terized by clusters of developmental defects involving the heart and great vessels along with pharyngeal pouch derivatives and disfigurement of facial features. The observed association of thymus and heart defects and the incidental absence or decreased size of thyroid and parathyroids in some animals suggest that the etiology of these syndromes is related to failure of neural crest cells to migrate and interact in sufficient quantity to support development of the organs in question. The Di George syndrome provides the best clinical correlation for this array of anomalies (*12, 13*). Di George (*13*) originally described this syndrome as "congenital absence of the thymus (and parathyroids)." Micrognathia and hypertelorism have been described in association with heart defects in addition to the characteristic thymus and parathyroid deficiencies (*1*). Other combinations of thymic defects with heart (*1*) and thyroid (*15, 16*) defects have been described in the presence of parathyroids. Numerous examples of heart defects associated with anomalies of head and neck, with or without reported defects in pharyngeal pouch derivatives, have been described (*12, 13*). It is possible that an understanding of the neural crest's role in the development of these organs will lead to increased recognition of clusters of developmental anomalies.

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The Edge Cell, a Possible Intraspinal Mechanoreceptor

Abstract. *In the lateral edge of the "white matter" in the lamprey spinal cord, there is a group of nerve cells referred to as edge cells. The results of a combined physiological, light microscopical, and electron microscopical study suggest that these cells serve as intraspinal mechanoreceptors. Edge cells are depolarized on stretch of the lateral margin of the spinal cord, and they have nestlike ramifications in this region oriented in a rostrocaudal plane. These cells exhibit a close structural similarity with the crayfish stretch receptor.*

Retzius has described a group of nerve cells in the spinal cord of the cyclostome located far out in the "white matter" along the lateral edge (*1*). These cells in the lamprey were further described by Kolmer (*2*), Tretjakoff (*3*), and Rovainen (*4*) and are now referred to as edge cells. They have "dendrites" extending toward the lateral margin in a rostrocaudal plane and an axon ascending on the ipsi- or contralateral side for some segments, and they receive synaptic input from a few sources. It has recently been demonstrated that mechanosensitive elements

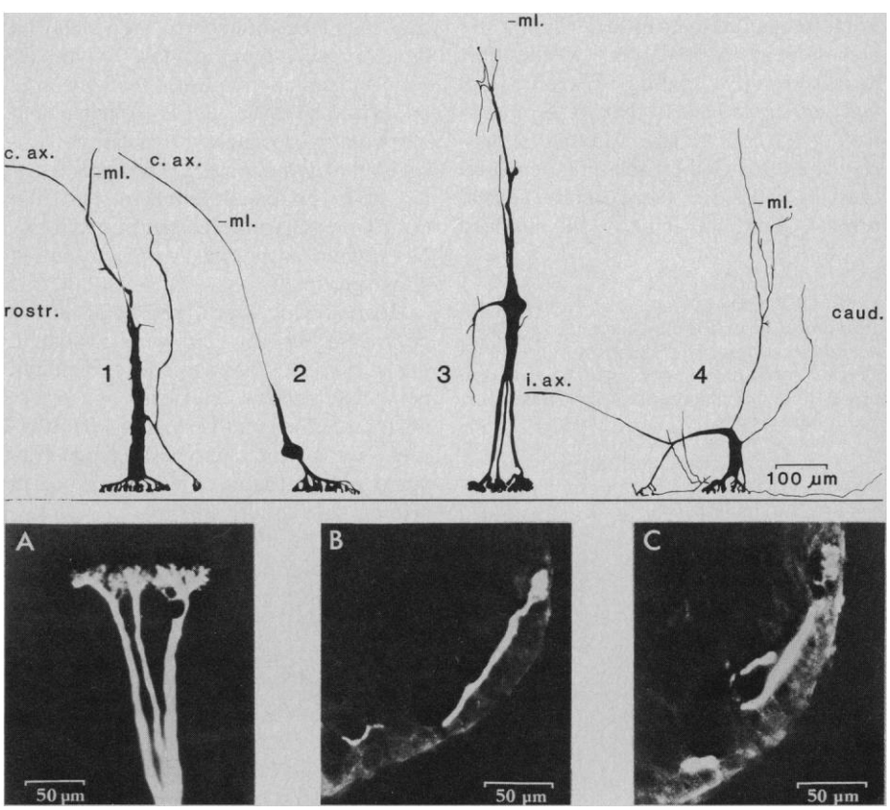

Fig. 1. Reconstruction of edge cells. The neurons were reconstructed (dorsal view) from four different Lucifer yellow preparations (1 through 4) and are oriented along the lateral margin (downward). The midline (*ml.*) is at different distances from the lateral margin because of differences in the sizes of the spinal cords. Rostral (*rostr.*) is to the left. Axons projecting to the ipsi- or contralateral side of the spinal cord are indicated with *i.ax.* or *c.ax.*, respectively. (A, B, C) Photographs of the lateral ramifications of cell 3. (A) Photograph showing the nests of fine terminals (Lucifer yellow, dorsal view). (B and C) Photographs of the same lateral processes in transverse section. Note the close proximity to the lateral edge and how the ramifications course among the individual axons.

exist in the spinal cord (*5*). Based on indirect evidence, it was suggested that edge cells might serve as mechanoreceptors. We undertook our study to describe the morphology of edge cells injected intracellularly with Lucifer yellow or horseradish peroxidase (*6*) and to investigate whether they might serve as mechanoreceptors.

The spinal cords of adult lampreys (*Petromyzon marinus* or *Ichthyomyzon unicuspis*) were dissected, and pieces consisting of eight to ten segments were pinned down with the ventral surface upward in a Sylgard-lined dish. The meningeal sheath (meninx primitiva) was stripped over the ventrolateral surface to facilitate microelectrode penetration. The physiological solution (*6*) was cooled to ~ 9°C. With the use of a dissection microscope and transillumination of the spinal cord, the cell bodies of the edge cells could as a rule be seen along the lateral edge. Microelectrodes were filled with Lucifer yellow or horseradish peroxidase (*6*), and the dyes were injected electrophoretically into the edge cells (N = 34). In some cases a small stretch was applied to the spinal cord segment near the edge cell before injection. For this purpose a coarse electrode was attached to the lateral margin of the spinal cord about 0.5 to 1.5 mm rostral or caudal to the cell. After impalement of the edge cell with the dye-filled electrode, this coarse electrode could be moved in 2-μm steps in either direction along the longitudinal axis of the spinal cord and thereby stretch or unload part of the spinal cord margin.

All neurons had very characteristic lateral ramifications. Four reconstructed edge cells are shown in Fig. 1 (1 through 4). The lateral processes are lined up along the lateral margin and do not terminate as ordinary dendrites. Instead they appear in low magnification as coarse and relatively large and blunt specializations. The three micrographs (Fig. 1, A–C) are from cell 3. Figure 1A shows that the lateral processes form nestlike formations of what appear to be very fine processes. Figure 1, B and C, show the same processes in transverse section. The branches course beween the axons of the white matter to terminate just at the very lateral aspect of the spinal cord. The processes are lined up very close to the lateral margin (upward) (Fig. 2A, dorsal view, electron microscopy), and the lateral processes are shown under higher magnification in Fig. 2, B and C. One very characteristic feature is apparent: on each lateral process there is a large number of thin (< 1 μm) fingerlike processes. Such processes have been described in the crayfish stretch receptor neuron and referred to as dendritic tips (*7*). The general type of arrangement closely resembles that of crayfish stretch receptor neurons, which have nests of dendritic tips that ramify along the structure that is stretched (*7*). In addition, chemical synapses are present on the lateral ramifications (Fig. 2B_1), as inferred from the simultaneous presence of synaptic vesicles and synaptic junctions. The crayfish stretch receptor neuron also has inhibitory synapses in this location (*7*). The lateral processes of the edge cells contain a large number of mitochondria (Fig. 2).

From the functional point of view, it is presumably important that the diameters of the branches from the lateral edge to the cell body are large. In this way a low-resistance path is created, so that electrical events at the lateral margin will be effectively transmitted to the cell body and the spike initiation zone of the cell.

The general shape of the cell body is quite variable. For instance, the soma may be ribbonlike and extend almost to the lateral margin (Fig. 1, cell 1), or the lateral processes may be longer, or the soma may be bent and give off lateral branches from both ends (Fig. 1, cell 4). Part of this difference can probably be attributed to the location of the cells among the bundles of axons. It is our impression that the shape of the cell body is influenced by the surrounding axons, which may, for instance, compress the cell body in some parts (note indentations of cell body 1 in Fig. 1). Some of the synaptic contacts located on the soma were observed to be of the en passant type made from large axons (seen with electron microscopy, not illustrated).

On the cell body there is an axon hillock, which rapidly tapers to a very thin process that subsequently (after 0.1 to 0.5 mm) increases in diameter again, that is, the axon (Figs. 1 and 3). The

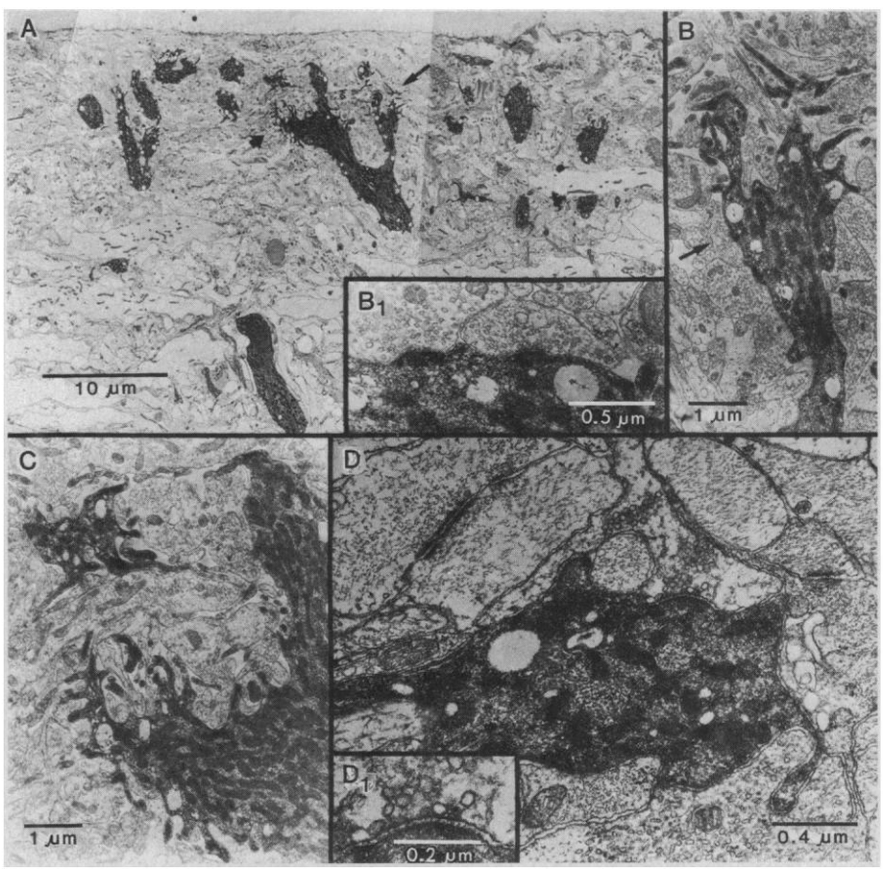

Fig. 2. Ultrastructure of lateral ramifications. (A) Overview of the lateral ramifications. The lateral margin is upward. Note the short distance between the margin of the cord and the lateral processes (dark color resulting from horseradish peroxidase injection). (B and C) Two different lateral processes in (A) (indicated by a short and a long arrow) shown under higher magnification. Note the abundance of fine fingerlike processes (dendritic tips). (B_1) Two synapses in the left central part of (B) (arrow) shown in higher magnification. (D) A bouton containing spherical synaptic vesicles in contact with the proximal part of an edge cell dendrite (dark color). (D_1) The synaptic complex.

axon may initially go toward the midline to turn rostrally, or it may continue on the ipsilateral side (*8*) (Figs. 1 and 3). In one instance (Fig. 3A) a contralateral descending branch was observed. In addition to the axon and the thick lateral branches, there are in most cells ramifications at the level of the cell body, which often go in the medial direction to the level of the dorsal column and the medial Müller axons (Figs. 1 and 3). Sometimes branches will also cross to the contralateral side (Fig. 1, cell 4). Other branches will ramify at the level of the ipsilateral gray matter. They may originate either from the cell body or in some cases from the lateral branches (Fig. 1, cell 4) and may extend for several hundred micrometers in a rostrocaudal or medial direction. Branches in this category taper gradually in diameter, in contrast to the case for the axon and the lateral processes. Input synapses occur on these processes (Fig. 2D), but no output synapses, that is, from edge cells to other neurons, have been observed so far.

The lateral processes of the edge cells are arranged along the lateral margin of the spinal cord, which is flat (Fig. 3). Whenever the body bends sideways, as during swimming or turning, the spinal cord will also bend. As an area of lateral margin changes shape, from an inside curve to an outside one, the processes will move relative to each other and be stretched rostrocaudally. The percentage change in length of the lateral margin during swimming or turning has been estimated to be up to about 5 percent (*8*).

Ten edge cells were tested for such rostrocaudal stretch sensitivity. A probe was attached to the lateral margin 0.5 to 1.5 mm away from the edge cells chosen for recording (Fig. 3D). The spinal cord on the other side of the edge cell was fixed to the Sylgard-lined dish by a fine pin. In some cases a gash was made in the spinal cord to minimize transmission of stretch to the cell body (Fig. 3D). After the cell had been impaled, the lateral margin and thereby the lateral processes of the edge cell were stretched in controlled steps of up to about 5 percent of the distance between the pins. The upper two recordings in Fig. 3C are from the edge cell reconstructed in Fig. 3B. When the lateral edge was stretched slowly in 10-μm steps (total 100 μm), the cell depolarized and began to spike; when it was released from stretch, the membrane potential returned to the previous level. A smaller stretch gave rise to a smaller maintained depolarization (middle record, Fig. 3C). All edge cells were depolarized on stretch and repolarized with release, although in some neurons spikes were not generated. The responses are similar to those of a mechanoreceptor with a moderate dynamic sensitivity. In some instances the neuron would discharge with one spike per 10-μm stretch (representing about a 1- to 2-μm stretch in the dendritic region).

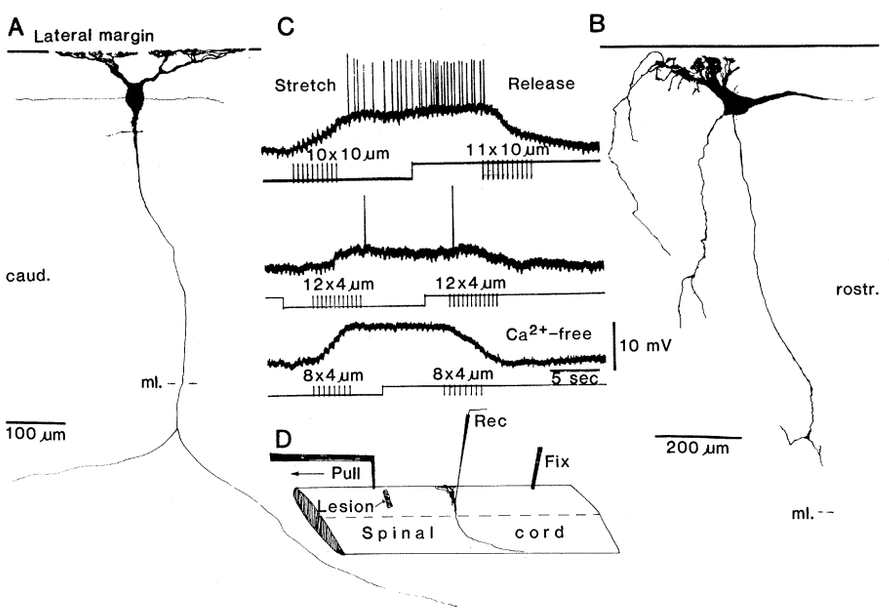

Fig. 3. Depolarizing responses to stretch of the spinal cord margin. The upper two sets of recordings in (C) are from the edge cell in (B) (reconstruction, dorsal view as in Fig. 1, Lucifer yellow); the lower set in (C) is from the cell in (A). The upper traces in (C) are intracellular records (time and voltage calibration). The lower trace in (C) indicates the length change of the lateral margin. Each vertical line in (C) indicates a brief 10-μm stretch or a 4-μm stretch. Over a period of 4 to 5 seconds, several 10- or 4-μm stretches were applied as indicated (C); the release of stretch is indicated in an analogous way. (D) The general experimental arrangement with the pull applied in the longitudinal direction along the lateral margin. The cell potential is depolarized on each stretch and returns to the resting level on release from stretch. The rest length of the cord corresponds approximately to the length in situ. The axon of the cell in (A) crosses the midline and has a descending branch; the cell in (B) has an axon ascending on the ipsilateral side.

To exclude the possibility that the edge cells were activated indirectly by other neurons rather than being stretch-sensitive themselves, we tested the stretch sensitivity in Ca^{2+}-free Ringer, which should prevent chemical synaptic transmission. The bottom record in Fig. 3C is from the cell reconstructed in Fig. 3A, and a stretch-evoked depolarization is still elicited in a Ca^{2+}-free solution.

It could be argued that the stretch-evoked depolarization is a mechanical artifact resulting from microelectrode movements in relation to the cell. This hypothesis is unlikely, however, since (i) the effects are graded, are reversible, are reproducible, and always have the same sign; (ii) other impaled neurons that have ordinary dendrites within the lateral aspect of the spinal cord were not depolarized; and (iii) extracellularly recorded axons postulated to originate from stretch receptors along the lateral margin can be activated by bending movements of the cord (*5*). In the latter case the cell somata obviously remained unperturbed by any electrode.

The edge cells have a morphology which appears to be different from that of all other neurons in the central nervous system that have been studied. In particular, the lateral processes are distinctive with their nestlike formations, consisting of fine dendritic ramifications. When stretch is applied along the lateral margin of the spinal cord, as during swimming, the processes extending along the lateral margin will be deformed, presumably causing a stretch-evoked depolarization of the edge cells. All the evidence taken together suggests that the edge cell acts as an intraspinal mechanoreceptor that is activated during normal movements and that also receives synaptic input from other sources (*9*) (Fig. 2, B_1 and D_1).

Mechanoreceptors have been demonstrated in the crustacean ventral nerve cord (*10*), but in the vertebrate nervous system the presence of mechanoreceptors has, to our knowledge, only been tested in the present series of studies. In an animal like the lamprey, the eel, or the snake, the spinal cord will be bent each time the body is bent. It is apparent that mechanoreceptors at the lateral

margin could be used to signal such movements in the lateral direction. Signals from intraspinal mechanoreceptors are used in the feedback control of the interneuronal network that generates the undulatory locomotor movements of the lamprey (*5*). Whether neurons of this type are found only in the lamprey or are widespread in the vertebrate phylum is unknown at present, but "marginal cells," located in the white matter of the lateral spinal cord, have been described in widely different groups, such as reptiles and birds. No function has been ascribed to them (*11*).

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6. The physiological solution used was as described by W. D. Wickelgren, *J. Physiol. (London)* **270**, 89 (1977). Microelectrodes were filled with Lucifer yellow (5 percent in 0.1 m*M* LiCl) or horseradish peroxidase (HRP) (25 percent in 1*M* KCl and 0.1*M* NaOH). All the edge cells (N = 34) were stimulated for about 30 minutes with 1.5 to 4 nA, as a rule pulsed at 5 Hz with 100-msec pulses [see K. Sigvardt and S. Grillner, *Soc. Neurosci. Abstr.* **7**, 362 (1981); S. Cullheim and J.-O. Kellerth, *J. Comp. Neurol.* **178**, 537 (1978)]. After completion of the injection, not more than 2 hours elapsed until the preparation was fixed in 4 percent formaldehyde (Lucifer yellow) or 5 percent glutaraldehyde (HRP) in a 100-mos*M* phosphate buffer (T. Carlstedt, personal communication). The Lucifer yellow preparations were dehydrated and cleared in methyl salicylate and mounted as "whole mounts" [W. W. Stewart, *Cell* **14**, 741 (1978)]. The HRP preparations were processed in Hanker-Yates solution without NaCl and mounted in Vestopal W [S. Cullheim and J.-O. Kellerth, *J. Comp. Neurol.* **178**, 537 (1978)] for light microscopical analysis. The preparations were then sectioned (ultrathin) for electron microscopy.
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Kainic Acid Induces Sprouting of Retinal Neurons

Abstract. *The neurotoxin kainic acid caused dose-dependent morphological changes in horizontal cells of the retinas of adult cats and rabbits. High concentrations of kainic acid killed the cells, but when exposed to sublethal doses they contracted their dendritic fields and sent sprouting processes into the inner retina. It appears that kainic acid can induce neuronal growth as well as degeneration and that the potential for morphological plasticity is still present in neurons of the adult mammalian retina.*

Many neurons in the mammalian retina degenerate after intraocular application of kainic acid (KA), an excitotoxic drug widely used to produce specific lesions in the central nervous system (*1*). We have studied the effects of KA on horizontal cells. These cells have their cell bodies at the outer edge of the inner nuclear layer (INL) and send their processes into the outer plexiform layer (OPL), where they contact the photoreceptor pedicles together with bipolar cells (*2*). In whole-mounted retinas stained with a neurofibrillar method (*3*), the A-type horizontal cells can be visualized consistently and quantitatively (*4*).

Kainic acid was injected into the vitreous body of adult cats and rabbits, which were then maintained for 3 to 11 months so that any KA-induced changes could develop fully and stabilize (*5*). This ensured that any observed effects were not transient stages in a process of degeneration.

The first morphological changes in the A-type horizontal cells of the cat retina became apparent at KA doses of 70 to 100 nmole. The density of the cells was normal, but each cell had a contracted dendritic tree. In Fig. 1 the plexus of A-type cells in a normal retina (Fig. 1a) is compared to that of a retina treated with 100 nmole of KA (Fig. 1b). Both areas are at the same eccentricity and contain the same number of cells, but the dendritic overlap of neighboring cells in the treated retina is greatly reduced. Although the primary dendrites are slightly stouter, the dendritic branching pattern is normal. The mean overlap, or coverage factor (*6*), drops from 2.7 in Fig. 1a to 1.6 in Fig. 1b. Contraction of the dendritic fields was uniform over large retinal regions but was dose-dependent, and high KA concentrations reduced the mean coverage factor to less than 1.0.

After exposure to medium doses of KA (100 to 200 nmole), the A-type horizontal cells remained at normal density, contracted their dendritic fields, and produced sprouting processes that descend-

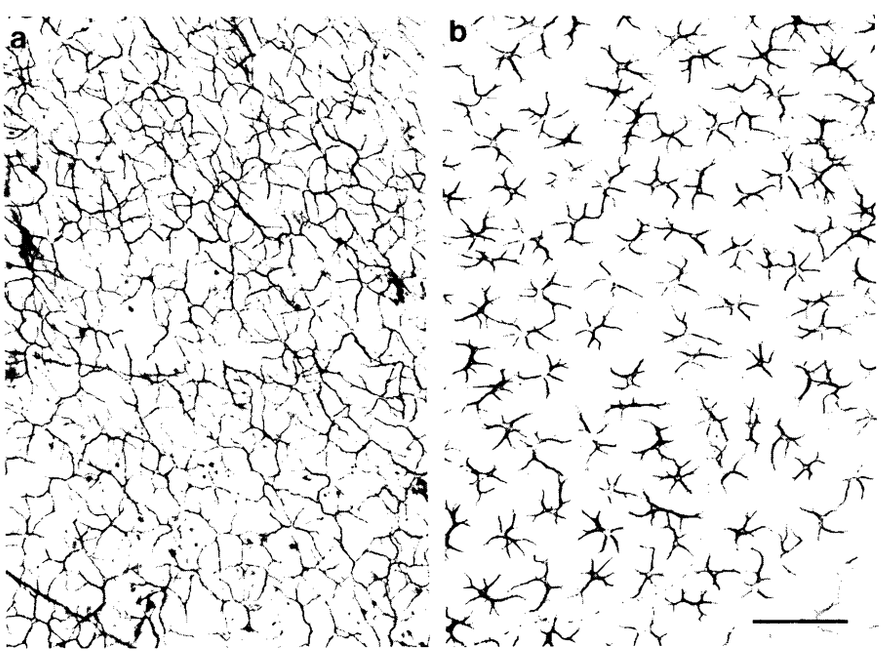

Fig. 1. Reduced overlap of horizontal cells in cat retina after KA treatment. (a) Plexus of A-type horizontal cells in a normal retina. Some axons and neurons of other retinal layers are out of focus. (b) Plexus in a retina treated with 100 nmole of KA. Here the dendritic trees are contracted. The micrographs are from equivalent positions in peripheral superior retina at a density of 95 horizontal cells per square millimeter and have the same magnification (scale bar, 200 μm).