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Postnatal Neuronal Plasticity of the Pyramidal Cells of CA1 Area of the Hippocampus as a Reaction to Neurotoxic Damage

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Summary

Kainic acid (KA) was injected into both lateral ventricles of the brain of adult laboratory rats with the aim of verifying whether damage to afferent fibres in the hippocampal CA1 area would also be reflected in changes in the dendritic arborization of the neurones after maturation of these structures was completed. A significant proportion of the afferent fibres ending in area CA1 comes from CA3-4. The neurodegenerative effect of KA on the neurones in CA3-4 thus leads to marked reconstruction of the dendritic network of the pyramidal cells in the CA1 area. In the CA1 area of the experimental animals, there are fewer segments in the proximal part of the basal dendrites and in the lateral branches of the apical dendrites. The total number of segments in the apical dendrites is smaller and the higher order segments are likewise reduced. In the experimental group, the segments of both the basal and the apical dendrites are shorter. In the experimental animals, dendritic spine density in the lateral preterminal branches, the distal part of the apical shaft, the terminal segments of the lateral branches and the apical preterminal branches are smaller than in the controls, whereas in the segments proximal to the soma of the pyramidal cells it is greater. It can be seen from the results that area CA1 of the hippocampus is endowed, even in adulthood, not only with high functional plasticity, but also with surprisingly high morphological plasticity.

Key words

Kainic acid – Neuronal plasticity – Hippocampus – Morphology of dendrites and spines – Transsynaptic stimulation

Introduction

Owing to the regular distribution of its neurones and their dendritic fields and to thorough descriptions of its afferent and efferent pathways, the hippocampus is becoming an ideal model for studying general neuronal functions (Pokorný 1982).

The aim of this study was to verify to what extent damage to the afferent fibres in CA1 area of the hippocampus would be reflected in changes in the

structure of the dendritic networks of the neurones if it occurred after maturation of these structures was completed.

Kainic acid (KA), a partial structural analogue of glutamate, is a chemical used in neurobiology for its neurodegenerative effects after local (i.e. chiefly intracerebroventricular) and systemic administration (McGeer *et al.* 1978, 1981). The sensitivity of hippocampal structures to the action of KA increases parallel to the maturation of glutamatergic systems, in particular glutamatergic synapses and receptors (Wolf and Keilhoff 1984). The main mechanism of the effect of KA is evidently binding to the presynaptic membrane receptors of glutamatergic and aspartatergic synapses, which leads to an increase in permeability for Ca^{2+} ions (the opening of Ca^{2+} channals). The result is depolarization of the membrane, followed by the release of excitatory amino acids, aspartate and glutamate, which are an important component of KA toxicity (Pastuzsko *et al.* 1984). Conversely, striking limitation of the toxic effect on the hippocampal region CA3-4 and the relative resistance of other parts of the hippocampus with glutamatergic transmission (CA1 area) after the intracerebroventricular administration of KA indicate that further mechanisms probably participate in the neurotoxic effect of this substance (Franck *et al.* 1988). The ependymal cells in the ventricle do not display any discernible signs of degeneration after the administration of KA (Wolf and Keilhoff 1984). That means that it is not direct contact with the target cell that is decisive for the toxic effects of KA, but the presence of specific receptors in the cell membrane and excitability of the latter. Olney (1979) ranked KA among the "excitatory" amino acids, which, when present in excess, kill the nerve cell by over-exciting it.

In the present study we used KA to destroy the population of large neurones in the CA3-4 hippocampal area and thereby to eliminate a significant portion of afferent connections ending in the CA1 area. The pyramidal cells in CA3 project (mainly by way of Schaffer collaterals) to CA1, where their axons terminate on the dendrites of pyramidal neurones, chiefly in the stratum oriens, radiatum and lacunosum. A further important projection from CA3 terminates in the lateral nuclei of the septum by way of the fornix (Teyler and DiScenna 1984).

Material and Methods

Three experimental and three control male laboratory rats (Wistar strain, our own breed) were used for the experiments. At the age of 50 days, using a stereotaxic apparatus, 0.4 μg KA was injected into both lateral brain ventricles of the experimental animals and 60 days after the operation they were killed under ether anaesthesia. The intact controls, not subjected to surgery, were killed at the age of 90-120 days.

The brains of the control and the experimental animals were impregnated according to the Golgi-Cox method and frontal sections 150 μm thick were sectioned. With this staining method, 60 days after the operation, the injection canal was no longer distinctly detectable. The specimens were analysed with an optic computer microscope (Pokorný *et al.* 1988) and a search was made for whole, well-stained pyramidal cells in CA1 area of the hippocampus.

We measured the length and determined the number of segments in the basal and apical dendrites and the total length of the dendritic tree in ten cells from the experimental animals and ten from the control group.

We also determined the number of dendritic spines per 10 μm dendrite in ten parts of the dendritic tree (Fig. 1).

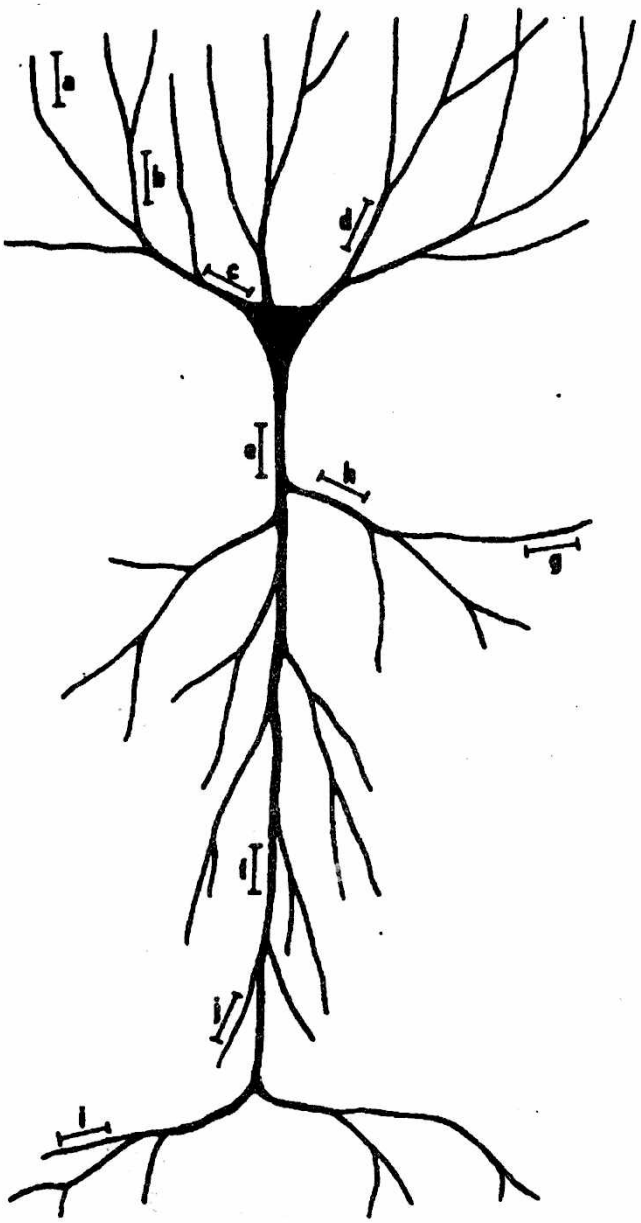

Fig. 1
Scheme of arborization of a typical pyramidal cell in CA1 area of the hippocampus, showing the area in which dendritic spine density was evaluated. a) basal terminal dendrites, b) basal preterminal dendrites, c) 1st order basal dendrites, d) 2nd order basal dendrites, e) shaft, proximal, f) shaft, distal, g) 2nd order and further apical lateral dendrites, h) 1st order apical lateral dendrites, i) apical terminal dendrites, j) apical preterminal dendrites.

The data were processed statistically and evaluated by the t-test. A three-dimensional reconstruction of the dendritic arborizations of individual neurones was carried out at the same time (Fig. 2).

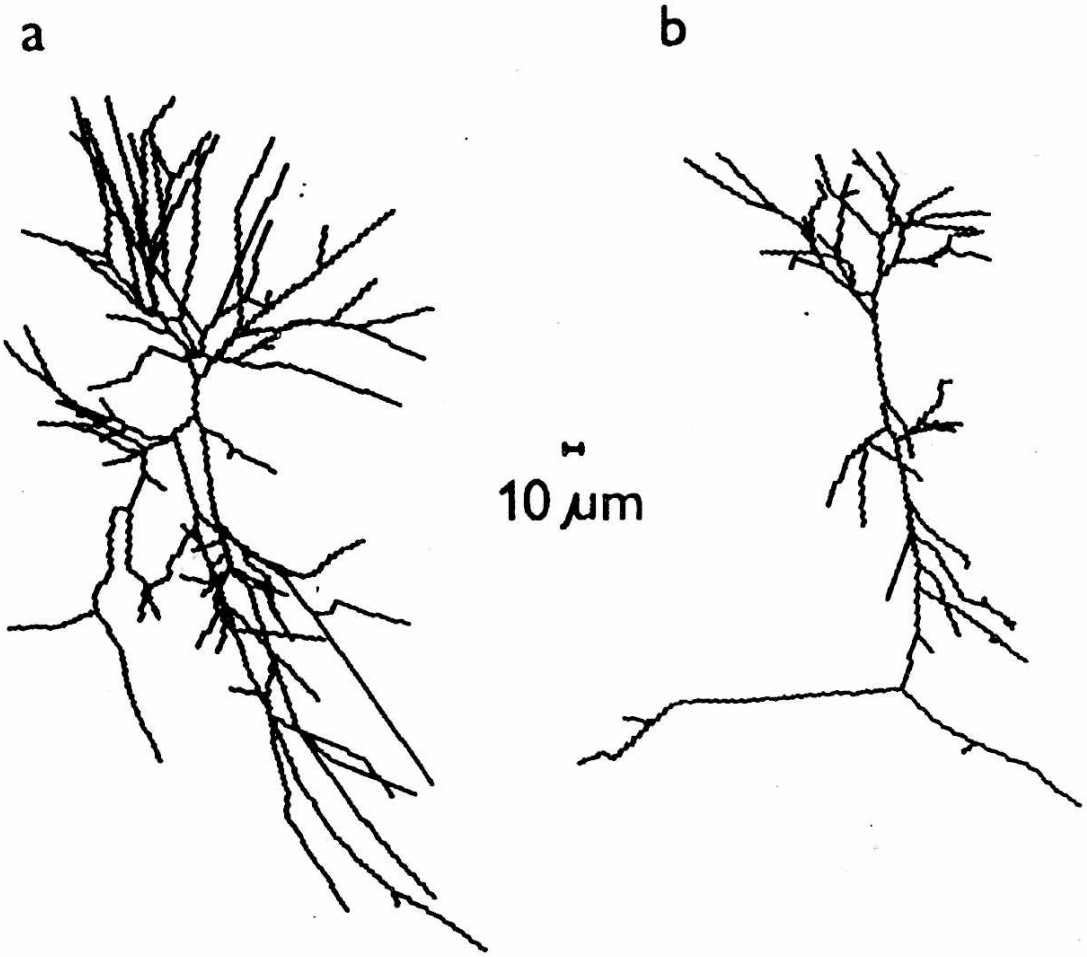

Fig. 2
Three-dimensional reconstruction of dendritic arborization of pyramidal cells in CA1 area of the hippocampus. a) neurone of a control animal, b) neurone of an animal in which kainic acid was injected into both lateral ventricles. The scale corresponds to 10 μm.

Results

The intraventricular injection of KA eliminated the great majority of large neurones in the CA3-4 hippocampal area.

Among the pyramidal cells in CA1 area of the hippocampus, differences in the number of segments were manifested in 4th and 5th order ($p<0.01$), 10th order ($p<0.05$) and 11th order ($p<0.01$) apical dendrites (Fig. 3) and in the 1st order ($p<0.01$), 2nd order ($p<0.05$), 3rd, 4th and 5th order ($p<0.01$) and 6th order ($p<0.05$) basal dendrites (Fig. 4). In both cases the values were lower in the group of experimental animals.

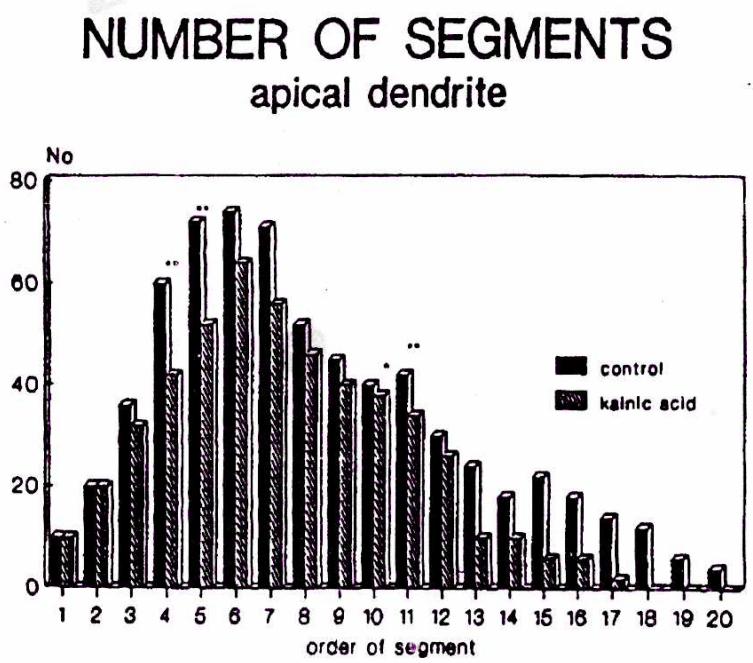

Fig. 3
Number of segments in the apical dendrites in the experimental animals and the controls.

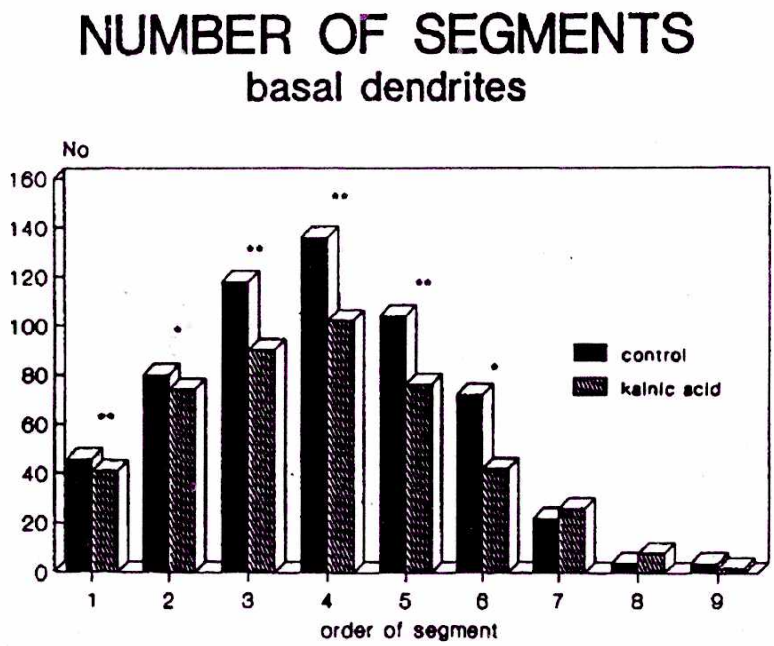

Fig. 4
Number of segments in the basal dendrites in the experimental animals and the controls.

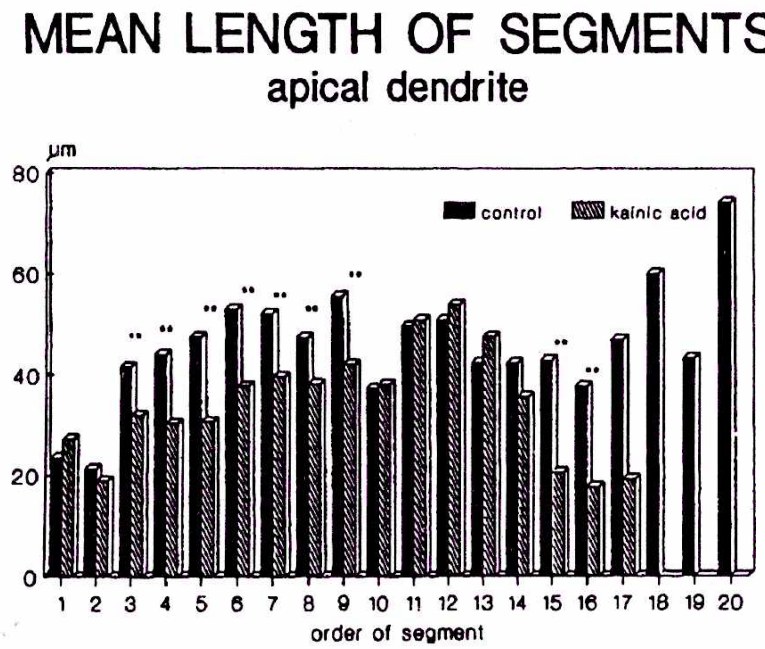

Fig. 5
Mean length of the apical dendrite segments in the experimental animals and the controls.

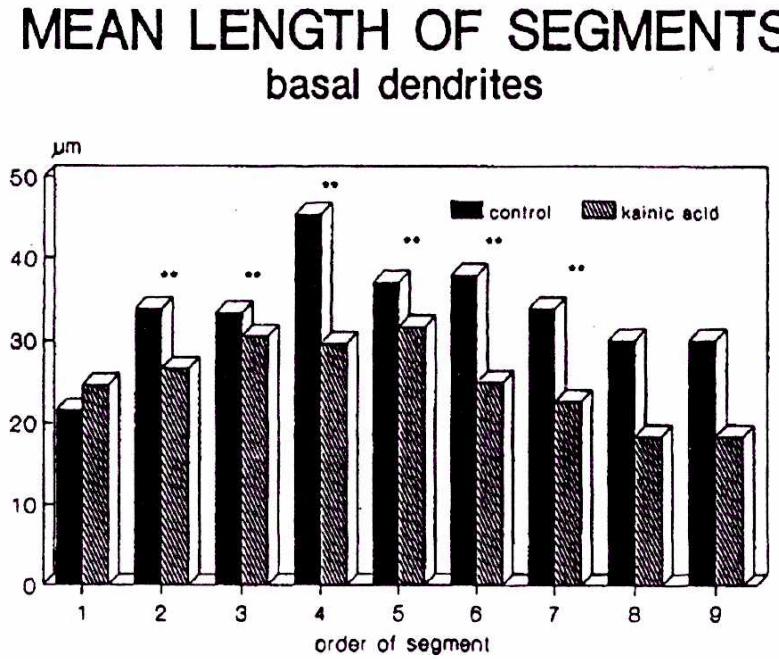

Fig. 6
Mean length of the basal dendrite segments in the experimental animals and the controls.

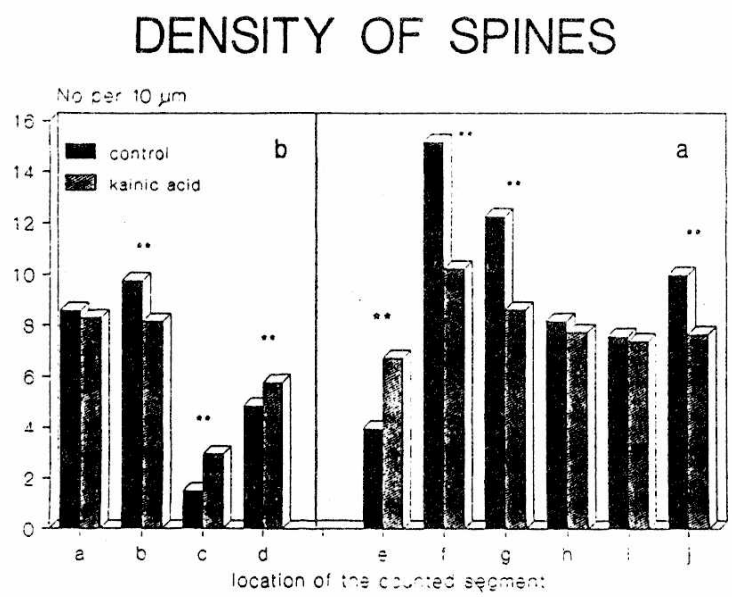

Fig. 7
Number of dendritic spines per 10 µm dendrite in the experimental animals and the controls. For the evaluation scheme see Fig. 1.

Differences in mean segment length were demonstrated in 3rd to 9th and in 15th and 16th order special dendrites ($p<0.01$) (Fig. 5) and in 2nd to 7th order basal dendrites ($p<0.01$) (Fig. 6). In both cases the values were again lower in the experimental animals.

The controls showed a constant incidence of higher order (17th and upwards) apical dendrites, but none were demonstrated in the experimental animals.

In the experimental rats, greater dendritic spine density was demonstrated in the region of 1st and 2nd order basal dendrites ($p<0.01$) and the proximal part of the shaft of apical dendrites ($p<0.01$). Fewer spines – compared with the controls – were found in the experimental animals in the region of the preterminal branches of the basal dendrites ($p<0.01$) and the distal part of the shaft of the apical dendrites ($p<0.01$), its lateral branches from 2nd order upwards ($p<0.01$) and its preterminal branches ($p<0.01$) (Fig. 7).

Discussion

The dendrites in the rat hippocampus branch and grow up to about the 24th day of postnatal life (Pokorný *et al.* 1981). Dendritic spine density also changes; it attains the maximum between the 25th and 30th day of life and then falls (Pokorný *et al.* 1981, Juraska 1982). Persistence of the dendritic tree is dependent on afferentation (Jones *et al.* 1982, Hámori 1973). Commissural and association fibres can already be demonstrated in the hippocampus in the first week after birth (Pokorný 1981). Dendrites stripped of afferent fibres during development or in

adulthood are destroyed or begin to turn towards a substitute source of afferent input (Berry *et al.* 1980). The existence of dendritic spines is further dependent on transsynaptic stimulation. The destruction of afferent systems also leads to loss of spines in adult animals (Hámori 1973). The response of the complicated systems of neuronal circuits to injury is by no means confined to the site of local brain damage, but also includes the reorganization of neuronal connections in more remote areas (Nadler *et al.* 1978). It is likewise assumed that there is a given deafferentation threshold value which must be overstepped before synaptic reconstruction can be initiated (Hoff 1986).

In bilateral intracerebroventricular administration, KA induced necrosis of the nerve cells in the CA3-4 hippocampal area and thereby impaired the afferent supply to the CA1 area, especially in the stratum oriens, radiatum and lacunosum. We likewise cannot rule out the possibility that KA damages the metabolism of nerve cells in the CA area directly, although we failed to find any signs of degenerative changes there.

The regions in which, in experimental animals, we demonstrated a reduction in the number of dendritic spines compared with the controls corresponded roughly to the stratum oriens, radiatum and lacunosum, i.e. to the layers in which afferentation from the inferior part of the hippocampus (CA3-4 area) plays an important role.

Conversely, in the proximal parts of the dendritic tree we found greater dendritic spine density, testifying to a compensatory increase in afferentation from diencephalic structures – evidently from the septum or from layers II and III of the entorhinal cortex.

We also recorded generally poorer branching of the apical and basal dendrites of cells in the experimental animals and shortening of the mean length of the individual dendritic segments. These changes correlate with the presumed destruction of branches stripped of afferentation.

The results thus unequivocally demonstrated that the pyramidal cells in CA1 area of the hippocampus are endowed not only with high functional plasticity (Landfield *et al.* 1986), but also, surprisingly, with high morphological plasticity even in adulthood. These findings must therefore be taken into account when interpreting certain electrophysiological and behavioural changes in animals to which kainic acid had been administered.

However, it likewise follows from the findings that the morphological plasticity of the adult brain – and hence its ability to re-form and switch neuronal pathways – is much greater than has hitherto, in general, been supposed. This mechanism evidently plays an important role in repair and regeneration processes in the central nervous system and it is even possible that it influences processes taking place in and around transplanted brain tissue.

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