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# Interplay Between the Dendritic Trees of Alpha and Beta Ganglion Cells During the Development of the Cat Retina

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ABSTRACT

We have analyzed the effect of a small lesion to the retina of a two-day-old kitten and observed that after degeneration of ganglion cells whose axons were severed, a restricted region of the retina remained depleted of cells. Cells located near the borders of the depleted zone showed an abnormal elongation of dendrites towards the bare area. By means of a computer-aided system, we analyzed the whole population of cells at the two borders, and in agreement with previous data found that the effect was most prominent at the border and progressively decreased to eventually disappear at a distance of approximately 500 μm. The distance from the border, however, is not the only factor to influence the degree of asymmetry; with comparable distances, the vicinity of an α-cell reduces the projection of the β-cell dendrites toward the empty area. We suggest that the organization of the adult retinal pattern is also influenced by interactions occurring between dendrites of different classes of ganglion cells. 

**Key words: HRP, image analysis**

In the adult retina of the cat, the dendritic fields of a well-defined physiological subclass of ganglion cells (i.e., α-on or α-off) forms a network that uniformly covers the retina. Cell bodies of each population are organized in a regular mosaic with little overlap among territories of dendritic arborizations. Cells of each subclass relate only to their physiologically homologous neighbors and provide a homogeneous coverage of the retina (for review, see Wässle and Boycott, 1991).

It has been suggested that this pattern results from specific interactions occurring during development (Perry and Linden, 1982; Eysel et al., 1985). This conclusion is supported by an experiment in which a small lesion was produced on a restricted area of the retina. The degeneration of ganglion cells whose axons were severed by the lesion leaves a small region in the retina depleted of cells. Neurons located near the borders of the depleted zone show an abnormal elongation of the dendrites projecting towards the bare area (Perry and Linden, 1982; Eysel et al., 1985; Perry and Maffei, 1988). This phenomenon occurs only if the lesion is performed within a limited period after birth (15 days in rat and 60 days in cat).

The conclusion is that the neuron's capability of extending its dendritic arborization is controlled by the presence of neighboring dendrites. Since these interactions are likely to direct the organization of the retinal mosaic, it follows that they must be absent between cells belonging to different classes (Wässle and Boycott, 1991).

However, other evidence obtained on the retinogeniculate projections during development shows that the terminal axon arbors of α- and β-cells do interact to reach the adult configuration (Sur et al., 1984; Sretavan and Shatz, 1986; Ramoa et al., 1988; Sur, 1988). Specifically, the mature extension of β-axon terminals is shaped by α-axons which develop later from the same eye. If the development of the terminal arbors requires reciprocal interactions between α- and β-cells, it seems reasonable to suppose that similar interactions shape the dendritic arborization also at the retinal level.

In the present study, we tested this hypothesis by looking at the effect of a small retinal lesion performed at birth. The analysis of the ganglion cell population at the border of the depleted zone shows that dendrites of different cell classes interact during development. Some of these results have already been reported in abstract form (Deplano et al., 1991).

## METHODS

A small retinal lesion was made in the left eye of a two-day-old kitten under Ketalar (Parke-Davis, Milan) anesthesia (45 mg/kg) by means of a needle (0.45 mm

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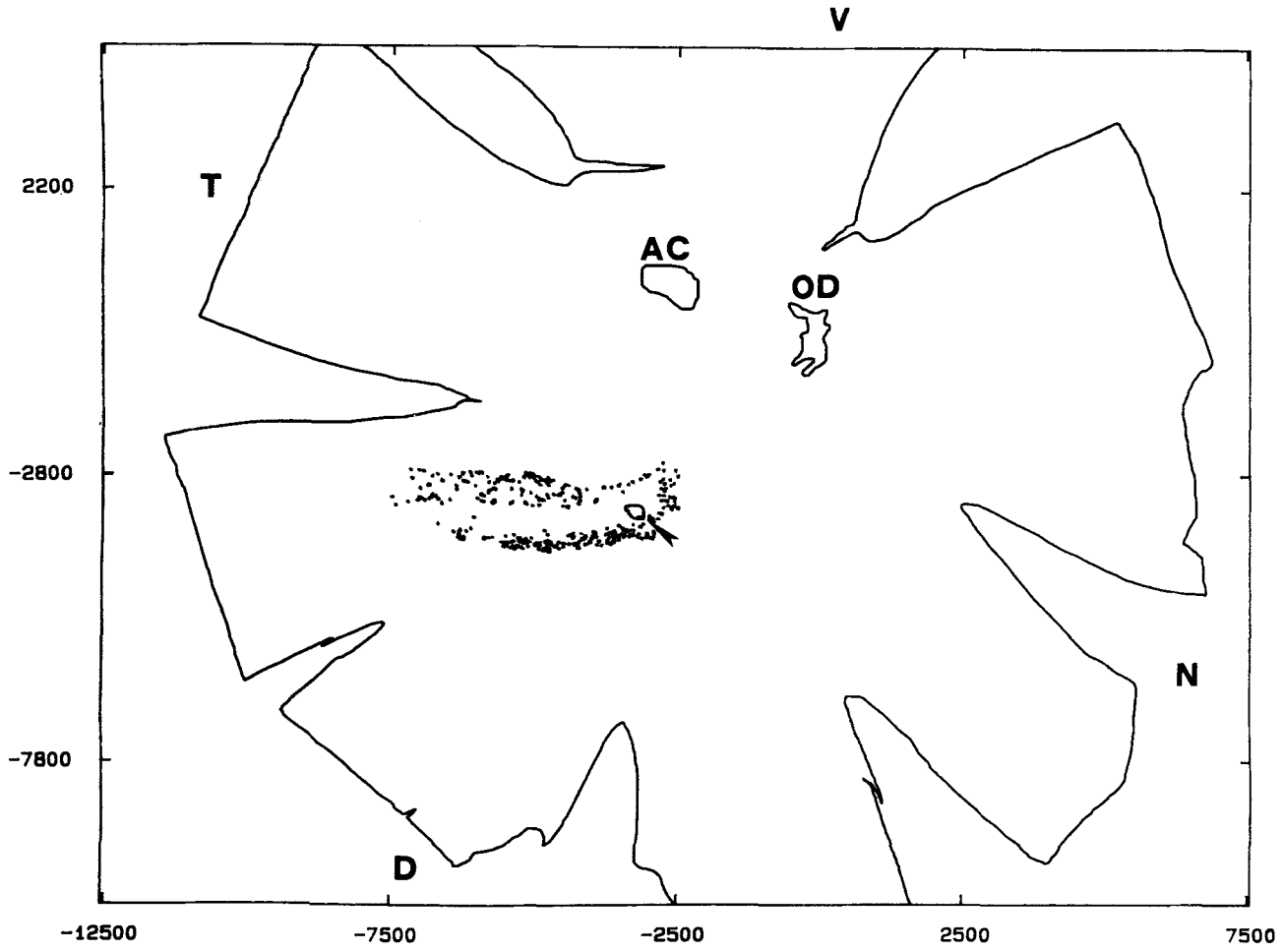


Fig. 1. Computer reconstruction of the whole mounted retina (left eye). Dots in the figure are the computer drawings of each neuron at the border of the lesioned area. The arrow indicates the location of the lesion. AC, area centralis; OD, optic disk; N, nasal; T, temporal; V, ventral; D, dorsal.

diameter). At eight months of age, the animal was anesthetized with an injection of Ketalar (30 mg/kg) and additional doses of pentobarbital (0.2 ml/kg/hr). The position of the lateral geniculate nucleus (LGN) was determined stereotactically and by recording visual evoked responses through a glass micropipette. The electrode was removed and a Hamilton syringe (10 μl filled with 30% horseradish peroxidase [HRP; Sigma, St. Louis, MO] in 2% dimethylsulfoxide [DMSO; Sigma] and 0,5% poly-L-ornithine) was positioned at the same depth. A total of 27 injections (3 μl each) were performed in nine positions of both LGN (three injections at each position one millimeter apart in depths). After two days, a lethal dose of Ketalar was injected; the cat was perfused, the retinae were removed, processed for HRP following the protocol developed by Hanker et al. (1977) and modified by Perry and Linden (1982), and whole mounted. The analysis of the whole population of cells at the two borders was conducted by means of a computer-aided system. The camera lucida drawing of each neuron's dendritic arborizations was digitized by a graphic tablet and combined with the position of the retinal landmarks obtained directly by the scanning stage of the microscope (for further details, see Ratto and Usai, 1991). This procedure enabled us to maintain the correct spatial relationship among the reconstructed neurons (Fig.1).

## RESULTS

We have analyzed the retina of an adult cat which was lesioned at two days of age. The extent and the location of the depleted zone is shown in Figure 1. The whole population of ganglion cells at the two borders of the depleted zone has been outlined by means of a camera lucida and subsequently these "small" profiles were redrawn in an x-y plotter, our software being capable of maintaining the correct spatial relationship among the profiles. Different color codings are used for different subclasses (Fig. 2). Photomicrographs of two β-ganglion cells at the border of the depleted area are shown in Figure 3.

In agreement with previous data (Eysel et al., 1985), we found that the dendritic elongation was most prominent at the border and progressively decreased to eventually disappear while moving away to a distance of approximately 500 μm. The distance from the border, however, is not the only factor influencing the degree of asymmetry; two aspects in the cells distribution have to be taken into account: the

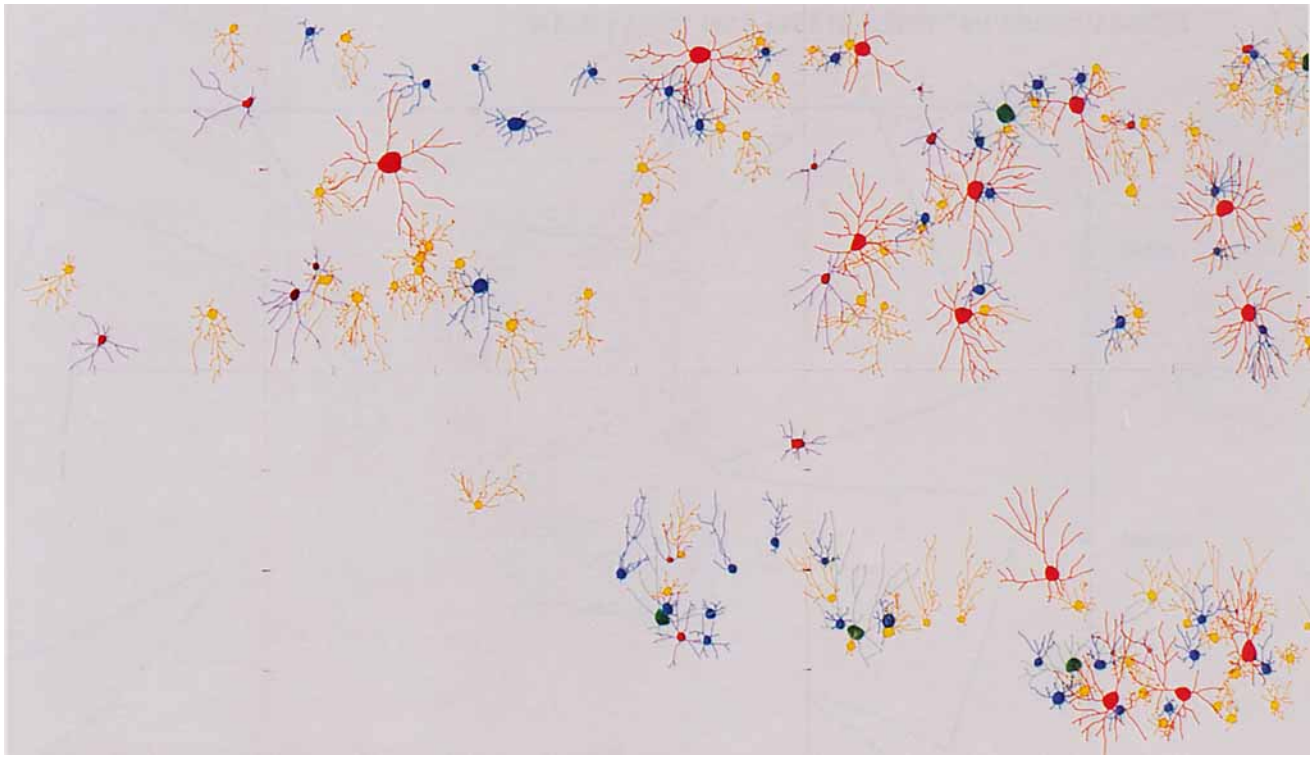

Fig. 2. Computer reconstruction of the whole population of ganglion cells at the two borders of the depleted zone. The asterisk indicates the location of the lesion. Different color codings are used for different subclasses. α on-cell, green; α off-cell, red; β on-cell, blue; β off-cell, orange; γ-cell, yellow.

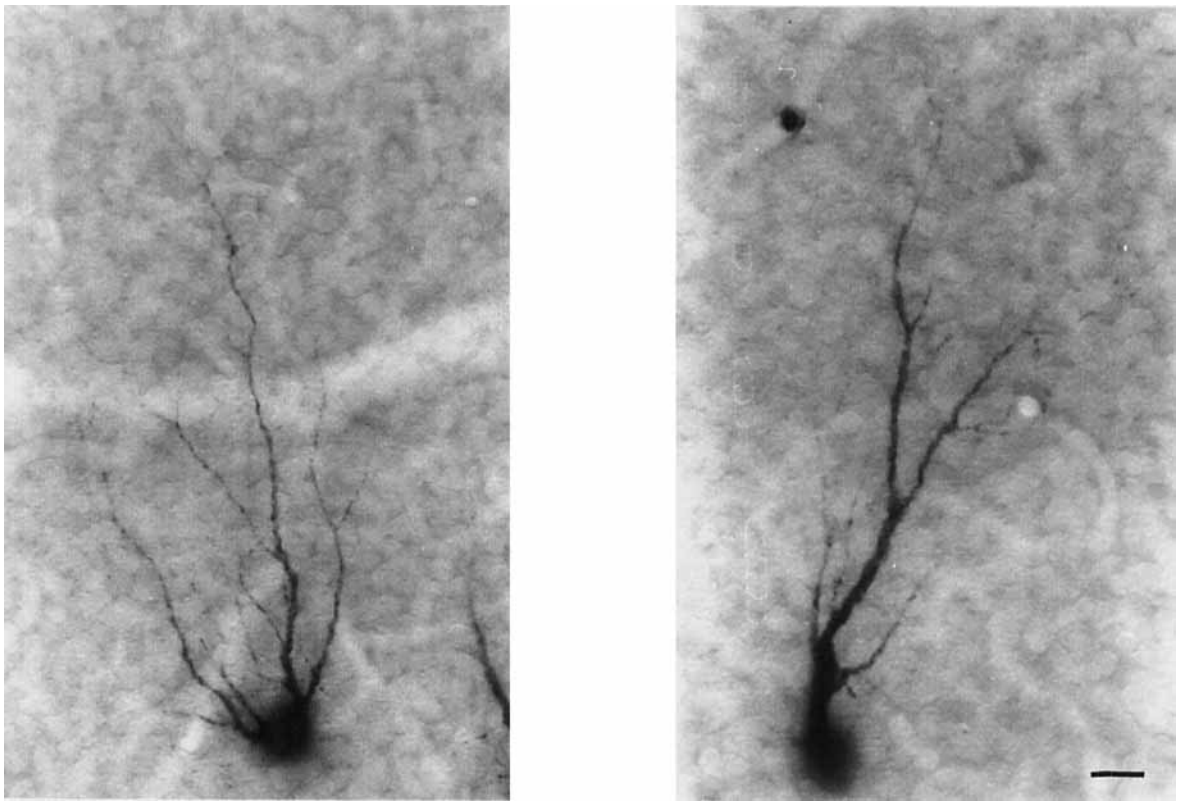

Fig. 3. Photomicrographs of two neurons at the border of the depleted area. Bar = 10 μm.

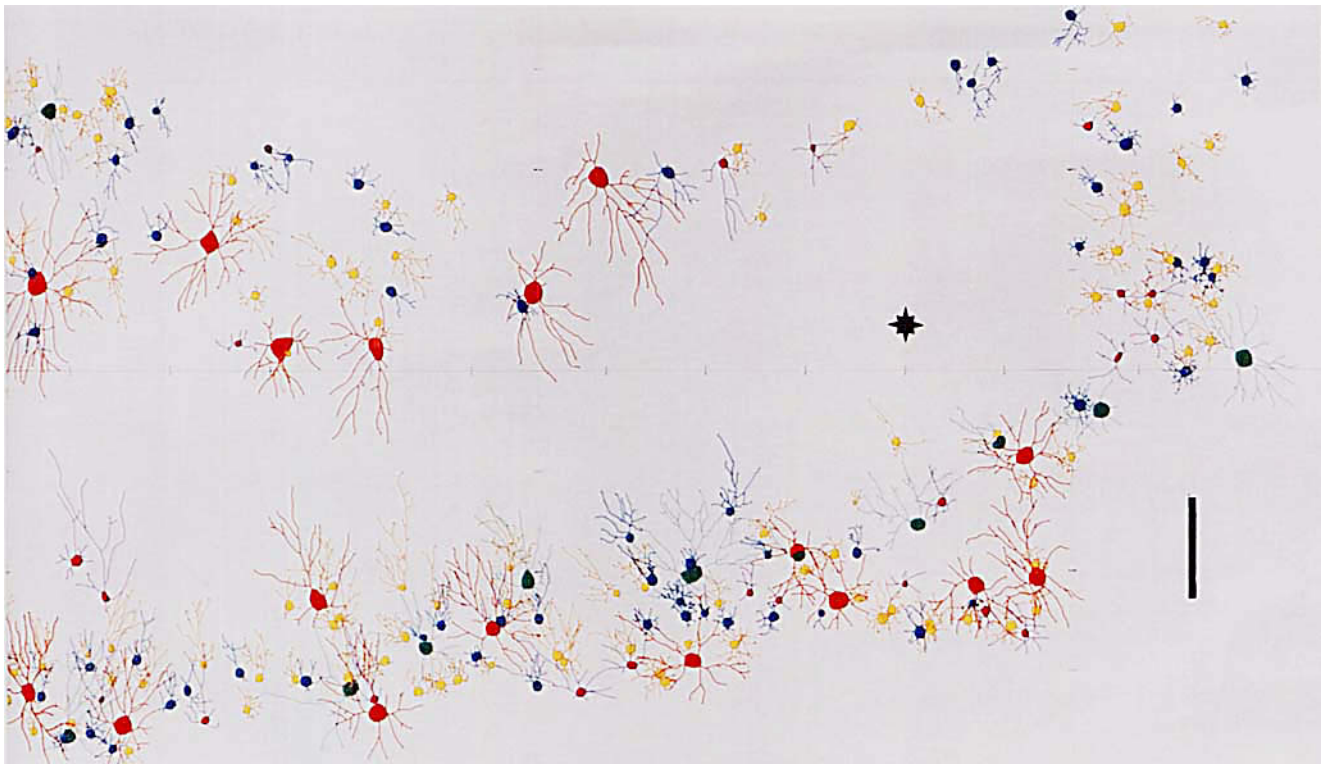

Figure 2. continued

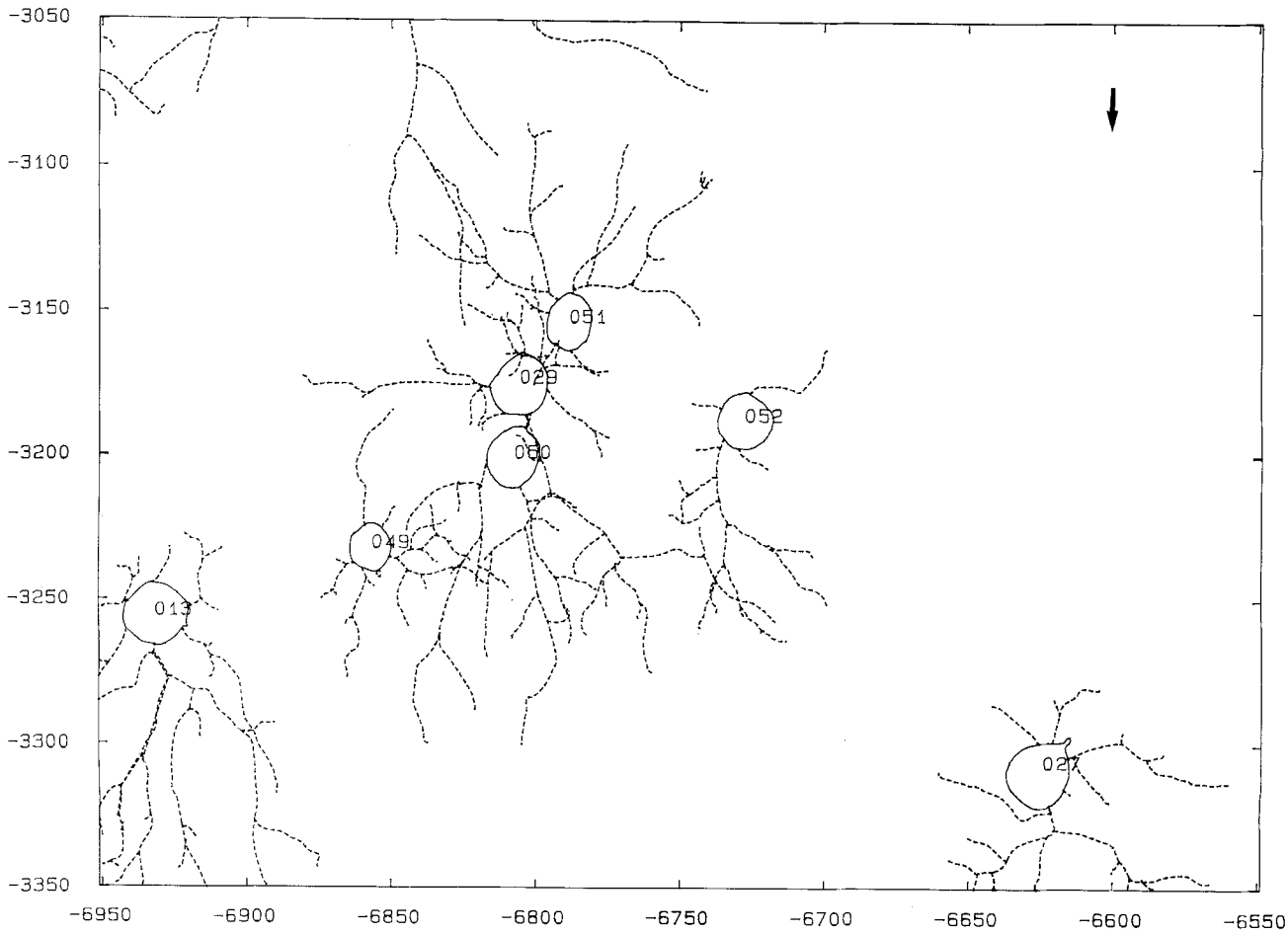


Fig. 4. Details of the reconstruction of Figure 2 centered on a population of off β-cells. It can be noted that dendrites of different neurons seem to interact to give rise to a "mosaic-like" organization. The arrow points to the border of the bare area.

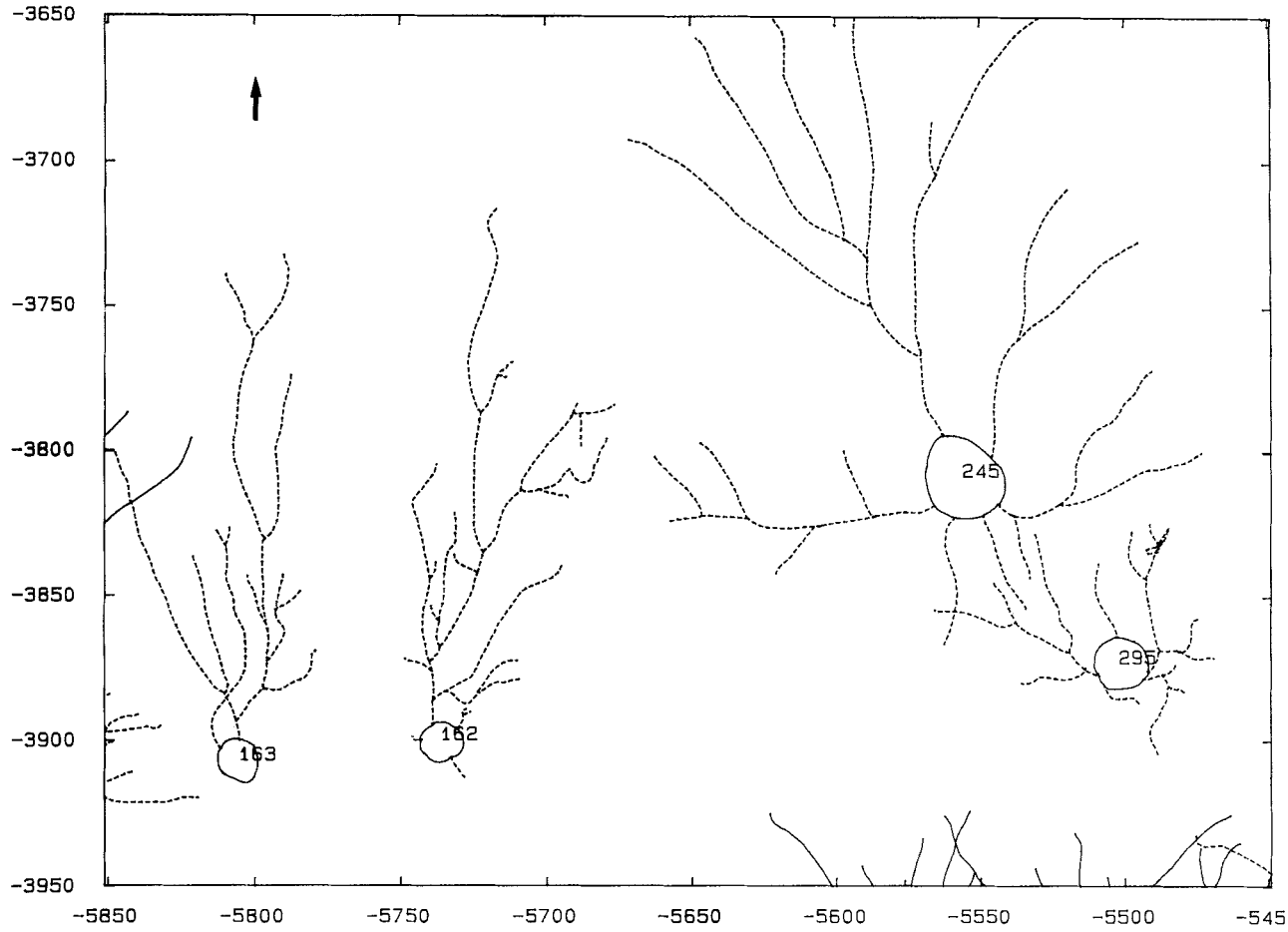


Fig. 5. Details of the reconstruction of Figure 2 centered on the α-cell 245. It can be noted that the distance from the bare area is not the only factor which affects the degree of asymmetry of the β-cell population. The relative position with respect to an α-cell is an important factor. Compare the β-off cell 295, beyond the off α-cell 245, with the two off β-cells (163, 162). The arrow points to the border of the bare area.

vicinity of cells belonging to the same subclass, and the relative position of a β-cell with respect to an α-cell.

## Interactions between cells of the same subclass

It is well known that cells of the same subclass are organized in a regular mosaic, which is supposed to develop early in life following specific interactions. Evidence of such an interaction taking place during development is illustrated in Figure 4, where the details of four β-off ganglion cells near the border are shown. It is evident that the dendritic territories of these cells are influenced by the presence of a depleted zone, but the relative position of a homologous cell also plays a significant role as it can be judged by the almost absent overlap of the dendritic fields.

## Interactions between α- and β-cells

The analysis of ganglion cells at the border of the bare area has shown that the distance from the border is only one of the factors in determining the degree of asymmetry of the dendritic field. At comparable distances, the vicinity of an α-cell reduces the projection of the β-cell dendrites toward the empty area. Figure 5 shows a computer reconstruction of ganglion cells located near the border. Note that the dendrites of the β-cell located close to the α-cell appear reduced in length when compared with dendrites of other β-cells located farther apart from α-cells. A more complicated pattern is illustrated in Figure 6 where three α-cells and several β-cells are drawn. Compare cell 170 with cells 62, 60, 131, etc. It is interesting to note that the interaction takes place regardless of the sublamina to which the α- and β-cells belong (solid line on-cell, broken line off-cell).

In order to evaluate the degree of influence of α-cells on the β-cell population, we determined the extent of the dendritic arborization for each α-cell by connecting the extreme points of all dendrites. We considered all the surrounding β-cells whose dendritic arborization extended inside the area delimited by the polygon. The procedure we used is illustrated in Figure 7 for one α-cell. We located the center of the cell body at the center of a polar plane where the 90° axis is perpendicular to the border of the depleted zone. For each surrounding β-cell we measured the distance (in microns) of the body from the center, the angle (in degrees), and the ratio between the major axis and the axis crossing perpendicularly at the body center. All collected data were plotted on two diagrams in polar coordinates; first and second row refer to the location of the α-cell with

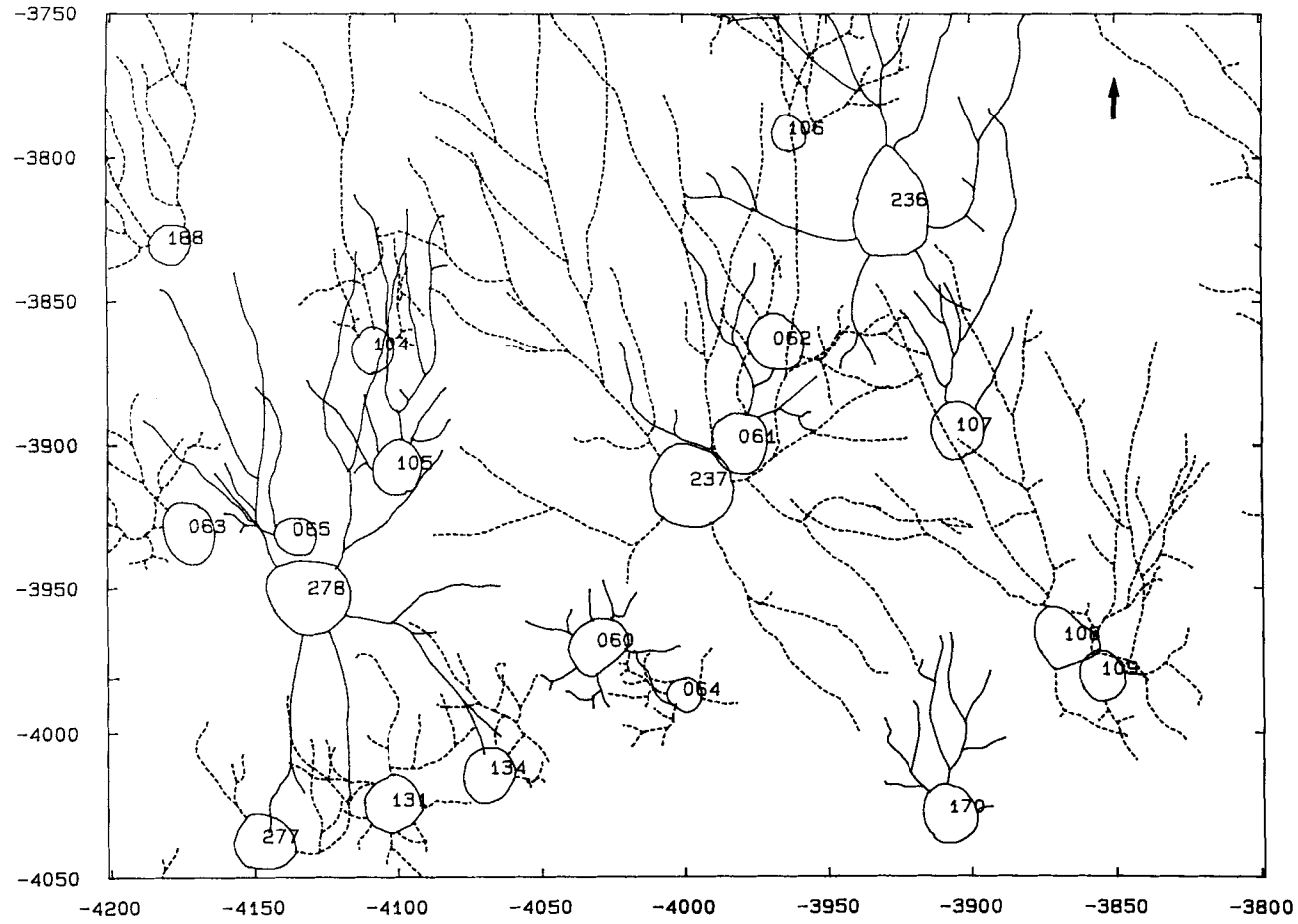


Fig. 6. Details of the reconstruction of Figure 2 centered on three α-cells (278, 237, 236) and β-cells population surrounding them. Compare cell 170 with cells 62, 60, 131, etc. The arrow points to the border of the bare area (continuous line, on-cells; broken line, off-cells).

respect to the border. We superimposed data obtained separately for each α-cell, the center of the plot corresponding to the center of the bodies of the α family (20 cells for the first row and 17 cells for the second row). We divided the β-cell population (79 cells in the first row and 62 cells in the second row) into three classes according to the degree of asymmetry: class 1 (filled circles in polar plot of Fig. 8 and Table 1) with a ratio between 1 and 1,3; class 2 (crosses) with a ratio between 1,3 and 3; and class 3 (squares) with a ratio more than 3. The three classes are distributed differently with respect to the α-cell bodies; cells of group 1 (the most symmetric) are more numerous behind the body, whereas the group 3 (the most asymmetric) are more frequent in front of it. It is interesting to note that this is true for both plots, without respect to the absolute distance from the border (Fig. 8). The spatial distributions of asymmetric and symmetric cells are statistically different with a degree of confidence of $P > 0.98$ (first row) and $P > 0.96$ (second row) at t-test.

To determine whether the degree of asymmetry is mainly due to an abnormal elongation of a few dendrites, we measured the angle formed by the primary dendrites of each cell with the axis crossing the border of the depleted zone at 90°. All the data are pooled together for the two classes (symmetric: ratio $< 1.3$ and asymmetric: ratio $> 3$) in the polar plot of Figure 9. It is seen that while asymmetric cells possess primary dendrites mainly oriented towards the lesion, the dendrites of symmetric neurones originate instead at all angles. It is important to note that this result is not influenced by incomplete filling of the distal dendrites.

## DISCUSSION

The main finding reported here is that the dendritic reorganization taking place at the border of a depleted zone is a function of both distance from the border and relative position with respect to cells of either the same or different subclass. Specifically, we showed that the elongation of a β-cell dendritic arborization toward the bare area can be highly influenced by the presence of an interposed α-cell.

It is well known that at the time we made the retinal lesion (P2), the ganglion cell population is still in a developing stage (Eysel et al., 1985). Spines at birth are present on both α- and β-cells at all retinal eccentricities; they begin to disappear starting from P5 and the phenomenon is completed at around P21 (Ramoa et al., 1988). The number of dendritic processes is largely reduced by the end of the first postnatal month, but at that time the final extension of the dendritic arborization and the soma size are not yet reached. Moreover, ganglion cells in the peripheral retina *continue*

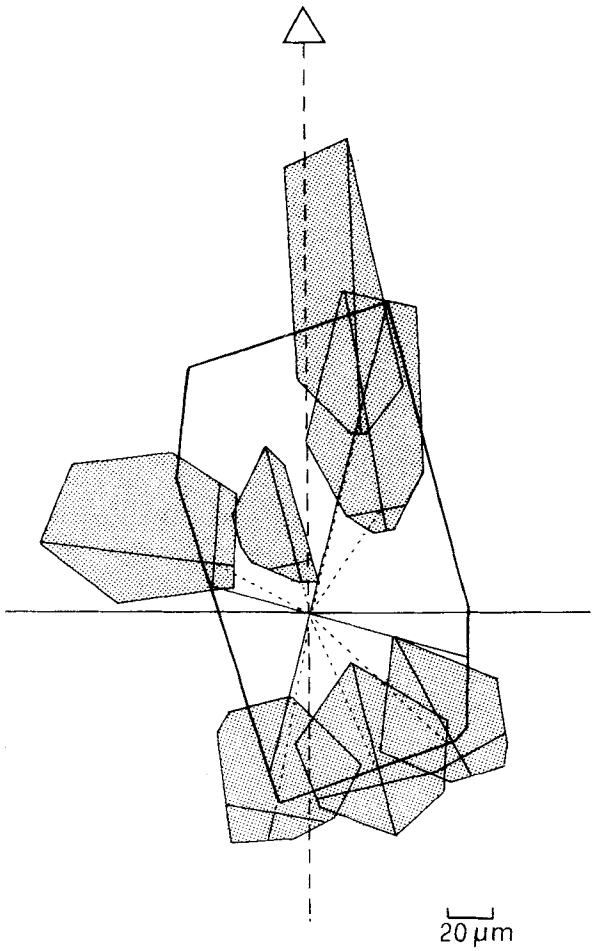


Fig. 7. Schematic explanation of the analysis of cell 278 (α on). The arrow indicates the position of the depleted area. See text for details.

TABLE 1. Mean Distance from the Body Center of the α-Cell Family of the Three Classes of the β-Cell Population[1]

| | First Row | | |
|---|---|---|---|
| | Mean (µm) | n of cells | SEM (µm) |
| •(1-1.3) | −19.6 | 12 | 10.4 |
| X(1.3-3) | 0.4 | 44 | 9 |
| □(>3) | 29.4 | 23 | 27.2 |
| | **Second Row** | | |
| | Mean (µm) | n of cells | SEM (µm) |
| •(1-1.3) | −21.4 | 16 | 12 |
| X(1.3-3) | 10.8 | 37 | 10.8 |
| □(>3) | 29.6 | 9 | 23 |

[1]Symbols as in Figure 8 (details in the text).

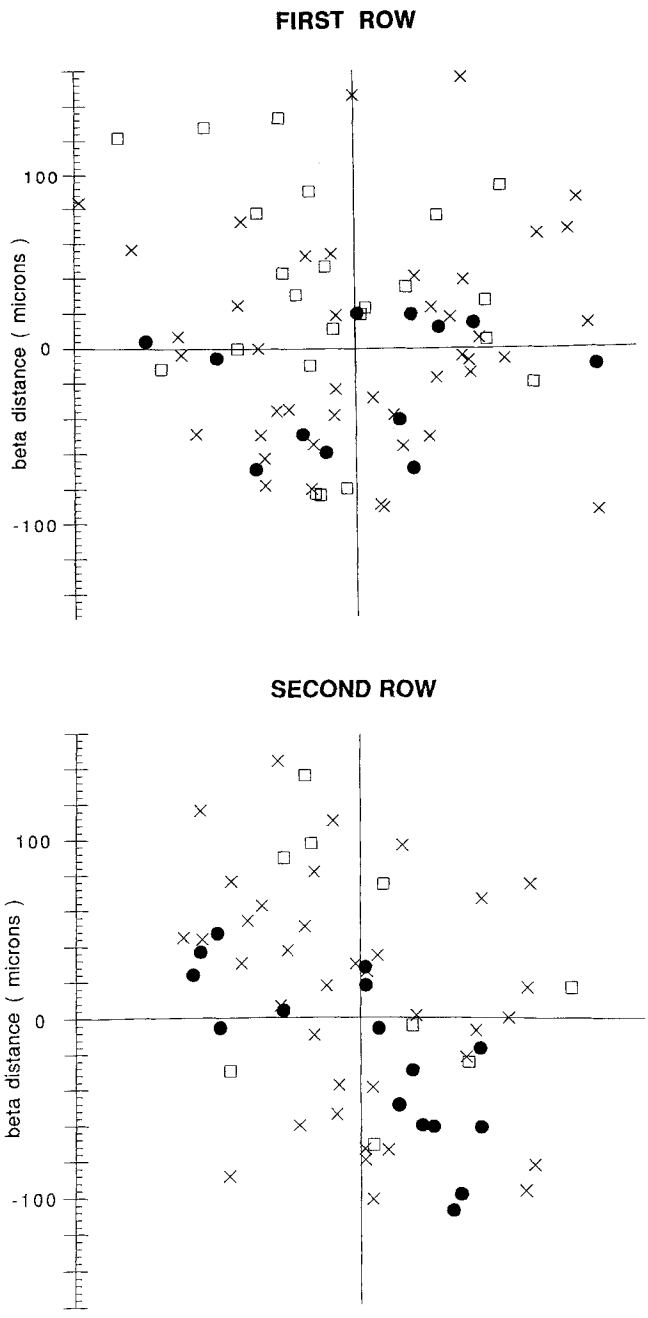


Fig. 8. Polar plots of β-cells distribution inside α-cells dendritic arborization.

their development while the area centralis is already mature (Maslim and Stone, 1986; Dann et al., 1988). All these remodelings suggest the conclusion that the synaptic contacts with bipolar and amacrine cells can be modified during the first postnatal weeks.

Several hypotheses have been advanced on the mechanism responsible for the elongation of cell dendrites in lesioned retinae (Perry and Linden, 1982; Eysel et al., 1985; Perry and Maffei, 1988). It has been proposed, for instance, that dendrites compete either for synaptic space or chemical factors. In both cases, however, the interplay is likely to occur among cells of the same subclass. It is generally assumed that these interactions lead to the adult organization of retinal coverage through a self-regulatory mechanism. Present results show that an interaction also occurs among neurons of different subclasses. This is suggested by the degree of asymmetry, which is related not only to the absolute distance from the lesion, but also to the relative position with respect to other neurons of either the same or different class. It is interesting to note that the interposi-

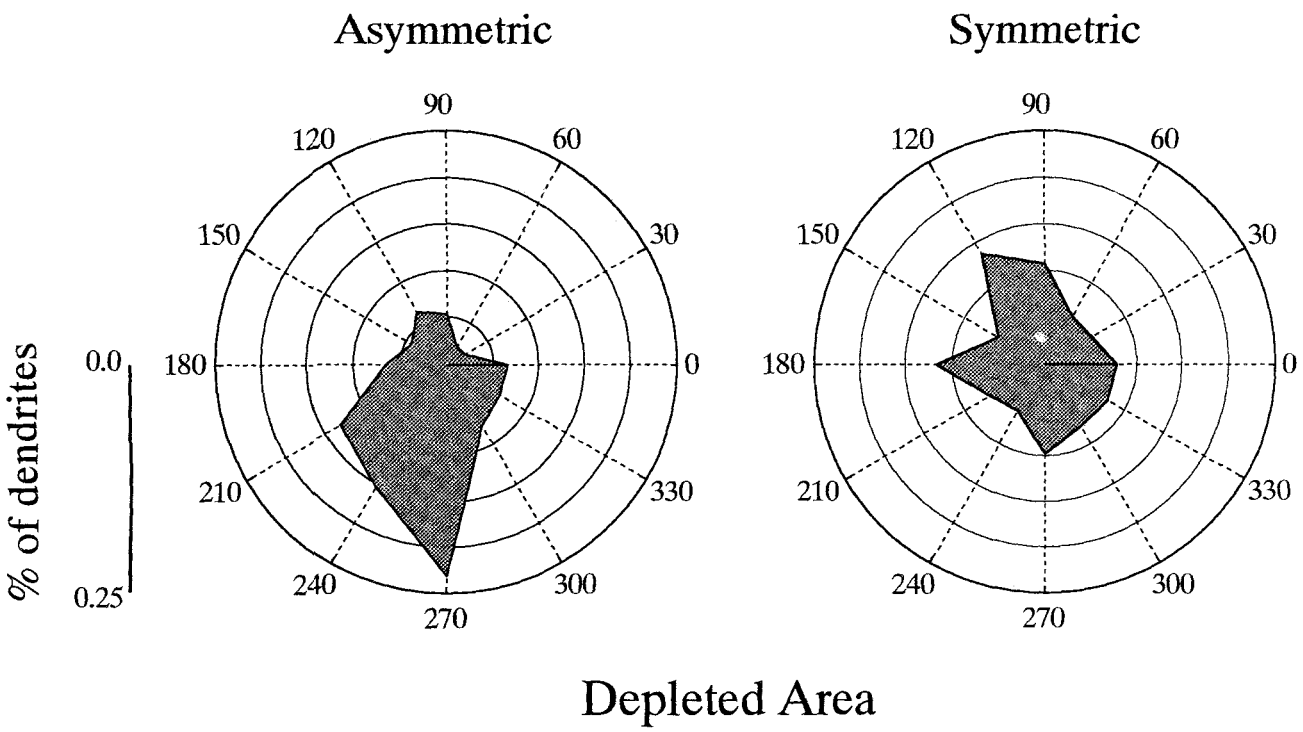


Fig. 9. Polar plots of the origin of primary dendrites in asymmetric and symmetric β-cells.

tion of an α-cell between the lesion and a β-cell is not a condition sufficient to reduce the degree of asymmetry; it is also necessary that the dendritic arborizations of the two cells are intermingled. This observation supports the hypothesis that dendrites of α- and β-cells interact during the first stage of development before reaching the adult pattern of organization. Similar interactions take place in the LGN where the axonal arborizations of β-cells are pruned by the later development of the α-cell axons (for review, see Ramoa et al., 1988).

We have also shown that dendritic fields terminating in both inner plexiform layer (IPL) sublaminae can be influenced by the presence of an α-cell. The possibility that neurons belonging to different sublaminae do interact is compatible with the data of Dann et al. (1988), who showed that it is possible to find α-cells with a bilaminar stratification up to 15 days after birth.

In agreement with our results, Ault et al. (1993) have reported data showing that non-class specific interactions take place in the cat retina. They provided evidence that the development of α-cell soma size is influenced by the surrounding β-cell population.

An interesting question is the functional relevance of the interaction between α- and β-cells during development. We do not have a clear answer to that, but we can make some suggestions on the basis of available anatomical data. It is known, for instance, that there is a temporal concomitance between remodeling of dendritic arborizations and synaptogenesis at the IPL level (Maslim and Stone, 1986). At all eccentricities, β-cells are born earlier than α-cells, and therefore are likely to be the first to form synapses, thus causing the α-cells to compete for synaptic space. At this stage, however, α-cells must have some advantage in forming synapses; this is suggested by our observations which show that a β-cell located near an α-cell does not exclusively orient its dendrites towards the bare area. In our sample we have neurons that do not orient properly and become asymmetric in the opposite direction. Another interesting way in which neurons belonging to different classes communicate may be through side branches and collaterals of ganglion cell axons, which are known to be present up to 15 days (axon collaterals) and 31 days (side branches) after birth (Ramoa et al., 1988). A remote control of the synaptic activity of the presynaptic elements (bipolar and amacrine cells) may be exerted through these lines.

## ACKNOWLEDGMENTS

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