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THESIS OF DISSERTATION FOR CANDIDATE DEGREE

THE GLYCINE LABELLING AND ITS APPLICATION IN THE CENTRAL NERVOUS SYSTEM

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The incorporation of amino acids into proteins of the central nervous system was studied in various functional states. The most typical experiment was the following: filter paper strip soaked in isotopically labelled amino acid solution was placed on some selected area of the cerebral contrex (or spinal cord) and kept there for 1—2 hours. During incubation the nervous structures, underlying to the applied amino acid, were stimulated in physiological way: the acoustic cortex was stimulated with click impulses or pure tones via the ears; the somatosensory cortex was stimulated with electric shocks applied to different points of the body, and the visual cortex was excited by flash stimuli given to one or both eyes. After the incubation period the cortical structures were excised, rapidly fixed in Karnowsky or Bouin solution and processed forther for light and/or electron microscopic autoradiography. The exposition time was 11 weeks.

Experiments were carried out also on the cerebellar cortex, hippocampus and spinal cord. Cats, rats and frogs were used in the experiments.

After application of labelled glutamic, aspartic acids and leucin a diffuse incorporation was found in case of glutaraldehyde fixation. The same was seen with glutamic, aspartic and gamma aminobutyric acids after Bouin fixation. Cellural localization of the incorporated amino acids was seen in case of leucin and glycine with Bouin fixative. Stimulation of the respective cortical area decreased the incorporation of leucine and enhanced the incorporation of glycine. For further studies therefore glycine was choosen, because its incorporation seemd to be in good correlation with the functional state. After Bouin fixation glycine remained incorporated only in nerve cells, while after glutaraldehyde fixation some labelling also in glial cell could be observed. First the effect of waking state was systematically studied on glycine incorporation into the cat cerebral cortex, then it was examined at different levels of anaesthesia. In waking state 32,5% of the neurons, stainable with haematoxylin-eosin, appeared labelled in the acoustic cortex of the cat, without any sensory stimulation. In light barbiturate anaesthesia this proportion was 20,7%, at medium depth of narcosis 10,1%, in chloralose anaesthesia this made 4,4%. In such spontaneous activity the labelling of upper layers was more intensive, that of the deeper (IV—VI) layers was less intensive than the overall proportion of labelled cells. This tends to show that impulses maintaining the resting activity of the cortex are mediated by the superficial layers (I—III).

Sensory stimulation resulted in an unaequivocal increase of labelling in the somatosensory, acoustic, visual and motor cortices, either.

In the somatosensory cortex stimulation of the skin of elbow or vibrissae led to significant labelling of layers IV—VI, especially of pyramidal cells in layer V and labelling of layer I—III became more intensive. In case of vibrissal stimulation the columnar organization of cortex was reflected by the autoradiographic labelling. Electrophysiological records taken from focal and extrafocal points of the somatosensory cortex were parallelled by the extent of glycine incorporation.

At very moderate electrical activity the motor cortex exhibited a considerable autoradiographic labelling in resting state. During stimulation of the ventrolateral nucleus (VL) of the thalamus triphasic evoked potentials appeared on the cortical surface. The glycine incorporation became enhanced in all cortical layers. During pyramidal tract (PT) stimulation the cortical electrical activity was depressed and glycine incorporation seemd to be restricted to some pyramidal cells and a small group of interneuron in layers II and III. This might be the sign of an irradiated cortical inhibition caused by the recurrent pyramidal axon collaterals. The experiment was performed both in barbiturate and chloralose anaesthesia and cell counts obtained with PT and VL stimulation gave an overall picture about extension of excitation and inhibition during the first steps of information processing.

Stimulation of the auditory cortex with pure tones in the frequency range of 0.33 to 30 kHz gave opportunity to construct the tonotopic map with the aid of the glycine labelling method.

Application of this procedure helped to differentiate two polysensory association areas in the suprasylvian gyrus of the cat.

During stimulation of the VA nucleus of thalamus a widespread labelling was encountered in the primary sensory, motor and associational areas of the cat cortex.

Stimulation of the inferior olive resulted in a well defined inhibition in the cerebellar cortex and in appearance of Purkinje cells in regular distanses.

Combination with the experimental paradigm of long term potentiation in the rat hippocampus led to the conclusion that the intensification of glycine incorporation in the dentate gyrus may be the morphological equivalent of the long term potentiation.

The glycine labelling method was successfully applied also for investigation of neuronal protein transport and of the information processing in the spinal cord.