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Morphological Changes of the Presynaptic and Postsynaptic Element in Excitatory Synapses during Kindling

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Summary

In a previous study of a kindling model using stimulation of the entorhinal cortex we found a redistribution of synaptic vesicles into the close vicinity of the active zone of synapses of Type I (Gray 1959) in the hippocampal gyrus dentatus. In this paper, ultrastructural studies of the same model are being continued using planimetry of the synaptic apparatus. A significant increase of the postsynaptic apparatus, area enlargement by 53 %, increase of the perimeter by 28 % and shape irregularity are being reported. No changes in shape or in size have been demonstrated in presynaptic structures or in the morphology of presynaptic mitochondria. These findings are discussed in relation to increased functional readiness of the synapses as signs of active reconstruction of the synaptic apparatus.

Key words

Kindling – Hippocampus – Synapse – Postsynaptic element – Presynaptic bag

Introduction

The mechanism and the significance of the kindling phenomenon for the function of the nervous system still remains obscure (Goddard *et al.* 1969, Goddard and Douglas 1975). In analogy to the problems of potentiation (Bliss 1979), three basic problems are mainly discussed, i. e. whether kindling proceeds in the presynaptic or in the postsynaptic nervous configuration or whether it occurs through an inhibitory mechanism. Contrary to most published studies indicating that there are no basic changes in the structure of active nervous cells during the course of kindling (e.g. Racine *et al.* 1975, Goddard and Douglas 1975) we succeeded in showing very substantial changes during the initial phases of kindling (Langmeier *et al.* 1980, 1983). To follow up this finding, we decided to analyse excitatory synapses in the hippocampal dentate gyrus in the classical kindling model by stimulation of the entorhinal cortical area. Experimentally we thus utilized the anatomically well-defined entorhinodentate pathway (Nastad 1967, Andersen *et al.* 1966a, b, Andersen 1975, Lömo 1971a, b).

In a previous paper we have shown a marked redistribution of synaptic vesicles into the vicinity of the synaptic cleft "a shift into a strategic position" two weeks after termination of the stimulation. At such a time the increased excitability of the tissue still persists. The relative number of vesicles in the described location increases, although their absolute number remains unchanged (Hovorka *et al.* 1989).

We wanted to ascertain whether some other changes could be demonstrated under the same experimental conditions in the structure of excitatory synapses. Planimetric evaluations of involved structures were therefore made.

Methods

Ultrastructural analysis of synaptic structures was performed in two experimental and two control adult Wistar rat males from our own breed, weighing 230-250 g. All surgical procedures were conducted under pentobarbital (Nembutal R, 50 mg/kg i.p.) anaesthesia. A double-barrel stimulation electrode was inserted into the entorhinal cortex of the right cerebral hemisphere whereas the registration electrode was introduced into the dentate gyrus of the left, i.e. contralateral hippocampus. The homolateral dentate gyrus was left intact for electron microscopic analysis. Electrodes were introduced under electrophysiological

control using the coordinates of Fifková and Maršala (1960). Their localization was verified by histological examination after the termination of the experiment. All electrodes were attached to a connector and fastened to the skull with dental acrylic Duracrol R Spofa. Stimulation of the right entorhinal cortex with 1-ms monopolar rectangular pulses of 50 Hz frequency lasting 1 s was started 3 weeks after the operation. Animals were stimulated daily at regular 24-hour intervals. In the course of the experiment there was a gradual progress of the electrographic response in the registered area of the contralateral dentate and also a progress of paroxysmal behavioural responses up to stage 5 after Racine (Racine 1972). The stimulation was terminated after three subsequent stage 5 seizures. Two weeks later the brains of the experimental animals were perfused transaortally with Karnovsky's solution (Palay and Chan-Palay 1974) under ether anaesthesia. In control animals, the electrodes were stereotactically introduced only; all manipulations were the same as with the experimental animals except that they were not stimulated.

For the electron microscopic analysis the tissue was prepared in a standard manner (Langmeier *et al.* 1983). Brain fixation was completed and the blocks containing all layers of the hippocampal system were cut from the right (homolateral) hemisphere at the anteroposterior level from 2 to 3.5 mm. Postfixation of the blocks was accomplished with OsO_4 followed by contrasting with uranyl acetate, the dehydration and embedding in Epon 812. Ultrathin sections of standard thickness were prepared with Tesla BS 490 A ultramicrotome in such a way that they contained the stratum moleculare of the dentate gyrus. Sections were stretched onto nets with a Formvar film and were contrasted with uranyl acetate and lead citrate treatment.

Randomly selected axo-dendritic (axo-spine) synapses of Type I after Gray (1959) from the central area of stratum moleculare of the right homolateral dentate gyrus were photographed with an electron microscope Tesla BS 500 at an enlargement 32 000 x. Total enlargement of the positive photographs was 96 000 x. From experimental animals 153 synapses and from control animals 142 synapses were evaluated.

With the use of Morphomat 30 planimeter Opton the parameters of surface area A, perimeter P, maximum diameter D_{max}, minimum diameter D_{min} and the Form factor were evaluated in presynaptic and postsynaptic structures and in the mitochondria of presynaptic structures (Fig. 1). Furthermore, the distribution of presynaptic and postsynaptic structures was evaluated with respect to the orientational angle formed by the maximum diameter D_{max} and the plane of the active zone (Fig. 2). For statistical evaluation the t-test and χ^2 test were used.

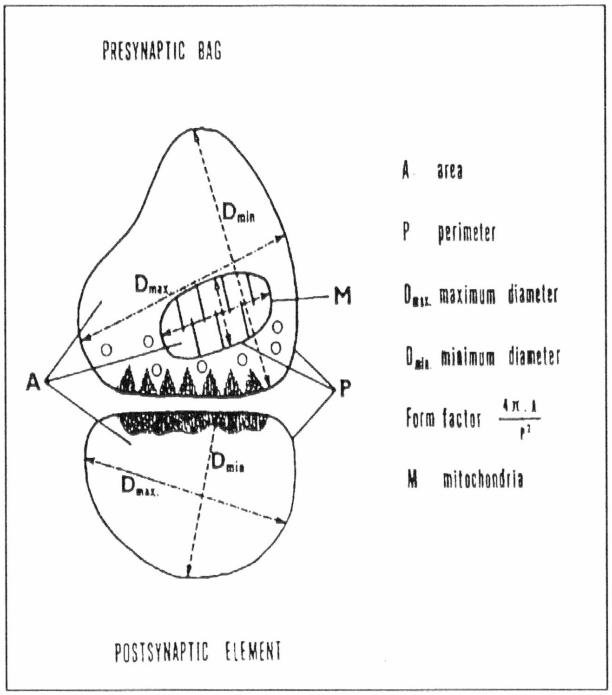

Fig. 1.
Evaluation scheme for individual ultrastructural parameters of cortical synapses.

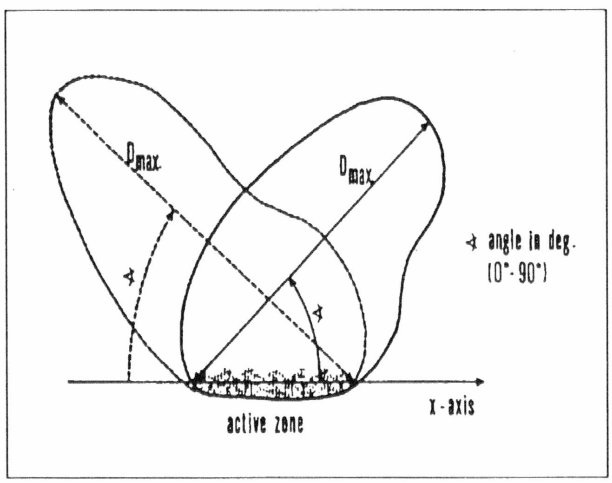

Fig. 2.
Evaluation scheme for angle of orientation. Presynaptic and postsynaptic structures were evaluated with respect to the orientational angle formed by the maximum diameter D_{max} and the plane of the active zone.

Results

No significant differences of the studied parameters were found in presynaptic structures and their mitochondria between experimental and control animals (Fig. 3, 4) as evaluated planimetrically.

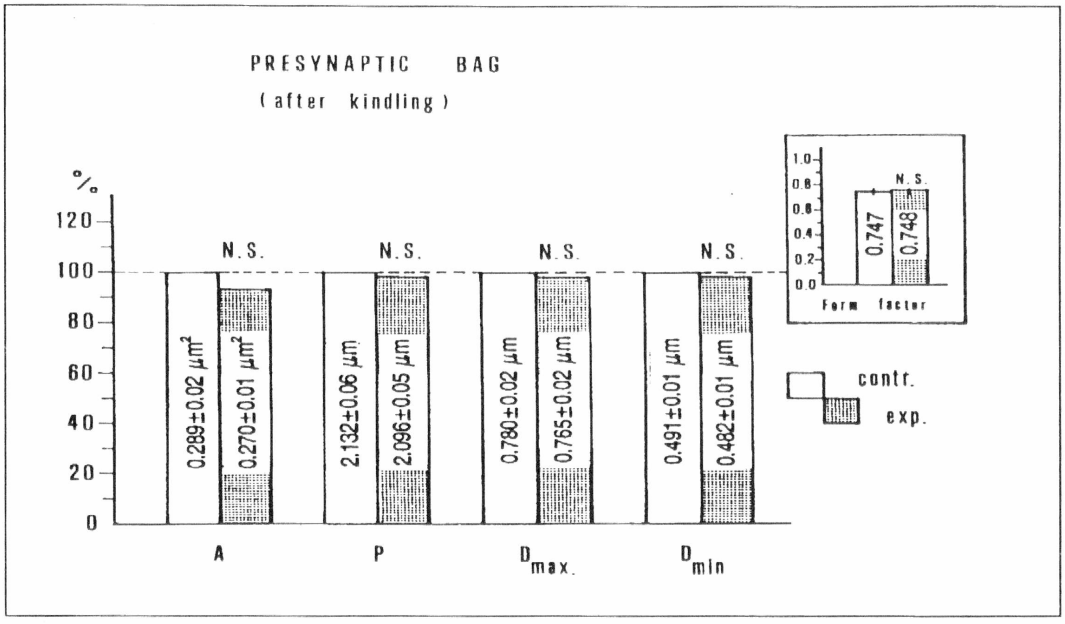

Fig. 3.
Changes in the chosen presynaptic bag parameters. (A) section area, (P) perimeter, D_{max} maximum diameter, D_{min} minimum diameter, form factor ($4p.A/P^2$). Percentage differences and mean absolute values (M ± S.E.M.) are given. See Fig. 1 for evaluation scheme.

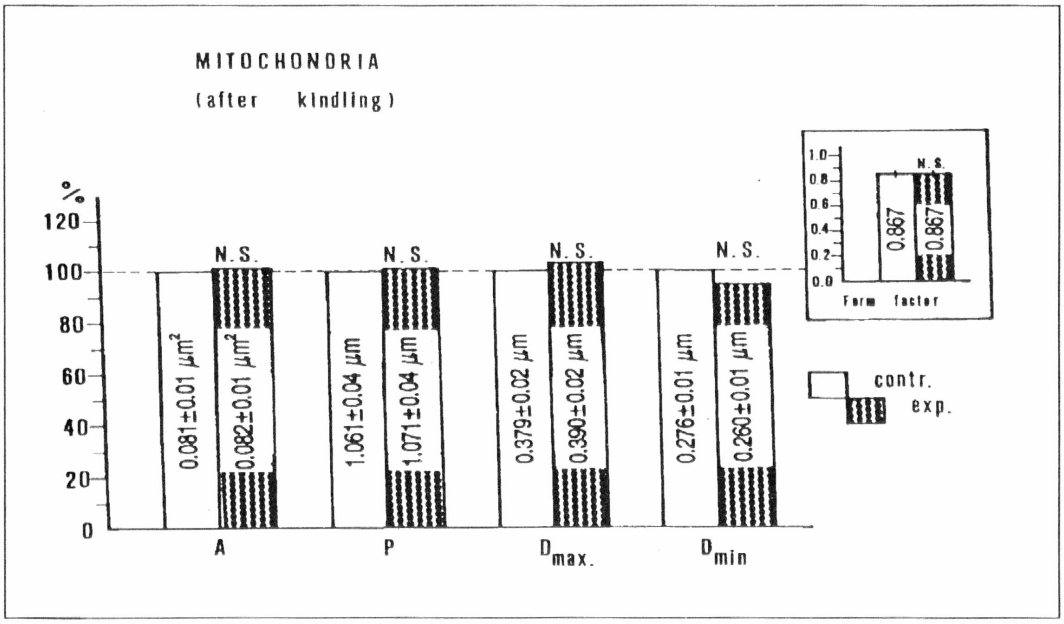

Fig. 4.
Changes in mitochondrial test parameters in the presynaptic bag. (A) section area, (P) perimeter, D_{max} maximum diameter, D_{min} minimum diameter, form factor ($4p.A/P^2$). Percentage differences and mean absolute values (M ± S.E.M.) are given. See Fig. 1 for evaluation scheme.

However, statistically significant changes in the postsynaptic structures were observed in the experimental animals. The area increased from 0.149 lm^2 to 0.228 lm^2, an increase of 53 % ($p < 0.001$), the perimeter increased from 1.566 lm to 2.000 lm, an increase of 28 % ($p < 0.001$), and the form factor changed from 0.714 to 0.663 ($p < 0.01$). Postsynaptic structures were thus enlarged and their shape became more irregular (Fig. 5).

No significant differences were found between the experimental and control animals as regards the distribution of presynaptic and postsynaptic structures according to the orientation angles (Fig. 6, 7).

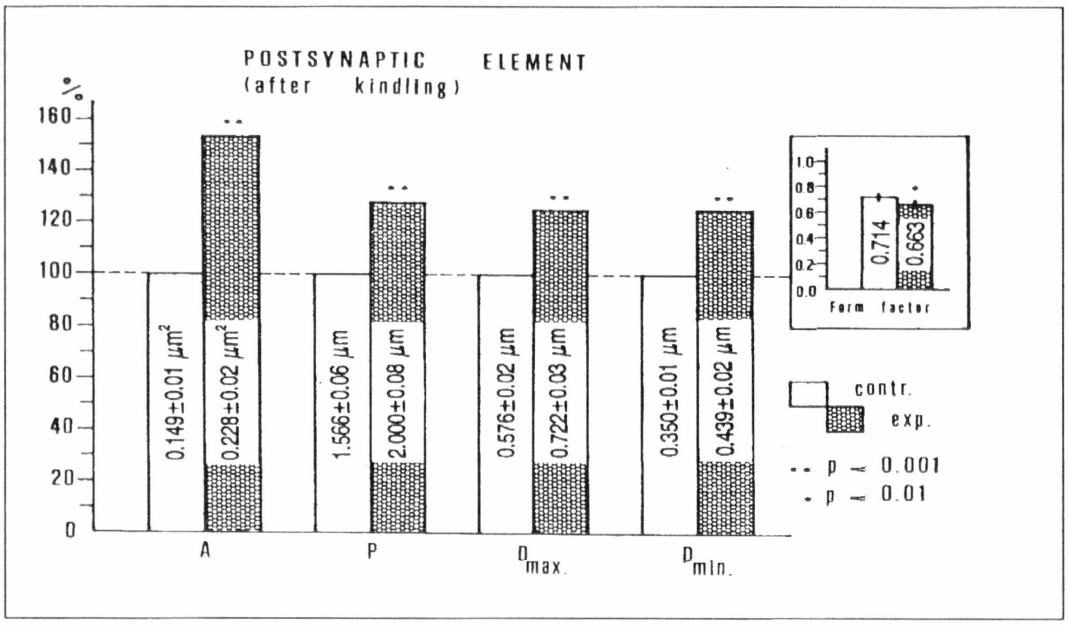

Fig. 5.

Changes in postsynaptic element test parameters. (A) section area, (P) perimeter, D_{max} maximum diameter, D_{min} minimum diameter, form factor ($4p.A/P^2$). Percentage differences and mean absolute values (M ± S.E.M.) are given. See Fig. 1 for evaluation scheme.

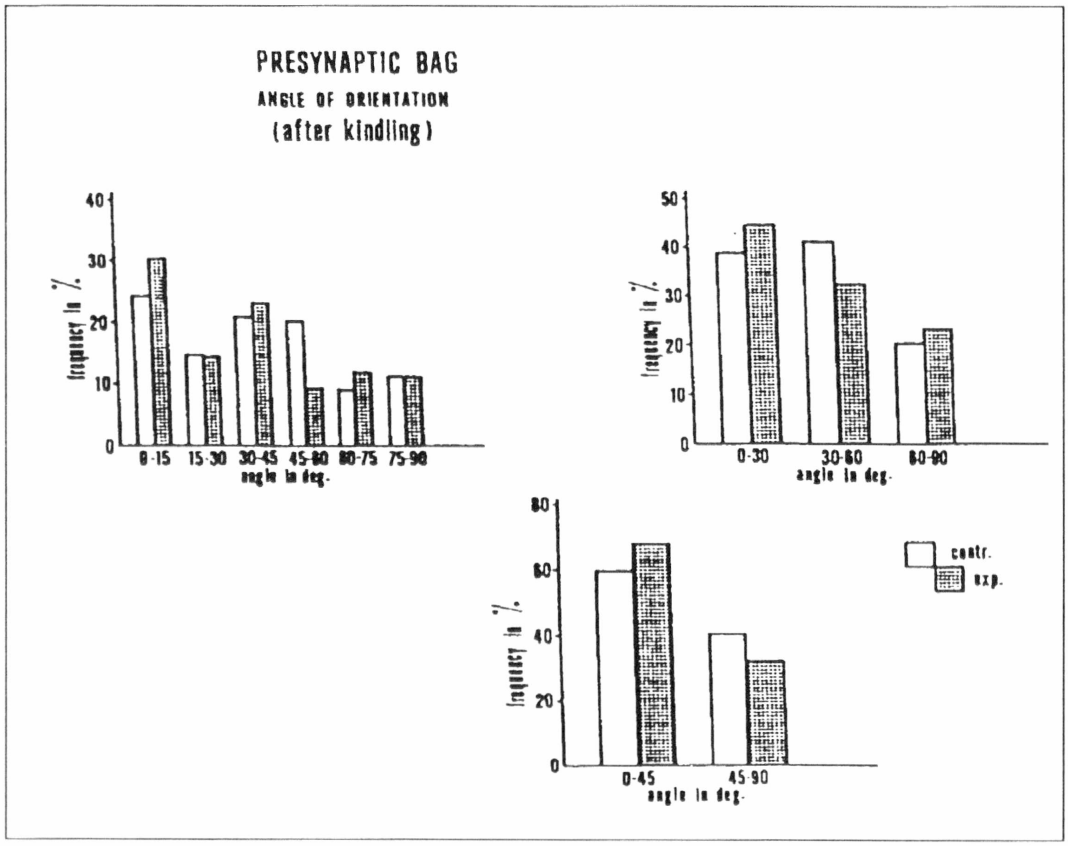

Fig. 6.

Percentage distribution of presynaptic bags according to angle of orientation. Differences in the distribution of the synapses are not statistically significant. See Fig. 2 for evaluation scheme.

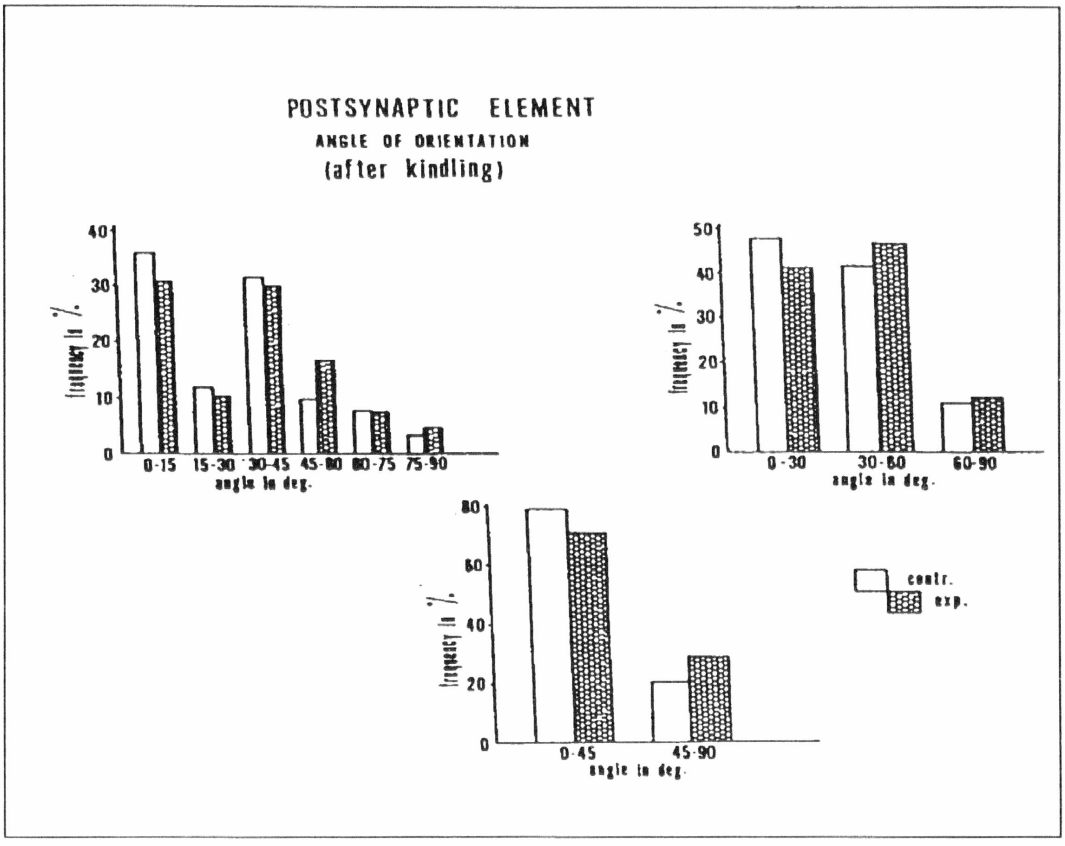

Fig. 7.

Percentage distribution of postsynaptic elements according to angle of orientation. Differences in the distribution of the synapses are not statistically significant. See Fig. 2 for evaluation scheme.

Discussion

Although the general opinion about the morphological basis of kindling is rather sceptical (Racine *et al.* 1975, Goddard and Douglas 1975, Brotchi *et al.* 1978, Crandal *et al.* 1979, Ribak 1986) we previously described (Langmeier *et al.* 1980a, b, Langmeier and Mareš 1984) a significant participation of the synaptic apparatus in increased excitability. To explore the initial phases of kindling, we studied the dynamics of the changes in the number and distribution of synaptic vesicles in the presynaptic terminal. There was a decrease in the number of vesicles in the presynaptic structure (Langmeier *et al.* 1983) one minute after the termination of a self-sustained after-discharge (SSAD), but we observed an increase of their number only after ten minutes and at the same time their redistribution into the vicinity of the active zone (Langmeier *et al.* 1980a). One hour after the termination of the seizure we could describe only changes in the size of the synaptic vesicles (Langmeier *et al.* 1986). We assume that during the initial phase of kindling there is some change in the distribution, but primarily a change in the number, size and shape of the synaptic vesicles.

Our findings concerning the classical model of kindling, however, show a completely different picture of the presynaptic apparatus of the excitatory synapses than that which can be observed only tens of minutes after the termination of the seizure. First, there is no change in the total number of synaptic vesicles two weeks after the last stimulation. It appears that there is "a shift into strategic position" by redistribution of the synaptic vesicles, as we have a relative increase in their number (Hovorka *et al.* 1989).

In the initial phases of kindling presynaptic structures in the rat cortex increased in size one minute after termination of SSAD (Langmeier and Mareš 1984). Simultaneously, we found that exocytotic and endocytotic activity in the presynaptic membrane increases and so does the postsynaptic apparatus and the number of mitochondria in the presynaptic structure. Ten minutes after SSAD the area of presynaptic structures and also the active zone of the synapses were increased (Langmeier *et al.* 1980b). These changes are being explained as a consequence of osmotic and/or metabolic changes (Dietzel *et al.* 1980, 1982). A similar acute swelling of dendritic spines was

observed in the hippocampus by van Harreveld and Fifková (1975) in a model of posttetanic potentiation in mice shortly after the termination of stimulation of the entorhinal cortex.

The changes in size of the presynaptic apparatus are also discussed as a part of the recyclization theory (Heuser and Reese 1973) in relation to recyclization of vesicles and of the activation of the synaptic membrane.

In amygdala kindling, Goddard and Douglas (1975) reported an increase in the size of synaptic terminals. Similarly, Racine and Zaide (1978) described an enlargement of presynaptic structures in the rat cortex two weeks after the termination of classical kindling. In our model there was no change of planimetric parameters and of the mitochondria in presynaptic structures of the excitatory synapses, but the postsynaptic structures were enlarged (area by 53 %, circumference by 28 %) and their shape became more irregular. These changes were found two weeks after the last stimulation, at which time acute metabolic and osmotic changes can be excluded. An additional mechanism may thus be involved in a more permanent rebuilding of the postsynaptic structure. An analogous enlargement of dendritic spines in the molecular layer of the mouse dentate gyrus was found by Fifková *et al.* (1982) four and ninety minutes after termination of tetanic stimulation of the entorhinal cortex. Applying anisomycin, a proteosynthetic inhibitor, fifteen minutes before stimulation, such enlargement of dendritic spines was not observed at four minutes, but occurred at ninety minutes at which time the inhibitory action of anisomycin did not persist any more. In a model of hippocampal kindling in the frog, Morrell *et al.* (1979) succeeded in abolishing the rogressive prolongation of a subsequent discharge and the development of spontaneous discharges with cycloheximide, another proteosynthetic inhibitor. This supports the view that proteosynthesis is involved in secondary epileptogenesis. Proteosynthesis and subsequent distribution of newly formed proteins into the spines are therefore possible factors in posttetanic swelling of dendritic spines. Some such mechanisms are perhaps involved in the kindling process and in the genesis of the described changes of postsynaptic structures, the participation of which is stressed in the development of the kindling phenomenon by Morrell *et al.* (1986). Pongracz (1985) hypothesized some theoretical possibilities of the modulation of synaptic transmission by changing the structural dimensions of the dendritic spine. He stressed the influence of morphological changes of the spines on the changes of their longitudinal resistance, electroresponsivity and regulation of the plasmatic flow of intracellular substances from the maternal dendrite into the vicinity of the synaptic active zone. Probably, an important regulative role of Ca^{2+} dependent mechanisms can be expected. The quantity or extended action of a mediator as well as the properties of dendrites can influence the excitatory postsynaptic potential (EPSP). Viewed differently, the changes of morphology of dendritic spines could influence the accessibility of the receptors for the transmitter substance in the synaptic cleft. Equally, changes of activity of contractile elements in the dendritic spines are able to modify their structural dimensions.

The significance of changes in size of the synaptic apparatus and their relation to hyperactivity remains unclear. We may conclude from our findings that the enlargement of postsynaptic structures need not just be a passive consequence of induced hyperfunction, but may, jointly with changes in the presynaptic structure, represent processes of active rebuilding of the synapse to serve increased functional demands.

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