THE JOURNAL OF COMPARATIVE NEUROLOGY 405:262–270 (1999)

Electrical Activity Regulates Dendritic Reorganization in Ganglion Cells After Neonatal Retinal Lesion in the Cat

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ABSTRACT

During the first month of postnatal life, the dendritic arborizations of cat retinal ganglion cells continue to develop and undergo a substantial remodeling. Mechanical and pharmacological interferences with the normal development induce, during this period of time, substantial modifications in ganglion cell morphology. Specifically, the degeneration of those neurons whose axons were severed by a neonatal retinal lesion leads to a zone depleted of ganglion cells. Neurons at the border of the depleted area develop an abnormal elongation of the dendritic trees toward the empty space. In the present paper, we report data showing that this dendritic reorganization can be prevented by blocking the electrical activity with repeated tetrodotoxin injections into the eye during the whole critical period. Our analysis was performed on neurons filled with horseradish peroxidase. J. Comp. Neurol. 405:262–270, 1999.

Indexing terms: tetrodotoxin; retina; dendritic arborization; development

The development of ganglion cell dendritic trees has been extensively studied in the cat retina (for a review, see Wingate, 1996). Each arbor undergoes phases of growth and remodeling, so that in adulthood, the dendritic arbor of a single morphological subclass (α on, α off, β on, β off) is organized in a very precise mosaic that shows little overlap of different dendritic arborizations (Wässle and Boycott, 1991). The dendrites also stratify in different sublaminae of the inner plexiform layer (IPL), ON cells in the inner portion of the IPL and OFF cells in the outer portion (Famiglietti and Kolb, 1976; Nelson et al., 1978).

This adult pattern of organization is reached during the first month of postnatal life. During this period, pharmacological and mechanical manipulations of the retina induce a remarkable rearrangement in the dendritic morphology. Inhibiting glutamate release from the afferent bipolar cells blocks the segregation of β-cell dendrites (Bodnarenko and Chalupa, 1993; Bodnarenko et al., 1995). Increasing ganglion cell density by prenatal unilateral enucleation (Kirby and Chalupa, 1986) results in smaller dendritic arbors at a given retinal eccentricity. Similarly, decreasing ganglion cell density by pathway lesions results in larger dendritic arbors (Leventhal et al., 1988; Ault et al., 1993). After a small retinal lesion producing the resection of axon bundles, a ganglion cell-free area can be observed, at the border of which is an abnormal growth of dendrites oriented toward this cell-depleted region (Perry and Linden, 1982; Eysel et al., 1985; Deplano et al., 1994b). These experiments provided evidence that interactions among developing dendrites play an essential role in the making of congruous mosaics. However, no attempt has ever been made to define the factors responsible for the dendritic reorganization itself.

A possible candidate in this process is electrical activity, which has been shown to play an essential role during the development of the nervous system either directly by contact inhibition or indirectly by regulating the release of some diffusible factors (Costantine-Paton et al., 1990).

Experiments in which the activity was blocked by tetrodotoxin (TTX) treatment during prenatal life have shown that the axonal segregation of retinal projections to the lateral geniculate nucleus (LGN) is abolished (Shatz and

Grant sponsor: National Research Council (Integrated Program University/CNR); Grant sponsor: MURST.

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Received 31 January 1997; Revised 7 October 1998; Accepted 8 October 1998

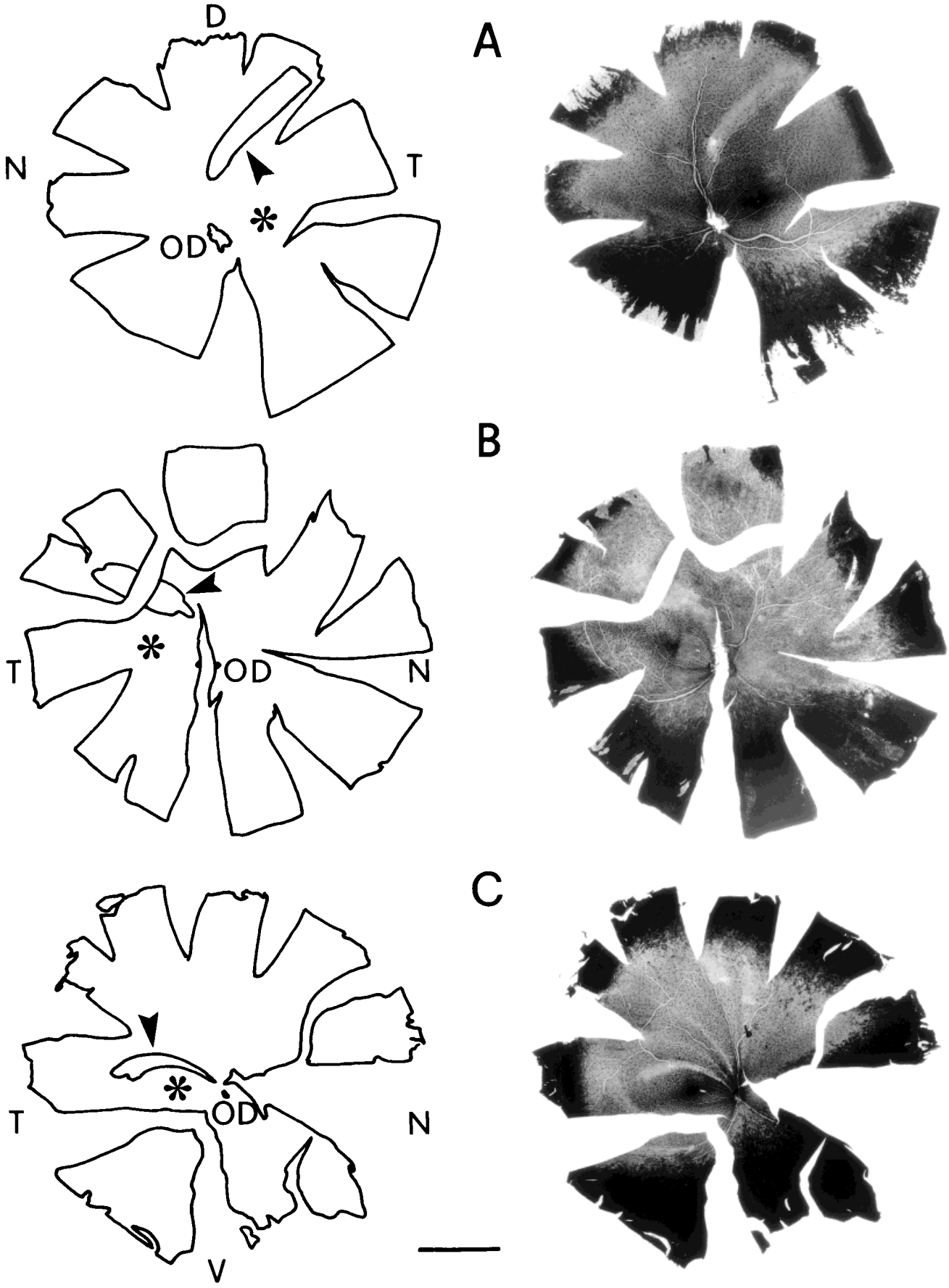

Fig. 1. Retinal wholemounts from one control (**A:** postnatal day 7; P7) and two tetrodotoxin-treated (**B:** P14, **C:** P7) retinae. On the left of the figure are respective camera lucida drawings with the indication of the orientation parameters. The arrowheads indicate the depleted area, and the asterisks the area centralis. Retinal ganglion cells were filled with horseradish peroxidase injected into the lateral geniculate nucleus. D, dorsal; N, nasal; T, temporal; V, ventral; OD, optic disc. Scale bar = 5 mm.

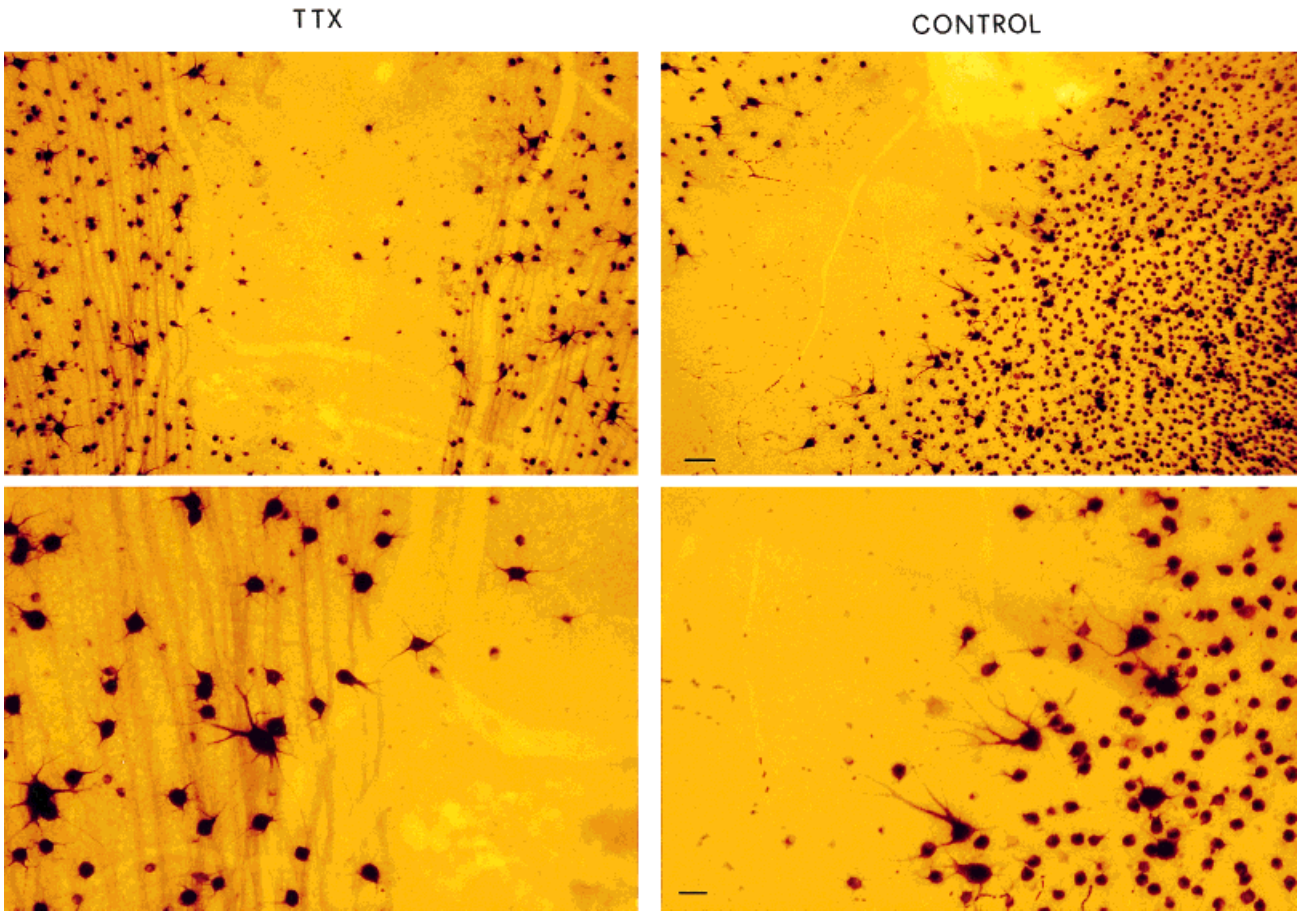

Fig. 2. Photomicrographs taken near the border of the bare area in tetrodotoxin (TTX)-treated (left) and control (right) retinae. Retinal ganglion cells were filled with horseradish peroxidase injected into the lateral geniculate nucleus. Scale bars = 100 μm in top panels, 20 μm in bottom panels.

Stryker, 1988; Sretavan et al., 1988). Similar results have been reported in primary visual cortex, where the axonal segregation in ocular dominance columns is prevented by TTX treatment during the critical period (Stryker and Harris, 1986). But surprisingly, the blockade of electrical activity in the developing retina has little effect on the remodeling of ganglion cell dendritic arborizations, which takes place during the first month of postnatal life (Wong et al., 1991).

The aim of the present study was to define the role of electrical activity in the reorganization of ganglion cell dendritic arborizations taking place at the border of a zone depleted of cells by means of a small lesion performed early postnatally.

To eliminate spike activity, we injected TTX into the eye throughout the whole critical period (Stryker and Harris, 1986). Our results show that blocking electrical activity prevents dendrites from elongating toward the bare area, with no major effect on the remaining ganglion cell population. Some of these results have been reported in abstract form (Deplano et al., 1994a).

MATERIALS AND METHODS

Eye lesion and TTX injections

The experiments were performed in 15 cats in accordance with Institutional Guidelines for Animal Care and the Policy on the Use of Animals in Neuroscience Research. Kittens were anesthetized with 4% halothane in oxygen during surgery. In 11 kittens, a small lesion was made to the retina (six kittens at postnatal day 7 [P7] and five kittens at P14) by means of a needle (0.45 mm in diameter) while blocking retinal activity with repeated intravitreal TTX injections over the whole critical period. Four kittens, used as controls, were damaged at P7 (three kittens) and P14 (one kitten) and treated with repeated saline injections. We followed the protocol of administration, concentration, and volume of TTX described by Stryker and Harris (1986). Briefly, continuous retinal blockade was produced by repeated injections of 1–10 μl of TTX (5×10^{-3} M). TTX injections were given every 3 days, with an initial dose of 1 μl, which increased by 5–10% with each injection. The photic reflex was checked routinely to confirm the block of the electrical activity along the optic nerve. The block of retinal activity in the injected eye was also confirmed by records of the light response from the lateral geniculate nucleus (LGN) in two 3-week-old kittens. The total number of injections was eight in kittens that started at P7 and six in kittens that started at P14. All injections were performed through the initial hole that produced the retinal lesion. The kittens were then allowed to reach adulthood.

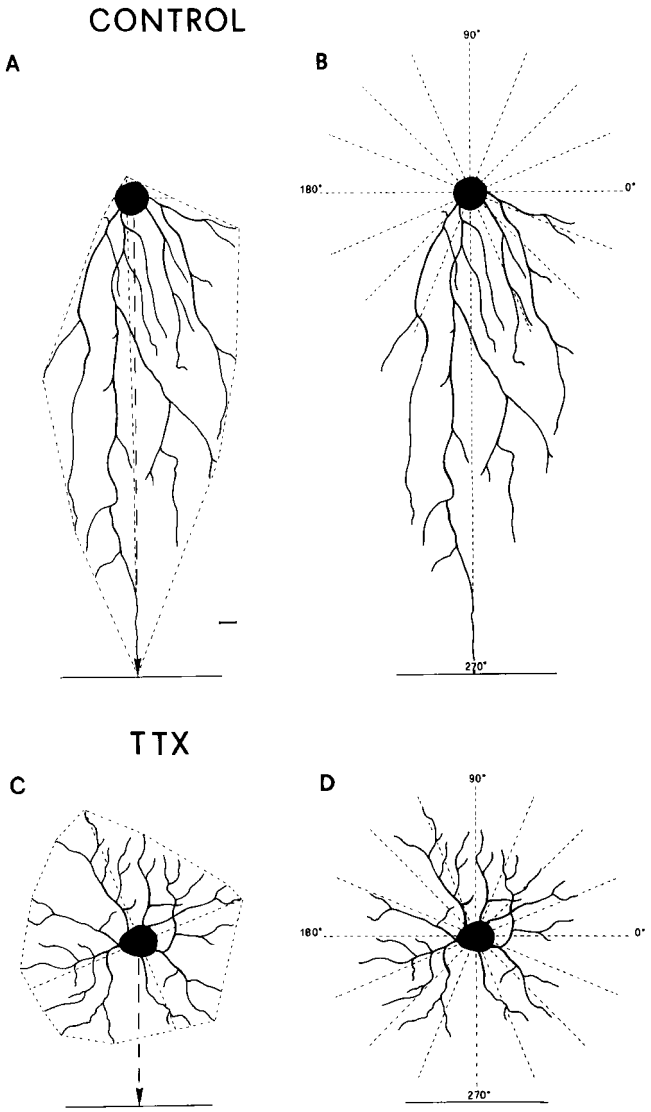

Fig. 3. Camera lucida drawings of two cells in control and tetrodotoxin (TTX)-treated retinae. The procedure for the evaluation of the degree of asymmetry is illustrated in **A** (>3) and **C** (≈1). The border of the bare area is indicated by the horizontal line. **B,D:** The drawings are presented in a polar diagram to evaluate the orientation of primary dendrites with respect to the border (270°). Scale bar = 10 μm.

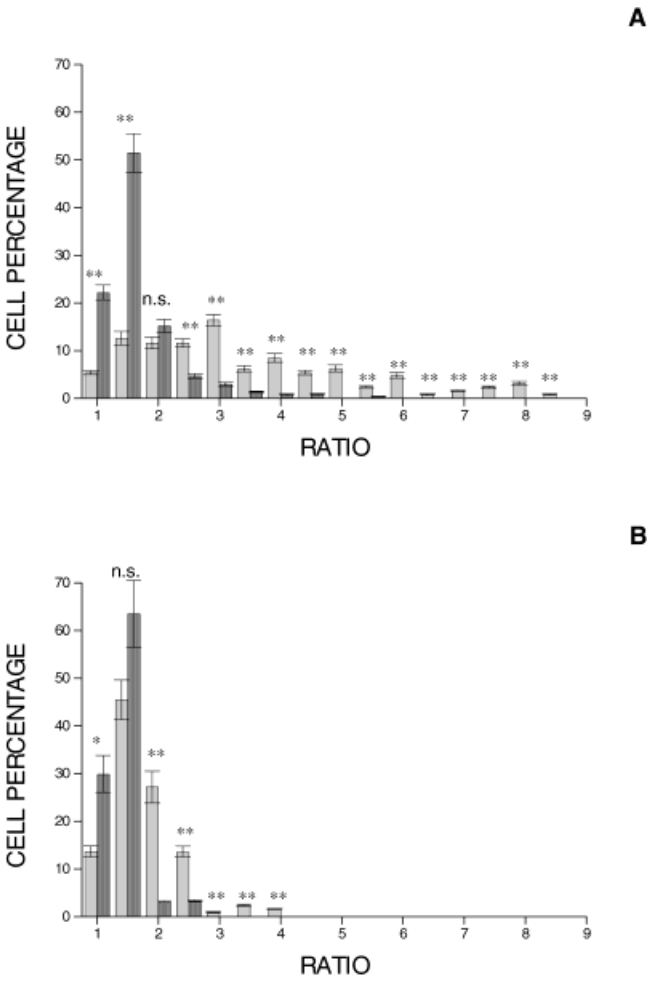

Fig. 4. Mean percentage of β (**A**) and α (**B**) cells ± S.E.M. (error bars) for control (open bars) and tetrodotoxin-treated (shaded bars) retinae as a function of the degree of asymmetry calculated as a ratio between the major axis and the axis perpendicularly crossing the center of the cell body. $P > 0.05$, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$. See Results for further details.

Horseradish peroxidase (HRP) injection

Animals were anesthetized with an injection of Ketalar (30 mg/kg) and additional doses of pentobarbital (0.2 ml/kg/hour). The position of the LGN was determined stereotaxically and by recording visual evoked responses through a glass micropipette. The electrode was removed, and a Hamilton syringe (10 μl, filled with 30% HRP in 2% DMSO 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 LGNs (three injections at each position 1 mm apart in depth).

After 2 days, a lethal dose of Ketalar was injected; cats were perfused, and the retinae were removed and processed for HRP by following the protocol developed by Hanker et al. (1977), as modified by Perry and Linden (1982), and wholemounted. We analyzed the whole population of cells at the two borders of the depleted area, and the dendritic arborizations of each neuron were drawn with the aid of a camera lucida (Deplano et al., 1994b). Although it has been reported that dendritic arborization is affected up to 500 μm from the border of the depleted zone (Eysel et al., 1985), we limited our analysis to the first row of cells, which is free from interference because of the presence of other neurons (Deplano et al., 1994b). Examples of the location and extension of the depleted area are shown in Figure 1 for one control (P7, Fig. 1A) and two TTX-treated (P14, Fig. 1B; P7, Fig. 1C) retinae. The quantitative analysis was performed in retinae, where both the position of the empty area and the quality of the HRP reaction products were comparable (four control and six TTX-treated retinae). Because we did not observe any major difference between lesions at P7 and P14, we pooled all data to perform the statistical analysis.

RESULTS

The main difference between TTX-injected and control retinae is a reduced elongation of the dendritic arboriza-

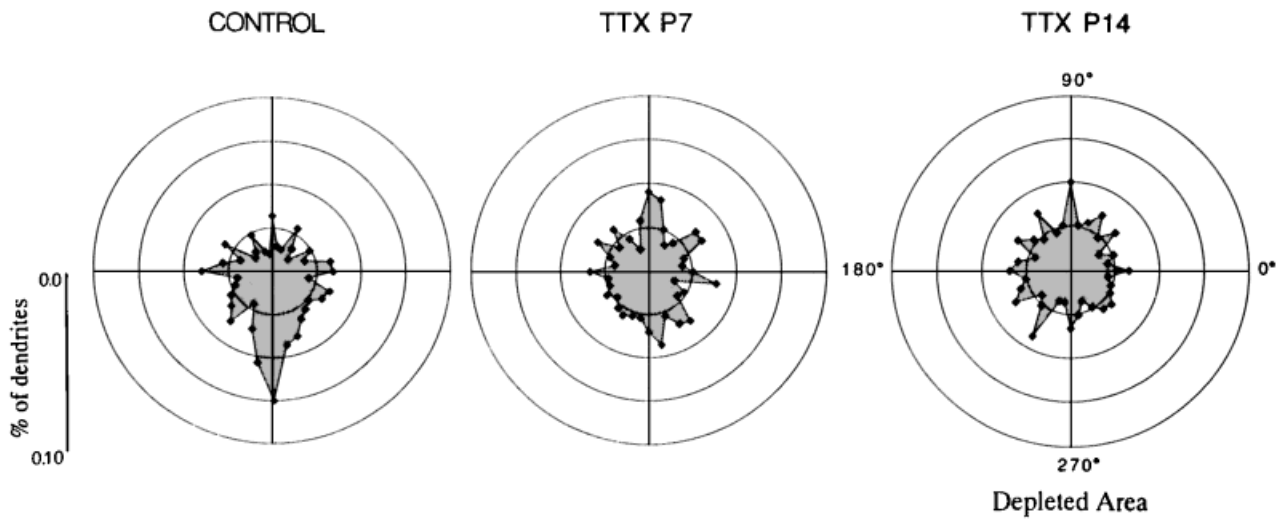

Fig. 5. Polar plots of the origin of primary dendrites from β cells located in the first row near the border of the bare area in one control retina and two tetrodotoxin (TTX)-treated retinae. Injections started at postnatal day 7 (P7) and P14. The dots indicate the percentage of primary dendrites found at the different orientations.

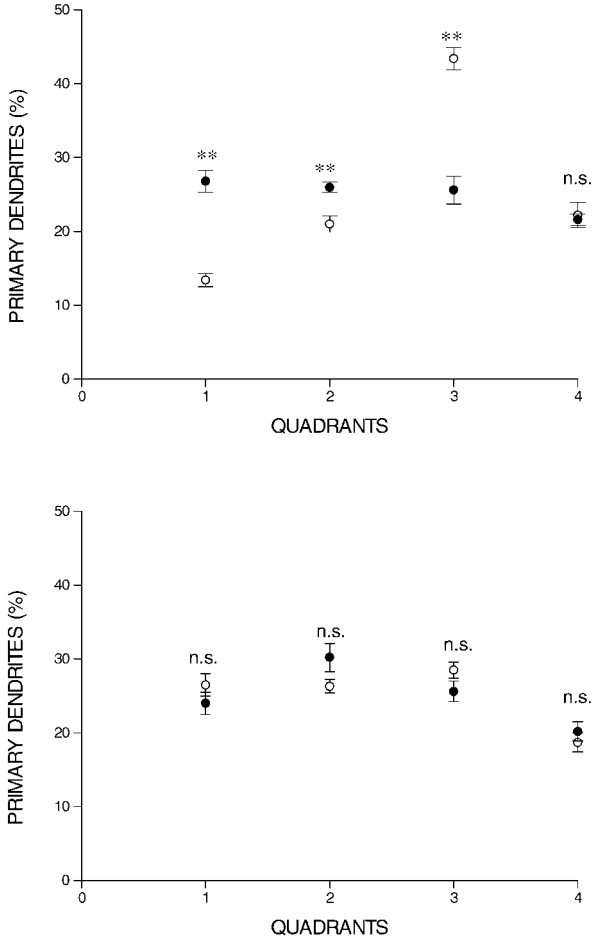

Fig. 6. Percentage of primary dendrites ± S.E.M. (error bars) for control (open symbols) and tetrodotoxin-treated (filled symbols) retinae found in the four quadrants of the polar plot. Quadrant 1, 45–135°; quadrant 2, 135–225°; quadrant 3, 225–315°; quadrant 4, 315–45°. **A:** β cells. **B:** α cells. $P > 0.05$, not significant (n.s.); $^{**}P < 0.01$.

tion of α and β cells at the border of the depleted zone in the TTX-treated retinae, as can be seen in the photomicrographs in Figure 2 (right: control, left: TTX treated). An incomplete filling of distal dendrites may be responsible for this effect. However, the quality of HRP reaction products appears to be quite similar in the two retinae, and the thickness of the smallest distal dendrites is similar in the two populations.

In an attempt to quantify this qualitative observation, we analyzed the whole population of α and β cells at the border in four control and six TTX-treated retinae. We considered two parameters of dendritic morphology: the degree of asymmetry of dendritic arborization and the origin and orientation of primary dendrites with respect to the border. The procedures we followed (see Deplano et al., 1994b) are schematized in Figure 3. Briefly, we defined the extension of dendritic arborization by connecting the extreme points of all dendrites. The degree of asymmetry was estimated by taking the ratio between the major axis and the axis perpendicularly crossing the center of the cell body (Fig. 3A,C). A ratio of 1 corresponds to a symmetric distribution of dendritic arborization. For each cell we also plotted the origin of the primary dendrites and their orientation with respect to the border (Fig. 3B,D); an orientation of 270° indicates dendrites that arise toward the bare area.

Statistical analysis was performed on a sample of 59 α/417 β cells in control and 99 α/598 β cells in TTX-treated retinae.

Data on dendritic arborization collected from four normal and six TTX-treated retinae were pooled together and are presented in Figure 4 for the β (Fig. 4A) and α (Fig. 4B) cell populations. The percentage of cells in a control (open bars) and in a TTX-treated (shaded bars) retina is reported as a function of the ratio between the two axes. In TTX-treated retinae, the majority of β ganglion cells qualified as symmetrical, with an axis ratio of 1–2; in control retinae, only a small number of cells showed a similar degree of symmetry. However, ganglion cells with a degree of asymmetry above 3 were fewer in TTX-treated than in control retinae. The extreme degree of asymmetry evaluated in our sample corresponded for β cells to a ratio

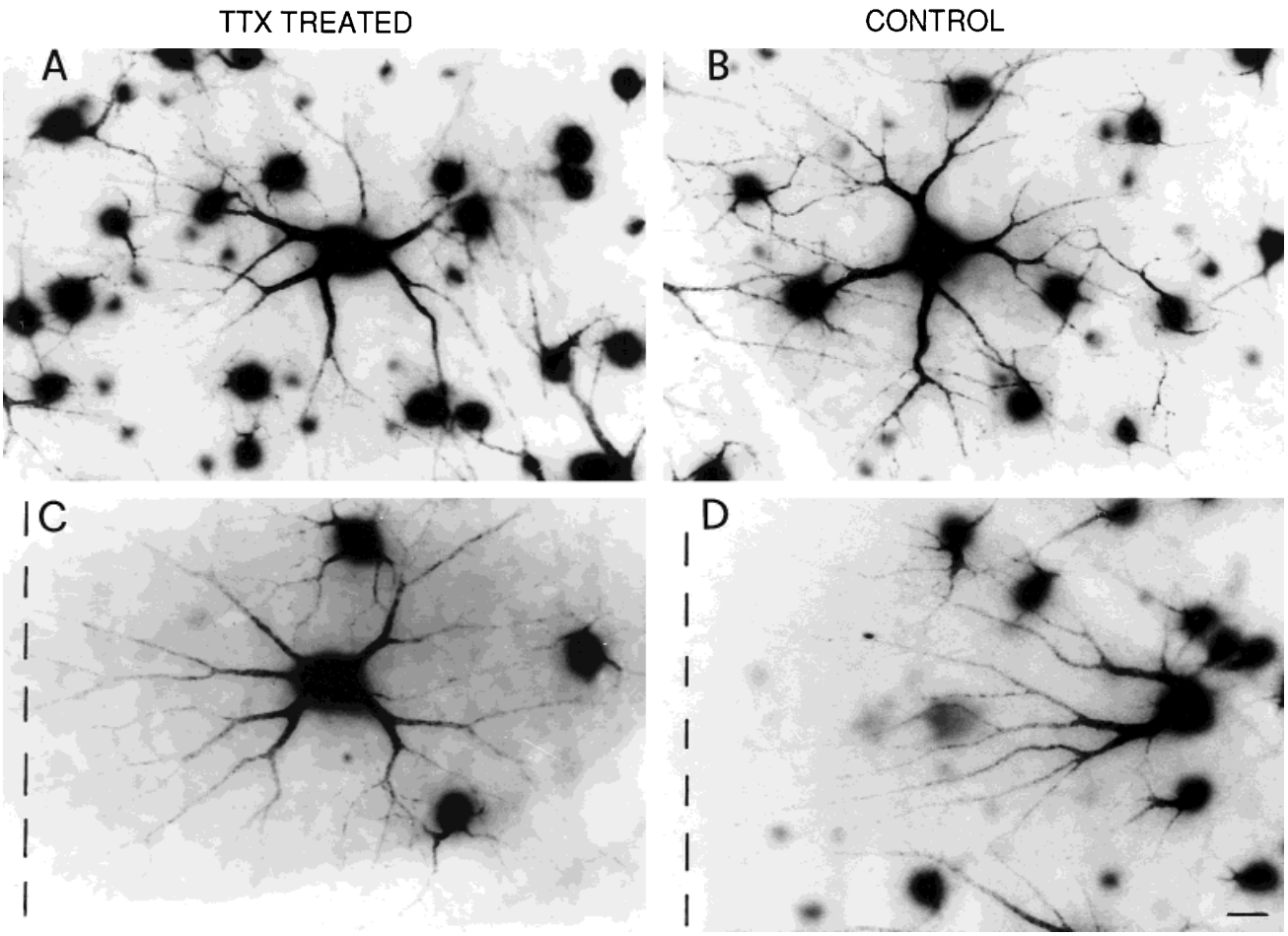

Fig. 7. Photomicrographs of α cells located in corresponding areas of the retina far from the bare area in tetrodotoxin (TTX)-treated (**A**) and control (**B**) retinae and at the border of the bare area in TTX-treated (**C**) and control (**D**) retinae. The broken line on the left-hand side of C and D indicates the plane of the bare area. Retinal ganglion cells were filled with horseradish peroxidase injected into the lateral geniculate nucleus. Scale bar = 20 μm.

of 8.5 in control retinae (see also Fig. 5 in Eysel et al., 1985). The different distribution of treated and untreated neurons in the various classes was statistically significant (analysis of variance, ANOVA). Data from all α cells were pooled together and are presented in Figure 4B. As for the β cells, there was an increase in the number of symmetrical neurons and a loss of asymmetrical neurons in TTX-treated retinae. For a substantial class of cells (intermediate degree of symmetry), however, the effect of TTX was not statistically significant.

Figure 5 shows the plot in polar coordinates the origin of primary dendrites of β cells from three retinae, one control and two TTX-treated, in which the two injections were made starting at P7 and P14, respectively. In agreement with previous data (Deplano et al., 1994b), the majority of primary dendrites in control animals emerged perpendicular to the border and oriented toward the empty space. There was no clear preference in retinae treated at P7 or at P14 with regard to the emergence of primary dendrites, except for a slight tendency to develop in the direction opposite to the border. The origin of primary dendrites was calculated for all α and β cells sampled in control and TTX-treated retinae. The percentage of dendrites found in the four quadrants of the polar plot (quadrant 3, 225–315°, corresponds to the position facing the bare area) is shown in Figure 6 (Fig. 6A, β cells; Fig. 6B, α cells).

The statistical analysis shows that the origin of primary dendrites is extensively modified by TTX treatment in β cells. We also calculated the mean number of primary dendrites (Dp) that originate from both populations of α and β cells in the two experimental conditions and observed that Dp in β cells increase after TTX treatment (Dp in control α 4.5 ± 0.2, β 2.9 ± 0.2; Dp in TTX-treated α 4.7 ± 0.2, β 4.1 ± 0.1; ANOVA α 0.42, $P > 0.05$, not significant; ANOVA β 32.15, $P < 0.01$). However, the mean dendritic field area of β cells remained constant with or without TTX. This point was checked by measuring the dendritic field area of β cells at the border of the lesion in control and TTX-treated retinae. Special care was taken in comparing cells at corresponding distances from the optic disk. The values obtained by this procedure were: at 5 mm from the optic disk, 0.0071 ± 0.0005 mm^2 in control (32 cells) and 0.0070 ± 0.0004 mm^2 in TTX-treated (48 cells) retinae, ANOVA 0.024, $P > 0.05$, not significant; at 8 mm from the optic disk, 0.0110 ± 0.0020 mm^2 in control (24 cells) and 0.0123 ± 0.0018 mm^2 in TTX-treated (33 cells) retinae, ANOVA 0.23, $P > 0.05$, not significant.

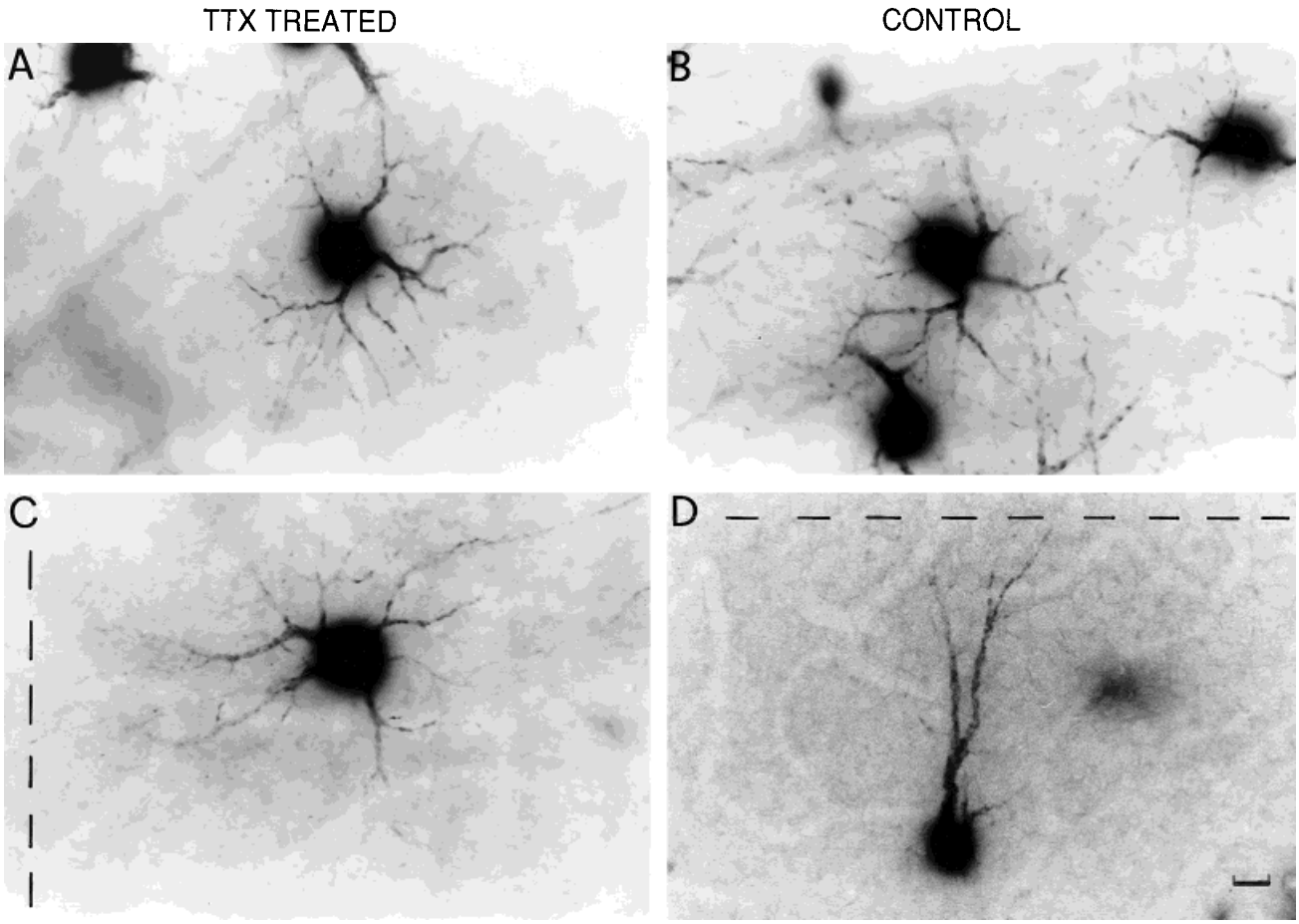

Fig. 8. Photomicrographs of β cells located in corresponding areas of the retina far from the bare area in tetrodotoxin (TTX; **A**)-treated and control (**B**) retinae and at the border of the bare area in TTX (**C**) and control (**D**) retinae. The broken line in C and D indicates the plane of the bare area. Retinal ganglion cells were filled with horseradish peroxidase injected into the lateral geniculate nucleus. Scale bar = 10 μm.

It seems reasonable to conclude that blocking the electrical activity had a substantial effect on the dendritic reorganization that usually takes place after a small retinal lesion.

Effects of TTX treatment on dendrite remodeling

To check the possibility that, in addition to the effect on dendritic reorganization near the lesion, there might also be a general effect on ganglion cell morphology, we extended our analysis to the population of ganglion cells far from the lesion. Photomicrographs of α and β cells taken near the border in control (Figs. 7D, 8D) and TTX-treated (Figs. 7C, 8C) retinae were compared with photomicrographs of cells from the same class taken far from the depleted area, but in corresponding positions in the two retinae (Fig. 7A,B for α cells, Fig. 8A,B for β cells). It can easily be noted that the ganglion cell population near the depleted area shows a different morphology in treated and untreated retinae following an insult. By contrast, no major difference is observed in the ganglion cell population far from the empty area, as illustrated in Figure 9, where somal and dendritic field sizes in four normal and four TTX-treated retinae are reported as a function of the distances from the optic disc (a total of 218 α and 832 β cells in control and 194 α and 739 β cells in TTX-treated retinae). In both α and β cell populations, the distributions of normal and TTX-treated somal and dendritic field areas overlap extensively across the retinal regions analysed. Thus, we conclude that, whereas electrical activity plays a key role in dendritic rearrangement, it has only a marginal role, if any, in dendritic remodeling, as has already been pointed out (Wong et al., 1991).

DISCUSSION

The main finding reported in the present study is that the injection of TTX modifies the developmental behavior of ganglion cells in damaged retinae. Specifically, the dendritic elongation induced by an early retinal lesion is prevented by repeated injections of TTX during the critical period. This is the first instance in which a morphological effect of TTX has been shown in the retina.

The present results also show that the rearrangement of arborization is associated with a reduction in the number of primary dendrites of β cells, which probably lose those dendrites that orient away from the depleted zone. It is perhaps important to note that overall extension of the

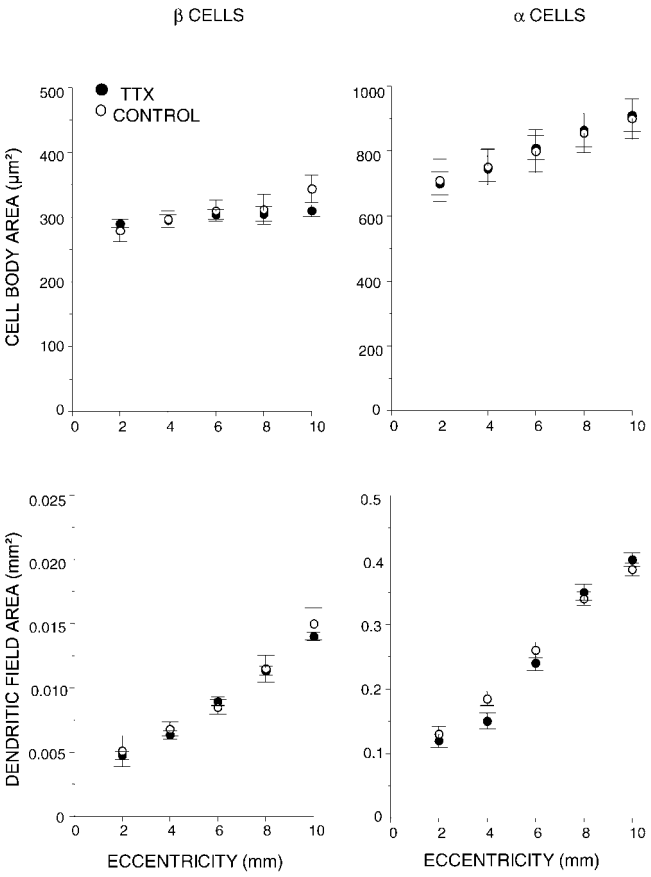

Fig. 9. Mean cell body area ± S.E.M. (error bars) as a function of eccentricity for β and α cells (top) in control (open circles) and tetrodotoxin (TTX)-treated (filled circles) retinae. Mean dendritic field area ± S.E.M. (error bars) as a function of eccentricity for β and α cells (bottome) in control (open circles) and TTX-treated (filled circles) retinae.

dendritic arborization remains constant in all conditions. These observations suggest that the effect of the lesion is to select properly oriented dendrites rather than instructing their development in a given orientation. Also, the loss of dendrites is prevented by TTX. Interestingly, no such reduction is present in the α-cell population.

In agreement with previous results (Wong et al., 1991), in the absence of damage, blocking of the electrical activity for the whole critical period has no effect on the dendritic remodeling that normally takes place during the first month of postnatal life. This result does not support the hypothesis that a correlated activity is essential in limiting the extension of dendritic arborization during development of the ganglion cell mosaic (Wässle and Boycott, 1991). This mechanism, however, has been shown to operate in axonal segregation at both the LGN (Shatz and Stryker, 1988) and the cortical level (Stryker and Harris, 1986).

Our results suggest that remodeling and rearrangement could be mediated by different mechanisms. Whereas the remodeling may follow a developmental program genetically predetermined, the rearrangement that follows an early lesion may result from an attempt to fill the gap left by the death of ganglion cells and signaled by the absence of a visually driven response in a sector of the visual field.

The cellular mechanisms responsible for the effect of TTX are not known; one may speculate that the rearrangment that follows an early lesion is determined from the electrical activity in a way similar to that described for the axonal segregation at LGN (Shatz and Stryker, 1988). The observation that TTX-sensitive Na^+ channels are also present on astrocytes (Sontheimer et al., 1996) and Muller cells from retinae of various species (Chao et al., 1994) may also suggest that TTX interrupts a communication line by which messages are exchanged between glial cells and neighboring neurons.

A close relationship between ganglion cell axons and astrocytes in the cat retina is supported by data showing that degeneration of ganglion cells causes death and morphological rearrangement in astrocytes (Karschin et al., 1986). Recent results (Bisti et al., 1997) have shown that intraocular injection of TTX in cats during the first month of postnatal life modifies the morphology of astrocytes, which are no longer associated with the axon bundles.

The hypothesis has also been proposed that diffusible factors are responsible for the dendritic growth toward the empty area (Perry and Maffei, 1988). The availability of these factors might be increased by a reduced competition because of the death of ganglion cells following the resection of axons bundles passing through the damaged area. The reason for suggesting a diffusible factor is based on the observation that dendritic elongation is not limited to the population at the border of the empty area, but it extends over a distance of 500 μm.

There are no indications about the sequence of events leading to the abnormal elongation of dendritic trees, but supposing that a diffusible factor is involved in dendritic rearrangement, the present results indicate that this is under the control of a TTX-sensitive mechanism.

ACKNOWLEDGMENTS

We thank Professors L. Cervetto and L.M. Chalupa for helpful discussion and critical reading of the manuscript, Bruna Margheritti for helping in the preparation of the figures, Alberto Bertini for photographic support, and Danila Moriconi for invaluable help in animal care.

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