

# SPATIAL DISTRIBUTION OF POTENTIAL IN A FLAT CELL

## APPLICATION TO THE CATFISH HORIZONTAL CELL LAYERS

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**ABSTRACT** An analytical solution is obtained for the three-dimensional spatial distribution of potential inside a flat cell, such as the layer of horizontal cells, as a function of its geometry and resistivity characteristics. It was found that, within a very large range of parameter values, the potential is given by

$$V(r, z) = K \cdot J \int_0^{\infty} \frac{(\mu - \gamma)e^{-\mu(2\delta - z)} + (\mu + \gamma)e^{-\mu z}}{(\mu + \gamma)^2(1 - e^{-2\mu\delta})} \sin \mu J_0(\mu r) d\mu, \quad (A)$$

where  $r = \rho/\rho_0$ ,  $\bar{z} = z/\rho_0$ ,  $\gamma = (R_i/R_m) \cdot \rho_0$ ,  $\delta = h/\rho_0$ ;  $K$  is a constant;  $J$  is the assumed synaptic current;  $\rho$ ,  $z$  are cylindrical coordinates;  $\rho_0$  is the radius of the synaptic area of excitation;  $h$  is the cell thickness; and  $R_i$ ,  $R_m$  are the intracellular and membrane resistivities, respectively. Formula A closely fits data for the spatial decay of potential which were obtained from the catfish internal and external horizontal cells. It predicts a decay which is exponential down to about 40% of the maximum potential but is much slower than exponential below that level, a characteristic also exhibited by the data. Such a feature in the decay mode allows signal integration over the large retinal areas which have been observed experimentally both at the horizontal and ganglion cell stages. The behavior of the potential distribution as a function of the flat cell parameters is investigated, and it is found that for the range of the horizontal cell thicknesses (10–50  $\mu$ ) the decay rate depends solely on the ratio  $R_m/R_i$ . Data obtained from both types of horizontal cells by varying the diameter of the stimulating spot and for three widely different intensity levels were closely fitted by equation A. In the case of the external horizontal cell, the fit for different intensities was obtained by varying the ratio  $R_m/R_i$ ; in the case of the internal horizontal cell it was found necessary, in order to fit the data for different intensities, to vary the assumed synaptic current  $J$ .

### INTRODUCTION

It was noticed by early investigators that the amplitude of the horizontal cell response (a potential usually referred to as S potential) to a fixed intensity light flash

increased as the illuminated area on the retina was expanded (Tomita et al., 1958; Oikawa et al., 1959; Gouras, 1960). This phenomenon was referred to as the "area effect" by Tomita et al. Studying the lateral spread of the horizontal cell response in a *Cyprinidae* fish, the tench, Naka and Rushton (1967) arrived at two conclusions. First, the spread of potential was not due to scatter of light stimulus but was due to spread of potential inside the retina (cf. Watanabe and Tosaka, 1959). Second, the structure responsible for the lateral spread of the potential could be approximated by a large flat cell which they referred to as the S space.

In the analysis of the horizontal cell responses in the tench, it was assumed that the decay of the potential inside the S space as a function of the distance of illumination from the recording site could be approximated by a single exponential function. During the last few years, however, working with the catfish horizontal cells, we have noticed that the decay of potential inside the S space, over a larger distance, cannot be fitted simply by a single exponential function although potential decay over a shorter distance could be approximated by an exponential function as proposed by Naka and Rushton (1967) and by Naka (1972). Negishi and Sutija (1969) have also found a considerable deviation from a simple exponential decay in the spread of potential in other teleost fish.

In this study, the electrical field problem posed by the flat cell geometry and the excitation conditions is solved and the solution is compared with the results obtained from the catfish horizontal cells. The solution predicts that the decay is not a simple exponential function. Moreover, due to the geometry of a large flat cell, like the S space, the form of the solution for large distances is such that potentials can be integrated over a far larger area than that predicted if the data were fitted with an exponential function which is the solution for a cable geometry. Experiments were performed in the catfish horizontal cell layers (S space), and the results have confirmed these theoretical predictions. This agreement is very significant because the receptive field of the retinal ganglion cell is known to extend up to 5 mm in diameter (Daw, 1968) and it was shown by Naka and Nye (1971) that such a large receptive field is subserved by the S space.

#### PROBLEM DEFINITION AND SOLUTION

The layer of horizontal cells is modeled by a flat cell of finite width and extending infinitely in the other two dimensions. The cell is bounded by two infinite plane parallel membranes of high resistivity  $R_m$  (ohms  $\times$  square millimeters) and between them there is a medium of low resistivity  $R_i$  (ohms  $\times$  millimeters) (see Fig. 1). The resistivity of the external medium  $R_e$  (ohms  $\times$  millimeters) is taken to be zero as is commonly done in solving problems of this type (for example, Eisenberg and Johnson, 1970, Minor and Maksimov, 1969, Shiba and Kanno, 1971). The reasons for this approximation are:

- (a) In nerve cells  $R_i$  is of the same order of magnitude or smaller than  $R_m$ . If  $R_m$

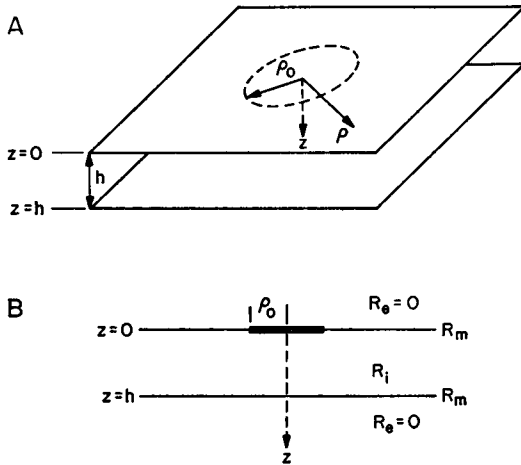


FIGURE 1 Schematic diagram of a flat cell (or S space) showing the exciting disc of radius  $\rho_0$  on the top membrane and the resistivities of the various media. Cell thickness is  $h$ .

is much larger than  $R_i$  and  $R_e$ , the potential distribution pattern within the cell remains practically unchanged by taking  $R_e = 0$ . This relation between  $R_m$ ,  $R_i$ , and  $R_e$  is almost always true for neurons. In our particular case the condition to be satisfied, so that the assumption  $R_e = 0$  is a good approximation, becomes

$$R_m \gg h \cdot R_i, \tag{B}$$

provided that  $R_e$  and  $R_i$  are of the same order of magnitude. As described in Results the ratio  $R_m/R_i$  varies from about 1 to 10 mm and  $h \simeq 0.05$  mm. Therefore condition B is certainly satisfied and the assumption is justified.

(b) If condition B is satisfied but the ratio  $R_e/R_i$  is not small, the deviation due to the approximation appears only for large distances from the excitation site at which the potential has attenuated to a small value (Minor and Maksimov, 1969). Since electrophysiological measurements are plagued with low signal-to-noise ratios the measurement of small potentials at large distances is inaccurate anyway.

(c) The assumption is also made for the sake of mathematical simplicity since, as discussed in (a) and (b) above, the error is small.

The excitation of a horizontal cell by the receptors is modeled in terms of an ideal conducting flat disc injecting constant current  $J$  into the intracellular space. The disc is placed against the inside surface of the top membrane.

We seek the potential distribution in the intracellular space. A cylindrical system of coordinates is introduced in a way such that the center of the disc is at point ( $\rho = 0, z = 0$ ) and the  $z$  axis is perpendicular to the membrane surfaces. The potential  $V(\rho, z, \theta)$  is found as the solution of Laplace's equation in cylindrical coordinates in the intracellular space. Because of the symmetry in the geometry of the

problem, the potential is not a function of  $\theta$  and the equation reduces to

$$\left(\frac{\partial^2}{\partial \rho^2} + \frac{1}{\rho} \frac{\partial}{\partial \rho} + \frac{\partial^2}{\partial z^2}\right) V(\rho, z) = 0. \quad (1)$$

Furthermore, due to the problem geometry (azimuthal symmetry) the solution of equation 1 is of the form

$$V(\rho, z) = \int_0^\infty [\phi(\nu)e^{\nu z} + \psi(\nu)e^{-\nu z}] J_0(\nu \rho) d\nu, \quad (2)$$

where  $J_0(\nu \rho)$  is the Bessel function of the first kind of zero order and  $\phi(\nu), \psi(\nu)$  are functions which will be determined by the boundary conditions.

The first boundary condition expresses the continuity of current flow from the intracellular medium through the membrane and perpendicular to the plane  $z = h$ . We ignore any current flowing through the membrane at other than a right angle to it. This boundary condition is expressed by

$$\frac{1}{R_i} \frac{\partial V(\rho, z)}{\partial z} \Big|_{z=h} = - \frac{V(\rho, z)}{R_m} \Big|_{z=h}. \quad (3)$$

The boundary condition at the plane  $z = 0$  is a mixed one. For the portion outside the charged disc it expresses the continuity of current flow through the membrane,

$$\frac{1}{R_i} \frac{\partial V}{\partial z} \Big|_{z=0} = - \frac{V}{R_m} \Big|_{z=0}, \quad \rho_0 \leq \rho < \infty. \quad (4)$$

It can be shown (Sneddon, 1966) that, in the case of a charged conducting disc immersed in a homogeneous medium of resistivity  $R_i$ , the current density on the surface of the disc is given by

$$\frac{\partial V}{\partial z} \Big|_{\text{disc surface}} = - \frac{JR_i}{4\pi\rho_0} \frac{1}{\sqrt{\rho_0^2 - \rho^2}}, \quad 0 \leq \rho < \rho_0, \quad (5)$$

where  $J$  is the current flowing from the disc and  $\rho_0$  is the disc radius.

In the case of the disc of our problem, we approximate the current density by a similar dependence on  $\rho$ ,

$$\frac{\partial V}{\partial z} \Big|_{z=0} = - KJ \frac{1}{\sqrt{\rho_0^2 - \rho^2}}, \quad 0 \leq \rho < \rho_0, \quad (6)$$

where the constant  $K$  (whose units are in ohms) is introduced to account for the approximation made. In general,  $K$  is a function of  $h, \rho_0, R_i, R_m$  and can be picked after we have solved for  $V(\rho, z)$  and in order to make the total current flowing through the membrane into the external medium equal to  $J$ .

Thus, in conclusion, the potential should satisfy the following two boundary conditions:

$$\frac{1}{R_i} \frac{\partial V}{\partial z} \Big|_{z=h} = - \frac{V}{R_m} \Big|_{z=h}, \quad (7)$$

$$\frac{\partial V}{\partial z} \Big|_{z=0} = \begin{cases} -KJ \frac{1}{\sqrt{\rho_0^2 - \rho^2}}, & 0 \leq \rho < \rho_0 \\ -R_i \frac{V}{R_m}, & \rho_0 \leq \rho < \infty. \end{cases} \quad (8)$$

From the boundary conditions stated by equation 7 and letting  $R_i/R_m = \beta$ , we obtain

$$\int_0^\infty [(\nu + \beta)\phi(\nu)e^{\nu h} - (\nu - \beta)\psi(\nu)e^{-\nu h}]J_0(\nu\rho) d\nu = 0. \quad (9)$$

Since equation 2 must hold for all  $\rho$ , we must have

$$\phi(\nu) = \frac{\nu - \beta}{\nu + \beta} e^{-2\nu h} \psi(\nu). \quad (10)$$

The boundary condition stated by equation 8, in principle, determines  $\psi(\nu)$ . Trying to determine  $\psi(\nu)$  from this boundary condition and taking into account equation 10 results in the following dual integral equations:

$$\int_0^\infty \nu \left[ \frac{\nu - \beta}{\nu + \beta} e^{-2\nu h} - 1 \right] \psi(\nu) J_0(\nu\rho) d\nu = -K \cdot J \cdot \frac{1}{\sqrt{\rho_0^2 - \rho^2}}, \quad 0 \leq \rho < \rho_0, \quad (11)$$

$$\int_0^\infty (\nu + \beta)(e^{-2\nu h} - 1)\psi(\nu) J_0(\nu\rho) d\nu = 0, \quad \rho_0 \leq \rho < \infty. \quad (12)$$

In general, the solution of such dual equations is difficult and involved.

We solve the pair of equations 11 and 12 by making certain approximations. First, we make the following normalizations and changes of variables:  $\mu = \nu\rho_0$ ,  $r = \rho/\rho_0$ ,  $z = z/\rho_0$ ,  $\delta = h/\rho_0$ ,  $\gamma = \beta\rho_0$ ,  $\psi_1(\mu) = \psi(\mu/\rho_0)$ . Then, equations 2, 11, and 12 become, respectively,

$$V(r, z) = \frac{1}{\rho_0} \int_0^\infty \left[ \frac{\mu - \gamma}{\mu + \gamma} e^{-2\mu\delta} e^{\mu z} + e^{-\mu z} \right] \psi_1(\mu) J_0(\mu r) d\mu, \quad (13)$$

$$\int_0^\infty \mu \left[ 1 - \frac{\mu - \gamma}{\mu + \gamma} e^{-2\mu\delta} \right] \psi_1(\mu) J_0(\mu r) d\mu = K \cdot J \cdot \rho_0 \cdot \frac{1}{\sqrt{1 - r^2}}, \quad (14)$$

$$0 \leq r < 1,$$

$$\int_0^\infty (\mu + \gamma)(1 - e^{-2\mu\delta})\psi_1(\mu) J_0(\mu r) d\mu = 0, \quad 1 \leq r < \infty. \quad (15)$$

In the Appendix we show that for a large range of parameters, including the range of the horizontal cell parameters (cell thickness of 10–50  $\mu$  and spot diameters from 0 to 5 mm) the solution of equations 14 and 15 can be closely approximated by

$$\psi_1(\mu) = KJ\rho_0 \frac{\sin \mu}{(\mu + \gamma)(1 - e^{-2\mu\delta})} \quad (16)$$

Thus, the expression of the potential distribution becomes

$$V(r, z) = KJ \int_0^\infty \frac{(\mu - \gamma)e^{-\mu(2\delta - z)} + (\mu + \gamma)e^{-\mu z}}{(\mu + \gamma)^2(1 - e^{-2\mu\delta})} \sin \mu J_0(\mu r) d\mu, \quad (17)$$

where, as stated before, the arbitrary constant  $K$  can be fixed from the additional condition that the total current flowing through the membrane into the external medium should be equal to  $J$ .

## METHODS

The eyecup preparation of the channel catfish, *Ictalurus punctatus*, was used for the experiment. Details of preparation and experimental procedure have been described (Naka and Nye, 1970). The horizontal cells from which responses were obtained were identified by injecting a procion dye. The computations were done by a digital computer.

## RESULTS

Equation 17 predicts the decay of potential in a large flat cell such as the S space. In Fig. 2 A the decay function for a cell with  $h = 0.05$  mm and  $\alpha = 2$  mm ( $\alpha = R_m/R_i$ ) is shown as a continuous line, while the dashed line with circles shows an exponential decay function whose space constant is picked such that it would fit the initial decay portion of the function derived from equation 17. As seen in Fig. 2 A the initial parts of both potential decay curves can be approximated by a simple exponential function (as originally proposed by Naka and Rushton, 1967) down to about 40% of the maximal response or up to 0.5 mm from the recording site. The decay for larger distances, however, shows a considerable deviation from the exponential decay; the exponential decays much faster for large distances. Recalling that the potential decays exponentially in the case of a cable structure we note that the different decay characteristics are due to the differing geometries of a cable and a flat cell structure. The spatial potential decay in the layers of horizontal cells was measured in experiments whose results are shown in Fig. 2 B. A small spot of light (0.5 mm in diameter) was moved on the retinal surface (at 0 mm the electrode was at the center of the spot as manifested by a maximum potential response) and the evoked horizontal cell potential was measured by an intracellular electrode. It should be noted that both the shape and dimensions of the horizontal cells are such that the lateral spread of potential, as described here, spans many individual cells

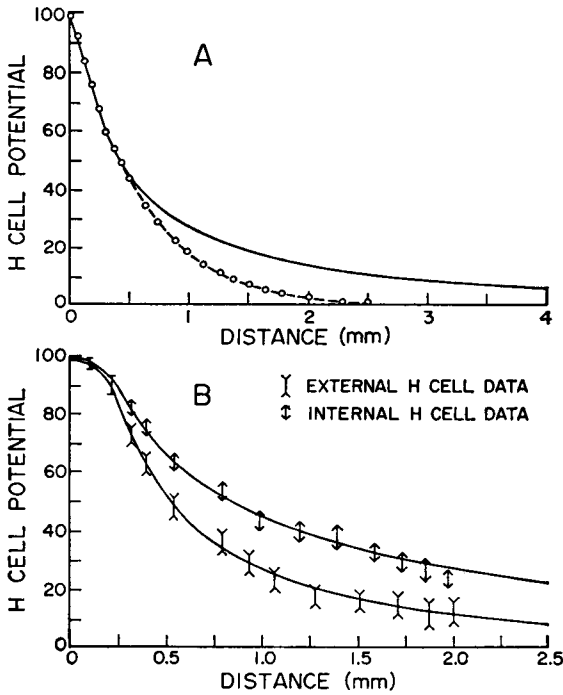


FIGURE 2 (A) Potential decay curves computed from equation 17 (solid line,  $h = 0.05$  mm,  $\alpha = 2$  mm,  $\rho_0 = 0.025$  mm) and from an exponential,  $e^{-z/\lambda}$ , (circles,  $\lambda = 0.6$  mm). (B) Data of potential decay from external and internal horizontal cells fitted by equation 17 for  $\rho_0 = 0.25$  mm,  $\alpha = 1.7$  and  $6.2$  mm, respectively. The vertical bars indicate the range of data for different experimental runs. The solid lines are the predictions of equation 17 for the same parameter values. The radius of the light spot is also  $0.25$  mm.

(i.e., it is across many contacts or junctions of adjacent horizontal cells; unpublished results).

The results of these experiments fall into two distinct groups: one set of data points, exhibiting a faster decay with distance, was found (by procion dye injection) to originate from the external horizontal cell layer; while the other set of points, exhibiting a slower decay, was recorded from the internal horizontal cell layer. Although the cell was not identified, the decay curve shown in Fig. 3 A of Naka (1972) was evidently from the external horizontal cells. No extensive record of potential decay in the intermediate horizontal cells has been obtained, but it was noticed that the decay of potential in this layer was very similar to that observed in the external horizontal cells.

These decay functions, obtained experimentally from the internal and external horizontal cell layers, were fitted with the theoretical predictions of equation 17 for a disc of  $0.5$  mm in diameter (similar to the spot used in the experiments). The parameter values for which these fits were obtained are  $h = 0.05$  mm,  $\alpha = 6.2$  mm (internal

H cell) ( $\alpha = R_m/R_i$ ), and  $h = 0.05$  mm,  $\alpha = 1.7$  mm (external H cell). For small distances we note a potential plateau which is a consequence of the disc dimensions. This plateau also exists in the experimental data. All curves shown here and computed from equation 17 were for  $z = h/2$ , i.e., the potential is calculated at the mid-plane between the two membranes.

The solid curve of Fig. 2 A has similar decay characteristics (i.e., similar parameter values of  $h$  and  $\alpha = R_m/R_i$ ) as the curve in Fig. 2 B which fits the data for the external horizontal cell layer. Thus, if the data are fitted with a simple exponential function, considerable error will be made for large distances, even though the initial decay portion of the curve can be closely approximated. It is exactly this slow decay for large distances (characteristic of a flat cell structure) that allows the S space to perform its integrating function over large receptive fields. For example, Norton et al. (1968) have reported that in the carp horizontal cells potential integration could be seen up to a spot diameter of 10 mm. The theoretical decay functions of Fig. 2 for a small spot (0.5 mm in diameter) can be obtained equally well whether we adopt a disc current source or a disc voltage source as the excitation site in our S space model. The voltage disc model, however, fails to predict the results of experiments in which the diameter of a light spot is expanded (Figs. 4 and 5).

The values of  $\alpha$  (i.e.,  $R_m/R_i$ ) for which the experimental data were fitted are rather small if we assume that the membrane and intracellular resistivities of the S space are similar to those of neural tissue such as the squid axon or frog muscle. There are at least three prominent possibilities, any one or a combination of which could be responsible for the low values. (a) In our idealization of the S space in terms of a laminar structure the equivalent  $R_i$  could be higher than that encountered in other neural tissue due to the structure of the horizontal cell layer, that is, the existence of the intercellular resistive junctions between cells. (b) The effective  $R_m$  may be lower than that of other neural tissue. (c) The equivalent external resistance  $R_e$  (which we assumed to be zero) may not be negligible. Possibility *a* is the most probable one.

Fig. 3 A shows potential decay curves for different spot radii as calculated from equation 17. It is noted that the initial plateau of the decay function becomes less prominent for spots of smaller diameter. Fig. 3 B shows the extent of potential variation in the  $z$  direction for two extreme cases; one for  $z$  nearly zero (corresponding to the case in which potentials were calculated close to the top membrane and active disc) and the other for  $z = h$  (potentials calculated at a point very close to the membrane lying opposite the active disc). Calculations were performed for two different  $\alpha$ 's;  $\alpha = 1$  mm ( $b_1, b_2$  in Fig. 3 B) and  $\alpha = 8$  mm ( $a_1, a_2$  in Fig. 3 B). For the lower  $\alpha$ , the decay in the  $z$  direction is larger, but still the potential decay across the entire thickness of the flat cell is only 5% of the potential value near the surface of the active disc. The potential variation in the  $z$  direction becomes negligible for distances larger than the disc radius.

Fig. 3 C shows potential decay curves for flat cells of different thickness  $h$  in which the maximum potential value is normalized. We conclude that, for the range of  $h$ 's



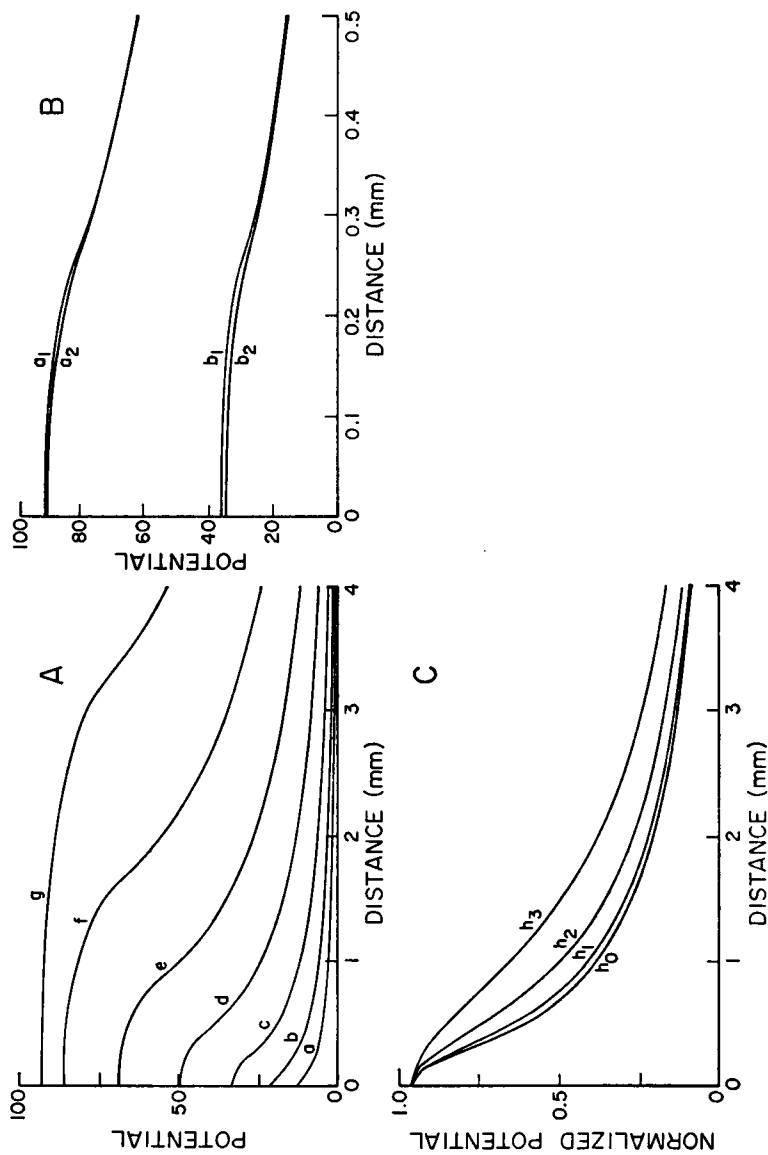


FIGURE 3 (A) Potential decay curves for different radii as computed from equation 17. Radius of spots was 0.05 mm for a, 0.10 mm for b, 0.20 mm for c, 0.40 mm for d, 0.80 mm for e, 1.60 mm for f, and 3.20 mm for g.  $h = 0.05$  mm and  $\alpha = 4$  mm. (B) Maximum potential variation in  $z$  direction (spot radius = 0.05 mm) for  $\alpha = 8$  mm ( $a_1, a_2$ ) and for  $\alpha = 1$  mm ( $b_1, b_2$ ), as computed from equation 17. Curves  $a_1, b_1$  are computed for  $z$  near 0 and  $a_2, b_2$  are computed for  $z$  near  $h$ . (C) Potential decay curves for various thicknesses  $h$  as computed from equation 17. Maximum value of potential is normalized to 1.  $h_3 = 2$  mm,  $h_2 = 1$  mm,  $h_1 = 0.5$  mm, and  $h_0 = 0.25$  mm. For  $h \geq h_0$  the curves are almost identical with the one for  $h = h_0$ .

that the catfish horizontal cells exhibit ( $10\text{--}50\ \mu$ ) and the range of  $\alpha$  in which we are interested, the spatial decay is fairly independent of thickness  $h$ . Only for very large  $h$ 's (such as  $h_3$  which corresponds to a thickness of 2 mm) does the spatial decay slow down, as shown in Fig. 3 C. Therefore, in our S space model, the difference in spatial decay (found experimentally) between the internal and external horizontal cell layers is not due to a difference in equivalent cell thicknesses, but it is solely due to different values for  $\alpha$  ( $= R_m/R_i$ ).

Fig. 4 A shows the results of experiments in which the potential was recorded at the center of a light spot for several spot radii and at several intensity levels. The recordings were from the external horizontal cell layer. The solid curves are calculated using equation 17 for the same range of spot radii and for increasing values of  $\alpha$  for increasing light intensities. In fact, increasing  $\alpha$ , which corresponds to an increase in the membrane resistance, suffices to fit the data for increasing intensity levels. For small values of the "space constant"  $\alpha$ , saturation occurs for smaller spot diameters, while for larger values of  $\alpha$  saturation occurs for larger spot diameters. The latter is a necessary condition for a mechanism that integrates signals over a large area.

In Fig. 4 B the data and model curves of Fig. 4 A are normalized so that the amplitude of the response produced by the largest light spot is set to unity for all the different intensity levels. This shows more clearly the extent of area integration performed by the external horizontal cell layer at the different intensity levels

Curves A, B, C, and D correspond to the following values of  $\alpha$  ( $= R_m/R_i$ ): A,  $\alpha = 7$  mm; B,  $\alpha = 3$  mm; C,  $\alpha = 1.2$  mm; D,  $\alpha = 10$  mm.

Since curves A and D are nearly identical, we conclude that increasing  $\alpha$  beyond the value of about 7 mm does not change the area-integrating characteristic of the S space for spots of various radii. This is, again, a natural consequence of a mechanism that integrates signals over an area.

The results shown in Fig. 2 B (obtained with a spot of light) indicate that, for the internal horizontal cells,  $\alpha$  is about 6.2 mm. Therefore, we expect, because of the argument just made, that the normalized decay data (and calculated model curves) will show no difference for the higher intensity levels by further increasing  $\alpha$ . In fact, as shown in Fig. 5 B, they exhibit the same area-integrating characteristics as curves A and D of Fig. 4 B.

Fig. 5 A shows the unnormalized data for the internal horizontal cell. Since, in this case, a variation in  $\alpha$  will not fit the data for the different intensity levels (or will not increase the saturation level), we assume that an increase in the intensity of the light stimulus results in an increase in the postulated synaptic current  $J$ . In fact, the data show that the effect of increasing the intensity of the light seems to introduce a multiplicative constant in the potential response data. We note that in equation 17  $J$  is a multiplicative constant for the potential. As seen in Fig. 5 A, the data from the internal horizontal cell layer can be fitted by assuming an increasing synaptic current  $J$  for an increasing intensity light stimulus. It should be noted that the argu-

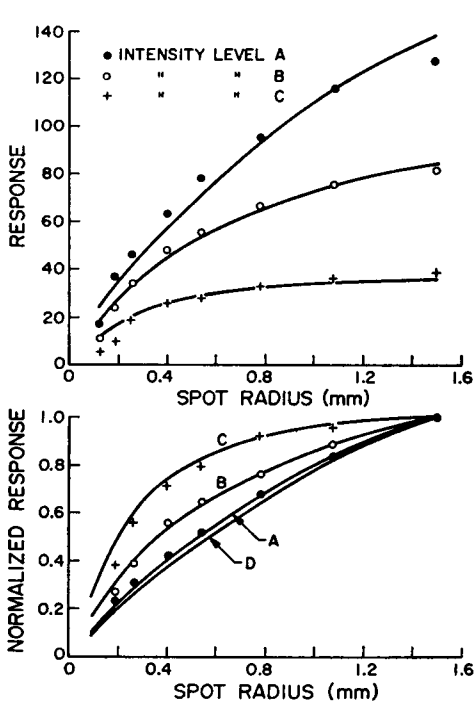


FIGURE 4

FIGURE 4 External horizontal cell. Data points (circles and crosses) and predictions (solid lines) of equation 17 for different intensity levels (A, B, C). Both unnormalized and normalized curves are shown. The response is computed for  $\rho = 0, z = h/2$ . Curves A, B, C computed for  $\alpha = 7, 3, 1.2$  mm, respectively. A potential response of 30 mv corresponds approximately to 100 of the ordinate units.

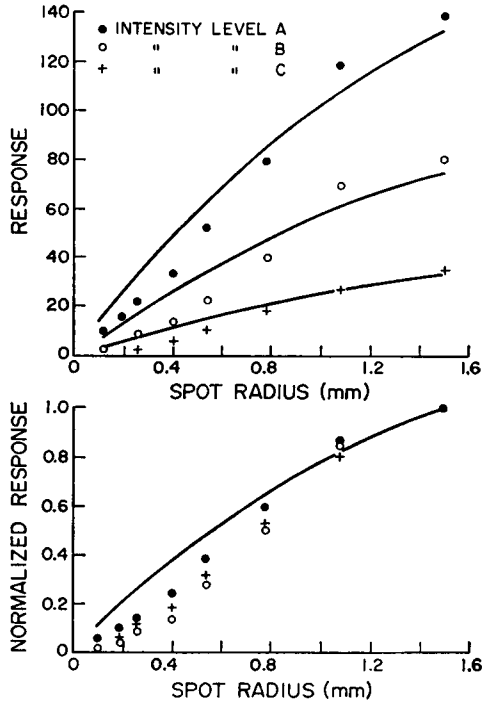


FIGURE 5

FIGURE 5 Internal horizontal cell. Data points (circles and crosses) and predictions (solid lines) of equation 15 for different intensity levels (A, B, C). Both unnormalized and normalized curves are shown. The response is computed for  $\rho = 0, z = h/2$ . All curves computed for  $\alpha = 6.2$  mm. A potential response of 30 mv corresponds approximately to 100 of the ordinate units.

ment presented above does not exclude the possibility that, in addition, light may induce an increase of the membrane resistance of the internal horizontal cells.

In the catfish retina a positive identification of the intermediate horizontal cell is far more difficult than in the case of the external or internal horizontal cells (Adomian, Matsumoto, and Naka, unpublished results) because the intermediate horizontal cells have to be identified by their long dendrites and by their location relative to the external horizontal cells. This has prevented us from making a satisfactory analysis of the spread of potential in the intermediate horizontal cells. Data we have obtained, however, indicate that the spatial decay and area-integrating characteristics are very similar to those of the external horizontal cells, namely, that a larger area of integration results for spots of higher intensity. This might have been expected,

as both the external and intermediate horizontal cells are very similar in their structural organization.

## DISCUSSION

The catfish retina is a horizontal cell-preponderated retina with the inner nuclear layer being often occupied entirely by the layers of horizontal cells. There are three layers of horizontal cells: the external, intermediate, and internal horizontal cells. Both the external and intermediate horizontal cells take a form of polygonal bricks, while the internal horizontal cells are tubular in shape (diameter 5–10  $\mu$ , length up to 500  $\mu$ ). According to Stell (1971) the external and intermediate horizontal cells are "true horizontal cells" in the sense that they receive synaptic inputs from the receptors while the internal horizontal cells do not seem to have any synaptic inputs. Their cytoplasmic structures also show a remarkable difference (Adomian, Matsumoto, and Naka, unpublished results). In the goldfish Kaneko (1970) has shown that the S potentials arise from two classes of horizontal cells; one class is the polygonal external horizontal cells and the other is the tubular internal horizontal cells. He further noted that the internal horizontal cells integrate potentials from a larger area than the external horizontal cells, an observation confirmed in this study for the catfish retina.

Although the electrical properties of a cable or axon have been a subject of extensive theoretical treatments starting with the one by Hodgkin and Rushton (1946), similar attention has not been paid to the electrical properties of a flat cell or laminar structure except in rare cases (Minor and Maksimov, 1969; Eisenberg and Johnson, 1970; Shiba and Kanno, 1971). In those treatments, however, one of the following simplifying assumptions was made: (a) a point current source was stipulated as the source of excitation, or (b) the exciting source was placed at the midplane between the two parallel membranes. Our model stipulates that a current-injecting disc on one side of two membranes is the source of excitation. This is a more realistic assumption, as whatever the generating mechanism of the horizontal cell potential may be, it is reasonable to postulate at least in the case of the external horizontal cells that the dendritic portion of the horizontal cell receives the signal from the photoreceptors and is the site of presumed synaptic current generation. Also, it is apparent that the effect of a spot of light can better be simulated by an active disc rather than a point source. Furthermore, assuming an S space (flat cell) model of the horizontal cell layer, our mathematical formulation makes no a priori assumptions on the potential decay mode, such as the exponential decay in the Naka-Rushton model, and is derived from principles based on the physical geometry of a flat cell and the electrical properties of the membrane and intracellular media.

Spatial spread of the horizontal cell response has been a subject of several recent papers. Norton et al. (1968) and Dowling and Ripps (1971) have treated the subject

trying to establish physiological mechanisms for Ricco's law while Naka and Rushton (1967), Negishi et al. (1968), and Negishi and Sutija (1969) have directed their attention to the establishment of a model or mechanism which would account for the spread of potential. The Caracas group concluded that the spread of potential involved metabolic chain reactions and that their experimental results could not be explained simply by the S space model. There are, however, two independent observations which favor the view that the horizontal cell layer can be approximated by a large flat cell, namely an S space; first, morphologically the fish horizontal cells are coupled by "gap" junctions implying a low resistance electrical passage among the cells (Yamada and Ishikawa, 1965; Witkovsky and Dowling, 1969; Witkovsky, personal communication), and second, the dogfish horizontal cells are electrically coupled (Kaneko, 1971).

Besides the horizontal cell layer in fish the approximation of a syncytium of cells as a single cell has been proposed for certain other types of syncytia such as the cardiac muscle (Woodbury and Crill, 1961; Tanaka and Sasaki, 1966). Certain aspects of it have also been discussed by George (1961). Such an approximation makes the problem mathematically manageable and allows the derivation of an explicit solution for the potential distribution. Moreover, approximate numerical solutions (by computer) for the syncytium of cells showed that the solution varies insignificantly provided that the intercellular junction resistances are sufficiently small.

It should be noted that the existence of the intercellular resistive junctions between cells is manifested, in our model, by the high values of  $R$ , needed to fit the data. Thus, in some respect, these junctions are taken into account in our model of the horizontal cell layer. Agreement between the theoretical predictions based on this laminar structure model and experimental results further justify our assumed model of the S space.

The receptive field diameter of the teleost retinal ganglion cells extends up to 5 mm (Daw, 1968). In addition, evidence is available showing that the area of integration in the horizontal cell layers can be as large as 10 mm in diameter (Norton et al., 1968). Naka and Nye (1971) have concluded that in the catfish, the signal responsible for the creation of the large, integrating ganglion cell receptive field has to be transmitted laterally through the S space. If in the S space, however, the potential decays exponentially from the site of excitation, a signal produced at larger distances (such as 2.5 mm away) can hardly show its effect at the receptive field center given the space constant which is needed to fit the initial decay portion of the data curve. As seen in Fig. 2 A the deviation from the exponential decay is more pronounced for large diameter spots (cf. Naka, 1972, Fig. 2) and at large distances.

Thus, the mathematical formulation presented in this paper has advantages over that of Naka and Rushton (1967) in that: (a) it makes no a priori assumptions on the potential decay mode, (b) it can fit better the potential decay data obtained in the

catfish, and (c) it can explain signal integration over large areas which has been observed experimentally (cf. Norton et al., 1968), and which cannot be obtained by the solution proposed for the tench data.

In addition, the analysis made in this study has shown the following:

(a) The potential decay data for small spot (0.5 mm in diameter) can be predicted equally well by our mathematical formulation whether we adopt a constant voltage or a constant current source at the excitation site. The data on the area integration of the potential for spots of various radii, however, could only be fitted by the constant current-disc model.

(b) In the external (and most likely in the intermediate) horizontal cells, an increase in the membrane resistance could account for the increase in the integration area with a brighter light. This agrees well with the observation that in some horizontal cells resistance increases in proportion to the amplitude of the catfish horizontal cell responses (Adomian, Matsumoto and Naka, unpublished results). According to Toyoda et al. (1969) a similar increase in the resistance could be seen in some *Necturus* horizontal cells and such increase was in proportion with the hyperpolarizing potential change.

(c) In the internal horizontal cells a simple increase in the resistance could not account for the data in which the spot diameter was increased. A reasonable fit could be obtained, however, by assuming an increase in the magnitude of the presumed synaptic current with increasing intensity.

Results *a*, *b*, and *c* make certain implications on the synaptic mechanisms of excitation of the internal and external horizontal cells assuming that our S space model is valid. Thus far it had not been possible to detect any functional difference between the external (and also intermediate) horizontal cells and internal horizontal cells, although morphologically they differ markedly from each other (Naka and Nye, 1971).

It has been reported that the tench S potentials arising from a light spot and from a light annulus can sum linearly so that the potential obtained by a simultaneous presentation of a spot and an annulus is the algebraic sum of the responses evoked by each stimulus alone (Naka and Rushton, 1967). The results of the present study show that this simple relationship is true in the case of the internal horizontal cells while in the case of the external and intermediate horizontal cells the response obtained by a simultaneous presentation of a spot and annulus could be *larger* than the algebraic sum of the two responses evoked when each stimulus is presented alone. (The relationship is also true in the latter case provided that the intensity of the annular flash is sufficiently low, so as not to change significantly the value of  $\alpha$ .) Indeed, it was shown experimentally in the catfish, that the responses due to a spot and an annulus sum almost linearly in the case of the internal horizontal cells, while in the case of the external (and intermediate) horizontal cells the potential evoked

by the simultaneous presentation of a spot and annulus is larger than the sum of the two individual responses (Adomian, Matsumoto and Naka, unpublished results).

Naka and Rushton (1966) have described in tench an increase in the amplitude of the S potential when two color stimuli were delivered simultaneously. This is what the present analysis predicts for the external (and intermediate) horizontal cells if the tench horizontal cells received independent inputs from two classes of cones and each input gave rise to an increase in the membrane resistance.

In a recent paper Negishi (1971) has described in the fish L type potential, an enhancement of the response to a spot in presence of an annular background stimulus. Based on the findings he concluded that the fish L units (horizontal cells) do not behave as "simple excitation pools" as suggested by Naka and Rushton (1966, 1967). We have shown in this paper, however, that it is possible to explain such an enhancement of the potential in terms of a rather simple model.

Thus, while morphologically the S space seems to be a very simple model of the horizontal cell layer, functionally it appears, so far, to be quite accurate.

#### APPENDIX

To solve the dual integral equations 14 and 15 we make some approximations in order to simplify them.

Consider,  $\mu_0 \gg \gamma$ , say,  $\mu_0 = 10\gamma$ . Then equation 14 can be written

$$\int_0^{\mu_0} \mu \left( 1 - \frac{\mu - \gamma}{\mu + \gamma} e^{-2\mu\delta} \right) \psi_1(\mu) J_0(\mu r) d\mu + \int_{\mu_0}^{\infty} \mu (1 - e^{-2\mu\delta}) \psi_1(\mu) J_0(\mu r) d\mu = KJ\rho_0 \frac{1}{\sqrt{1-r^2}}, \quad 0 \leq r < 1. \quad (\text{A } 1)$$

On the left-hand side, we add and subtract the term

$$\int_0^{\mu_0} \mu (1 - e^{-2\mu\delta}) \psi_1(\mu) J_0(\mu r) d\mu,$$

and then collect terms to get

$$\int_0^{\mu_0} \frac{2\gamma\mu}{\mu + \gamma} e^{-2\mu\delta} \cdot \psi_1(\mu) J_0(\mu r) d\mu + \int_0^{\infty} \mu (1 - e^{-2\mu\delta}) \psi_1(\mu) J_0(\mu r) d\mu = KJ\rho_0 \frac{1}{\sqrt{1-r^2}}, \quad 0 \leq r < 1: \quad (\text{A } 2)$$

Now we add and subtract (on the left-hand side) the term

$$\int_0^{\infty} \gamma (1 - e^{-2\mu\delta}) \psi_1(\mu) J_0(\mu r) d\mu,$$

to obtain

$$\int_0^{\mu_0} \frac{2\gamma\mu}{\mu + \gamma} e^{-2\mu\delta} \psi_1(\mu) J_0(\mu r) d\mu - \int_0^{\infty} \gamma(1 - e^{-2\mu\delta}) \psi_1(\mu) J_0(\mu r) d\mu + \int_0^{\infty} (\mu + \gamma)(1 - e^{-2\mu\delta}) \psi_1(\mu) J_0(\mu r) d\mu = KJ\rho_0 \frac{1}{\sqrt{1-r^2}}, \quad 0 \leq r < 1. \quad (\text{A } 3)$$

We assume that the sum of the first two terms is small compared with the third term (or the total on the right-hand side, which is  $1/\sqrt{1-r^2}$ ). We will justify this assumption shortly.

Neglecting the first two terms of equation A 3 and recalling the equation,

$$\int_0^{\infty} \sin \xi J_0(\rho\xi) d\xi = \begin{cases} \frac{1}{\sqrt{1-\rho^2}}, & 0 \leq \rho < 1 \\ 0, & 1 \leq \rho < \infty, \end{cases} \quad (\text{A } 4)$$

we find that the resulting dual integral equations A 3 and 15 have the solution,

$$\psi_1(\mu) = KJ\rho_0 \frac{\sin \mu}{(\mu + \gamma)(1 - e^{-2\mu\delta})}. \quad (\text{A } 5)$$

Now, let us justify the assumption made above about the smallness of the sum of the first two terms of equation A 3. First let us consider intermediate values for the (catfish horizontal cell) parameters:  $\alpha = 5$  mm,  $\rho_0 = 0.3$  mm, and  $h = 0.05$  mm. These parameter values correspond to  $\gamma = 0.06$  and  $\delta = 0.17$ .

The first integral (let us call it  $I_1$ ) is

$$I_1 = \int_0^{\mu_0} \frac{2\gamma\mu}{(\mu + \gamma)} e^{-2\mu\delta} \frac{\sin \mu}{(\mu + \gamma)(1 - e^{-2\mu\delta})} J_0(\mu r) d\mu, \quad 0 \leq r < 1, \quad (\text{A } 6)$$

and, considering that  $\mu_0 = k\gamma$  ( $k = 10$ ) and  $0 \leq r < 1$ , we can get an estimate of integral  $I_1$  by expanding the sine, exponential, and Bessel functions in a series. Thus integral  $I_1$  is approximated by integral

$$\frac{\gamma}{4\delta} \int_0^{10\gamma} \frac{\mu(4 - \mu^2 r^2)}{(\mu + \gamma)^2} d\mu, \quad (\text{A } 7)$$

which, finally, gives the estimate,

$$I_1 = \left(\frac{\gamma}{\delta}\right) \cdot (1.4 - 9\delta^2 r^2). \quad (\text{A } 8)$$

The second integral (let us call it  $I_2$ ) is

$$I_2 = \int_0^{\infty} \gamma \frac{\sin \mu}{(\mu + \gamma)} J_0(\mu r) d\mu, \quad 0 \leq r < 1, \quad (\text{A } 9)$$



which we can write as

$$I_2 = \gamma \int_0^{\mu_0} \frac{\sin \mu}{\mu + \gamma} J_0(\mu r) d\mu + \gamma \int_{\mu_0}^{\infty} \frac{\sin \mu}{\mu} J_0(\mu r) d\mu, \quad 0 \leq r < 1, \quad (\text{A } 10)$$

or

$$I_2 = \gamma \int_0^{\mu_0} \left[ \frac{1}{\mu + \gamma} - \frac{1}{\mu} \right] \sin \mu J_0(\mu r) d\mu + \gamma \int_0^{\infty} \frac{\sin \mu}{\mu} J_0(\mu r) d\mu, \quad 0 \leq r < 1. \quad (\text{A } 11)$$

The second term of expression A 11 is equal to  $\gamma(\pi/2)$  (Gradshteyn and Ryzhik, 1965, p. 744).

Considering that  $\mu_0 = 10\gamma$  and  $0 \leq r < 1$  we get an estimate of the first term by expanding in series the sine and Bessel functions. Thus  $I_2$  is approximated by

$$\gamma \frac{\pi}{2} - \frac{\gamma^2}{4} \int_0^{10\gamma} \frac{4 - \mu^2 r^2}{\mu + \gamma} d\mu, \quad (\text{A } 12)$$

which, finally, gives the estimate,

$$I_2 = (1.5\gamma - 2.4\gamma^2) + 9\gamma^4 r^2. \quad (\text{A } 13)$$

Thus we obtain an estimate of the magnitude of the neglected terms (within the parameter range considered) from  $\epsilon = I_1 - I_2$ . It is easily checked that, indeed,  $\epsilon$  (of order  $10^{-1}$ ) is small compared with  $1/\sqrt{1-r^2}$  ( $0 \leq r < 1$ ).

The error is of the same order ( $10^{-1}$ ) for  $\delta$  as large as 1. If  $\delta$  is much larger than 1, the approximations used in evaluating integral  $I_1$  are not quite valid, but the error is still of the same order ( $\sim 10^{-1}$ ). This can be deduced by approximating  $I_1$  for large  $\delta$ . It will be noted that  $I_1$  has a smaller value.

In the case of the catfish horizontal cell, the maximum spot diameter of interest (where saturation occurs) is about 3 mm, and  $h$  is about 0.05 mm.

Clearly, for large  $\delta$ , that is, small spot radii, the error is small. For very small  $\delta$ , however, the error can become large. This corresponds to the case of very large diameter spots. Numerical approximations done by computer have shown that quantity  $(I_1 - I_2)$  is small compared with  $\{1/\sqrt{1-r^2}, 0 < r < 1\}$ , within the horizontal cell parameter range, for spot diameters in the range of 0-5 mm. For larger (than 5 mm) diameters, the solution will have some error which may be significant.

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