antibody to mouse γ-globulin, and rabbit antibodies to ferritin were prepared by conventional procedures [C. A. Williams and M. W. Chase, *Methods in Immunology and Immunochemistry* (Academic Press, New York, 1967), pp. 371–72]. Purified antibody to ferritin was prepared by acid elution from glutaraldehyde-aggregated ferritin [C. A. Williams and M. W. Chase, *ibid.*; S. Avrameas and T. Ternyneck, *Immunochemistry* **6**, 53 (1969)]. Peripheral blood leukocytes were isolated by $NH_4Cl$ lysis of whole blood [K. Hummler, T. N. Harris, N. Tomassini, M. Hechtel, M. B. Farber, *J. Exp. Med.* **124**, 255 (1966)].
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10. Supported by funds provided for biological and medical research by the State of Washington initiative measure No. 171, and PHS general research support grant FR 5465. Electron micrographs were taken in the Electron Microscope Facility of the Animal Disease and Parasite Research Division, U.S. Department of Agriculture, College of Veterinary Medicine, Washington State University. We thank Donald Gayman for technical assistance.

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## Lateral Interactions at Inner Plexiform Layer of Vertebrate Retina: Antagonistic Responses to Change

Abstract. *Lateral interactions at the inner plexiform layer of the retina of the mudpuppy were studied intracellularly after they were isolated from interactions at the outer plexiform layer with a special stimulus. The isolation was confirmed by recording no surround effect at bipolar cells under conditions that elicited a strong surround effect at ganglion cells. It appears that amacrine cells, which respond to spatiotemporal change at one retinal region, inhibit the response to change in on-off ganglion cells at adjacent sites.*

As the visual message passes through the retina it is modified by systems of lateral interneurons that relate activity elicited at adjacent regions of the visual field. Investigations at the outer plexiform layer of the retina have revealed a significant functional role for the horizontal cells as lateral interneurons; when driven by receptors at one region they act to reduce the efficacy of transmission at adjacent receptors (*1*). As a result, the receptive field for each receptor (*2*) and for each bipolar cell, which is driven by a small group of receptors (*3*), seems to be embedded in an antagonistic field mediated by laterally oriented horizontal cells.

The inner plexiform layer also contains a system of lateral interneurons, the amacrine cells, but their functional role is not yet clear. Like horizontal cells the processes of each amacrine cell extend laterally for a few hundred microns (*4*) and appear capable of reciprocal feedback synapses with the cells that drive them (see Fig. 1). Unlike horizontal cells, which respond with sustained hyperpolarizing potentials that are graded with intensity, amacrine cells depolarize and respond only to changing stimuli. A major difficulty in studying lateral interactions at the inner plexiform layer alone is that light-evoked activity there has already been affected by horizontal cell interactions. In this report a special stimulus was used to isolate and characterize lateral interactions at the inner plexiform layer and to determine the role of these interactions in processing the visual message.

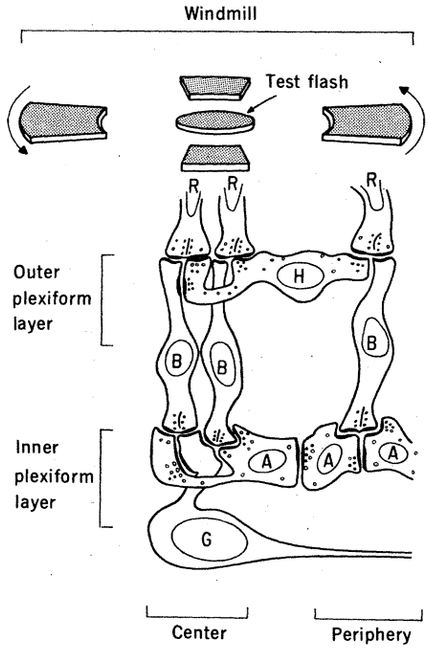


Fig. 1. Synaptic sites for lateral interactions in the retina. Structures are abstracted from electron microscopic study of the mudpuppy retina. Synaptic regions are indicated by thickening of membranes and clustering of synaptic vesicles in the presynaptic cytoplasm. Cell types are indicated by initials: *R*, receptors; *H*, horizontal cells; *B*, bipolar cells; *A*, amacrine cells; *G*, ganglion cells. The stippled areas represent the lighted parts of the stimulus. The four square elements represent the spinning vanes, inside diameter is 1 mm, outside diameter is 1.5 mm. The central disk represents the locus of the central test flash, diameter 300 μm. Windmill spins at ¼ rev/sec, so the vanes have a mean tangential velocity of about 1 mm/sec. Intensities were within 2 log units of ganglion cell threshold.

The retina of the mudpuppy, *Necturus maculosus*, was used because each cell type can be studied intracellularly (*1*), and because the synaptic structures found in the mudpuppy retina (*4*) are characteristic of most other vertebrates (*5*). The greatest variation in retinal structures between different vertebrates lies in the relative number of synaptic contacts by amacrine cells in the inner plexiform layer; the number in the mudpuppy lies intermediate between the very complex retina of the frog and the relatively simple retina of the cat (*6*). Techniques for recording, identifying, and stimulating cells have been reported previously (*1*).

Lateral interactions at the inner plexiform layer were isolated by taking advantage of the difference in response properties for horizontal and amacrine cells; horizontal cells respond to steady levels of illumination with sustained potentials whereas amacrine cells respond only to change. To illustrate this difference, the activities were recorded in each cell type as a vane-shaped stimulus was moved across the receptive fields at 1 mm/sec (Fig. 2). While the stimulus was moving the horizontal cell hyperpolarized and the amacrine cell depolarized over a region spanning about 1 mm. When the vane was stopped within the receptive field of these cells the horizontal cell remained polarized, but the amacrine cell activity was lost and the membrane potential returned to the resting state. This indicates that horizontal cells are polarized by the presence of the vane, but amacrine cells respond only to its movement. To isolate activity at the inner plexiform layer, four vanelike segments were incorporated into a "windmill" stimulus. Its presence activated horizontal cells, but only its spin activated amacrine cells. Activity elicited by the vanes was measured in cells lying at the center of the windmill because lateral effects from all four vanes tend to converge there, and the effects of lateral interactions were tested by a disk of illumination flashed at the center (see Fig. 1). The intensities of the disk and windmill were not critical as long as they were below saturating levels for the cells studied.

It is first necessary to show that there

is a population of cells at the inner plexiform layer that is unaffected by interactions at the outer plexiform layer; this is the region in which interactions at the inner plexiform layer can be studied in isolation. The response of a central bipolar cell to the flashing disk alone was recorded (dotted curve in Fig. 3A). When a stationary windmill was introduced, the bipolar cell response to the same flash was reduced in magnitude (solid curve in Fig. 3A). The magnitude of response was probably reduced by the action of horizontal cells that form an antagonistic region surrounding the bipolar cell as described above. When the windmill was spun (Fig. 3B), the resting potential of the bipolar cell was unchanged and the magnitude of response to the central flash remained the same as in the nonspinning case (Fig. 3A). Rates of spin between 0 and 4 rev/sec gave similar results in bipolar cells. This indicates that the central bipolar cells are affected by the presence of the windmill but not its spin. An explanation can be found in the form of horizontal cell activity (Fig. 2A); whether the windmill was spinning or not, some horizontal cells were always polarized and therefore capable of antagonizing the central response of the bipolar cell. As a result, activity which is elicited by the spinning windmill and recorded from cells proximal to the central bipolar cells, cannot be signaled by the central bipolar cells. This activity must, therefore, travel through lateral connections at the inner plexiform layer alone.

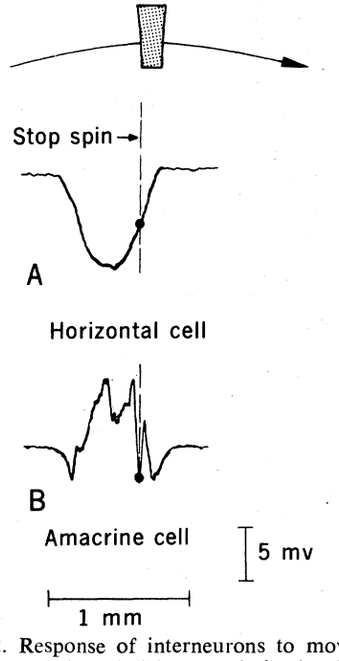


Fig. 2. Response of interneurons to moving vane. (A) and (B) are polarization in horizontal and amacrine cells beneath the moving vane as a function of distance (not time) across their receptive fields. Large dots represent the steady response maintained after the vane stops in the receptive field. Vane is drawn approximately to scale, it moves at 1 mm/sec in the direction indicated by the arrow.

Amacrine cells normally respond to flashing stimuli with transient depolarizations at on and off (Fig. 3C). When the windmill was spun the central amacrine cells were depolarized to a new, sustained level (Fig. 3D). The amacrine cells beneath the spinning vanes were depolarized by the movement (Fig. 2B), but these cells are 500 $\mu$m away from the center and their processes extend only 200 $\mu$m or so toward the center (*4*). It is therefore likely that peripheral amacrine cells beneath the vanes drive the central amacrine cells through excitatory synaptic connections (Fig. 1). The breadth of the amacrine cell receptive field, as estimated from either Figs. 1B or 3D, appears to be greater than 1 mm in response to moving stimuli. Light scattering toward the center from the spinning windmill could also account for the depolarization of the central amacrines, but this is less likely because central illumination actually reduced the level of depolarization (Figs. 3D and 2B), and in another experiment a small windmill spinning at the center to simulate the scatter did not evoke a depolarizing response. For amacrine cells a spin rate of about ¼ rev/sec was optimal; faster and slower spinning still elicited steady polarizations but of smaller magnitude.

It remains unclear just how the steady polarization observed in the bipolar cell (Fig. 3A) is processed at the bipolar to amacrine cell synapse to form a transient response (Fig. 3C), but the results indicate that at least two antagonistic components may be involved; amacrine cells appear to be depolarized by amacrine cell input (steady level in Fig. 3D) and by bipolar cell input (transients in Fig. 3C), but bipolar cells also seem to elicit an antagonistic effect (response to flash in Fig. 3D).

Amacrine cells make reciprocal synaptic contact with bipolar cells (Fig. 1), but the effect of amacrine activity on the bipolar cell response when the windmill is spinning cannot be measured as Fig. 3B indicates. This is unfortunate because the role of the reciprocal synapse remains obscure, but the recordings support the hypothesis that recorded bipolar cell activity is formed at the outer plexiform layer alone (*1*).

Amacrine cells also make feedforward contact with ganglion cells where their postsynaptic effect can be measured. The depolarizing response of an on-off ganglion cell to a flashing stimulus at the center of its receptive field in the presence of the stationary windmill was measured (Fig. 3E). Like amacrine cells, the on-off ganglion cells are driven only by change, and it has been shown previously that this type of cell in the mudpuppy (*3*) is often responsive to movement across its receptive field. When the windmill was spun (Fig. 3F), the ganglion cell membrane was hyperpolarized to a new, sustained level, and the number of spikes in response to the central flash was reduced. The windmill fell well outside the dendritic field for ganglion cells in the mudpuppy (*4*), and, as indicated above, the bipolar cells within the dendritic field were unaffected by the spin. These results suggest that the excitatory region of the on-off ganglion cell receptive field is embedded in a broader an-

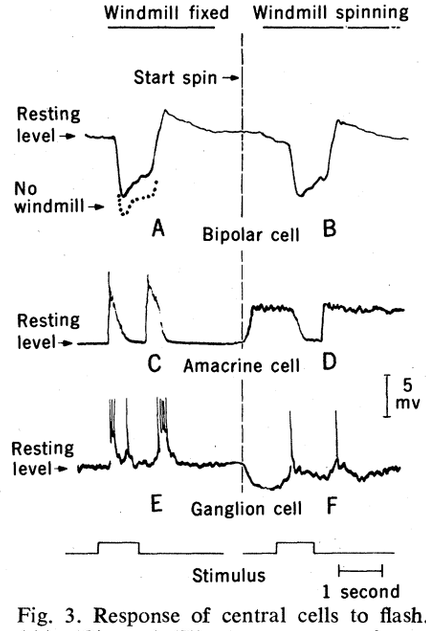


Fig. 3. Response of central cells to flash. (A), (C), and (E) are responses of central cells to central flash with windmill stopped. Dotted curve in (A) is response of bipolar cell with windmill absent. (B), (D), and (F) are responses of the same central cells to central flash with windmill spinning. Intracellular recordings from different cell types were made at different times but under similar conditions. These represent consistent results from about 20 bipolar, 10 amacrine, and 10 ganglion cells. Numerous extracellular recordings from ganglion cells confirm the intracellular findings.

tagonistic field mediated by amacrine cells; activity in both regions is elicited exclusively by spatiotemporal change.

Another major class of ganglion cells in the mudpuppy, the on-center and off-center units, generate a sustained discharge in response to maintained contrast across their concentric, antagonistic receptive fields (*1*). The central response in these units was reduced by the presence of the windmill, but they were not further affected by its spin, much like the bipolar cells described above. It appears, therefore, that the dichotomy between sustained and transient activity in the different classes of ganglion cells applies to both the center and antagonistic surround. Units driven by steady central illumination are antagonized by a steady surround; those responding to change at the center are antagonized only by change in the surround (*7*).

The retinas of animals with elaborate synaptic connections at the inner plexiform layer display many movement-sensitive functions at the ganglion cell level. For example, in frog (*8*), rabbit (*9*), and pigeon (*10*) there is a class of ganglion cells that responds best to small moving targets. These units represent a good example of a movement-activated receptive field with antagonistic zones; activity is suppressed when the size of the moving targets is increased past a critical dimension. My results suggest that the large targets extend beyond the central excitatory zone of the ganglion cell, so their movement elicits activity in peripheral amacrine cells which in turn antagonize the central response to movement.

The directionally selective movement detectors described in pigeon (*10*), rabbit (*9*), and mudpuppy (*3*), to mention only a few animals, represent an even more complex movement-sensitive system. It has been shown in each of these systems that directional selectivity is mediated by inhibition when the target is moving in the "null" direction and that, in the mudpuppy, the inhibition is measured as a hyperpolarization of the ganglion cell membrane. The mechanisms described above, however, are not sufficient to account for the directionally selective function; this represents a problem for further investigation.

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# Lack of Enhanced Oxygen Consumption by Polymorphonuclear Leukocytes on Phagocytosis of Virulent Salmonella typhi

Abstract. *Human polymorphonuclear leukocytes exhibit an enhanced rate of oxygen consumption during phagocytosis of relatively avirulent strains of* Salmonella typhi *or* Staphylococcus aureus. *However, phagocytosis of a virulent strain of* Salmonella typhi *is not associated with augmented oxygen consumption. The ability of a bacterial strain to alter the postphagocytic rate of oxygen consumption of polymorphonuclear leukocytes may be related to its in vivo virulence.*

Phagocytosis and bacteriocidal activity by polymorphonuclear leukocytes have been classically associated with an enhanced rate of their oxygen consumption. The biochemical mechanisms responsible for this increased rate are not clearly defined, although several schemes have been proposed which involve the hexose monophosphate shunt, glutathione peroxidase, and pyridine nucleotide oxidases which are cyanide-insensitive (*1*). The virulence of certain *Salmonella typhi* strains has been attributed to an interaction between specific antigenic determinants of the bacterial cell wall and factors associated with serum bacteriocidal activity (*2*). However, the relation between antigenic virulence factors and cellular host defense mechanisms has not been clearly established although the influence of *Salmonella* cell wall structure upon phagocytosis has been studied (*3*). We report that phagocytosis of a virulent *Salmonella* strain (Quailes) is not accompanied by an augmented oxygen consumption but ingestion of either a less virulent strain (O-901) or of the relatively avirulent *Staphylococcus aureus* 502A is associated with the classical postphagocytic increase in oxygen uptake. This alteration of the normal metabolic bacteriocidal reactions of phagocytes may contribute to the in vivo virulence of *S. typhi*.

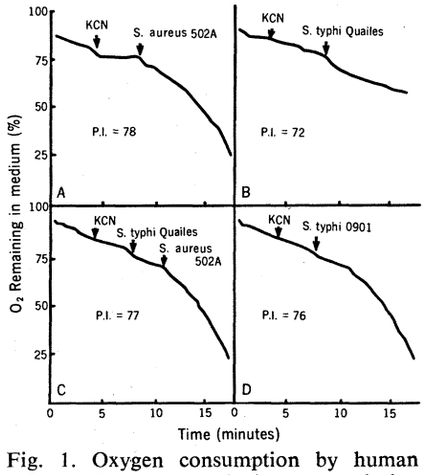


Fig. 1. Oxygen consumption by human polymorphonuclear leukocytes during phagocytosis of *Staphylococcus aureus* 502A, *Salmonella typhi* Quailes, and *Salmonella typhi* O-901. Phagocytic index (*P.I.*) is the percentage of polymorphonuclear leukocytes containing bacteria.

Normal human polymorphonuclear leukocytes were isolated from blood by the technique of dextran-enhanced sedimentation of erythrocytes. The leukocytes were then washed with modified Hanks solution (HBG) (*4*) by two low-speed (180*g*) centrifugations. The contaminating erythrocytes were eliminated by hypotonic lysis and centrifugation (*5*). The leukocyte pellet was then suspended in HBG, the cells were enumerated in a standard hemocytometer and in smears with Wright stain, and the cell suspension was adjusted with HBG to a final concentration of $10^7$ polymorphonuclear leukocytes per milliliter.