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Constancy of synaptic ribbon numbers in the retina of the arctic charr, *Salvelinus alpinus*

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Synopsis

At high latitudes, such as in Iceland, the daily photoperiod varies from almost continuous darkness in winter to virtually constant light in summer. Previous studies of detailed retinal structure in vertebrates have shown significant daily and annual effects of photoperiod. We sampled arctic charr in Iceland during the summer, including fish that were both light- and dark-adapted, during both day and night. We observed retinomotor responses characteristic of light- and dark-adaptation, but found no difference in the number of synaptic ribbons in the retina. The morpho-physiological changes, appearing as retinomotor responses, are thus not expressed at the synaptic level.

Introduction

The effects of light on the retina and photoreceptors of vertebrates have been actively studied for a number of years, but it is only in recent years that a broader, comparative approach has been used in an attempt to understand the functional aspects of retinal structure (Ali & Klyne 1985). Fishes are particularly useful for such studies since they form the largest taxon, with a great diversity of habitats and life histories and are readily available. The charrs have received a great deal of attention mainly due to their economic and recreational importance and there is a good deal of basic descriptive information known about retinal structure in these species (Ali 1965, Ali & Wagner 1980, Ali et al. 1984). We studied the retinal structure of the arctic charr, *Salvelinus alpinus,* from Thingvallavatn, the largest lake in Iceland. At high latitudes, such as in Iceland, they are subjected to extremes of annual photoperiod from essentially continuous light in midsummer to almost continuous darkness in midwinter in this lake (approximately 64° north latitude). Furthermore, there are four distinct morphs of arctic charr in Thingvallavatn: small benthivore, large benthivore, piscivore, and planktivore (Sandlund et al. 1987). These differ considerably in life history and habitat utilization, but all are very similar genetically (Magnusson & Ferguson 1987).

The photoreceptor cells of the vertebrate retina are connected to bipolar and horizontal cells by a particular type of synapse, characterized on the presynaptic side by the presence of electron-dense non-membranous structures called synaptic ribbons (sr) surrounded by many synaptic vesicles. Structures similar to sr have also been reported in synaptic terminals of bipolar cells in the vertebrate

retina, and cells of pineal organs and parietal eyes in lower vertebrates. The presence, absence or number of sr indicates the state of activity of the photoreceptors and thus serves as an index.

Quantitative studies have demonstrated that there is no variation in the number of synaptic vesicles in the cell bases of mammals regardless of whether they had been subjected to external stimuli ranging from continuous light to total darkness (Mountford 1963, Cragg 1972, Ball & Dickson 1983). Quantitative changes, however, have been reported by Monaghan & Osborne (1975) and Osborne & Monaghan (1976) in the amphibian *Xenopus laevis*. After being subjected to several days (up to 9) of constant light, there was an increase in the number of vesicles surrounding the sr in rods while no similar build-up of vesicles was seen in cones.

In fishes (Wagner 1973, 1975) as well as in higher vertebrates (Kuwabara & Funahashi 1976, Abe & Yamamoto 1984, Williams et al. 1985), the sr show either cyclic morphological changes induced by light or a dependence on an endogenous circadian cycle. Their number decreases in the dark. The aim of the present study was to determine whether or not, during the virtually continuous summer daylight in Iceland, there is any variation in the number of sr in fish that are sampled during day and night and have either been kept in continuous light or continuous darkness. The small benthivore morph was chosen for this study because large numbers can easily be collected in the lake, and its habitat is well known.

Materials and methods

Adult specimens of the small benthivore morph were collected from Thingvallavatn on 27 July, 1986 (day length approximately 22 h) in small mesh gillnets set overnight (water temperature approximately 11° C), and brought alive to holding facilities at the University of Iceland in Reykjavik. Fish were held in the laboratory for a few days (12 : 12 photoperiod, water temperature approximately 5–6° C) and sampled under four conditions: light-day, light-night, dark-day and dark-night. The left eyes of the fish (7 to 9 mm diameter) were fixed in a solution of 2% glutaraldehyde, 1% paraformaldehyde and 3% sucrose in phosphate buffer (0.1 M, pH 7.2), then dissected into small pieces in Montreal. For this study only the dorso-central parts of the eyes were studied.

After a double rinse in phosphate buffer containing 7% sucrose, the material was post-fixed in 1% osmium tetroxide. It was subsequently dehydrated in a gradual ethanol series (40 to 100%), and rinsed twice in propylene oxide before overnight infiltration in a mixture of propylene and Spurr resin (1 : 1). After embedding in fresh Spurr resin, thin (0.5–1.0 μm) and ultrathin (70–90 nm) sections were cut on an ultramicrotome (LKB II). Methylene blue was used as the stain for light microscope sections, while sections for electron microscopy were stained with uranyl acetate and lead citrate (Venable & Coggeshall 1965). Sections were collected at each 10 μm (minimum) for a total length of approximately 50 μm. The synaptic ribbons of five cones and five rods were counted in each section. The mean sr counts for each fish were compared among treatments by a two-tailed t-test for unpaired samples (Sokal & Rohlf 1981).

Results

In light microscopy, transverse sections of the retina sampled in light conditions and total darkness, showed the typical retinomotor responses characteristic of such adaptations, but did not show any differences between samples taken during the day and the night. In light, the epithelial retinal pigment (ERP) and the rods were extended and the cones were retracted, while in darkness the reverse situation was found. In dark-adapted retinas both the pigment and the rods were contracted to a narrow band, while the cones were expanded and positioned closer to the epithelial retinal pigment layer (Fig. 1).

Electron microscope observations of transverse sections of the cone pedicles and rod spherules located at the level of the external plexiform layer showed numerous vesicles and large spinules. The latter are digitiform extensions of horizontal cells

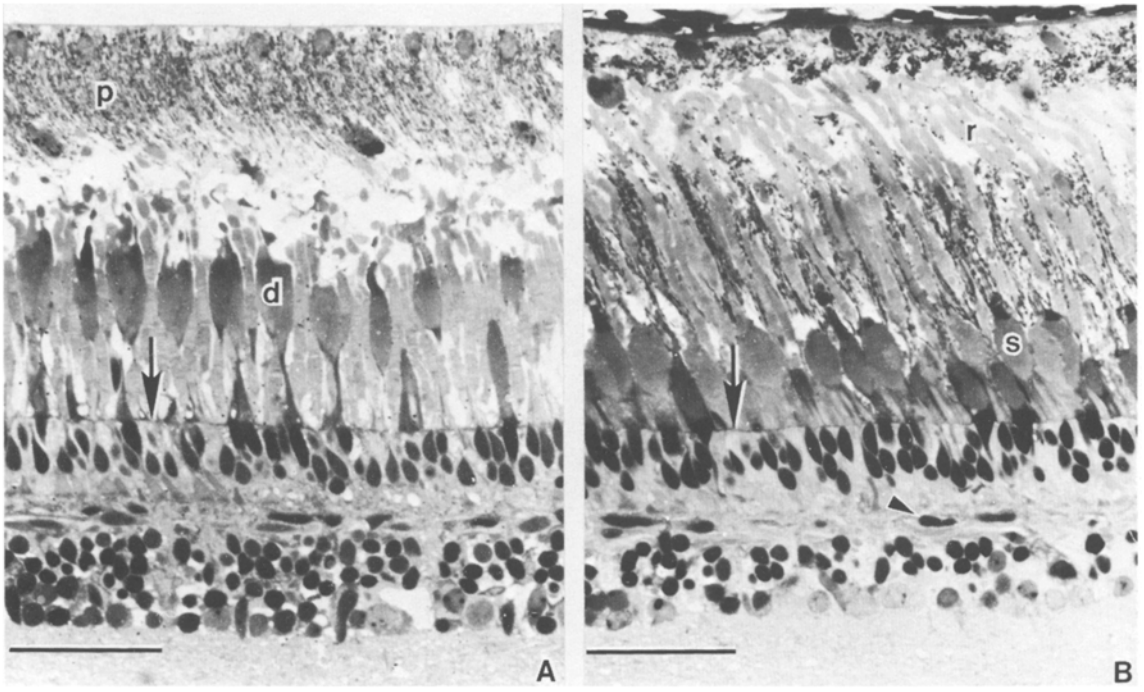

Fig. 1. Transverse sections (1 μm) of dark- (A) and light- (B) adapted retinas. *d* – double cone, *p* – pigment epithelium, *r* – external segment of rod, *s* – single cone. The arrows indicate the external limiting membrane and the arrowhead shows a horizontal cell. Bars represent 50 μm.

into the bases of the photoreceptors. While these spinules were abundant among the cones, they were almost absent from the rods (Fig. 2). Similarly, while the cones possessed a mean of three to four synaptic ribbons, the rods usually had only one. The sr consists of a non-membranous, electron-dense, lamellar structure drawn into a short extension of the cytoplasm of the receptor terminal, the synaptic ridge. At the distal extremity of the latter appears a characteristic feature seen as a circular, electron-dense structure, the arciform density (Ladman 1958). As for the post-synaptic element, it has a triform configuration composed of elongations of one bipolar and two horizontal cells (Dowling & Boycott 1966).

The number of sr in the cone pedicles in retinas adapted to light showed a normal distribution. The means for diurnal and nocturnal samples adapted to light were not significantly different ($p > 0.05$; 3.70 and 3.28 sr per cell, respectively). The same conclusion applies to the dark condition (normal distribution, with no significant difference between day and night samples, 3.16 and 3.34, respectively).

When the number of sr in light- and dark-adapted states is compared it is seen that there are up to eight in the light-adapted state (Table 1). In the dark-adapted specimens however they mostly do not exceed five (Table 2). However, in both cases the mode was three sr per cell and there was no statistically significant difference ($p > 0.05$) between the mean number of sr in the light (3.56) and dark (3.22) (Table 3).

Discussion

The retina of the small benthivore charr shows the retinomotor responses characteristic of light- and dark-adapted states (Ali 1965). However, it does not appear that this adaptation can be accounted for at the synaptic level by a variation in the number of synaptic ribbons (sr) in light or dark. In the

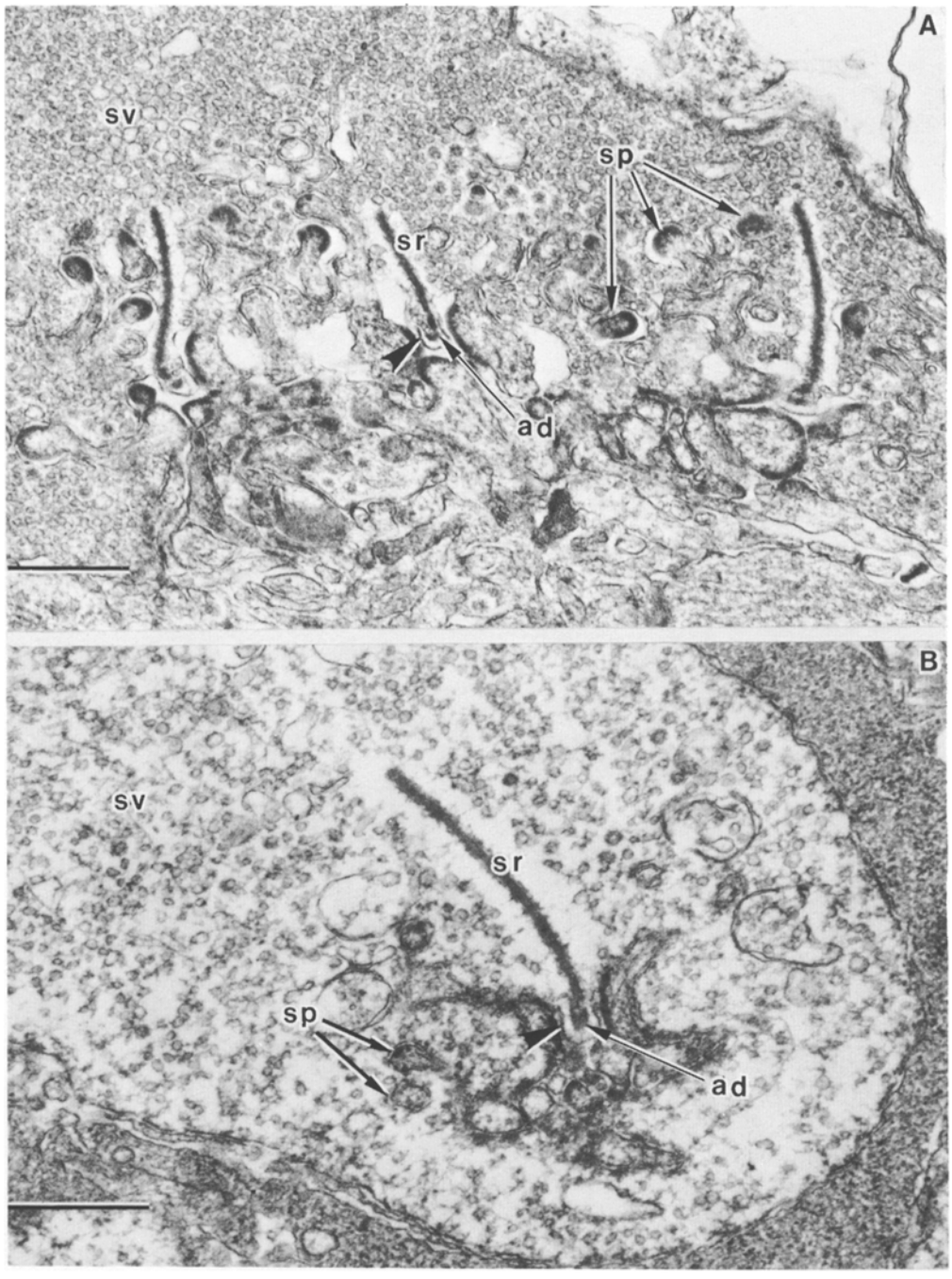

Fig. 2. Electron micrographs of cone pedicle (A) and rod spherule (B). Note that spinules (*sp*) are more numerous and dense in the cone pedicle. *sv* – synaptic vesicle, *sr* – synaptic ribbon, *ad* – arciform density, arrowhead – synaptic ridge. Bars represent 0.5 μm.

Fig. 2. Electron micrographs of cone pedicle (A) and rod spherule (B). Note that spinules (*sp*) are more numerous and dense in the cone pedicle. *sv* – synaptic vesicle, *sr* – synaptic ribbon, *ad* – arciform density, arrowhead – synaptic ridge. Bars represent 0.5 μm.

Table 1. Frequency distribution of the number of synaptic ribbons in the pedicles of dark-adapted cone cells of arctic charr, sampled during the day and during the night.

Number of synaptic ribbons	Day sample		Night sample		Total
	fish no. 1	fish no. 2	fish no. 1	fish no. 2	
1	1	1	1	1	4
2	4	7	4	4	19
3	13	7	13	8	41
4	4	7	5	6	22
5	3	3	2	4	12
6	–	–	–	2	2
7	–	–	–	–	–
8	–	–	–	–	–
Total	25	25	25	25	100

Table 2. Frequency distribution of the number of synaptic ribbons in the pedicles of light-adapted cone cells of arctic charr, sampled during the day and during the night.

Number of synaptic ribbons	Day sample		Night sample	Total
	fish no. 1	fish no. 2	fish no. 1	
1	2	–	–	2
2	1	9	9	19
3	5	8	7	20
4	6	5	6	17
5	5	1	1	7
6	5	1	1	7
7	1	1	–	2
8	–	–	1	1
Total	25	25	25	75

Table 3. Mean number of synaptic ribbons, and results of paired t-tests between light-adapted and dark-adapted, and between day sample and night sample fish. x = mean + standard error, n = number of observations, t = value of t statistic, df = degrees of freedom, p = probability value of t statistic.

Group	x (+ s.e.)	n	t	df	p
Light	3.56 (1.509)	75	– 1.72	173	0.087
Dark	3.22 (1.106)	100			
Light-day	3.70 (1.529)	25	1.139	73	0.259
Light-night	3.28 (1.458)	50			
Dark-day	3.16 (1.037)	50	– 0.827	98	0.410
Dark-night	3.34 (1.136)	50			

teleostean retina, one typically finds the number of sr to be cyclic. These cycles are often clearly dependent, not on exogenous stimuli, but on an endogenous circadian rhythm (Wagner 1975).

While the precise function of the synaptic ribbon, let alone its development has not yet been determined, the presence of this proteinaceous material (Bunt 1971, Williams et al. 1985) is an essential part of the formation of the 'b' wave of the electroretinogram (ERG) (Nilsson & Crescitelli 1969, 1970). Furthermore, the presence of microtu-

bules lying in close association with the sr (Gray 1976) corroborates the hypothesis that the sr are 'orienting structures to channel synaptic vesicles in an orderly, conveyor belt fashion to the plasma membrane for transmitter release' (Bunt 1971). Finally, according to another theory, the sr may be involved in the storage of transmitter substances that are released at a maximal rate in the dark. Under these conditions, there is a continual release of transmitters by the cones, whereas in the light this flux of transmitters is reduced (Dowling 1974).

An important decrease in the number of sr has previously been observed in a tropical cichlid (*Nannacara anomala*) where this fluctuation is independent of the external light conditions (Wagner 1975). In contrast, *Salvelinus fontinalis,* a temperate and supposedly arhythmic species, reveals no endogenous control of retinomotor reactions and little change in the number of sr (Wagner & Ali 1977). Nonetheless, in both these fishes, the number of synaptic ribbons does vary between light and dark conditions when a comparison is made of the percentage of sr found within a synaptic ridge.

It would be particularly interesting to examine the other arctic charr morphs in Thingvallavatn. They have different lifestyles which are reflected in their electroretinographic (ERG) responses (Thorarensen 1987). The small benthivore charr tends to spend long hours within crevices and cavities in the porous volcanic substrate of the littoral zone (Sandlund et al. 1987). This behaviour could account for the present results in that the small benthivore charr may spend most of its time, both on a daily and annual basis, in a microhabitat with low light intensity.

There appears to be some difference between light- and dark-adapted fish. In fact, in the former, the sr are generally distributed up to eight per cell base, while in the latter there are rarely as many as six sr per cell base.

Observations of Raynauld et al. (1979) of the external plexiform layer of the goldfish, *Carassius auratus,* were interpreted by the authors in terms of an adaptation to light and dark, and not in terms of a variation in the number of sr. This was due to a diminution of the spinules (Wagner 1980) as seen clearly in tangential sections. Spinule formation and degradation are also partially under endogenous control but they need light for full expression (Douglas & Wagner 1983). At the electrophysiological level, the spinules act to mediate chromatic feedback in cones (Raynauld et al. 1979, Weiler & Wagner 1982). In the case of the planktivore morph of the arctic charr, previous observations (Ali et al. 1984) have shown that there was not a circadian rhythm, either at the level of the photoreceptors or the spinules during the summer months when light is practically constant. However, under conditions of total darkness, arctic charr show a decrease in the number of spinules in comparison to the condition in light, indicating that they are still capable of retinomotor responses throughout the summer. Additional material is required to verify this. Preliminary observations do not show a decrease in the number of spinules in darkness but they were made with transverse sections. As mentioned above, tangential sections provide clearest results.

Even though the synaptic activity of the cones does not vary during the summer (either between light and dark or between day and night), it would be interesting to examine it in material sampled on summer and winter solstice when photoperiods are at their extreme. For the present, we may presume that the small benthivore morph of arctic charr in Thingvallavatn does not have a circadian rhythm, but a considerable annual variation in cone synapses.

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