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Responsiveness and Receptive Field Size of Carp Horizontal Cells Are Reduced by Prolonged Darkness and Dopamine

Abstract. *In the fish retina the interplexiform cells contain dopamine and provide a centrifugal pathway from the inner plexiform layer to horizontal cells of the outer plexiform layer. Dopamine application reduced the responsiveness and receptive field size of cone horizontal cells, as did a prolonged period of complete darkness. Other results suggest that the interplexiform cells may release dopamine after a prolonged period in the dark. The interplexiform-horizontal cell system may modify the strength of the antagonistic surrounds of retinal neurons as a function of time in the dark.*

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In 1957 Barlow *et al.* (*1*) reported that the strength of the antagonistic surround of cat retinal ganglion cell receptive fields is severely reduced or eliminated after a prolonged period in the dark. This change in receptive field organization did not relate to a switch from cone to rod vision (*1*) and the mechanisms that underlie the phenomenon have remained unknown. Evidence presented here suggests that interplexiform cells, neurons whose perikarya lie among amacrine cells and whose processes extend into both plexiform layers of the retina (*2*), may mediate such a phenomenon in fish.

In the fish retina, interplexiform cells contain dopamine and their processes make numerous synaptic contacts onto horizontal cells in the outer plexiform layer (*2*). Application of dopamine to the retina results in an accumulation of adenosine 3′,5′-monophosphate (cyclic AMP) in horizontal cells (*3*) and a reduction in the response of these cells to full-field illumination (*4*). Furthermore, dopamine, cyclic AMP analogs, and forskolin, an adenylate cyclase activator, reduce the receptive field size of horizontal cells by decreasing the electrical coupling among these cells (*5–7*). Horizontal cells provide receptor, bipolar, and many ganglion cells with their receptive field surrounds (*8, 9*); thus a reduction in horizontal cell responses or receptive field size would be expected to affect the surround antagonism observed in receptor, bipolar, and ganglion cells. In support of this, application of dopamine to the goldfish retina decreases the antagonistic surround responses of bipolar and receptor cells (*4*).

We report that, following a prolonged period in the dark (100 to 110 minutes), L-type (H1) cone horizontal cells in the carp showed alterations in responsiveness and receptive field size very similar to those induced by dopamine. Furthermore, exogenous dopamine no longer had effects on the cells. These observations suggest that interplexiform cells in fish release dopamine after prolonged darkness and thus modulate inhibitory surround effects mediated by horizontal cells as a function of time in the dark.

Experiments were performed on superfused retinas from carp *(Cyprinus carpio)* maintained on a 12:12-hour light: dark cycle. During the light phase whole retinas were dissected in dim red light from fish kept in the dark for 20 minutes (control) or 90 minutes and were mounted receptor side up in a superfusion chamber. On average it took 10 to 20 minutes after the initial dark period for the preparation to be set up and for a satisfactory cell to be impaled. Thus, total time in the dark or dim red light for control preparations was 30 to 40 minutes, compared to 100 to 110 minutes for preparations kept in prolonged darkness. The superfusion medium consisted of Ringer's solution containing 110 m*M* NaCl, 2.5 m*M* KCl, 20 m*M* $NaHCO_3$, 20 m*M* glucose, 0.1 m*M* $CaCl_2$, and 0.1 m*M* $MgSO_4$, maintained at ~19°C and *p*H 7.6 and aerated with a mixture of 97 percent O_2 and 3 percent CO_2 (*10*). The medium flowed by gravity at a rate of 1.5 ml/min into a superfusion chamber (volume, 0.5 ml). Test drugs were added to the medium.

Membrane potentials and light-evoked responses of cone-driven H1 horizontal cells were recorded intracellularly. The cells were identified by their responses to monochromatic spectral stimuli, by response waveform, and by the depth of the penetrated unit (*4, 10*). The resting membrane potential after both 30 to 40

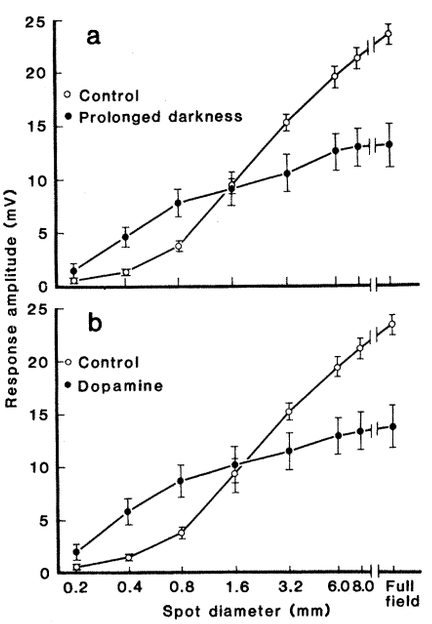

Fig. 1. Average H1 horizontal cell response amplitudes as a function of stimulus spot diameter. (a) Comparison of average response amplitudes of cells to spot stimuli after 30 to 40 minutes in the dark (control, $n = 16$) and after 100 to 110 minutes in the dark ($n = 8$). (b) Comparison of average response amplitudes of cells to spot stimuli after application of 25 μ*M* dopamine ($n = 8$) and after 30 to 40 minutes in the dark (control, $n = 16$). Dopamine application and 100 to 110 minutes in the dark caused average response amplitudes to small spot stimuli to be significantly larger and average response amplitudes to large spot stimuli to be significantly smaller. Each data point represents the mean ± standard error.

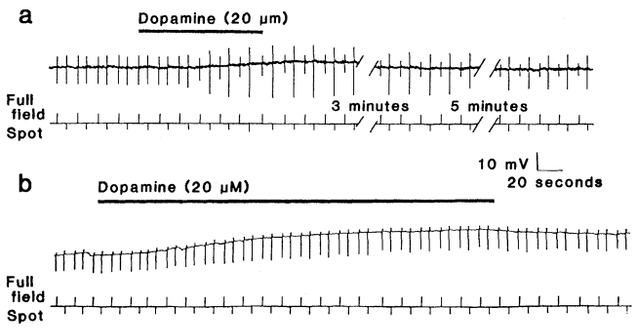

Fig. 2. Effects of dopamine application on the responses of H1 horizontal cells to spot and full-field white light stimuli for a cell from a control retina kept in the dark for 30 to 40 minutes (a) and for a cell from a retina kept in the dark for 100 to 110 minutes (b). In the control preparation, dopamine application after 30 to 40 minutes in the dark caused the response to the spot stimulus to increase in amplitude and the response to the full-field stimulus to decrease in amplitude. After a retina was kept in the dark for 100 to 110 minutes, dopamine application had little or no effect on the relative response size to spot and full-field stimuli.

minutes and 100 to 110 minutes in the dark was approximately −30 mV.

The receptive field profile of H1 horizontal cells was determined by recording an area response series (*11*). That is, light stimuli (500 msec) of various diameters and constant intensity were centered on the receptive field of a horizontal cell and presented sequentially. The receptive field center was determined initially by moving a slit of light across the retina in orthogonal directions until a maximum response was generated. The intensity of light chosen for the area-response series was that which gave a half-maximal response when a full-field stimulus was used.

Figure 1a shows area-response functions for H1 horizontal cells after 30 to 40 and 100 to 110 minutes in the dark. After the longer period of darkness, the average response amplitudes to large spot stimuli (>1.6 mm) were considerably smaller than were the response amplitudes after 30 to 40 minutes of darkness. On the other hand, the response amplitudes to small spot stimuli (<1.6 mm) were larger after prolonged periods in the dark. For three-spot (0.4, 0.8, and 8.0 mm) and full-field stimuli, the differences in average response amplitude were significant ($P < 0.01$, Mann-Whitney U test). With full-field stimuli the average response amplitudes after prolonged darkness decreased by over 40 percent (*12*).

Figure 1b shows the effect of dopamine on the area-response function of H1 horizontal cells (*13*) after 30 to 40 minutes in the dark. The effects of 25 μM dopamine were almost exactly like those of prolonged darkness. In fact, average response amplitudes after dopamine application and 100 to 110 minutes in the dark were not significantly different at any stimulus diameter ($P > 0.05$). After dopamine application, average response amplitudes to small spot stimuli were increased significantly, while average response amplitudes to large spot and full-field stimuli were decreased significantly. The increase in the responses to small spot stimuli is similar to that reported earlier (*5, 6*), and is expected if dopamine acts by decreasing electrical coupling between horizontal cells. The decreased responsiveness to large and full-field stimuli after dopamine application has also been reported (*4, 5*), but cannot be explained simply on the basis of decreased electrical coupling between the horizontal cells. That is, the responses of horizontal cells to large and full-field stimuli should not depend on the extent of electrical coupling between the cells. Thus the decreased responsiveness of H1 horizontal cells to these stimuli after dopamine application is probably due to some other effect of the drug.

The change in the receptive field area–response function after prolonged darkness may be due to the endogenous release of dopamine (Fig. 2) (*14*). Dopamine (20 μM) was applied to H1 cells exposed to darkness for 30 to 40 minutes (control preparation) and to cells exposed to darkness for more than 100 minutes. Full-field and spot (0.8 mm) stimuli were initially adjusted in intensity to give reponses of similar amplitudes and were alternately presented to the retina. In the control preparation, 2 minutes of dopamine application increased response amplitudes to spot stimuli and decreased response amplitudes to full-field stimuli. Recovery was very slow, requiring about 15 minutes. If additional dopamine was applied to the retina during the early part of the recovery period, then there were small or no effects. After prolonged darkness, 5 minutes of dopamine application had virtually no effect on the relative responses to full-field and spot stimuli, suggesting that endogenously released dopamine had already produced a maximum effect (Fig. 2b). As occurred in about half of the cases in which dopamine was added to the superfusion medium, the cells in both preparations depolarized somewhat (*4*), indicating that the drug reached the retina in both instances.

These results provide a possible explanation for the earlier observation that, with prolonged time in the dark, the antagonistic surrounds of ganglion cells are reduced or eliminated (*1, 15*). As noted earlier, the elimination of the surround antagonism of cat ganglion cells with time in the dark was not related to a switch from cone to rod vision. This is because receptive field surrounds could be demonstrated initially in the dark under both photopic and scotopic conditions. Barlow *et al.* (*1*) thus argued that a laterally arranged cell system, such as horizontal or amacrine cells, must underlie the dependence of the ganglion cell surround on time in the dark. Our evidence suggests that the interplexiform cells acting on the horizontal cells could be part of that lateral system in the fish retina (*16*).

References and Notes

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12. Addition of dim continuous background lights reduced the response size of H1 horizontal cells to small and large spot stimuli proportionately with no suggestion of a change in receptive field size. This result is different from that obtained with prolonged darkness and can be accounted for by a decrease in receptor sensitivity due to the background illumination [J. E. Dowling and H. Ripps, *J. Gen. Physiol.* **58**, 163 (1971)].
13. Dopamine application also causes a reduction in the responsiveness and receptive field size of fish C-type (H2 and H3) cone horizontal cells [T. Teranishi, K. Negishi, S. J. Kato, *J. Neurosci.* **4**, 1271 (1984); S. C. Mangel and J. E. Dowling, in preparation]. However, because of insuffient data, we do not know whether prolonged darkness mimics the effects of dopamine on these cells. It is also not known whether dopamine or prolonged darkness affect rod horizontal cells, but evidence suggests that fish interplexiform cells do not contact these cells (*2*).
14. In carp and goldfish there is indirect evidence that flickering lights release dopamine in the retina [(*4*); J. E. Dowling and K. J. Watling, *J. Neurochem.* **36**, 569 (1981)]. A reexamination of this question with a direct measurement of dopamine release indicates that substantial dopamine release occurs after prolonged darkness (P. O'Connor, S. Dorison, K. J. Watling, J. E. Dowling, in preparation).
15. Prolonged darkness has also been reported to decrease the responsiveness and spontaneous activity of cone-driven goldfish color opponent ganglion cells and to eliminate spinules, the

fingerlike specializations that have been described on horizontal cell processes in the cone pedicles during light adaptation [J. P. Raynauld, J. R. Laviolette, H. J. Wagner, *Science* **204**, 1436 (1979)]. It is possible that the release of dopamine by interplexiform cells during darkness and the resultant increase in cyclic AMP in cone horizontal cells may play a role in this phenomenon.

16. It is unclear whether our findings will apply to other animals. Although interplexiform cells appear to occur in most, if not all, retinas, they may not be dopaminergic in many animals. In the cat, for example, only a few interplexiform cells are dopaminergic (C. W. Oyster *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press); most may contain γ-aminobutyric acid [Y. Nakamura, B. A. McGuire, P. Sterling, *ibid.* **77**, 658 (1980)]. It is possible that nondopaminergic interplexiform cells perform the same function as the dopaminergic ones do in fish. On the other hand, dopaminergic amacrine cells are observed in virtually all retinas, and it may be that these neurons alternatively or in addition modulate the center-surround organization of ganglion cells. In the cat retina it has been reported that dopamine reduces the strength of ganglion cell surrounds [P. Thier and V. Alder, *Brain Res.* **292**, 109 (1984)], whereas in the rabbit, dopamine antagonists reduce ganglion cell surround responses [R. J. Jensen and N. W. Daw, *J. Neurosci.* **4**, 2972 (1984)].

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Crystallographic Structure of the Octamer Histone Core of the Nucleosome

Burlingame *et al.* (*1*), have presented the results of their x-ray analysis, nominally at a resolution of 3.3 Å, of crystals of the isolated histone octamer (*2*). Their proposed structure is quite different in shape, size, and internal arrangement from that determined by us from a crystallographic analysis of nucleosome core particles (*3, 4*), which consist of histone octamers associated with their natural complement of DNA. All the same, Burlingame *et al.* argue that their structure for the isolated histone octamer is more relevant to the structure of chromatin than that of the octamer within nucleosome core particles, and go on to propose a different model for the way in which DNA associates with the octamer to form a nucleosome. Their analysis has led to a hydrated spongelike structure and a shape for the octamer which disagrees with the results of x-ray solution scattering on both the octamer and nucleosome core particles (*5–7*), and which cannot be fitted into the lattice of nucleosome core crystals. These large discrepancies suggest that the structure proposed by Burlingame *et al.* is wrong; we attribute this to deficiencies in their x-ray analysis.

First we deal with the relevance of our x-ray work on nucleosome core particles to the structure of intact chromatin which Burlingame *et al.* questioned:

1) Burlingame *et al.* argue that because core particles derive from nucleosomes that have lost histone H1 and the linker DNA, they have an altered structure or are artifacts. [The logic of Burlingame *et al.* (*1*) is baffling, because the isolated histone octamer, which has lost all its DNA, and is only stabilized by the use of high salt concentrations, might, by their argument, be expected to be even less representative of the state of the histones in chromatin.] What is the evidence that nucleosome core particles are present in chromatin? The answer comes from comparisons of the effects of the enzyme deoxyribonuclease I (DNAse I) on intact chromatin and nucleosome cores. Lutter (*8, 9*), using a high resolution gel electrophoresis technique, which can resolve single nucleotide steps in mixed sequence DNA, has shown that the characteristic cutting pattern of DNAse I on the DNA of core particles accounts quantitatively for both the length and frequency distribution of DNA fragments produced from nuclei. Thus the bulk of the chromatin in nuclei contains nucleosome core particles, and it is these that we have crystallized.

2) Could the nucleosome core particles have undergone an extensive structural change on crystallization? This is unlikely, since the crystallization conditions are mild and close to physiological ionic strength (*3*). The shape that emerged from our studies, a disk of diameter 110 Å and height 57 Å, is consistent with the low angle x-ray scattering studies in solution (*5–7*). These spacings are found in x-ray diffraction patterns of both chromatin and nuclei in vivo (*10, 11*) and arise from the packing of the nucleosomal disks in the 300 Å diameter filaments of chromatin (*12, 13*). Furthermore the higher angle x-ray spacings at 37 and 27 Å, which arise from the internal structure of the nucleosomes, are also found in the correct orientation in 300 Å filaments of intact chromatin (*13*), as calculated on the basis of our electron density map (*4*).

The histone octamer in the nucleosome core particles has the shape of a disk about 70 Å in diameter and 57 Å in height (*3, 4*). There is no way in which our electron density map could give the shape proposed by Burlingame *et al.* (*1*), namely a prolate ellipoid of diameter 70 Å and a length of 110 Å. The overall protein density in the nucleosome core particle is limited to 70 Å in two dimensions by the two-turn superhelix of DNA (which is clearly visible) and to less than 60 Å in the third dimension by the DNA in neighboring layers of core particles. The octamer in our crystals is so confined by this adjacent DNA that no significant density could extend beyond the disk.

The structure presented by Burlingame *et al.* also has curious physical chemical properties. The proposed ellipsoid has a volume of 280,000 $Å^3$, three times that of the dry volume of 82,000 $Å^3$ of the histone octamer in solution [as calculated from the molecular weight (108,000) and the partial specific volume of 0.77 at appropriately high salt concentrations (*14*)]. Being penetrated by numerous holes and channels like a sponge, the proposed octamer has an abnormally high water content. A simple calculation shows that even on the most favorable assumptions (*15*), the proposed ellipsoid would have a sedimentation constant of 3.7*S* compared with the experimental value of 4.8*S* (*14*). An octamer of the shape and volume found in the nucleosome core crystals leads to a value of 4.2*S*, more consistent with the observed value.

Despite these disagreements, it could be argued that the structure proposed by Burlingame *et al.* could be correct for the octamer in high salt (the nominal ionic strength of the crystallization buffer is of the order of 7*M*), even if it does not reflect the structure of the octamer when combined with DNA. This, too, must be discounted, since the shape and size of the histone octamer, deduced by image reconstruction from electron micrographs of helical aggregates of octamers prepared at similarly high salt concentrations (*16*), agrees with that present in nucleosome core particles, as determined by neutron diffraction contrast variation at low resolution (*17*) or x-ray analysis (*3, 4*).

We are thus led to the view that either Burlingame *et al.* (*1*) have misinterpreted their map, or that the map contains errors that have led to a structure of the histone octamer at variance with other, firm data. Despite their demonstration of two α-helical rods of density (in which amino acid side chains are not visible), we believe that their map is unreliable. First, it is surprising that the polypeptide chain has not been traced since this should be easily discernible at the resolution of 3.3 Å, but there are grave deficiencies in the crystallographic analysis. Only a single heavy atom derivative has been used, and this is reported to be located at a rather special position with fractional coordinates very close to (1/3,