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SCREENING-PIGMENT MIGRATION IN THE OCTOPUS RETINA INCLUDES CONTROL BY DOPAMINERGIC EFFERENTS

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Summary

The extent of screening-pigment (SP) migration in the intact octopus retina and the amplitude of the early receptor potential (ERP) correspond with the degree of adaptation to light or darkness. The light-adapted retina has SP granules concentrated in an apical layer, at the tips of the photoreceptor rhabdoms and supporting cells, and the ERP is barely detectable. In the fully dark-adapted retina, the SP granules are mostly at the base of the rhabdoms, and the ERP is at its maximum. Retinae at intermediate stages, between the fully dark- and light-adapted states, show corresponding intermediate stages of SP migration and ERP amplitude.

A series of experiments demonstrates the effects on SP migration of the efferent nerves, which form a subset of fibres in the optic nerves. When the optic nerves to one half of the retina have been severed, there is a dramatic difference in the distribution of SP in areas of the retina (of the dark-adapted eye) connected with severed or intact nerves: apical *versus* basal, respectively. On incubation of a light-adapted retina with $5\,\mu mol\,l^{-1}$ dopamine, but not with other catecholamines or other putative neurotransmitter substances, SP migrates basally and the ERP is significantly larger than for controls. In octopuses treated with reserpine, SP stays in an apical location and the ERP remains very small, regardless of the state of adaptation and of whether the optic nerves are intact. It is concluded that dopaminergic efferents from the optic lobes effect dark-adaptational SP migration in the cephalopod retina. The arrival in the retina of efferent signals that effect adaptational changes through the mediation of dopamine is a remarkable analogue of the vertebrate system.

Introduction

Octopuses are alert for long periods of the day, looking out at the open sea from a den during the daylight and occasionally foraging for food, and making long hunting trips at night (Kayes, 1974). So they are required to cope with a wide range of ambient light

Key words: dopamine, efferent innervation, octopus, reserpine, retina, screening pigment, early receptor potential.

intensities: possibly some 8 logarithmic units, from starlight to bright sunlight (e.g. Barlow *et al.* 1989). The dynamic range of photoreceptor responses is typically much narrower (approximately 3 log units; e.g. Fein and Szuts, 1982) so that some form of adaptation is necessary to provide appropriate changes in sensitivity.

Known adaptation mechanisms include (i) pupil constriction to reduce the amount of light entering the eye (e.g. Fein and Szuts, 1982); (ii) changes in the photoreceptor membrane (e.g. Tsukahara and Horridge, 1977; Hochstrate and Hamdorf, 1990); (iii) retinomotor movements (e.g. Kohler *et al.* 1990); and (iv) the effects of screening pigments (e.g. Stavenga, 1989). Movements of the latter have been observed for more than a century (see reviews in Young, 1963; Daw and Pearlman, 1974; Autrum, 1981; Stavenga, 1989) and have been used to investigate the course of light- and dark-adaptation in several of the major groups of experimental invertebrates: see, for example, Lo and Pak (1981; *Drosophila melanogaster*); Aréchiga *et al.* (1990; *Procambarus clarkii*); Kier and Chamberlain (1990; *Limulus polyphemus*); Daw and Pearlman (1974; *Loligo pealei*); and, for *Octopus vulgaris*, Young (1963).

It is known that the mechanisms controlling screening-pigment (SP) migrations for light-adaptation may be different from those governing dark-adaptation (e.g. Kier and Chamberlain, 1990). Further, the mechanism governing light-adaptation, for example, may be different among species of the same animal group, or there may be more than one mechanism within the same eye (Nilsson *et al*. 1989; Frixione and Perez-Olvera, 1991; Besharse and Iuvone, 1992; Nilsson *et al.* 1992). In coleoid cephalopod molluscs, SP migrates apically during light-adaptation in the isolated eye, i.e. in the absence of efferent innervation (Hagins and Liebman, 1962; Young, 1963). In the present paper, evidence is provided that, during dark-adaptation, dopaminergic efferent innervation controls the retraction of SP.

A role for efferent innervation as a means of effecting adaptation of the retina has been well demonstrated in other animal groups (e.g. Barlow *et al.* 1977; Zucker and Dowling, 1987; Aréchiga *et al.* 1990; Kier and Chamberlain, 1990). In *Limulus*, the radial dispersal of SP from the central rhabdom during dark-adaptation can be mimicked by stimulating optic efferents or by the addition of octopamine (Barlow and Chamberlain, 1980; Battelle *et al.* 1982; reviewed by Barlow *et al.* 1989). In the crayfish *Procambarus*, 5-hydroxytryptamine (5-HT) fulfils an analagous ‘dark response’ function (enhanced sensitivity and retraction of intra-receptor SP from the rhabdom; Frixione and Hernández, 1989; Aréchiga *et al.* 1990). Vertebrate efferents containing FMRFamide-like and LHRH-like peptides synapse onto interplexiform cells (Zucker and Dowling, 1987), which subsequently release dopamine (see reviews by Dowling, 1991; Witkovsky and Dearry, 1991; Besharse and Iuvone, 1992). This substance has a number of effects on retinal sensitivity, such as alterations to horizontal cells and movements of the photoreceptors, and on SP migration in the retinal pigment epithelium. Dopamine release in the vertebrate eye is involved (at different sites) in both dark- and light-adaptation (Besharse and Iuvone, 1992). A role for dopamine in the efferent control of retinal adaptation in cephalopods therefore provides yet another example of the remarkable physiological and anatomical analogies that exist between the cephalopods and vertebrates (cf. Packard, 1966, 1972; Budelmann and Bleckmann, 1988).

The relatively simple morphology of the cephalopod retina (Fig. 1) and the linear movement of SP within the (non-inverted) receptor cells make this a relatively straightforward experimental system. There appear to be only two types of peripheral interaction: one involving the fine collaterals of the photoreceptors, and the other involving a system of fine efferent fibres that arise in the outer layers of the optic lobe and end in the vicinity of the photoreceptor cell somata (Tonosaki, 1965; Yamamoto *et al.*

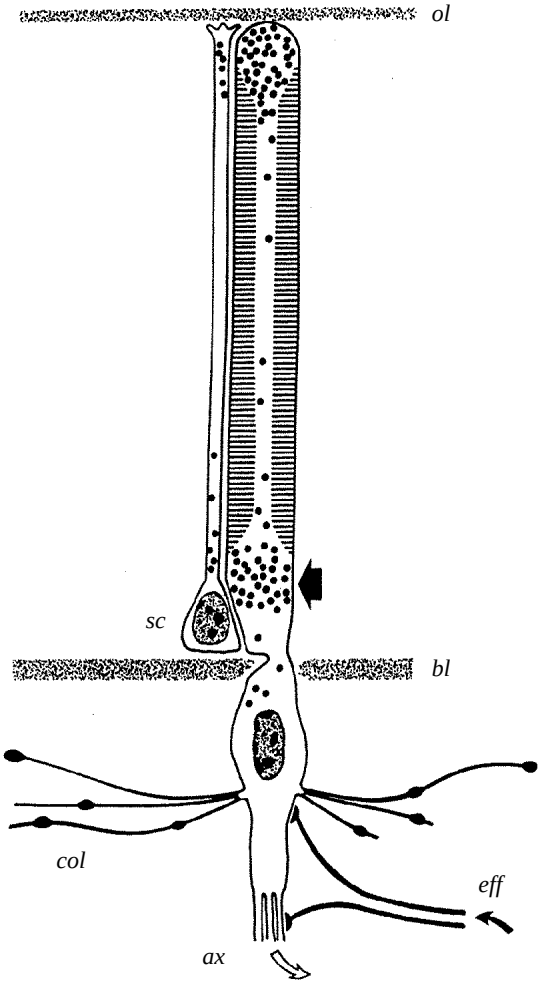

Fig. 1. Diagram of the main components of the octopus retina based on the electron microscopical investigations and diagrams of Tonosaki (1965), Yamamoto *et al.* (1965) and Yamamoto *et al.* (1976). The collaterals (*col*) are very fine processes arising near the base of the photoreceptor cell body. Varicosities on collaterals represent synapses onto other photoreceptor cells. Efferents (*eff*) arising in the optic lobe end in synapses with the photoreceptor cell bodies and their processes. *ax*, axon-like processes of the photoreceptor; *ol*, outer lamina; *bl*, basement lamina; *sc*, supporting cell. The large filled arrow indicates a layer of proximal screening pigment which does not migrate. The filled curved arrow indicates the path of afferents to the optic lobe; the open curved arrow indicates the path of efferents.

1965, 1976; Lund, 1966; Saidel, 1979; Tasaki *et al.* 1982; Silver *et al.* 1983). Lam *et al.* (1974) first suggested that the photoreceptor synapses are cholinergic and that the efferents that terminate in the retina are dopaminergic. Further experiments (i) confirmed the association between efferent endings and the presence of dopamine (Tasaki *et al.* 1982; Silver *et al.* 1983); (ii) demonstrated that both cholinergic collaterals and dopaminergic efferents have 'inhibitory' effects involved in 'on-centre'/'off-surround' retinal functioning (Tasaki *et al.* 1982; Suzuki and Tasaki, 1985); and (iii) showed that the efferents arise in the optic lobes (Tasaki *et al.* 1982) and fire in response to afferent stimulation (Patterson and Silver, 1983).

Young (1963) made a detailed study of SP migration in unstained sections of retinae from a cuttlefish, a squid and two genera of octopuses. He noted the basic trends of SP migration (towards the rhabdom base during dark-adaptation; apically in response to light) and compared responses for dorsal, central and ventral regions of the retina. He found differences in the velocity and extent of SP movements, both within the retina and among the four species studied. Young also recognized a central strip of longer photoreceptors, where apical SP migration during light-adaptation was minimal. In none of the species studied was a well-defined 'fovea' discernible, but the poor performance of apical SP migration in the central strip led to the suggestion that the receptors here may constitute a relatively insensitive photopic region (Young, 1963). In view of these findings, histological studies in the present paper are limited to the ventral pole of the retina.

Before fixation of the retinae for histology, measurements of the size of the early receptor potential (ERP) were taken, to obtain a quantitative indication of the extent of SP migration. The cephalopod ERP (or 'fast photovoltage') is a photochemically produced charge displacement within the rhodopsin molecules in the rhabdom plasma membrane (Hagins and McGaughy, 1967, 1968; see also Järvilehto, 1979). The amplitude of the ERP depends on the number of photoconverted rhodopsin molecules and is therefore expected to be related to the screening effects of SP migration. The lighting conditions (see below) for the experiments limit the metarhodopsin content for each animal to a maximum of approximately 24% of total visual pigment. Under these conditions, and since the area in contact with the ERP suction electrode is constant, responses from different retinae are much more easily comparable than electroretinogram (ERG) responses (the amplitude of the ERG being a combined response reflecting the effects of other adaptation mechanisms, in addition to SP migration).

Preliminary results were presented at the third International Congress of Comparative Physiology and Biochemistry.

Materials and methods

Animals

The species used was *Octopus fangsiao* d'Orbigny [1840 (=*O. ocellatus* Gray, 1849)], obtained locally and fed *ad libitum* on local bivalves. Only mature male octopuses were used (body masses 60–100g). They were maintained in an outdoor (covered) aquarium at

the Marine Biological Laboratory of Okayama University, Ushimado, Japan, where all experiments were performed. The aquarium was provided with a continuous open flow of aerated natural sea water at ambient temperatures (9–14˚C).

Dark- and light-adaptation

For adaptation to dark or light, octopuses were placed in indoor tanks. That for dark-adaptation was of heavy-duty black plastic, closed with a tightly fitting lid skirted with a light-trap of heavy black plastic sheeting, and kept in a dark room. For light-adaptation, an acrylic tank (38cm diameter) was backed with a reflecting layer of aluminium foil and the lid was a sheet of coloured transparent acrylic, which formed an orange cut-off filter with negligible transmission at wavelengths shorter than 540nm (Fig. 2). The water was 25cm deep and approximately 7cm below the lid. Both tanks (and the heat-sink jacket of the light-adaptation tank) were supplied with fresh sea water from the same source as the outside aquarium.

Animals were light-adapted by illumination with a single 500W bulb (Toshiba Photoreflector Flood, 100V) clamped 50cm above the orange filter, which together provided an illuminance of 5300lx at the water surface. The transmittance characteristics of the filter favour the accumulation of a uniform amount of rhodopsin in the light-adapted retina: approximately 76% of total photopigment, as estimated by inspection of Fig. 2 (since photo-conversions between rhodopsin and its metarhodopsins occur freely

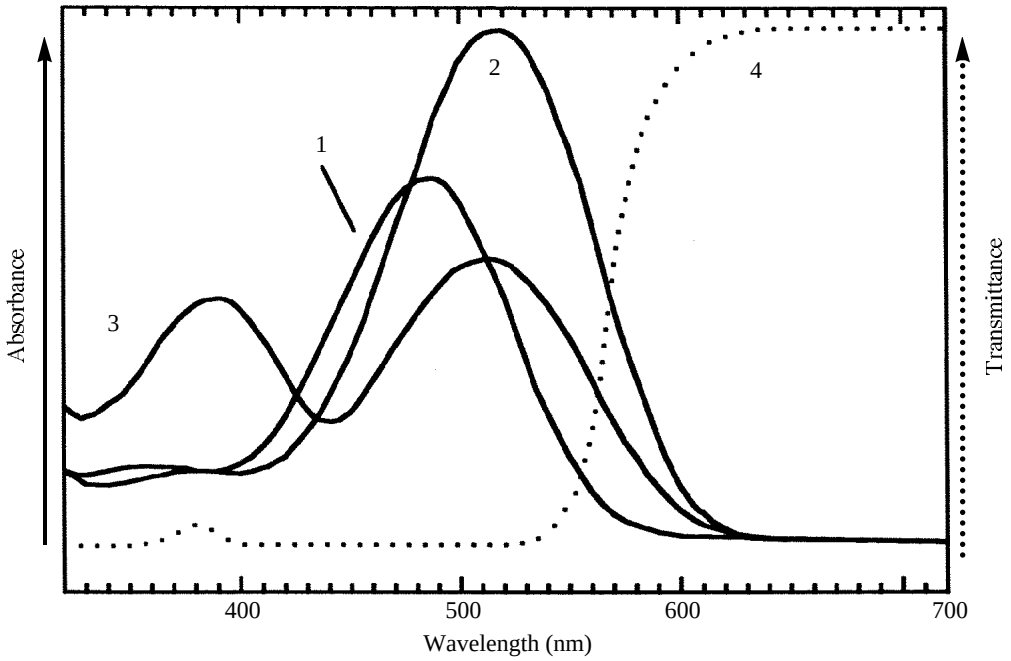

Fig. 2. Absorption spectra (curves 1–3) of the rhodopsin and transmission spectrum (curve 4) of the orange filter used in this study. Absorption spectra: curve 1, pure rhodopsin, pH7.2; curve 2, pure rhodopsin after irradiation with light of 410nm; curve 3, as for curve 2 but after adjustment to pH9.5.

within the cephalopod photoreceptor; Seki, 1984; Hara, 1988; Terakita *et al.* 1988). Octopuses maintained in these conditions for 3h were considered to be fully light-adapted, and this formed the starting point for following the progress of dark-adaptation.

For all experiments, intact animals were adapted for the stated times, after which they were killed by decapitation. Enucleation of the eyes and preparation of the retinae (for electrophysiology and/or histology) were performed with the aid of a dim deep-red safelight.

Electrophysiology

The method for recording the octopus ERP has previously been described in detail (Ohtsu and Kito, 1985; Ohtsu, 1989). Briefly, an eye was enucleated in deep-red light and the lens and anterior half of the eye were removed. This eye-cup preparation was pinned, scleral-side down, to a slab of silicone rubber mounted on an acrylic plate, which was slotted vertically into the recording apparatus. A hole through the silicone and the acrylic plate received a glass L-piece, of 3mm internal diameter, which, with gentle suction against the exposed sclera, sealed off a chamber for the reference electrode. The recording electrode was placed, out of the light path, in the circulating sea water (cooled and oxygenated) bathing the vitreous side of the retina. Both electrodes were chloridised silver plates, shielded from stray light by black lacquer.

The ERP was evoked by a bright flash of white light (Sunpak GX 340, delivered *via* a light guide) and recorded on film after conventional amplification and display. The preparation time for each retina was standardized by presenting the flash exactly 15min after the animal had been killed.

Histology

To see the extent of pigment migration, retinae were fixed in fresh 2.5% glutaraldehyde (in 0.45mol l^{-1} sucrose buffered with 0.1mol l^{-1} sodium cacodylate, pH7.4). Small pieces of tissue were trimmed from the same region of each retina (the ventral pole) to minimize the effects of any regional variations in SP distribution (see Young, 1963). Following dehydration through ethanol and epoxypropane, the pieces of retina were embedded in Taab resin. Sections of 1.0–1.5 μm were stained lightly with 1% Toluidine Blue.

Quantification of pigment migration

Light micrograph images were scanned with a Hamamatsu Percept Scope C3160 image analyzer. Measurements were taken of the percentage area occupied by SP within 20 consecutive (identical) rectangular grids, adjusted for each micrograph to range from the tips of the rhabdoms to the proximal band of non-migrating SP.

Anaesthesia

Octopuses used to investigate the effects of optic nerve sectioning were anaesthetized by immersion for 2min in sea water containing 2% ethanol. This induced general anaesthesia, characterized by (i) relaxation of the postural musculature and apparent loss of ‘consciousness’; (ii) widening of the pupils; and (iii) cessation of breathing

movements, but not of the heartbeat. Anaesthesia persisted for up to 15min, which was ample time to perform surgery, even in the event of complications. The octopuses all resumed breathing movements and recovered quickly on being placed in a bucket of fresh sea water.

Some octopuses were reserpinized over a 3-day period prior to final adaptation experiments. These animals were anaesthetized briefly in 2% ethanol for their daily intramuscular injection of reserpine ($1\,\mu g\,g^{-1}$ bodymass) at approximately 09:00h. All recovered quickly and showed no obvious signs of stress (e.g. no release of ink or violent 'jetting' response).

Surgery

The procedure for severing the optic nerves was modified from that of Young (1971). The skin and subcutaneous muscle were cut open in the midline, by making a small longitudinal incision above the cranial cartilage. This aperture was then manoeuvred laterally to lie just medial to the right eye. The exposed outer wall of the anterior chamber (a thick opaque white membrane) was then cut. A further cut was made to open the underlying extra-orbital sac, which was clamped open with small haemostats. The orbital sinus was then opened and the 'white body' (haemopoietic tissue) displaced to expose the optic nerves. A glass hook was used to gather the optic nerves gently and raise them slightly for careful severing with fine iris scissors. In the experiments reported here, only the anterior nerves of the right eye were severed.

The orbit was closed by one or two sutures in the cut edges of the outer wall of the anterior chamber. The skin and subcutaneous muscle were then replaced in their original central position, and the wound was encouraged to close by briefly pressing the damaged edges together with forceps (the musculature of the skin holds this wound closed, provided it is not too large).

Rhodopsin purification

Rhodopsin purification was performed by I.G.G. at Osaka University, courtesy of Associate Professor Yuji Kito. *Octopus fangsiao* rhodopsin was extracted from frozen eyes, solubilized in detergent L-1695 and purified by ion-exchange chromatography (DEAE cellulose) and affinity (concanavalin A) chromatography (Nashima *et al.* 1978; Hiraki *et al.* 1991). Absorption spectra were measured with a Union Giken SM-401 spectrophotometer.

Results

Light- and dark-adaptation

Animals that had been dark-adapted for several hours, or overnight, were placed in the light-adaptation tank for different periods to follow the course of light-adaptation. Fig. 3A shows the appearance of the fully dark-adapted retina, with only a few streaks of SP granules among the outer segments distal to the dense basal layer. SP granules are absent from the most distal regions. During the course of light-adaptation, SP granules migrate apically, quickly accumulating close to the vitreal surface of the retina

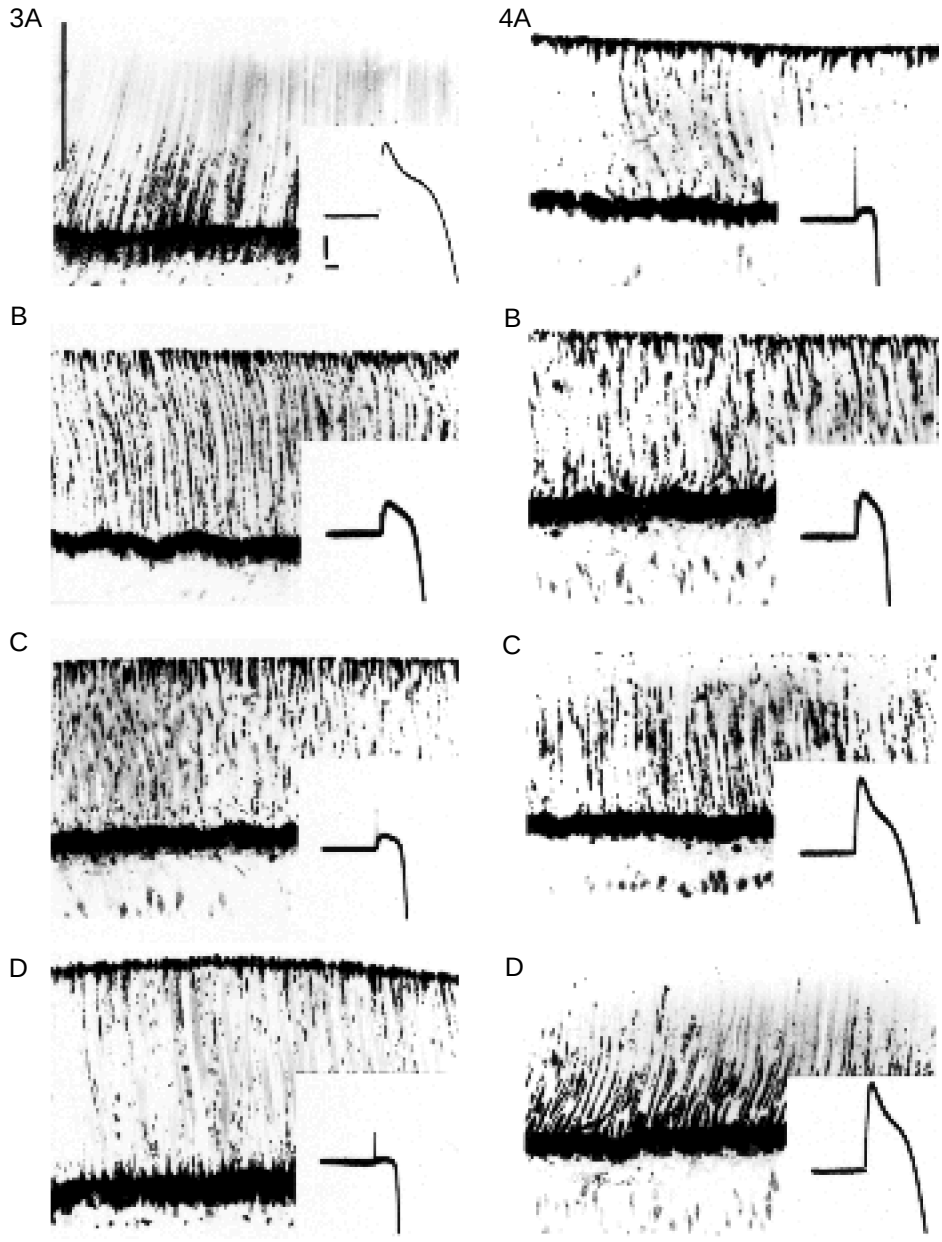

Fig. 3. The effects of light-adaptation on screening-pigment migration and ERP (insets). Octopuses were dark-adapted overnight and transferred (mid-morning) to the 'light-adaptation tank' (see text), using a dim deep-red safelight. They were then left undisturbed in the dark until 'lights-on' (t=0) in mid-afternoon. (A) t=0 (scale bars, vertical bar at left, 100 μm; inset vertical bar, 1mV; inset horizontal bar, 50ms); (B) t=5 min; (C) t=30 min; (D) fully light-adapted. The brief 'spike' visible on the ERP traces in B, C and D is a stimulus artefact.

Fig. 4. The effects of dark-adaptation on SP migration and ERP (insets). Octopuses were removed from the ambient L:D cycle at approximately 10:00h and 'fully light-adapted' in orange light (see text) for 3h before 'lights out' (t=0). (A) t=0; (B) t=20min; (C) t=50min; (D) fully dark-adapted.

(Fig. 3B,C) until, in the fully light-adapted state, they lie predominantly in a dense layer at the rhabdom tips (Fig. 3D; cf. Fig. 1). During this period, the ERP declines from just under 4mV until it is barely detectable (insets, Fig. 3A–D). Note that the light-adapting conditions (see Materials and methods) were such that only about 24% of the reduction in ERP amplitude can be accounted for by a reduction in the amount of rhodopsin.

Dark-adaptation of a light-adapted eye reverses these events: SP granules in the apical layer (Fig. 4A) migrate towards the base of the rhabdoms (Fig. 4B–D), and the ERP increases (insets, Fig. 4A–D). The light-adapting conditions permit an estimation that only 24% of the increase in ERP amplitude during dark-adaptation can be accounted for by increases in the total amount of rhodopsin present. The time course of dark-adaptation was much slower than that of light-adaptation at the light-adapting intensity used in these experiments (compare Figs 5 and 6).

Relationship between SP migration and ERP amplitude

The pattern of SP distribution revealed by image analysis allows the estimation of a pigment position index (PPI; cf. Rodríguez-Sosa and Aréchiga, 1982; Burnside *et al.* 1983). SP position was designated arbitrarily, as indicated by arrows in the examples shown in Fig. 7, and converted to PPI values (see Fig. 6, squares). Note that the PPI is a modal value among a fairly wide distribution of speeds of SP movement. However, the correspondence between ERP and PPI (Fig. 6) is sufficient to demonstrate that the ERP is a useful measure of the extent of SP migration (under the experimental conditions defined in this study).

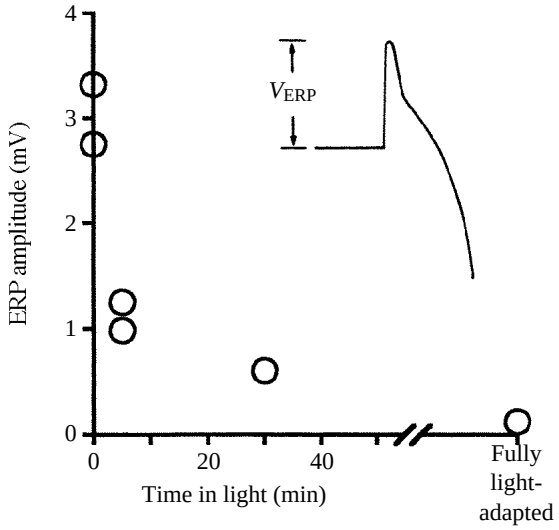

Fig. 5. ERP amplitude (V_{ERP}) *versus* the duration of light-adaptation. The inset describes the ERP amplitude measurement used. The data are from the animals used in Fig. 3. Data for both eyes are plotted for times 0 and 5min.

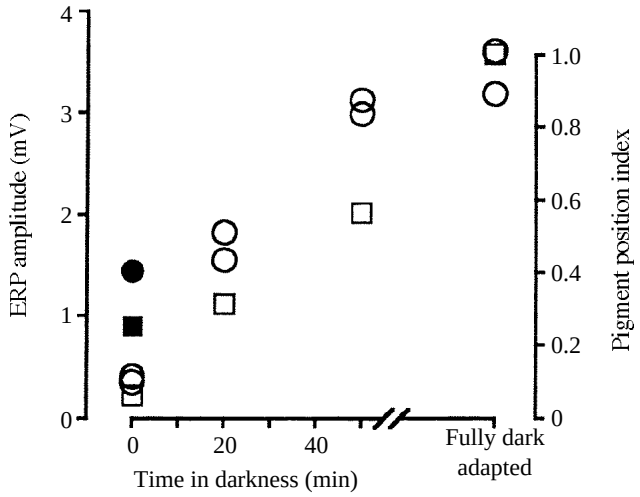

Fig. 6. Relationship between ERP amplitude (left ordinate; circles), pigment position index (PPI) (right ordinate; squares; see Fig. 7) and the duration of dark-adaptation. ERP data are for both eyes, from the animals used in Fig. 4. Filled symbols, ERP and PPI values from a dopamine-treated retina (see Fig. 9B), for comparison.

Effects of severing the optic nerves

After severing the optic nerves to the anterior half of the right eye, octopuses were maintained in the outdoor aquarium, under the ambient light–dark cycle, for 3 days. On the fourth day, following dark-adaptation since the previous dusk, the retinae were removed under dim red light. Intact and lesioned regions were then trimmed away and fixed separately. The results for one animal are shown in Fig. 8, which illustrates the striking difference between a region where the nerves were intact (Fig. 8A) and one where the nerves had been severed (Fig. 8B). The intact region resembles the normal dark-adapted retina (compare Figs 8A and 3A) but that to which the optic nerves had been severed shows a distinctive band of SP in the rhabdom tips, comparable with the appearance of a normal light-adapted retina (compare Figs 8B and 4A). This result has also been obtained in experiments with *Octopus sinensis* (? *O. vulgaris*) d'Orbigny (Y. Tsukahara, unpublished results).

Effects of the presence and absence of dopamine

Cutting the optic nerves mimics the effects of light adaptation or, rather, prevents the appearance of the characteristics of dark-adaptation. There is also strong evidence that the efferent nerves to the retina are dopaminergic (Tasaki *et al.* 1982; Silver *et al.* 1983; Suzuki and Tasaki, 1985). Therefore, isolated retinae from light-adapted animals were incubated for 15min in sea water containing dopamine (in the dark) to determine whether this would mimic the effects of optic nerve efferent activity. The control preparations (retinae from light-adapted animals, incubations in darkness for 15min) were: (i) the normal (unincubated) light-adapted retina; (ii) retinae incubated in plain oxygenated sea water; (iii) retinae incubated with noradrenaline or L-Dopa (catecholamines closely

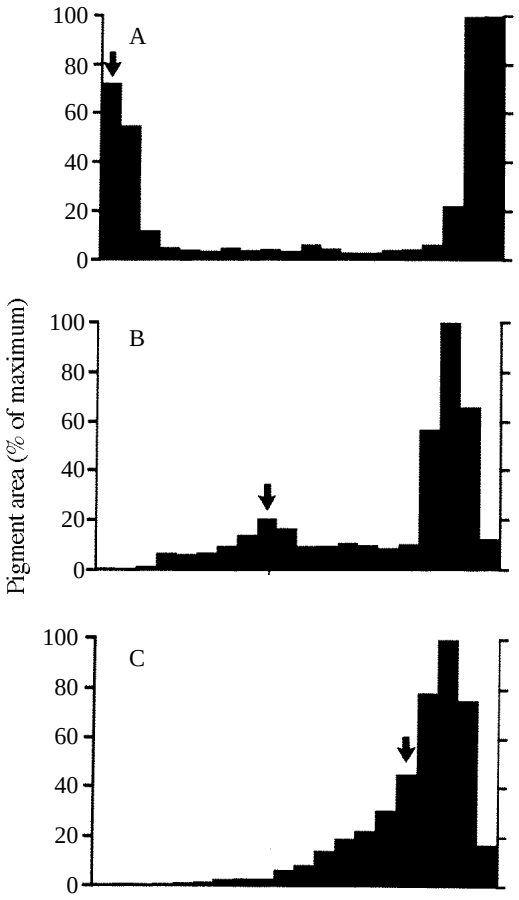

Fig. 7. Histograms showing the results of image analysis on three different retinae (cf. Fig. 4): (A) fully light-adapted; (B) after 20min of dark-adaptation; and (C) fully dark-adapted. Each histogram bar represents the percentage area of a rectangle occupied by SP, for 20 rectangles covering the distance between the rhabdom tips, at left, and the proximal (immobile) SP, at right. Arrows indicate modal values used to calculate PPI (see text).

related to dopamine), or with 5-HT; and (iv) retinae incubated with dopamine in the presence of SCH 23390 (a dopamine antagonist).

The apical SP layer retains its light-adapted position in the seawater-incubated control (Fig. 9A), with no sign of any SP migration despite a significant time in darkness, and appears little different from the normal light-adapted retina (cf. Fig. 4A). Dopamine incubation ($5\,\mu mol\,l^{-1}$), however, causes a marked proximal displacement of the SP granules, such that, after 15min, the apical regions of the visual cells are clear of pigment (Fig. 9B). The corresponding ERPs (see insets, Fig. 9) also show a dramatic change: incubation in plain sea water for 15min in the dark (Fig. 9A) produced a mean ERP of 0.53±0.09mV (S.E.M. N=5), which is in marked contrast to the significantly higher (P<0.05; independent t-test) mean of 1.67±0.18mV (N=6) recorded in the presence of

5 μmol l^{-1} dopamine (Fig. 9B). On incubation with other catecholamines, with 5-HT or with dopamine in the presence of 50 μmol l^{-1} SCH 23390, there were no obvious differences from the light-adapted control (results not shown; cf. Fig. 4A).

To demonstrate further the effects of dopamine, four octopuses were depleted of dopamine by treatment with a daily dose of reserpine (1 μg g^{-1} bodymass) for 3 consecutive days (maintained in the outdoor aquarium, under the natural light–dark cycle). On the evening of the third day, they were placed in the dark-adaptation tank, and were killed (under dim red light) during the afternoon of the fourth day. Fig. 10A shows a normal dark-adapted retina for comparison with the striking appearance of the retina from

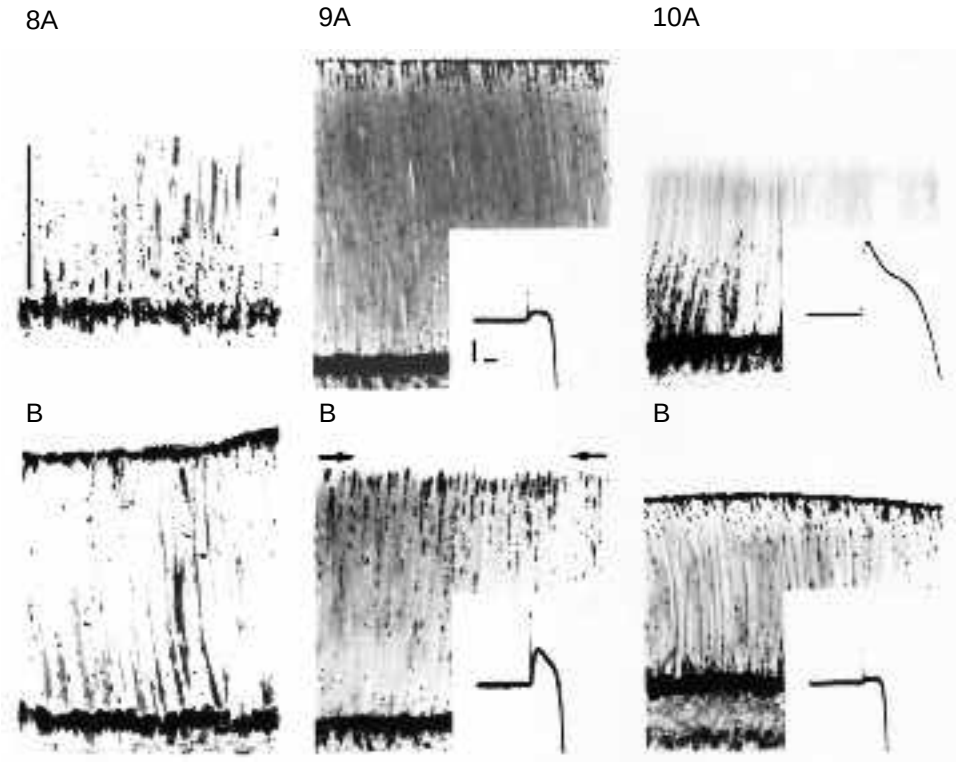

Fig. 8. Effects of severing the optic nerves (fixation in darkness, following dark-adaptation since dusk the previous day). (A) Intact retina, showing proximal distribution of SP granules (scale bar, 100 μm). (B) Region of retina to which the optic nerves had been cut 3 days previously. The dense apical layer of SP resembles that of the light-adapted retina.

Fig. 9. Effects of dopamine incubation on the light-adapted retina (incubations in darkness for 15min). (A) Aerated sea water control. (B) Aerated sea water containing 5 μmol l^{-1} dopamine. Note that SP has been displaced from the rhabdom tips (arrows). Insets: corresponding ERPs (scale bars, 1mV and 50ms).

Fig. 10. Effects of reserpine treatment (fixation in darkness, following dark-adaptation since dusk the previous day). (A) Normal dark-adapted retina. (B) Retina of an octopus given three once-daily intramuscular injections of reserpine in saline (1 μg g^{-1} bodymass). Note the intense apical layer of SP, resembling that of the light-adapted retina. Insets: corresponding ERPs.

a reserpinized octopus (Fig. 10B). Note that, despite some 20h of dark-adaptation, there is a distinct apical layer of SP aggregated in the tips of the outer segments of the reserpinized retina (Fig. 10B). The ERP of the reserpinized retina resembles that of the normal light-adapted animal (compare Fig. 10B with Fig. 4A).

Discussion

Dark- and light-adaptation in *Octopus fangsiao* show good correspondence with the same phenomena in *O. vulgaris* (cf. Young, 1963), both in the histological appearance of SP migration and in the characteristic times for migration (compare the ERP results in Figs 5 and 6 with the densitometer readings of the rhabdom tips of *O. vulgaris* by Young, 1963). The results in the present study show a close relationship between ERP amplitude, duration of adaptation to dark or light and the extent of SP migration and confirm the earlier findings of Young (1963) on the relationship between SP migration and the degree of light- or dark-adaptation in the intact retina.

The experiments of Young (1963) and Daw and Pearlman (1974) were performed mainly with intact animals. In one experiment, however, Young (1963) excised dark-adapted retinae and subjected them to further light or dark conditions for 1min. SP in the retina exposed to light showed dramatic distal migration (Young, 1963: Plate 2, Fig. 6). However, he reported no observations of dark-adaptation in isolated retinae and was therefore premature in his conclusion that SP movements 'do not depend on the efferent fibres' (Young, 1963).

The incubations of isolated light-adapted retinae, described in the present paper, argue forcefully that efferent innervation is involved in the withdrawal of SP during dark-adaptation. Note that 15min incubations (e.g. Fig. 9) followed the standard preparation period of 15min: i.e. a total of 30min in the dark before ERP measurements were taken and the tissue was fixed. Only in the presence of unblocked dopamine was there any significant withdrawal of SP. Furthermore, many of the results reported in the present paper could not have been obtained had dark-adaptative SP migrations occurred independently of the efferent input: the time-dependency of the SP movements shown in Figs 3–6, for example, would have been masked because of the subsequent 15min in darkness during preparation. Note, similarly, the close correspondence between results obtained from both eyes, where these are shown in Figs 5 and 6: there was a further delay of 15min before the ERP from the second eye was recorded.

The effects of 15min of dopamine incubation ($5\,\mu mol\,l^{-1}$) on the isolated light-adapted retina are similar to the effects of 15–20min of dark-adaptation in an intact octopus (compare ERP and PPI values; Fig. 6), but there are marked differences in these preparations. SP movement in the dark-adapting intact retina is diffuse, with a broad distribution of speeds of SP migration (compare Fig. 4 with the image analysis SP migration profiles in Fig. 7) and the rhabdom tips are freed of SP very gradually. In the dopamine-incubated retina, SP migration occurs in a more uniform fashion, with the rhabdom tips being cleared of pigment first (Fig. 9B). These qualitative differences are presumably explained by dopamine gaining direct access to the rhabdom tips in the incubated retina, whereas diffusion through the cell bodies and basement membrane is

rather slow. In the intact retina, conditions are just the opposite, with the efferents carrying dopamine directly to the nerve processes at the base of the photoreceptors.

Although dopamine is involved in retinal adaptation in both vertebrates and cephalopods, it is generally associated with light-adaptation in the former (e.g. Witkovsky and Dearry, 1991), but with dark-adaptation in the latter. The significance (if any) of this observation is difficult to interpret, given the remoteness of any common phylogeny and the fact that retinal functioning is fundamentally very different (e.g. the comparative silence of the photoreceptors in darkness and the depolarizing response to light by the cephalopod photoreceptors, *versus* the vertebrate hyperpolarizing response to light, which modifies the tonic background discharge present during darkness).

Suzuki and Tasaki (1985) discovered that brief electrical stimulation of the octopus optic nerve (efferents) markedly enhances the ERG obtained from the isolated retina, an effect that was mimicked in the presence of dopamine. In the present study, inspection of histological sections and measurement of the ERP have demonstrated that dopamine released from the optic nerve efferents can enhance photon capture by causing the withdrawal of SP, providing a possible explanation (at least partly) for these earlier observations. A direct effect of dopamine on the electrical properties of the photoreceptors is not excluded, however, especially in view of similar findings in the retinae of other animals (e.g. Barlow *et al.* 1989; Dowling, 1991). This requires further investigation. Recent experiments by Mondragón and Frixione (1992) emphasize that efferent innervation is not the only mechanism controlling SP migrations: in vertebrates (frog) and invertebrates (crayfish), under lidocaine anaesthesia *in vitro*, both light- and dark-adapted SP positions can be induced in the absence of local nervous input.

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