mental stage during which the survival of CG neurons seems to be regulated by target-derived trophic support (*2–4*). Crucial experiments necessary to verify the role of this material in vivo await the purification of the active factor or factors and the development of antibody against it. We have already found in preliminary experiments that this material is nondialyzable, thermolabile, and capable of being fractionated by chromatographic procedures (*9*). The large amounts and high specific activity of this material in the eye make eye tissue an excellent source for the purification of the active molecule or molecules involved.

Carcass extract also contains substantial amounts of soluble trophic agents for CG neurons, although at a low specific activity. The carcass is rich in tissues, such as skeletal muscle, that are normally innervated by neurons sharing the cholinergic nature of the CG neurons. In addition, detailed studies of some of these neurons, located in the lateral motor column of the spinal cord, have shown a well-defined period of developmental cell death (*10*), which is regulated by their peripheral territory of innervation, the limb bud (*11*). In culture, skeletal muscle cells support cholinergic activities of spinal cord neurons (*12*) as well as the survival of CG neurons (*5*). These parallel features in the development of CG and spinal cord neurons, together with the distribution of trophic activity for CG neurons reported here, suggest at least three hypotheses for the nature of the trophic factor or factors:

1) A single cholinergic neuronotrophic factor is present in both eye and carcass. Limb tissues would use the same factor to regulate survival of their motoneurons that the choroid and ciliary body use to regulate survival of CG neurons. The factor may be produced by all peripheral targets for cholinergic innervation, and specificity of interactions would be conferred only by the spatial and temporal relationships between neurons and peripheral targets. The high concentration of such a common factor in the intraocular periphery of CG neurons compared with all other putative cholinergic territories, however, would require additional hypotheses, for example, temporal differences in the accumulation of trophic activity in different target tissues.

2) A ciliary neuronotrophic factor is present in both eye and carcass. The low specific activity in the carcass may represent a low-level expression of the genetic information regulating the synthesis of this factor. The substantial total activity would merely reflect the large relative mass of the carcass at this stage of embryonic development. The high specific activity found in the eye would be due to some signal turning on the transcription of the gene in those tissues to be innervated by CG neurons.

3) A ciliary neuronotrophic factor is present in the eye and a spinal neuronotrophic factor is present in the carcass. The two factors would be distinct molecules, each used for regulating survival in the respective neuronal population. There would be enough cross-activity between the two molecules for the carcass factor to show an attentuated activity in the ciliary bioassay. Conversely, one may expect the ciliary factor to display some activity on spinal cord motoneurons, although its apparent specific activity on a motoneuron bioassay would be much lower than that demonstrated in the ciliary bioassay.

The three hypotheses are amenable to experimental verification; their relative validity can be ascertained by additional research.

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30 October 1978; revised 16 February 1979

Goldfish Retina: A Correlate Between Cone Activity and Morphology of the Horizontal Cell in Cone Pedicules

Abstract. *In the cone pedicules, the digitations of horizontal cell process lateral to the synaptic ribbon disappear after dark adaptation. This disappearance is correlated with the loss of color opponency and cone function shown in ganglion cell recordings in isolated retinas. Cone function and color-opponent responses are restored by reapplying background light.*

For most of the animal kingdom, the night phase of the circadian cycle constitutes a period of prolonged dark adaptation. During that period, the cones of duplex retinas are assumed to remain functional even though the rod system takes over control of the retinal output except at the fovea. It is further thought that no major synaptic modifications take place in the retina during this time (*1, 2*).

Since rod and cone inputs converge onto the same ganglion cells, it is very difficult, in whole-animal preparations, to test cone activity at the level of the optic nerve after dark adaptation because the rod receptor system comes quickly into play, masking the cone system. In the isolated retina, however, it is possible under special conditions to study cone function during dark adaptation without the intrusion of the rod system that would normally occur. After suitable bleaching, the rods will not regenerate their visual pigment in the absence of the enzymatic system present in the pigmented epithelium, which is left in the eyecup after retinal isolation (*3*). Under these conditions, cone function can be studied for an extended period under complete darkness without rod intrusion.

We report here that in the goldfish (*Carassius auratus*) the cone system becomes nonfunctional after prolonged dark adaptation, and that major synaptic modifications occur in the cone pedicules, which can be related to the change in activity observed at the ganglionic cell level and to the state of adaptation.

Comet goldfish were subjected to an artificial day-night cycle of 12 hours of light (125 lux, incandescent) and 12

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hours of darkness for a period of at least 3 weeks before the experiments. Three hours before dark, the retina was isolated under ambient room light (*4*), and put into a recording chamber maintained at 15° ± 3°C and into which flowed a moist gaseous mixture (95 percent O_2 and 5 percent CO_2 at 200 ml/min). Ganglion cell activity was recorded with tungsten microelectrodes, and the cell response was tested with a light spot 1 mm in diameter and an annulus (2.5 mm inside diameter and 5.0 mm outside diameter). During dark adaptation, testing was limited to 15-minute intervals. The quartz-iodine light source could be varied in wavelength from 350 to 700 nm with a monochromator and attenuated with neutral density filters. The maximum illuminance at 625 nm was 3.6×10^{12} quanta cm^{-2} sec^{-1}. The material used for electron microscopy came from two sources: (i) whole retinas taken at midday and midnight and (ii) retinas from which the receptive field center was dissected at the recording site with a 1.5-mm corneal punch. The material was fixed in 1 percent formaldehyde–2.5 percent glutaraldehyde and 1 percent osmium (1 hour each) at room temperature in a 0.06*M* phosphate buffer at *p*H 7.3.

Figure 1A illustrates the response properties of a typical double opponent (*5*) ganglion cell, being OFF to red and ON to green in the center of the receptive field and opposite in the surround. During dark adaptation, this cell lost its spontaneous activity as well as its opponent color response. Both stimuli that had been equated for equal absorption by the 625-nm red cones (*6*) generated a nearly identical response (Fig. 1C), thus indicating that the green cone input no longer had an effect on the ganglion cell response. Subsequently, the cell became completely insensitive to even the brightest stimuli regardless of the wavelength; this result eliminates the possibility that the response at 500 nm was attributable to the rods. Rather than move the microelectrode to another region as is the common practice, we instead turned the background light on. Shortly thereafter, this cell regained its color opponent response and eventually its sensitivity as well as its spontaneous activity.

Recordings from more than ten cells confirmed that normally within 1 hour after the onset of darkness, a color-opponent cell would lose its color opponency with no sign of rod intrusion. Its spectral sensitivity would then become determined by the 625-nm cones. During the next 2 hours, the cell usually became unresponsive to stimuli regardless of wavelength or intensity available from our light source (*7*). In some experiments the microelectrode was then withdrawn. With the aid of an infrared viewer, the center of the receptive field was dissected with a corneal punch, and the retina was fixed in the dark. In other experiments, after recording color opponent responses for an extended period, the receptive field was also punched and fixed with the rest of the retina in ambient room light.

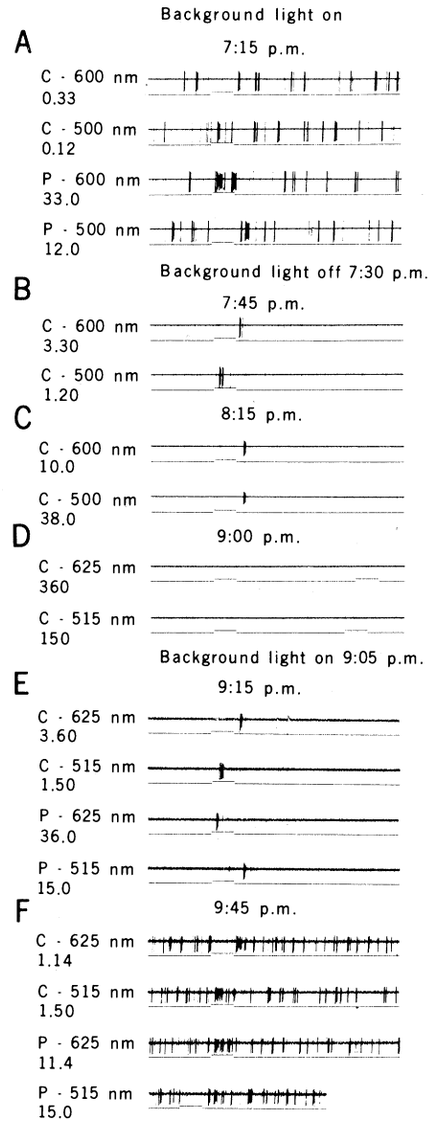

Fig. 1. Behavior of a ganglion cell through the background light sequence ON-OFF-ON; *C* denotes central spot stimulation and *P* peripheral stimulation with an annulus. The second trace indicates the stimulus pulse of 0.7 second duration. The multiplication factor for illuminance is $\times 10^{10}$ quanta cm^{-2} sec^{-1}. (A) The background, which has been ON since the isolation of the retina, is present and the cell behaves as a double opponent red-OFF cell with spontaneous activity. (B) After 15 minutes of darkness, the cell has lost its spontaneous activity but is still color opponent. (C) After 45 minutes of darkness, the cell has lost its color opponent behavior. (D) The cell is no longer responding even to the brightest flashes available. (E) After application of background light, the cell shows again a double opponent behavior but without spontaneous activity. (F) After 40 minutes of background light, spontaneous activity has returned and the cell behaves very much as in (A) some $2^1/_2$ hours earlier. The spikes have been retouched to make them more visible.

When examining our material for morphological differences that could be correlated with our electrophysiological findings, we at first failed to recognize any of the eye-catching changes we had expected to find somewhere around the synaptic ribbon complexes (*8, 9*). It was only when comparing tangential sections, of light- and dark-adapted retinas, where about six to ten synaptic ribbons were visualized simultaneously that we noticed the changes. The fingerlike extensions of the horizontal cell process and their paramembranous densities, flanking the synaptic ribbon and prominent in light-adapted material (Fig. 2A), were absent after dark adaptation (Fig. 2B); this was the case at all levels in the cone pedicule where synaptic ribbons were seen in both tangential and transverse sections (*10*) and was observed in all cone pedicules with few exceptions.

These structures have been described in detail by Stell for the goldfish retina (*11*) and can also be readily observed in published material of other fish (*12*), but they have not been reported in retinas of other animals. Using only the presence of these digitations and their densities in the cone pedicule as a criteria for light adaptation or cone function, one could easily classify the nearly 100 electron micrographs of our material in both transverse and tangential sections.

In an attempt to quantify the extent of these changes, we counted the digitations in more than 300 cone pedicules of different retinas and related them to the number of synaptic ribbons observed. In material taken from whole eyes, there are 1.9 digitations per synaptic ribbon in retinas fixed at noon, whereas in specimens fixed at midnight there are only 0.05 digitations per ribbon. In material subjected to electrophysiological recording (the punched-out receptive fields), the number of digitations per ribbon was 1.1 for retinas in which cone function was present and 0.1 in those which were no longer responding to stimulation after dark adaptation. These morphological changes, observed within such a short time, constitute another example of the plasticity of synapses (*2, 13*). Modifications of the cone plasmalemma itself have also been reported to occur with changes in the state of light adaptation (*14*).

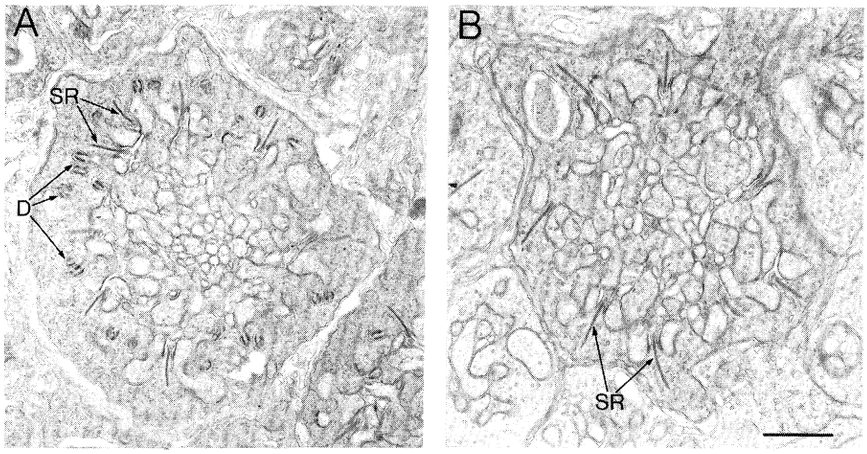

Fig. 2. Electron micrographs showing two cone pedicules cut in tangential section. (A) The retina was light-adapted. Note the digitations and the densities (*D*) in the horizontal cell processes flanking the synaptic ribbons (*SR*). (B) After dark adaptation, both the densities and the digitations have disappeared. By the amount of cone cytoplasm (material filled with vesicules), it can be judged that both sections were cut at approximately the same level in the pedicule. Horizontal bar represents 1 μm.

Although it is difficult to correlate anatomy and physiology, we think that the implication of the horizontal cells in color coding is well established (*15*). The digitations of the horizontal process that we report here as disappearing during proplonged dark adaptation are likely sites for the opponent color interactions; their disappearance is probably linked with our observations at the ganglion level. The formation of these digitations and their content during light adaptation may be explained in terms of an active process: The cone horizontal cells, which in the goldfish terminate as large fusiform structures in the inner nuclear layer instead of the outer synaptic layer as seen in other species (*16*), improve their connectivity with the cones when these are maximally active—that is, in daytime—by growing digitations in the cone pedicule. After dark adaptation or at night, when the cones serve no useful purpose and photomechanically extend into the pigment epithelium, they would relinquish this connectivity.

The fact that the cones become nonfunctional after long periods of darkness may not be as strange as it seems. Although it is established that the photoreceptor releases transmitter in the dark, if the cones were to function throughout the night, considerable transmitter substance would be lost to no useful purpose. Indeed, in the absence of artificial lighting, the chances that enough light would be available to activate the cone system are nil. As an energy-saving measure, goldfish cones would stop functioning during the night, thus economizing large quantities of transmitter substance.

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13 November 1978; revised 21 March 1979