This fact confirms the view that AN in rats does not participate in the synthesis of LH-releasing factor, although it is involved in the regulation of the gonadotropic function of the anterior lobe of the pituitary [5, 9].

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# INTERNEURONAL CORTICAL CONNECTIONS STUDIED BY KAINIC ACID INJECTION


G. P. Dem'yanenko



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Changes taking place in the cat cerebral cortex 1, 3, 7, and 14 days after injection of 0.2% kainic acid solution were studied. Kainic acid was found to cause local injury to bodies of neurons and their processes, by leaving intact afferent fibers entering this region. This was demonstrated both by the use of Golgi and Golgi–Kopsch methods and also by injecting horseradish peroxidase after kainic acid into the cerebral cortex. Survival for 3 days after injection of kainic acid provides the optimal time for studying interneuronal connections. Pericellular plexuses formed by afferent fibers were discovered.



KEY WORDS: kainic acid; cerebral cortex; interneuronal connections.


The use of microinjections of kainic acid (KA) — a cyclic analog of glutamate — is justified by the fact that KA causes degeneration of neurons which selectively accumulate glutamate, but does not injure axons passing through or terminating in the given region [1, 2, 6]. It has been shown by the use of KA-$^3$H that it possesses high affinity for glutamate receptors and has a powerful excitatory-toxic action on them [9]. After injection of 2 μg KA into the striatum activity of certain enzymes — glutamate decarboxylase, choline-acetyltransferase — is sharply reduced, and ultimately this leads to neuropathological changes similar to those observed in Huntington's chorea [5], the lateral hypothalamic syndrome [10], etc.; for that reason, KA is nowadays used to create models of these diseases. On the basis of biochemical, histological, and electron-microscopic investigations it has been concluded that KA has spe-


Laboratory of Neurohistology and Department of Physiology of Higher Nervous Activity, Leningrad University.


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cific neurotoxicity. More recently, however, work has been published by authors who used injection of KA into a tissue culture of the cerebellum [7, 8] and directly into the cerebellum [3]. On the basis of their observations it was suggested that the neurotoxic effect of KA is attributable not only to its action on glutamate receptors, but also to the involvement of other, less specific, mechanisms.

Nearly all experiments with KA have been carried out on subcortical formations. We have investigated the action of KA on the cerebral cortex; it was not known what changes take place in the cortex after injection of KA or whether nervous structures could be impregnated with silver after the action of KA.

## EXPERIMENTAL METHOD

Experiments were carried out on 22 cats. KA (kainic acid, from Sigma), in a dose of 2 μg in 1 μl sterile physiological saline, was injected into the parietal, visual, and motor areas of the cat's cortex in the course of 5 min from a Hamilton's syringe, connected to an apparatus ensuring regular injection of the solution. The quantity of solution injected was 0.83-0.87 μl. The needle remained implanted in the cortex for a further 10 min after the end of the injection. The animals were perfused with physiological saline 1, 3, 7, and 14 days after injection of KA, and then perfusedwith 10% formalin for subsequent staining by Nissl's method. Pieces of brain were impregnated by the Golgi and Golgi–Kopsch methods. Sterile physiological saline was injected under the same conditions into control animals (two cats).

## EXPERIMENTAL RESULTS

The general character of the action of KA on the cerebral cortex was investigated on preparations stained by Nissl's method. Nerve cells with the most severe injuries, an increase in the number of glial cells compared with the intact cortex, and more intensive staining of the blood vessels were observed 3 days after injection of KA to a depth of 2000-2500 μ, at a distance of up to 1000 μ from the position of the tip of the needle. Among the modified vessels and glial cells bodies of individual pycnotic neurons could be seen. Macrophages accumulated around them, forming a "residual nodule." In the focus of action of KA the macrophages carried out intensive phagocytosis of the remnants of the dead cells and their processes. At a distance of 1500-2000 μ from the track a gradual transition was observed from the focus of injury to intact and clearly defined cortex. With increasing distance from the site of injection the integrity of cells in layer II improved, at a distance of 1500-2000 μ some neurons in layers III-IV remained undamaged, and at a distance of 2000-2500 μ from the site of injection, all layers of the cortex remained completely free from injury. Depending on the degree of destruction of the cortical cells, three zones could thus be distinguished topographically: a zone with damaged nerve cells, an intermediate zone, and intact cortex. As a result of uneven diffusion of the injected KA the configuration of the zone of injury was not symmetrical relative to the track, and for that reason the three zones thus distinguished varied in their parameters, but the focus of destruction was approximately hemispherical in shape.

When KA was injected to a depth of 1000-1200 μ into the motor cortex neurons of the upper layers were destroyed whereas the giant pyramidal Betz cells remained intact. The reason for this was evidently that when KA was injected not very deep it was more easily washed out on to the surface of the brain by the flow of blood and lymph, so that these cells were not injured. Maximal destruction of neurons in all layers was observed after fractional injection of 0.83-0.87 μl of KA solution to different depths: Half of the solution was injected to a depth of 2000-2500 μ and the rest was injected, after withdrawing the needle, to a depth of 1000-1500 μ.

Existing methods of electrocoagulation and of surgical intervention have certain disadvantages, for they cause injury not only to the cell population of one particular structure, but they also destroy fibers both passing through and terminating there. The new method with injection of KA evidently creates certain advantages for the study of projections from different structures.

The use of KA provides wide opportunities for a qualitatively new approach to the study of interneuronal connections. A combination of this method with impregnation methods seems promising. The results obtained by the use of the latter, as we know, are extremely variable and success in the detection of nerve cells depends on a multiple of still largely unknown

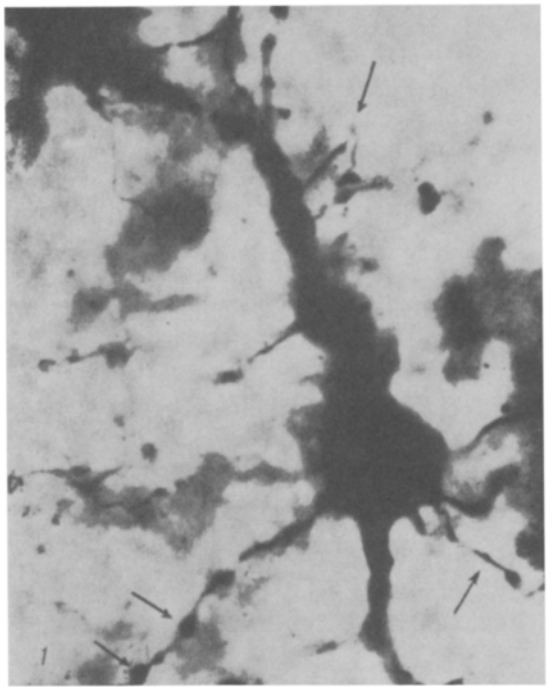

Fig. 1

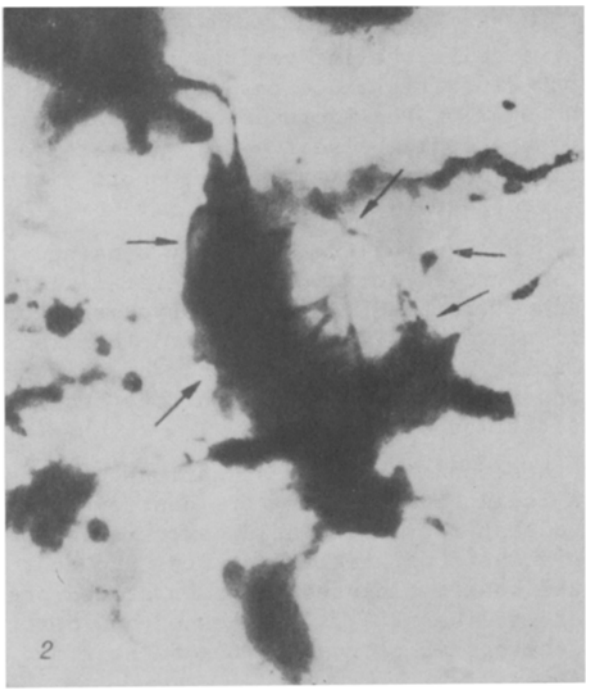

Fig. 2

Fig. 1. Pyramidal cell with destructive changes (arrows) of dendrites and axon. Golgi's method after injection of KA. 250×.

Fig. 2. Pericellular plexus around half-destroyed body of pyramidal neurons. Arrows indicate axons. Golgi's method after injection of KA, 250×.

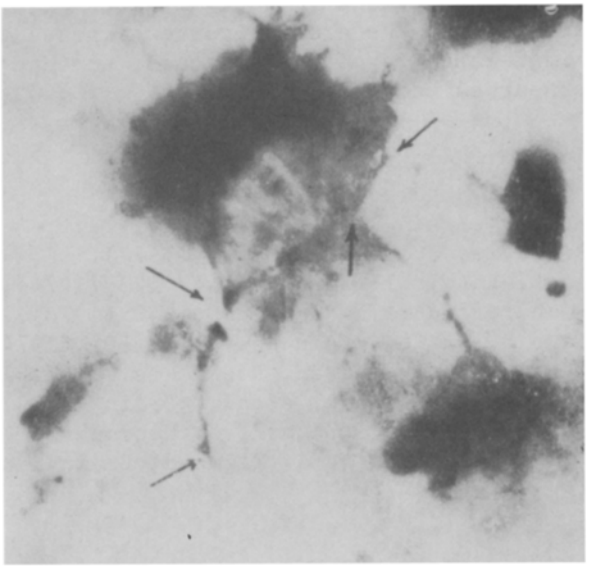

Fig. 3. Axon forming branches (arrows) on body of destroyed pyramidal cell. Golgi's method after injection of KA, 250×.

factors; for that reason, as a control for the investigation of sections stained by Golgi's method, the same sections in which the track from injection of KA with the consequences of its action (described below) could be seen together with intact cortex with well-impregnated neurons in all its layers, were used.

In preparations stained by Golgi's method the track from injection of KA was clearly

visible. Glial cells were seen in the track 3 days after injection of KA. Alongside the track nerve cells showing various stages of destruction could be seen. In the zone of action of KA both dendrites and axons of most nerve cells showed destructive changes (Fig. 1). Under high power of the microscope alternation of expansions and interruptions along the course of the fibers could be seen. Only in rare cases were single spines preserved on dendrites of pyramidal neurons on which, for short distances, the expansions caused by destructive changes were absent.

Only outlines of the cell body remained of some impregnated neurons and in some cases the half-destroyed neuron bodies pericellular plexuses formed by intact and degenerating axons became visible (Fig. 2). Convergence of several intact axons on the body of the same pyramidal neuron could be observed: The axons broke up into thinner and thinner collaterals and utimately wound around the body of this neuron in a plexus (Fig. 3). As many as 10 intact axons approaching the neuron body from different directions could be seen on pyramidal cells of the deep layers.

As these and other investigations by Nissl's method showed, practically all neurons within a radius of 1.5-2 mm were destroyed, as also were their axons. It can be tentatively suggested that the degenerating axons which were observed on the bodies of the pyramidal cells were axons of stellate neurons and that the intact axons were afferent. The dual origin of the basket plexuses has been stated previously: from axons of stellate basket cells and afferent fibers [4]. The method now described enables a differential analysis to be made of the basket plexus for the first time.

In the zone of action of KA numerous thin intact axons ran in different directions in all layers of the cortex. The intact axons in the lower layers and in layer I were more numerous than in the other layers. To prove that the intact axons were afferents, 0.2 μl of a 30% solution of horseradish peroxidase (HRP; type Sigma-6) was injected into the same track in the parietal association cortex simultaneously with, and 3, 4, and 14 days after injection of KA. HRP-positive neurons were found in the contralateral hemisphere in the parietal cortex and in n. lateralis posterior of the thalamus.

It can thus be concluded from the results of this investigation that KA causes local injury to neuron bodies and their processes in the cat cerebral cortex, while leaving intact afferent fibers entering this region; the area of cortex exposed to the action of KA does not lose its ability to be impregnated by Golgi's method, so that new opportunities are provided for the study of interneuronal connections.

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