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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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-

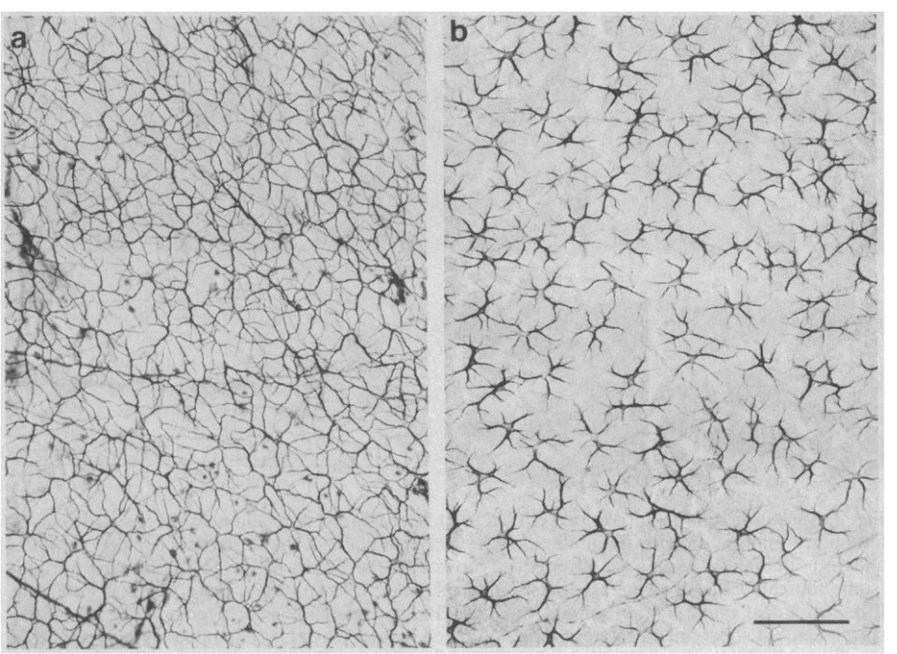

Fig. 1. Reduced overlap of horizontal cells in cat retina after KA treatment. (a) Plexus of A-type horizontal cells in a normal retina. Some axons and neurons of other retinal layers are out of focus. (b) Plexus in a retina treated with 100 nmole of KA. Here the dendritic trees are contracted. The micrographs 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).

ed into the INL and inner plexiform layer (IPL). Such processes are never observed in normal retinas or at lower KA doses. Sprout length was variable up to 100 μm, and a cell could have one or more. The proportion of sprouting cells was dose-dependent: in regions where the KA concentration was just sublethal, up to 90 percent of the cells produced inward-directed sprouts (*7*). In Fig. 2, a to c, are micrographs (taken at three focal levels) of a flat-mounted rabbit retina treated with 100 nmole of KA. They show how the horizontal cell sprouts branch off the main dendrites and descend into the retina, ending in brushlike structures. Sprouting horizontal cells in vertical sections are illustrated in Fig. 2, d to f.

In retinas that received large doses of KA (200 nmole or more), many horizontal cells underwent pathological changes. Their cell bodies and dendrites, now contracted to swollen stumps, showed strong argyrophilia. Above a critical KA concentration all cells degenerated and disappeared. We always found gradients of reaction over the retina corresponding to concentration gradients of the toxin. The transition zone between the region of sprouting cells at normal cell density and the region of total horizontal cell loss was, however, only a few cells wide, indicating that the lethal concentration of KA was the same for all cells of the population (see cover).

Experiments on fish and rabbits have shown a direct depolarizing effect of KA on horizontal cells (*8*), presumably mediated by the glutamate or aspartate receptors, and this may have induced the morphological changes described here. Alternatively, the changes could be induced transneuronally. Vertical sections of cat and rabbit retinas show that cell density in the INL is about halved with 100 nmole of KA, whereas the density of photoreceptor nuclei in the outer nuclear layer (ONL) is unchanged even at a 1000-nmole dose (*9*). Horizontal cell degeneration could therefore be caused transneuronally by degeneration of the bipolar cell component of cone pedicle triads, where A-type horizontal cells have their chemical synapses (*10*).

Whatever the mechanism of horizontal cell degeneration, the sprouting into the INL and IPL could be induced or potentiated by bipolar and amacrine cell loss, leaving free synaptic sites in the inner retina that could provide a target for horizontal cell sprouts. In other parts of the central nervous system such target sites may be invaded by neurons that have a potential for plasticity and sprouting (*11*). Seventy years ago it was reported that sectioning the optic nerve in the rabbit, when accompanied by inflammation of the eyeball, produces horizontal cell sprouting, and it was concluded that trophic factors were the cause (*12*). Although no inflammatory reactions occurred in our experiments, it is possible that KA treatment does have specific trophic action on some retinal neurons.

Our results indicate that the specificity of KA as a neurotoxin is dose-dependent and that at some concentrations this drug does not kill but induces a form of growth. This is an unusual case of morphological plasticity and sprouting in the central nervous system of adult mammals.

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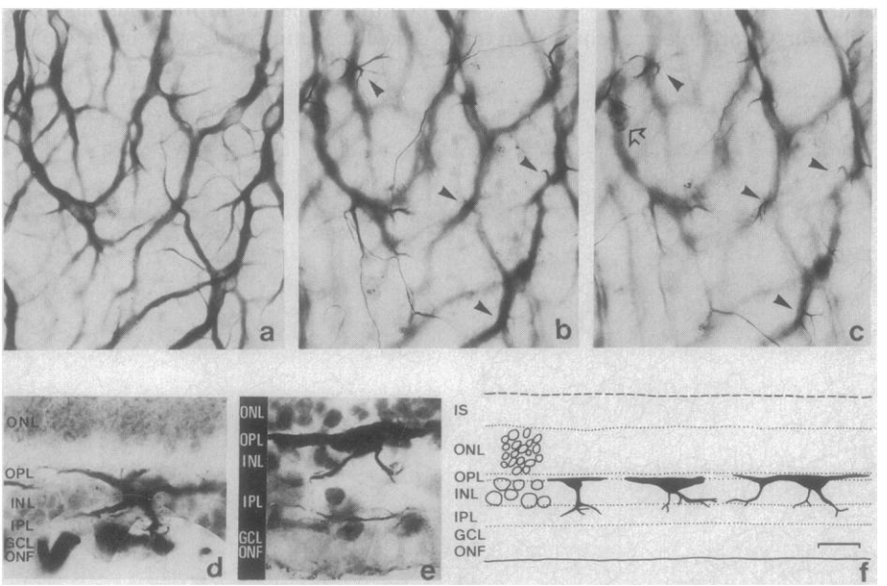

Fig. 2. Sprouting A-type horizontal cells. (a to c) Micrographs of a field near the visual streak of a rabbit retina treated with 100 nmole of KA. (a) Focal plane at the horizontal cell plexus (OPL and INL boundary); somata and dendrites in focus. (b) Focus at the INL, showing sprouts (some indicated by arrowheads) that branch off main horizontal cell dendrites and enter the INL. (c) Focal plane at the boundary of the IPL and ganglion cell layer (*GCL*), where the sprouts end in short fine branches (some indicated by arrowheads). The open arrow points to a ganglion cell. All horizontal cells in this field showed sprouting. (d and e) Sprouting horizontal cells in 35-μm vertical sections. Each horizontal cell shows two inward sprouts in addition to its normal OPL dendrites. The relative thickness of the layers in cat (d) and rabbit (e) retina differs. The ganglion cell layer and optic nerve fiber (*ONF*) layer are thin and disorganized due to KA-induced degeneration. (f) Schematic of vertical section of cat retina, showing sprouting horizontal cells. The OPL dendrites are incomplete where they leave the section. *IS*, photoreceptor inner segments. Scale bars: 20 μm (a to c and f) and 10 μm (d and e).

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6. The coverage factor, determined by multiplying dendritic field area by cell density, gives the number of cells that overlap at each point in the field (*4*). Each cell was drawn at ×400 magnification, and its dendritic field (area covered by the dendritic tree) was measured.
7. We consistently observed, in both species, that when the appropriate toxin concentration reached the central retina, nearly all cells there showed sprouts, whereas when this concentration reached the peripheral retina the proportion of sprouting cells was more variable, ranging from nearly all to less than one-third.
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