the Crucian Carp: The Effect of Stimulus Parameters upon both Neuronal Threshold and Response Magnitude

By

Yoshiaki Sato[1)]

Zoological Institute, Hokkaido University

(With 10 Text-figures)

One of the salient features of vertebrate visual systems is in their ability to adapt and function over an extremely wide range of light intensities. Psychophysical measurements show that the human eye can discriminate light intensities over a range of about 9 log units. Since Hecht (1937) proposed his photochemical theory that changes in the sensitivity of the visual system are directly ascribed to the bleaching and regeneration of the visual pigments, many experiments have been performed concerning the rivalry between photochemical and non-photochemical factors on visual adaptation (Lythgoe, 1940; Craik and Vernon, 1941).

The experiment on rivalry has ended by the outcome of the studies on bleaching of photo-pigments; there may be large changes in visual sensitivity without significant changes in the concentration of visual pigment, and the photochemical factors play certain limited roles in adaptation (cf. Rushton, 1961). A new question, however, arises whether the main part of visual adaptation can be attributable to the neuronal level of the retina or elswhere in the visual pathway. The responses of neurones throughout the entire retinal structure of the mudpuppy were recorded intracellularly by Dowling and Werblin (1969) and Werblin (1971). These results indicated that the main site of visual adaptation is in the bipolar cell level and that most of the adaptive processes appear to be completed at this level in case of light adaptation; i.e. the output gain from the cell is reduced, the antagonistic surround appears, and the resting potential is thus not altered appreciably by increasing level of background illumination.

These studies, however, were mainly done about the steady state properties of the eye after the adaptation to the various background. Scarcity of the knowledge of the temporal changes of properties during the light and dark adapta-

1) Present address; Department of Physiology, St. Marianna University School of Medicine, 2095, Sugao, Takatsu-ku, Kawasaki.

tion should be noted.

A short description of the temporal properties of the dark adaptation only was given in the previous paper (Sato and Niida, 1972). This paper will demonstrate to what extent the neural elements play the role in the processes of light and dark adaptation which are generally assumed to be attributable to the retinal processes; i.e. how the activity of a single tectal neurone is modified by light and dark adaptation, and how changes in intensity and duration of the light stimulus modify both the neuronal threshold and response magnitude during the light and dark adaptation.

Material and Methods

Preparation of the optic tectum of the crucian carp, *Carassius auratus langsdorfii* Temminck et Schlegel, was essentially the same as that described previously (Niida and Sato, 1972, 1972a, b; Sato and Niida, 1972). The fish was anesthetized by intraperitoneal injection of d-tubocurarine chloride (usually 6 mg/kg body weight). At this anesthetic level, the eyes remained immobilized. Then the fish was clamped and fixed with a suitable holder in normal position and kept outside the water. Continuous flow of tap water through the mouth and gill chamber of the fish was maintained by means of a mouthpiece of metal tube while the maxilla of fish was clamped with a holder attached to the tube. The fish's body was wrapped in a piece of the cheese-cloth of which wetness is maintained by water flowing out the gill. By means of this arrangement, the recording of the electrical activity from the same single tectal neurone could be maintained for more than 5 hours. The skull was trephined and the connective fatty tissue covering the brain was carefully removed to expose the right optic tectum. The opening was not filled with any solutions, because the cerebro-spinal fluid covering the brain is enough to prevent it from drying. The condition of animal was monitored by microscopic observation of blood flow velocity in the arteries and veins of the exposed tectal surface and also by electrocardiography. All experiments were performed at 18–22°C.

Activities of tectal neurones were recorded with tungstem microelectrode which was electrolytically sharpened to 1–5 μ in tip diameter and coated with polystyrene except the tip. The microelectrode was lowered perpendicularly down into the right optic tectum. with the aid of a micromanipulator. The signal derived from single tectal neurones, which mainly existed in a layer 50–200 μ proximal to the tectal surface, was led to ordinary amplifiers and then to a cathode ray oscilloscope and an auditory monitor. A phototransistor was used to monitor the stimulus. All electrical signals were permanently recorded on a magnetic tape.

Three tungsten incandescent lamps were separately used for photic stimulation; light of test flash, background illumination and bleaching illumination. The test flash was generated by an electromagnetic shutter, a modified solenoid relay, placed in the light path of one of the light sources. The shutter solenoid was energized by an electric stimulator. With this device, it was possible to turn on or off the flash with a minimal duration of 10 msec., and the time for completion of turning on and off is 1 msec. The illumination of the flash was controlled with interposing neutral density filters in steps of 0.3 or 0.4 log units covering 9 log units. Via a fiberoptic light guide, 2 mm. in diameter and 2 m. in length, the flash stimulus was led into a dark room in which the material was set up. A movable transparent lucite hemisphere of 60 cm. in diameter was positioned so that the fish's left eye was in the centre of the apparatus and the terminal face of the light guide

was placed perpendicular to the surface of the sphere with three plastic vacuum cups. Possible maximal intensity of test flash was 16000 mililamberts (mL) at the cross-sectional face of the terminal. For the background illumination, a tungsten diffused light which was positioned over the head at a distance of 80 cm. from the material was used. The light through a semicircular aperture of 21 cm. in diameter illuminated the entire room and its intensity was changed with reducing the aperture with a diaphragm in steps of 1 log unit covering over 4 log units. The maximal intensity was 30 lux near the point of the fish eye. For the bleaching light, a movable white circular screen of 3 cm. in diameter was placed at a distance of 3 cm. from the left eye of the fish and was illuminated with another tungsten lamp. Light intensity of the screen surface was kept 20 mL constantly.

First, single tectal neurone was picked up and roughly identified in the dark room with moving white spot according to the classification described in previous paper. The identification process took at least 10 min. in each neurone. The receptive field of the neurone was explored with the spot moved over the surface of the lucite hemisphere mentioned above. Neurones having their receptive fields in the nasodorsal area of the fish left eye were selected. The terminal of the light guide was placed at the centre of the receptive field of the selected neurone. The screen for bleaching light was positioned, then, illuminated for 5 min. and removed. Immediately following this, the material was subjected to the different intensities of background illumination or in the dark. At a constant interval thereafter, measurements and recordings of neuronal responses were made with test flashes of various threshold intensities and durations. (Detailed procedures of the measurements and records will be given later.) When a series of measurements and records were completed, the eye was bleached for 5–10 min. and neuronal activity was compared with that obtained prior to the test series. The entire experiment usually lasted 3–5 hours. At the end of an experiment, the data which had been stored on the magnetic tape was displayed on the oscilloscope, photographed for relevant data and analyzed.

Results

Part 1 General response feature of light and dark adaptation

By a sudden increase in illumination, the class 1 neurone responds with an on-discharge composed of an initial burst of impulses and steady ensuing discharge in a lower rate. Although the frequency of discharge decreases slowly, the maintained discharge never ceases as long as intensity of the light is kept steady. When the light is suddenly turned off, this process of light adaptation shifts into recovery process of sensitivity. Time course of this recovery process in the dark can be seen by the analysis of response magnitude and the pattern of impulse discharge which is elicited by a brief test flash of constant magnitude applied to the eye. An eye was illuminated by light for 5 min.; at various times after turning off this light, test flashes of constant intensity and duration were applied.

Remarkable changes in impulse discharge pattern were found as the dark adaptation proceeded. The first of the marked change was a dark-discharge appearing at 6–10 min. after the dark adaptation and disappearing at 40–60 min.; the frequency of this dark-discharge increased gradually, reached to a peak and declined as a function of time of the dark adaptation. The second was the appearance of a silent period after the on-discharge, followed by a sustained impulse

discharge of higher frequency than that of the dark-discharge. Since it was elicited by turning off the test flash, this response was defined as a rebounding-discharge, neither as a static- nor a dark-discharge. The frequency of this type of discharge increased rapidly in the earlier stage, and reached to a peak. The frequency of this peak was almost of the maximal response or saturation level of this class of neurone. The latency of the rebounding-response, on the other hand, showed very little change. Thirdly, the neurone responded to the short flash with a strong burst discharge lasting for a period of several seconds, namely a long-lasting-discharge. This response seemed to indicate final stage of the dark-adapted eye since no further marked change occurred in the discharge pattern even if the eye was kept in the dark further. Not only did the total number of impulses in this response increase as a function of time in the dark, but also the duration of the response lengthened and the frequency also increased.

As above mentioned, the changes of impulse discharge pattern of the class 1 neurone occurring while dark adaptation were represented fundamentally by three types of impulse discharge; (1) dark-discharge, (2) on-discharge and (3) rebounding-discharge. It is clear that the individual rise and fall of those three categories of response are resulted in the very complex pattern of discharges observed.

The class 2 neurone is defined as those tectal neurones which show a comparatively low frequency of irregularly maintained discharge in darkness (dark-discharge) and which respond with a complete suppression of impulse discharge to turning on the light and with large volley of impulses to turning off the light (off-discharge). The time course of this off-discharge is similar to that of the on-discharge of the class 1 neurone. Thus, the class 2 neurone responds to the comparatively prolonged illumination in an opposite manner to that of the class 1 neurone. It is, however, possible to investigate the recovery process by means of the same method as described before.

When an eye was kept in the dark and dark adaptation proceeded, the class 2 neurone responding to a short test flash showed a silent period after an off-discharge and then a sustained response, which was a train of impulses of higher frequency than that of the dark-discharge. Therefore, it was easy to identify this response as a rebounding-discharge, similar to that of the class 1 neurone. As dark adaptation progressed, the response magnitude of the off-discharge decreased gradually and disappeared in the later stage. The latency of the rebounding-discharge, on the other hand, increased rapidly and reached to the saturation in the final stage of dark adaptation.

In this way, the changes of impulse discharge pattern of the class 2 neurone were elementally represented by three types of discharges as dark adaptation proceeded; (1) dark-discharge, (2) off-discharge and (2) rebounding-discharge. Detailed characteristics and time course of these types of discharge of both classes of neurone will be described later.

Part 2 Threshold responses of single neurones

The first question which arises from the above observation is how the rebounding-discharge relates to a 'threshold' of the neurones in the dark adapted state. This part will present the results of the determination of the thresholds of the class 1 and class 2 neurones when the eye was kept at a particular adapting level of the background illumination and also in the dark.

(1) *Threshold under background illumination of different intensities*: Both classes of neurone showed an irregularly maintained discharge under a given background illumination (background-discharge), so that it is of first necessity to establish a criterion of 'threshold' response which can be observed in the presence of the background-discharge. This task was performed by the following procedures.

After bleached for 5 min., the material was immediately light-adapted under 30 lux background illumination for 10 min. and then, 'provisional' threshold was determined by changing the intensity of flash with a given duration. The minimal intensity of illumination eliciting a just detectable change in the on- or off-response which was cautiously monitored by the oscilloscope and loudspeaker was regarded as the provisional threshold. The determination process was usually completed within 30 sec. Flashes of fixed duration and interval but at three intensity levels, that is, of the provisional threshold and the ones of slightly lighter and lower than it were applied. The ten records were successively obtained at each intensity level. Then the flash duration was changed in five steps of 10, 50, 300, 500 and 1,000 msec. The test series of varying duration took approximately 10 min., in completion. When the series of the test under 30 lux background illumination was completed, the material was re-adapted under 3 lux background for 10 min. and the similar test series was repeated and completed. The background illumination was changed in four decade steps of 30, 3, 0.3 and 0.03 lux.

Fig. 1 shows each one of the representative records obtained from the class 1 and the class 2 neurones subjected to these test series. As is seen in this figure, the difference in responses between an intensity level at which a neurone showed no response and the higher intensity level at which the neurone showed a response was readily recognizable. The variance in the flash intensity required to produce such change in response was usually of 0.3 or 0.4 log units.

No marked change was found in the upper group of records of Fig. 1. In the middle one, the class 1 neurone responded to the flashes with a sustained on-discharge, followed by a pausing-period of short duration and after then recovering the background-discharge. At this illuminating level a rebounding-response was not observable. The class 2 neurone, on the other hand, responded with a complete suppression followed by a transient increase in impulse discharge (off-discharge) which declined gradually to the constant rate of the background-discharge. The response characteristics of these two classes of neurone were further demonstrated in the lower group of records of the Fig. 1. It should be noted that the

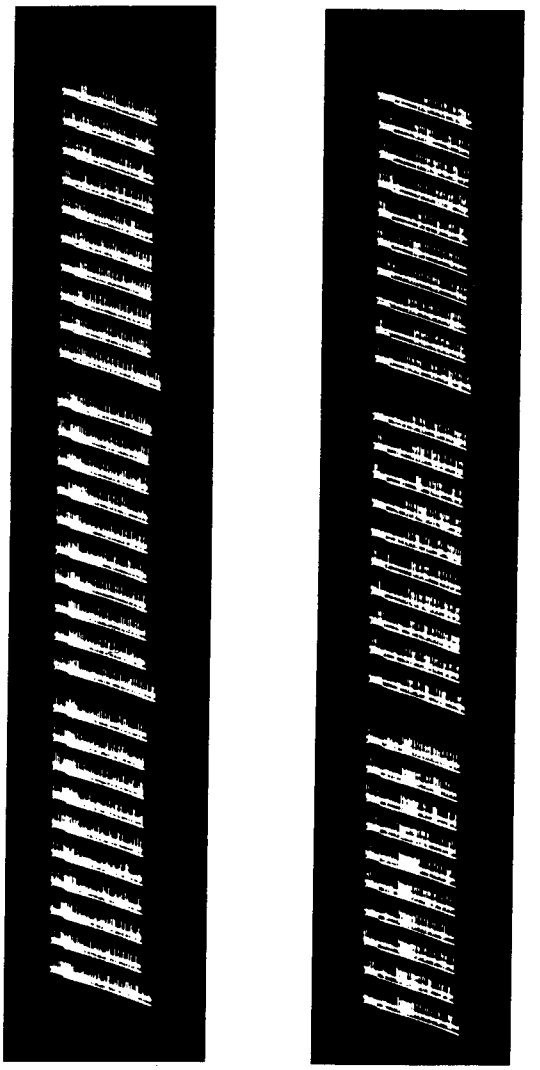

Fig. 1. Responses of the class 1 (records of left side) and the class 2 neurones (records of right side) to the test flashes of 300 msec. duration, after the material was kept under 0.03 lux background intensity for 10 min. The upper group of ten records of each neurone represents the responses to the test flashes at the lower intensity level than that of the provisional threshold (0.0016 mL on the both neurones), the middle one at the provisional threshold level itself (0.0032 mL) and the lower one at the higher intensity level (0.008 mL), respectively. In each record, the upward deflection of the second trace indicates the flash application and time signal is 10 cycles/sec.

timings of onsets of the on-response (or suppression-response) and pausing-response (or off-response) were almost exactly identical in all ten records.

Such response, i.e. the on-discharge and ensuing pausing period of the class 1 neurone and also the suppression period and ensuing off-discharge of the class 2 neurone, became clearly recognizable when the test flash duration exceeded 10 msec.. Eventually, the longer the flash duration was the clearer the response characteristics were. However, a test flash of exceedingly long duration may produce a mixed condition of light adaptation and simultaneous stimulation. In order to minimize this process of light adaptation, test flashes of less than 500 msec. duration were mainly used in determination of the threshold, as is described later.

Fig. 2 shows the response of the same neurones shown in Fig. 1 to the test flash of near threshold but of varying duration under various intensities of the

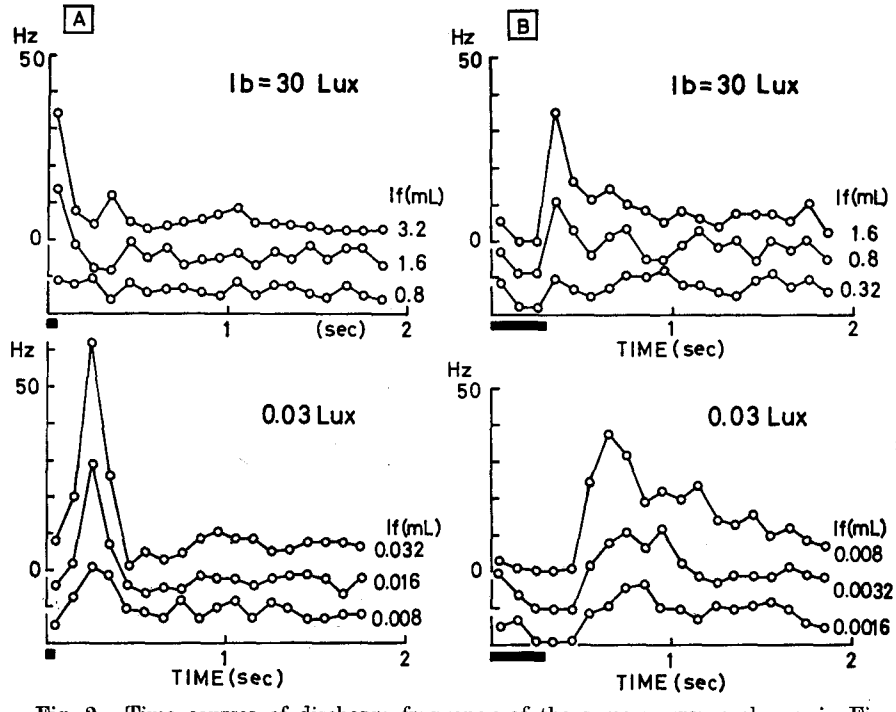

Fig. 2. Time courses of discharge frequency of the same neurones shown in Fig. 1 under different background intensities. A; the class 1 neurone, B; the class 2 neurone. The data obtained from the records of the test flash duration of 50 msec. on the class 1 and 300 msec, on the class 2 neurones are shown here. Frequency values were taken by counting impulse number within time intervals of 100 msec. and by normalizing ten records which were obtained in the same condition of stimulation. Countings were started from turning on the test flash. In each figure, lower rectangle indicates the flash application, and Ib and If the intensity of background (lux) and test flash (mL), respectively.

background illumination. At the given background intensity, the peak frequency values of the on-response and the off-response increased with the increase in flash intensity. Furthermore, latent periods of both types of response at the critical intensity levels increased with the decrease in background intensity and also time intervals reaching up to the maximal frequency of impulses were prolonged. On the other hand, durations of the on-response and the suppression-response of neurones depended upon the flash duration, while, durations of the pausing-response and the off-response were almost independent of the flash duration. These pausing- and off-responses terminated within 500 msec. from the end of the light stimulation in every records of both neurones.

In order to determine the threshold intensity, therefore, the on-response of the class 1 neurone and off-response of the class 2 neurone were selected and a Fp/Fs was calculated on the each data as an index of response amplitude, where Fp is peak frequency of these responses and Fs is background-discharge frequency which was obtained by counting the impulse number during 500 msec. starting from 1.4 sec. after the flash onset. Fs was almost constant in both neurones (4.6–14.8 impulses/sec.) even though the background illumination was changed in range of 4 log units.

The threshold plotted as a function of different background illumination is shown in Fig. 3. The data show an increase in sensitivity with decrease in background intensity with longer duration of test flashes. However, no change in

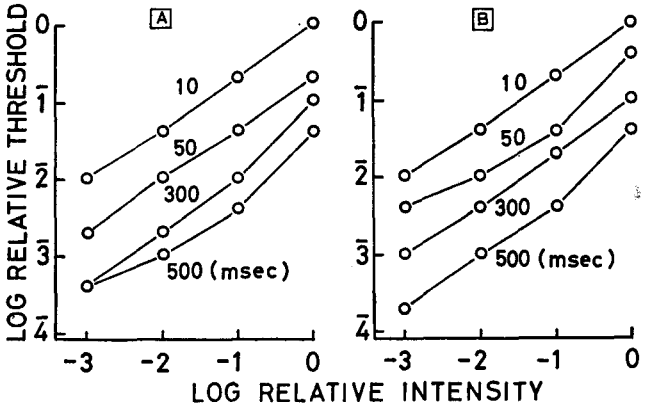

Fig. 3. Thresholds of the both neurones shown in the preceding figures plotted against a function of background intensity. A; the class 1 neurone, B; the class 2 neurone. The each threshold value shown in the graph was the minimal intensity which elicited the response of more than 3.0 in the index value (Fp/Fs) in the both neurones. The numeral beside each threshold curve is related to the flash duration (msec.) used in the threshold measurement. Although test of 1,000 msec duration is not presented in those graphs, the results were almost identical with those of 500 msec. The origins of the abscissae and ordinates represent 30 lux and 8 mL on the both neurones, respectively.

threshold intensity was found with test flash durations which exceed 500 msec.. In general, the curves had a slope of approximately 3/5 and the tendency to flatten out at the low intensity of the background in both neurones.

(2) *Changes of threshold under different background illuminations*: Since the determination of threshold by the method described in the former section took a considerably long period of time. This method may be unappropriate to measure points in early critical stages of background adaptation. In this section, threshold of the neurones for test flashes was quickly determined by audio-monitoring instead of photographic recording of oscilloscope.

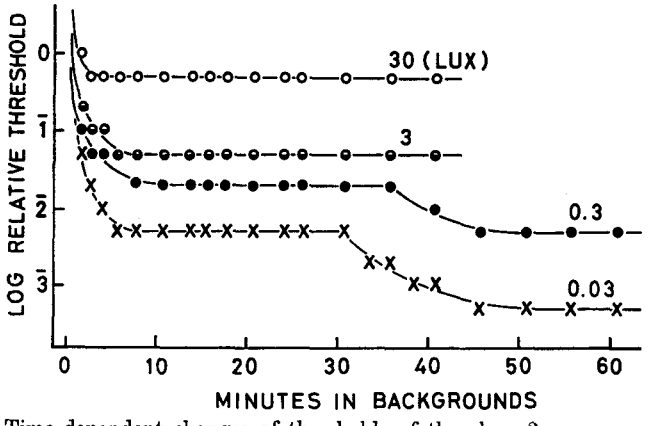

Fig. 4. Time dependent changes of thresholds of the class 2 neurone under different background intensities. Each threshold value was obtained by the application of the test flash of 300 msec. duration with intervals of 2 sec. The numeral beside each curve is related to the background intensity (lux). The origin of the ordinates is 0.16 mL.

Fig. 4 shows the time dependent changes of the thresholds of the class 2 neurone after placed under different background illuminations. As to the general characteristics of the curve thus obtained, a simple monotonous exponential function represents the data obtained with high intensity background illumination (30 and 3 lux), while those of the lower background illuminations are composed of two separate exponential functions (0.3 and 0.03 lux). The transition points of these two exponential functions were at 35 min. under 0.3 lux and at 30 min. under 0.03 lux of the background illumination.

These transitions of the low intensity curves are similar to ones which are, so called, a "Kohlrausch's notch" found during the dark adaptation of the human eye and can be regarded as representing a shift from the photopic to scotopic visions; it is safe to assume that left half of the curve of Fig. 4 shows changes in the sensitivity of cones, while the second right hand branch represents the activity of rods.

(3) *Changes of threshold in the dark*: The shift from the photopic to scotopic visions is clearly observable furthermore in Fig. 5, which represents the time dependent changes of the thresholds in the dark. When the eye was kept in the dark for 70 min., the threshold changed in ranges of 4 log units on the class 1 and also of 3 log units on the class 2 neurones. Furthermore, the data of both neurones show the similar initial rapid recovery in sensitivity, and after about 20 min. of the dark adaptation there are secondary decreases of which time courses differed between two classes of neurone. This recovery processes of each neurone showed an exponential decay, therefore, time constants of these curves were obtained by means of the least square method. From the data, time constant of the first recovering process was found to be almost equal in the two neurones, while those of the secondary decreases were –6 on the class 1 and also –4 on the class 2 neurones.

These data clearly establishes the fact that there is an adaptation process in tectal neurones themselves which will be shown as the sensitivity increase of over 3–4 log units in the dark. As the threshold becomes lower in the dark, the test flash will produce larger response of the recorded cells. Then, the flash of the same intensity becomes more effective as a stimulus. The rebounding-discharge can be regarded as an indication of the recovering process of adaptation which is temporarily broken with brief test flashes.

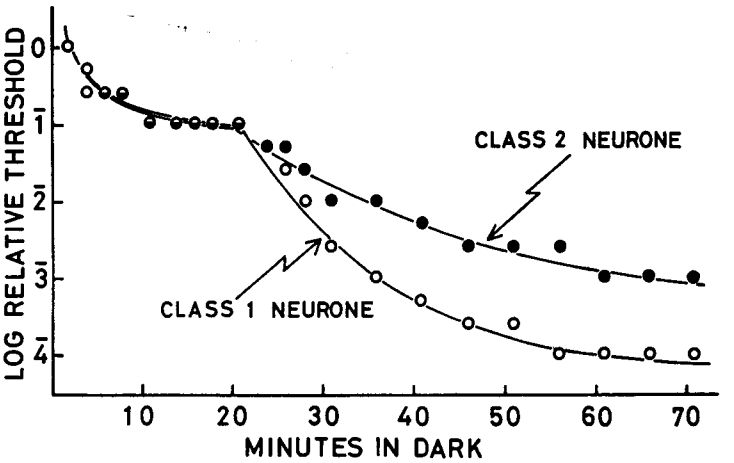

Fig. 5. Time dependent changes of thresholds of both class neurones during dark adaptation. The origin of the ordinates denotes 0.32 mL on the class 1 and also 0.032 mL on the class 2 neurones, respectively. Exponentially decaying curves of the figure were obtained according to the least square method. The primary curves are $I=t^{-0.8}$ on the class 1, $I=t^{-0.9}$ on the class 2, and the secondarys are $I=10^{6.0}t^{-6.0}$ on the class 1, $I=10^{3.6}t^{-4.0}$ on the class 2 neurones, respectively.

Part 3 Stimulus parameters that influence rebounding-response

It was described in Part 1 that a rebounding-response appears along the course of dark adaptation, and that the time of dark adaptation would be regarded as one parameter which strongly influences the response. This part of the paper will present systematically what sorts of parameters influence the rebounding-response of both classes of neurone. To evaluate the relative intensity of the test flash, the threshold was also determined immediately preceding the stimulation with test flash. The flash to detect the threshold was of 50 msec. duration and of 1–2 sec. interflash intervals.

(1) *Time in the dark (Td)*: Fig. 6 shows typical responses of both classes of neurone to short test flashes of an uniform intensity at given intervals. Thresholds of both neurones decreased gradually as time kept in the dark prolonged, and at 72–80 min. showed values of 2–3 log units lower than those at 1–2 min. of dark adaptation.

The first component of response of the class 1 neurone, on-discharge, was clearly seen in early stages of dark adaptation (Fig. 6A). While, in later stages, it became not clear because of existence of an ensuing rebounding-response. The impulse frequency of the on-discharge reached to a maximum with the test flash applied as early as 1 min. after the start of dark adaptation. The flash intensity applied here corresponded to 3 log units above the threshold. With further increase of the time in the dark, the frequency value did not increase any more, indicating a saturation level of response of the neurone was attained.

The second component of response of the class 1 neurone was a rebounding-discharge, which became noticeable after the material had been kept in the dark for longer than 7 min.. Here, the intensity of the test flash used was equivalent to 4 log units above the threshold. The rebounding-response was elicited by turning off the flash and was composed of a sustained impulse discharge which appeared after a short pausing period following the on-discharge.

Usually, the rebounding-response of this class of neurone did not appear as of completely separate entity from the on-discharge because of the continuation and fusion of these two components. The fused response began to appear after 17 min. from the beginning of the dark adaptation and was composed of a strong burst discharge lasting for some seconds, namely a long-lasting-discharge, which was elicited by a brief flash. A maximal frequency of this response would be reached within 35 min. of the dark adaptation. After then, the frequency value was almost constant in spite of increase in sensitivity as dark adaptation proceeded. While the duration of the long-lasting-discharge increased gradually with increase in the duration of dark adaptation till it reached longer than 7 sec. at 72 min. from the beginning of the dark adaptation. However, no further increase of duration of this discharge was observed beyond this maximum, indicating a saturation.

The class 2 neurone, on the other hand, responded to turning off of the test

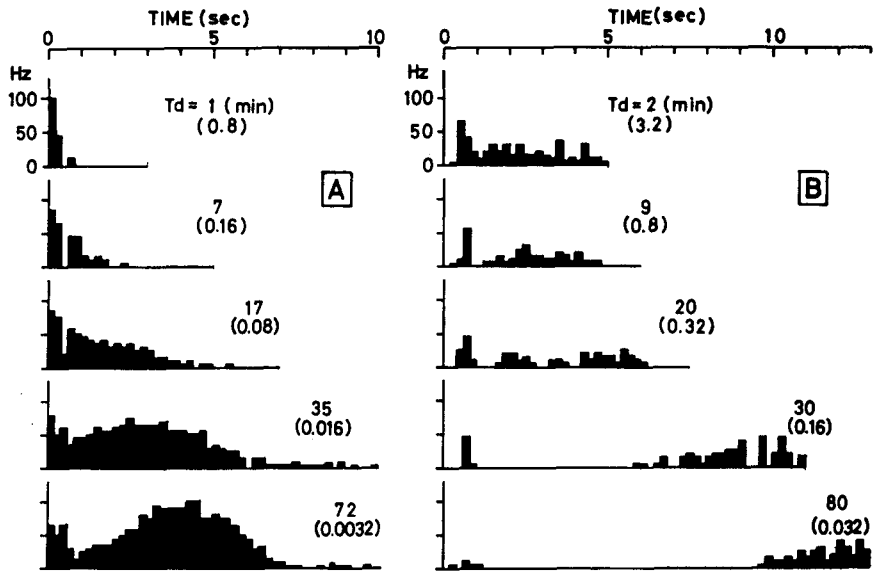

Fig. 6. Time courses of impulse discharge frequency of the both class neurones responding to test flashes of the fixed light intensity and duration interposed at different times of the dark adaptation. A; the class 1 neurone, B; the class 2 neurone. If=1,600 mL in A, 16,000 mL in B. Df=50 msec. in both. Frequency value was obtained from counting impulse number within time interval of 200 msec. The counting was made from turning on the test flash on the class 1 neurone and from turning off it on the class 2 neurone. The value in the blacket below the each Td value indicates a threshold (mL) of that time of the dark adaptation.

flash with off-discharge. The response magnitude of this discharge diminished gradually as dark adaptation progressed (Fig. 6B). Finally, the off-component almost disappeared at 80 min. of the dark adaptation. Such a disappearance of the off-component was generally found in the tests of changing both background and flash intensities. At all events, test flashes above 5–6 log units from the threshold elicited the phenomenon.

The second component of the response of the class 2 neurone, a rebounding-response which occurred after a short silent period following the off-discharge, became noticeable after the material was kept in the dark for more than 9 min.. At this point, the intensity of the test flash applied was approximately equivalent to 4 log units above the threshold and this value was equal to that of the class 1 neurone. Though the response magnitude of the rebounding-discharge increased as the dark adaptation progressed similarly to the class 1 neurone, the most marked event found in the class 2 neurone was an increase in latent period of the response. The latent period increased rapidly between 20–50 min. of the time kept in darkness, while in the earlier and later stages it showed a moderate increase or no change.

(2) *Illumination of the background (Ib)*: Effect of background illumination upon the rebounding-response of both classes of neurone was basically the same as the effect of the dark adaptation; decrease in illumination resulted in increase of duration of the response of the class 1 neurone and also of latent period of the class 2 neurone. Typical example of changing the background in step of 1 log unit is shown in Fig. 7. As all data were obtained after the material was kept under a given illumination for 10 min., in that state neurones seemed to be still in process of recovery in sensitivity. It appears that test flashes were applied before the transition points of time course curves of threshold was reached.

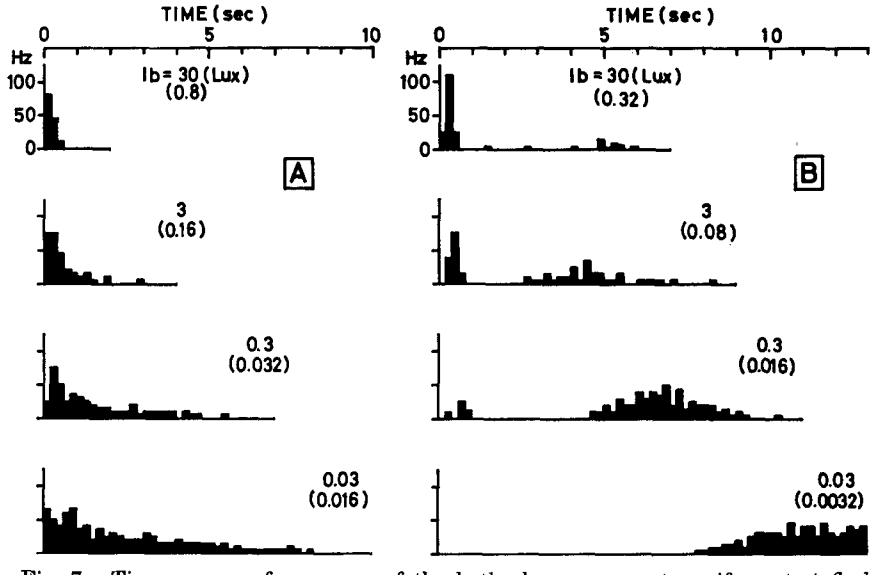

Fig. 7. Time courses of responses of the both class neurone to uniform test flashes applied at 10 min. from the beginning of different background adaptations. If=1,600 mL in A, 320 mL in B. The other expressions are the same as those of Fig. 6.

The rebounding-response of the class 1 neurone was caused by placing the material under the background illumination of 3 lux or less. The magnitude of the response increased with decrease in intensity of the background illumination. The duration of the long-lasting-discharge reached 7 sec. at 0.03 lux (Fig. 7A). Whereas, the rebounding-response of class 2 neurone appeared at 3 lux and the latency was 8 sec. at 0.03 lux of the background illumination (Fig. 7B).

These phenomena may be explained from the decrease in threshold with different background adaptations. The neurones shown here increased their sensitivities at least in amount of 2–3 log units at 0.03 lux more than those of 30 lux of the background illumination. Accordingly, it may be concluded that test flashes having the same original intensity produced the large effects of stimulation,

because of the added sensitivity of 2–3 log units which was gained during recovering processes of the adapting neurones.

(3) *Intensity of test flash* (If): As is mentioned in the previous two sections, the intensity of the test flash mainly appeared to determine the magnitude of the rebounding-response of both classes of neurone. Effects of varying intensity of test flashes at an equilibrium state under the given background illumination was tested. The results are graphically given in Fig. 8. The experiment showed that an increase in intensity of the test flash resulted in increase in magnitude of the response.

On the class 1 neurone, a rebounding response of duration of about 1 sec. appeared at 80 mL which was equivalent to an intensity of about 3 log units above the threshold (Fig. 8A). Following data of higher test flash intensities showed the fused responses, long-lasting-discharges, and their duration increased with the increase in intensity of the test flash. With 8,000 mL of the intensity, the duration of this response reached 10 sec. The rebounding-response of the class 2 neurone was also initially observable with 8 mL test flash which was approximately equivalent to an intensity of 3 log units above the threshold, and the latent period was 1.4 sec. (Fig. 8B). The latency of the response increased exponentially with logarithmic increase in intensity and reached longer than 10 sec. with 8,000 mL

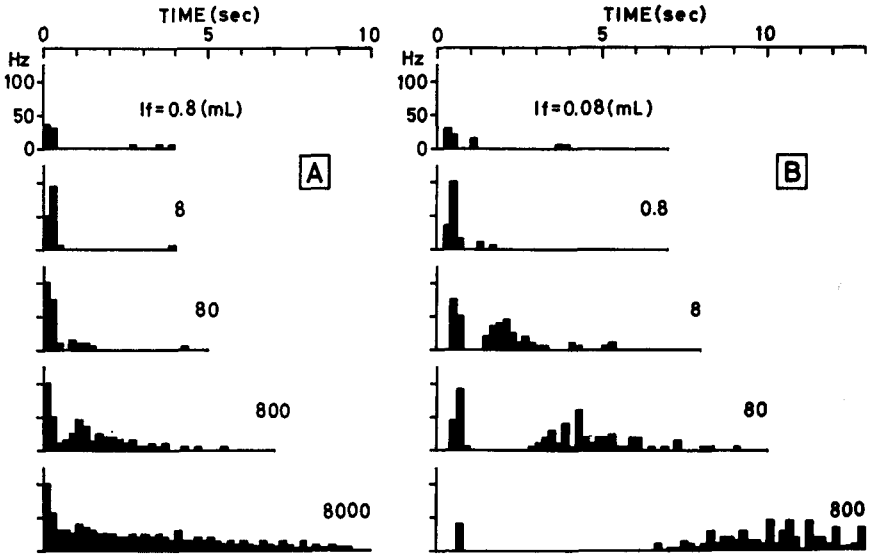

Fig. 8. Time courses of responses of the both class neurones to test flashes of different intensities and fixed duration (50 msec.). The data were obtained from recording successively at 10 min. after the material was placed under 0.3 lux background illumination. The threshold was 0.16 mL in A and 0.016 mL in B. The expressions are the same as those of Fig. 6.

test flash (not indicated in Fig. 8B).

In this way, neurones placed under the background intensity of less than 3 lux showed the rebounding-response with applications of test flashes of which intensity exceeded 3 log units above the threshold. The result was similar to the data which were obtained from the tests of changing Td and Ib. Although it is not shown here, the relationship between the threshold and the test flash intensity eliciting such rebounding-responses was tested also at the other background levels. The curve of the minimal intensities which elicited the response of each class of neurone plotted against the intensity of the background illumination ran approximately parallel to that of the thresholds, though at lower background level these two curves showed a tendency to approach each other. It was concluded that rebounding-responses of both classes of neurone were elicited by the photic stimulus of which intensity exceeds 3–4 log units above the threshold and that at this light level, 'on' and 'off' responses already appeared to be in saturation.

(4) *Duration of test flash* (Df): Effect of flash duration upon a rebounding-response was basically the same with those described in previous sections; increase in flash duration resulted in the increase in duration of the response of the class 1 neurone and also in latency of the class 2 neurone. The data are graphically shown

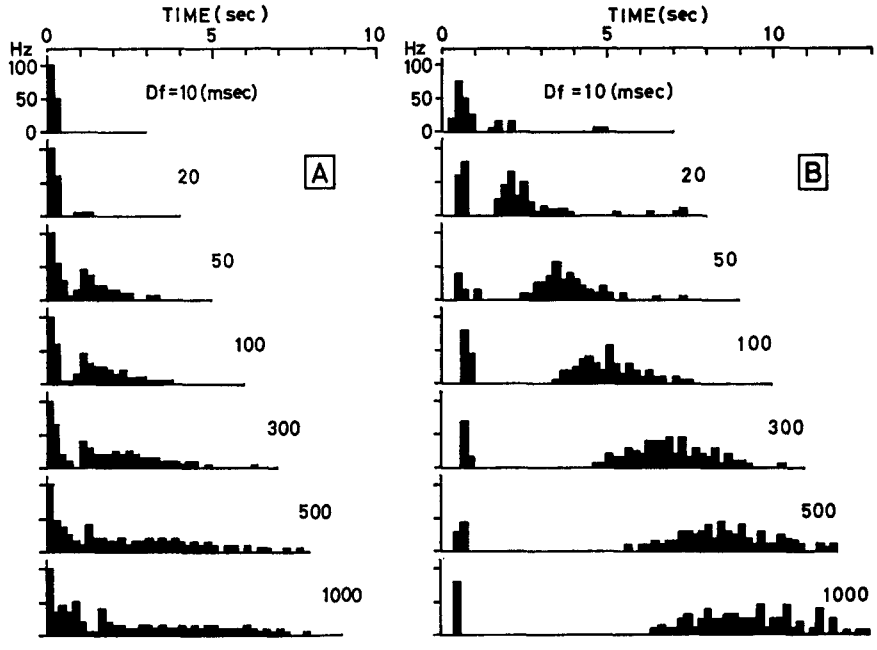

Fig. 9. Time courses of response of the both classes of neurone to test flashes of different durations and fixed intensity (80 mL). The data were obtained just after the test shown in Fig. 8 was completed. The expressions are the same as those of Fig. 6.

in Fig. 9 which was obtained from response to an uniform intensity of test flashes of varying duration in ranges of 10–1,000 msec.

Rebounding-responses of the two class neurones appeared with the test flash of 20 msec. in duration. At the longest duration of the test flash used in the present study (1,000 msec.), the duration of the response of the class 1 neurone as well as the latency of the class 2 neurone was 6–7 sec. The values of the duration or latency against a logarithm of flash duration between 20–500 msec. increased linearly, not exponentially as was found with varing flash intensity.

Discussion

Receptor input of tectal neurones recorded

It must be the first importance to find out whether each class of neurone studied here receives a pure cone or rod input or a mixed rod and cone input. Structural organization of the optic tectum in the carp, the goldfish and the crucian carp was investigated by Leghissa (1955), Ito (1970) and Niida and Sato (1972a). According to them, in the superficial layers down to about 200 μ from the surface, no marked cell was found except for thick myelinated fibres, which can be traced to the optic tract. Electrical activities in this study were obtained from neurones which located in a layer 50–200 μ from the tectal surface. No distinctly different types of neurones was found in this layer from those of the optic tract (Jacobson, 1968; Niida and Sato, 1972a). From these findings, it is safe to assume that the neurones recorded from the optic tectum in this study are the same ones observed in the optic tract, that is, retinal ganglion cells.

The crucian carp has a retina of mixed cone and rod population. The rod density in the posterior portion of the retina is richer than in the anterior (Takatuzi, 1939). The neurones having their receptive fields in the nasodorsal area, which is related to the posterior-ventral portion of the retina, showed a simple relationship of two components during dark adaptation; the first one is an initially rapid exponential function and second is an ensuing-comparatively slow exponential function. These results show that these neurones have input from both kinds of receptors, cones and rods, which undergo dark adaptation. The first component originates from the cones, while the second one from the rods. The dual components of recovering process of threshold in the dark are well known in ganglion cell responses of the other duplex retinas (Donner and Reuter, 1965; Dowling, 1967; Baumann and Scheibner, 1968).

Furthermore, facts that the ganglion cells in the fish retina receive the mixed cone and rod input are directly shown by means of the measurement of spectral sensitivity (Wagner et al., 1960; Daw, 1967; 1968; Witkovsky, 1967; Raynauld, 1969). In comparing response types in light adaptation with those in dark adaptation, Raynauld (1972) found in the goldfish retina that after light adaptation all on-centre ganglion cells in the dark turned into red on-centre, and off-centre cells into red off-centre cells. Undoubtedly, the class 1 neurone of the crucian

carp defined in the present study corresponds to the Raynauld's former ganglion cell and the class 2 neurone to the latter one.

Parameters eliciting and influencing the rebounding-response

A phenomenon that closely resembles the rebounding-response of the class 1 neurone found in the present study had been obtained from axones of the visual receptor cells of *Limulus* by Hartline and MacDonald (1947). They reported that exposure to light decreased the ability of the receptor to respond to a short test flash of light; during the subsequent stay in the dark the discharge of impulses elicited by a test flash of fixed intensity and duration increased, both in the number and the frequency of impulses in the burst. By making use of the response, they investigated mainly the effect of the light adapting exposure upon the recovering process of sensitivity in darkness, and obtained the result that the loss of sensitivity following exposure to light and the time required to regain dark adaptation were strongly affected by the duration and intensity of the exposure. In the test of strong light adaptation, furthermore, these two factors were interchangeable within a certain limit. However, recovery was faster following extremely short intense exposure than after very long weak ones. The fact found by Hartline and McDonald suggests that in an extent, effects of exposure intensity and duration upon the recovering process of sensitivity may differ a little.

As to a phenomenon similar to that of the class 2 neurone of the present study, a delayed response is observed in frog's ganglion cells by Pickering and Varjú (1967) and Pickering (1968). When the eye of the frog was stimulated under certain restricted conditions by flashes of light, a response pattern of retinal ganglion cells showed 'apparent latency' or 'delay time' which was long, and the response was not seen until 20 sec. after stimulus application. The duration of this silent period before the response was influenced by three parameters; (1) background illumination, (2) degree of dark adaptation and (3) stimulus flash intensity (Pickering and Varjú, 1969).

In the present study, on the other hand, rebounding-responses of both classes of neurone of the crucian carp were produced and modified by the four stimulus parameters; (1) time kept in the dark (Td), (2) intensity of background illumination (Ib), (3) intensity of test flash (If) and (4) duration of test flash (Df). Duration of the rebounding-response of the class 1 neurone and also latent period of the class 2 neurone increased with the increase in Td, If and Df and with the decrease in Ib.

Summarizing the stimulus parameters influencing these phenomena, discharge patterns and response magnitudes of visual sense cells during light and dark adaptation are affected by duration and intensity of light adapting exposure, time in darkness, intensities of the background and intensity and duration of the test flash.

Whether these parameters are independent each other or not is questionable. For example, Fig. 10 shows graphically a relationship between the light quantity

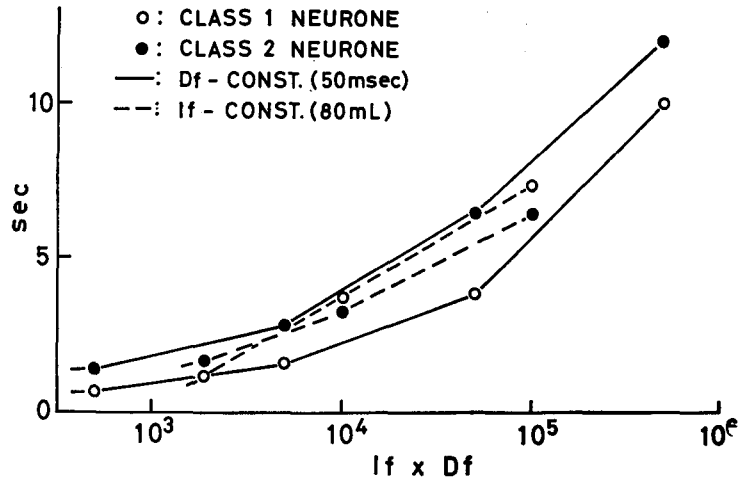

Fig. 10. A relationship between light quantities (intensity x duration) of the stimuli and magnitudes of the rebounding-responses of both class neurones. The data were calculated from the records shown in Fig. 8 and 9. The measurements are as follows; on the class 1 neurone, the time interval from the onset of the test flash to the end of the rebounding-discharge, and also on the class 2 neurone, the time interval from the end of the test flash to first impulses of the rebounding-discharge.

(If×Df) and the rebounding-response magnitude of both classes of neurone. The duration of the long-lasting-discharge of the class 1 neurone and also the latency of the rebounding-response of the class 2 neurone were measured as the criteria indicating the response magnitude. The responses from separate tests of constant intensity and constant duration performed on the same neurone increased with an increase in the light quantity in each neurone. Two curves, however, do not exactly overlap. On the test of the fixed duration and varied intensities, the response magnitude against the logarithm of light quantity increased exponentially, while linearly on the test of the reverse condition. This suggests that there is certainly a different mechanism between intensity and duration factors of the photic stimulus which causes the rebounding-response of the dark or light adapted tectal neurone, as was shown by Hartline and MacDonald. A quantitative description and discussion of the rebounding-response in detail shall be reported elsewhere (in preparation).

Origin of the rebounding-response

An original site causing the rebounding-response is almost unknown except that it may occur within the retina. Whether the response depends upon photochemical processes within receptor cells or upon neural adaptation processes of a retinal network is open to question. Pickering and Varjú (1969) tried to explain

the delayed response from assuming two converging retinal processes upon the ganglion cells, long-lasting excitatory and inhibitory processes. They concluded that the delayed response might easily be obtained by the summation of the two excitatory and inhibitory curves. Assuming that the rebounding-response of the present study may also be caused by the same mechanism to that on the frog's ganglion cells, it can be speculated that in which cells of the dark adapted retina such long-lasting excitatory and inhibitory processes are found.

According to the morphological study of the carp's retina by Witkovsky and Dowling (1969), a ganglion cell receives directly both bipolar and amacrine cells inputs. Bipolar cells of the carp and the goldfish were found to be classified into two opposing types, depolarizing and hyperpolarinzing types and amacrine cells were also divided into two types, transient and sustained types in photopic range (Kaneko and Hashimoto, 1969; Kaneko, 1970; Toyoda, 1973; Toyoda *et al.*, 1973). In dark adapted retina, on the other hand, bipolar cell responses saturate in its amplitude with flash intensities about 3 log units above threshold and form a prolonged plateau when the stimulus intensity exceeds the saturation level (personal communication with Toyoda). Therefore, at the neuronal level of bipolar cells two long-lasting excitatory and inhibitory processes are already proved to exist by an intracellular recording.

Next, bipolar cells receive directly cone and/or rod input. It is well known that both cones and rods in vertebrate retinas are hyperpolarized by light (Toyoda *et al.*, 1969, 1970). These cone and rod responses to photic stimulus differ throughly in their time courses. The cone response was saturated at relatively high intensity of light and showed a relatively fast recovery to the intense light. The rod response, on the other hand, was saturated in amplitude at relatively low light intensity and formed a prolonged plateau at this saturation level even when the intense light was turned off ('positive after-effect' by Toyoda *et al.*, 1970). Accordingly, it can be concluded that the main originating site of the rebounding-response may be a rod and that its positive after-effect is modified via a retinal network function (mainly bipolar cell function) and then converged upon the ganglion cell.

Physiological significance of the rebounding-response

Many psychophysical investigations have been done on 'afterimage' experienced by human (Brindley, 1959, 1961, 1962; Padgham, 1963, 1968; Barlow and Sparrock, 1964). If the eye is placed in complete darkness immediately after exposure to a strong bleaching light source, it is possible to see an image of the light for some seconds following exposure. It is a difficult question to answer whether or not a fish indeed 'percept' afterimages following the flash given in the present experimental condition. Behavioural studies appear to be necessary to answer this question.

Formerly, from behavioural observation, Swindle (1919) concluded that birds

might possibly have afterimages and that an owl's afterimage was of 40 sec. duration and that a cockatoo's was of 20 sec. duration. The present experienced results indicated that the long-lasting-discharge of the class 1 neurone was of several seconds or more and that the duration of some neurones reached few tens of seconds. Though the similarity could be superficial, these data of birds indicate that the duration of the long-lasting-discharge of the crucian carp is certainly of a magnitude which would suggest the similarity between two phenomena.

Possibility of the similarity between the rebounding-responses on the crucian carp and human afterimages can be also considered, in particular in the time courses of both phenomena after a brilliant flash was applied. It is well known that human afterimages pass through a variety of stages, or phases, in which both hue and brightness changes are quite evident. The afterimage of brightness may be positive, in which the light and dark parts correspond to those of the object, or negative, in which the light and dark parts reverse. These positive and negative phases alternately appear and fade with a certain periodicity. The present experienced results showed that the tectal neurones behaved to brilliant flashes of short duration, as if the light and dark images between a part exposed by the flash and the other non-exposed remained within the retina for a considerably long duration after the light flash was ceased.

The class 1 and the class 2 neurones have a receptive field consisted of a centre and antagonistic surrounding area and respond to an intensity difference of lights illuminated upon them; the class 1 neurone excites only when the light intensity in the centre exceeds that of the surrounding (centre>surrounding relation) and the class 2 neurone does when the oppsite relation is held. As is obvious in the present results of the timings of appearance of their rebounding responses, the class 1 neurone excited first and then did the class 2 neurone, although the test flashes were applied only to the centres of both classes of neurone. (Receptive fields of both types of neurone overlap and sometimes a simultaneous recording from those neurones can be obtained. The alternation of excitation of both neurones is clearly seen in such a recording). This fact suggests that after the test flash, the centre>surrounding image in brightness may be formed at the first stage, then followed by the centre<surrounding image in brightness within retina. It is possible to consider that a time during which the former image is formed may correspond with the positive phase and the latter one with the negative phase known in psychophysics. If such an association between the data of psychophysics and neurophysiology can be shown, the rebounding-response would have real significance on the animal vision; further experiments in electrophysiology and behaviour will be needed to produce a satisfactory explanation.

Summary

Single neuronal activity in response to light flashes of short duration was recorded with a tungsten microelectrode from the optic tectum of the crucian

carp, *Carassius auratus langsdorfii* Temminck et Schlegel, during light and dark adaptation.

When the brief and relatively intense test flash was applied upon the fish's eye during the course of dark adaptation, the class 1 neurone showed an initial brief on-discharge and a rebounding-discharge, which appeared after a short silent period ensuing to the on-discharge. This silent period gradually shortened depending on the time of the dark adaptation. In the later stage of the dark adaptation, the on- and rebounding-discharges fused together and became a high frequency impulse discharge lasting for several seconds. The class 2 neurone, on the other hand, showed usually a brief off-discharge and a rebounding-discharge appeared after a long latent period when the intense test flash was applied in the dark. As the dark adaptation proceeded, the silent period of this class neurone was gradually prolonged and reached longer than 10 sec. in the later stage of dark adaptation.

On the background adaptation, the threshold of both class neurones increased with decrease in the intensity of the background and the curves of the logarithmic intensity against the logarithmic threshold showed an approximately linear relationship having a slope of 3/5 on both classes of neurone. When the eye was placed under different background intensities after an exposure by the relatively brilliant illumination was completed, the time dependent change of thresholds of the class 2 neurone resulted in indicating one exponentially decaying curve under the higher intensities of the background illumination (30, 3 lux), while under the lower ones (0.3, 0.03 lux), it appeared to be separated into two curves. In the complete darkness, this tendency found in the class 1 neurone also appeared more markedly and the neurones recovered their sensitivites in ranges of 3–4 log units.

From detailed analyses of effects of light stimulation upon them, rebounding-responses of both classes of neurone were found to be caused by the four stimulus parameters; time kept in the dark (Td), intensity of the background illumination (Ib) and intensity (If) and duration (Df) of the test flash. Within them, 'If' was the most important factor and it was clearly observable that rebounding-responses of both class neurones were elicited by the test flash of which intensity exceeded 3–4 log units above the threshold under various conditions and that at this intensity level usual on- or off-response already reached to the saturation. On the other hand, duration of the response of the class 1 neurone and also latency of the response of the class 2 neurone increased with the increases in Td, If and Df, and with the decrease in Ib.

From the response types of cells composed of the retina observed, it could be concluded that the originating site causing the rebounding-response might be a rod and that its positive after-effect might be modified via a retinal function (perhaps a function of bipolar cells) and then converged upon the ganglion cell. Physiological significance of the rebounding-discharge was discussed mainly in relation to the time course of afterimages seen by man.

Acknowledgement: The author wishes to express his gratitude to Professor Mituo Tamasige, Dr. Mituhiko Hisada and Akiyoshi Niida, of Zoological Institute, Hokkaido University, for their expert guidance throughout the course of this work and also for their kindness in critical reading of the manuscript.

References

Barlow, H. B. and J. M. B. Sparrock 1964. The role of afterimages in dark adaptation. Science **144**: 1309–1314.

Baumann, C. and H. Scheibner 1968. The dark adaptation of single units in the isolated frog retina following partial bleaching of rhodopsin. Vision Res. **8**: 1127–1138.

Brindley, G. S. 1959. The discrimination of after-images. J. Physiol. **147**: 194–203.

———— 1961. Two new properties of foveal afterimages. *Ibid.* **158**: 20P.

———— 1962. Two new properties of foveal afterimages and a photochemical hypothesis to explain them. *Ibid.* **164**: 168–179.

Craik, K. J. W. and M. D. Vernon 1941. The nature of dark adaptation. Brit. J. Psychol. **32**: 64–81.

Daw, N. W. 1967. Goldfish retina: Organization for simultaneous color contrast. Science **158**: 942–944.

———— 1968. Colour-coded ganglion cells in the goldfish retina: Extension of their receptive fields by means of new stimuli. J. Physiol. **197**: 567–592.

Donner, K. O. and T. Reuter 1965. The dark-adaptation of single units in the frog's retina and its relation to the regeneration of rhodopsin. Vision Res. **5**: 615–632.

Dowling, J. E. 1967. The site of visual adaptation. Sience. **155**: 273–279.

Hartline, H. K. and P. R. McDonald 1947. Light and dark adaptation of single photoreceptor elements in the eye of *Limulus*. J. Cell. and Comp. Physiol. **30**: 225–253.

Hecht, S. 1937. Rods, cones, and the chemical basis of vision. Physiol. Rev. **17**: 237–290.

Ito, H. 1970. Fine structures of the carp tectum opticum. J. Hirnforsch. **12**: 325–354.

Jacobson, M. 1968. Physiology of fish vision. In "Central Nervous System and Fish Behavior" D. Ingle, Ed. (Univ. of Chicago Press, Chicago). 17–24.

Kaneko, A. 1970. Physiological and morphological identification of horizontal, bipolar and amacrine cells in goldfish retina. J. Physiol. **207**: 623–633.

———— and H. Hashimoto 1969. Electrophysiological study of single neurones in the inner nuclear layer of the carp retina. Vision Res. **9**: 37–55.

Leghissa, S. 1955. La struttura microscopica e la citoarchitectonica del tetto ottico dei pesci teleostei. Z. anat. Entwicklungsgesch. **188**: 427–463.

Lythgoe, R. J. 1940. The mechanism of dark adaptation. Brit. J. Ophthal. **24**: 21–43.

Niida, A. and Y. Sato 1972a. Single unit analysis of the optic tract and optic tectum of the fish, *Carassius carassius*. Zool. Magazine **81**: 16–31.

———— and ———— 1972b. An analysis of visual responses in the optic tract and tectum of the crucian carp. J. Fac. Sci. Hokkaido Univ. Ser. VI Zool. **18**: 371–386.

Padgham, C. A. 1963. The role of the retinal receptors in the formation of the positive visual after-image. Vision Res. **3**: 45–49.

———— 1968. Measurements of the colour sequences in positive visual after-images. *Ibid.* **8**: 939–949.

Pickering, S. G. 1968. The extremely long latency response from on-off retinal ganglion cells: relationship to dark adaptation. *Ibid.* **8**: 383–387.

——— and D. Varjú 1967. Ganglion cells in the frog retina inhibitory receptive field and long-latency response. Nature **215**: 545–546.

——— and ——— 1969. Delayed responses of ganglion cells in the frog retina: the influence of stimulus parameters upon the length of the delay time. Vision Res. **9**: 865–879.

Raynauld, J. P. 1969. Rod and cone responses of ganglion cells in goldfish retina: A microelectrode study. Ph. D. Thesis., Johns Hopkins Univ. Baltimore, Md.

——— 1972. Goldfish retina: Sign of the rod input in opponent color ganglion cells. Science **177**: 84–85.

Rushton, W. A. H. 1961. Dark-adaptation and the regeneration of rhodopsin. J. Physiol. **156**: 166–178.

Sato, Y. and A. Niida 1972. Rebounding discharge of tectal neurone of crucian carp in the dark adapted state. Zool. Magazine **81**: 165–168.

Swindle, P. F. 1916. Positive afterimages of long duration. Amer. J. Psychol. **27**: 324–334.

Takatuzi, M. 1939. Die sehzellen in der Netzhaut der Fische, besonders ihre Reihenordnungen. Kaibogaku Zassi **15**: 1–69.

Toyoda, J. 1973. Membrane resistance changes underlying the bipolar cell response in the carp retina. Vision Res. **13**: 283–294.

———, Hashimoto, H., Anno, H. and T. Tomita 1970. The rod response in the frog as studied by intracellular recording. *Ibid.* **10**: 1093–1100.

———, ——— and K. Ohtsu 1973. Bipolar amacrine transmission in the carp retina. *Ibid.* **13**: 295–307.

——— , Nosaki, H. and T. Tomita 1969. Light-induced resistance changes in single photoreceptors of *Necturus* and *Gekko*. *Ibid.* **9**: 453–463.

Wagner, H. G., MacNichol, E. F. Jr. and M. L. Wolbarsht 1960. The response properties of single ganglion cells in the goldfish retina. J. Gen. Physiol. **43**: 45–62.

Werblin, F. S. 1971. Adaptation in a vertebrate retina; Intracellular recording in *Necturus*. J. Neurophysiol. **34**: 228–241.

——— and J. E. Dowling 1969. Organization of the retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *Ibid.* **32**: 339–355.

Witkovsky, P. 1967. A comparison of ganglion cell and S-potential response properties in carp retina. *Ibid.* **30**: 546–561.

——— and J. E. Dowling 1969. Synaptic relationship in the plexiform layers of carp retina. Z. Zellforsch. **100**: 60–82.