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Incorporation of ^{3}H-Leucine into Brain Cells After Learning[1]

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POHLE, W. AND H. MATTHIES. *Incorporation of ^{3}H-leucine into brain cells after learning.* PHARMAC. BIOCHEM. BEHAV. 2(5) 573–577, 1974. – Immediately after a brightness discrimination ^{3}H-leucine was administered to rats intraperitoneally. One hour after injection the brains were prepared for microautoradiographical examination. In conditioned animals, as compared to the controls, the incorporation of leucine into neurons was increased in all structures of the hippocampal formation, in the visual cortex and cingulate cortex, whereas no increase in incorporation was found in other cortical structures as well as in thalamic and hypothalamic nuclei investigated.

Learning experiments Protein synthesis Leucine Labelled precursors Neurons Hippocampus Cortex

DURING and after acquisition of learning experiments an increased incorporation of precursors into the neuronal RNA of different brain regions has been observed by several investigators [1, 3, 6, 9, 13, 23], comparable findings being obtained in our own experiments on brightness discrimination in rats [20].

It can be assumed that this increased RNA synthesis may be followed by a change in protein synthesis indispensable in the consolidation of a memory trace. This hypothesis may be supported by the observations made for the amnestic effects of inhibitors of the protein synthesis administered during consolidation of memory [4,5].

To validate this assumption we investigated the incorporation of ^{3}H-leucine into the brain after the acquisition of a brightness discrimination in rats, which served in our work as a suitable learning model [17,19]. Microautoradiographic evaluation of the incorporation has been chosen to assess not only the general incorporation, but also the different participation of several brain structures in these processes.

METHOD

Male Wistar rats aged 11–12 weeks were trained in a Y-chamber to attain a brightness discrimination [19]. Immediately after having reached the criterion, 10 mCi of DL-4,5-^{3}H-leucine (Institute of Isotopes, Budapest, Hungary, specific activity 30 Ci/mmole) were administered intraperitoneally. The intraventricular injection was found to be unsuitable when using leucine, because the distribution in the brain was non-uniform. One hr after application the rats were decapitated, the brains removed, fixed in Formalin and prepared for microautoradiography [20]. Seven groups of animals, each containing a trained animal, an active and a passive control of equal body weight were investigated. The brains of one group were embedded in paraffin and sliced jointly in order to have identical conditions for microautoradiography. The silver grains were counted using coded microphotographs so as to avoid the experimental conditions in this procedure to be known. Figure 2 shows the brain structures investigated.

RESULTS

The smaller CA 1 and CA 2 pyramidal cells of the hippocampus showed less incorporated radioactivity than the CA 3 and CA 4 cells. However, in all 4 sectors of the hippocampus the pyramidal cells of the trained animals exhibited a significant increase in incorporation by 25 to 35% over the incorporation into the active controls. The latter, however, was only slightly elevated over that of the passive controls (Fig. 1).

A relatively high radioactivity was detected in the granular cells of the area dentata in the trained animals, the increase over the active controls amounting to nearly +50%. A significant increase was also revealed in the neurons of the cingulate cortex as well as of the dorsal cortex, this corresponding in our preparation to the frontal part of the visual cortex [14,21], when the trained animals were compared with the active controls, whereas no differences seemed to occur between the active and the passive controls. (In previous unpublished investigations during the runs of the learning experiment significant differences were found in labelling of gyrus dentatus, CA 3 and CA 4 sectors

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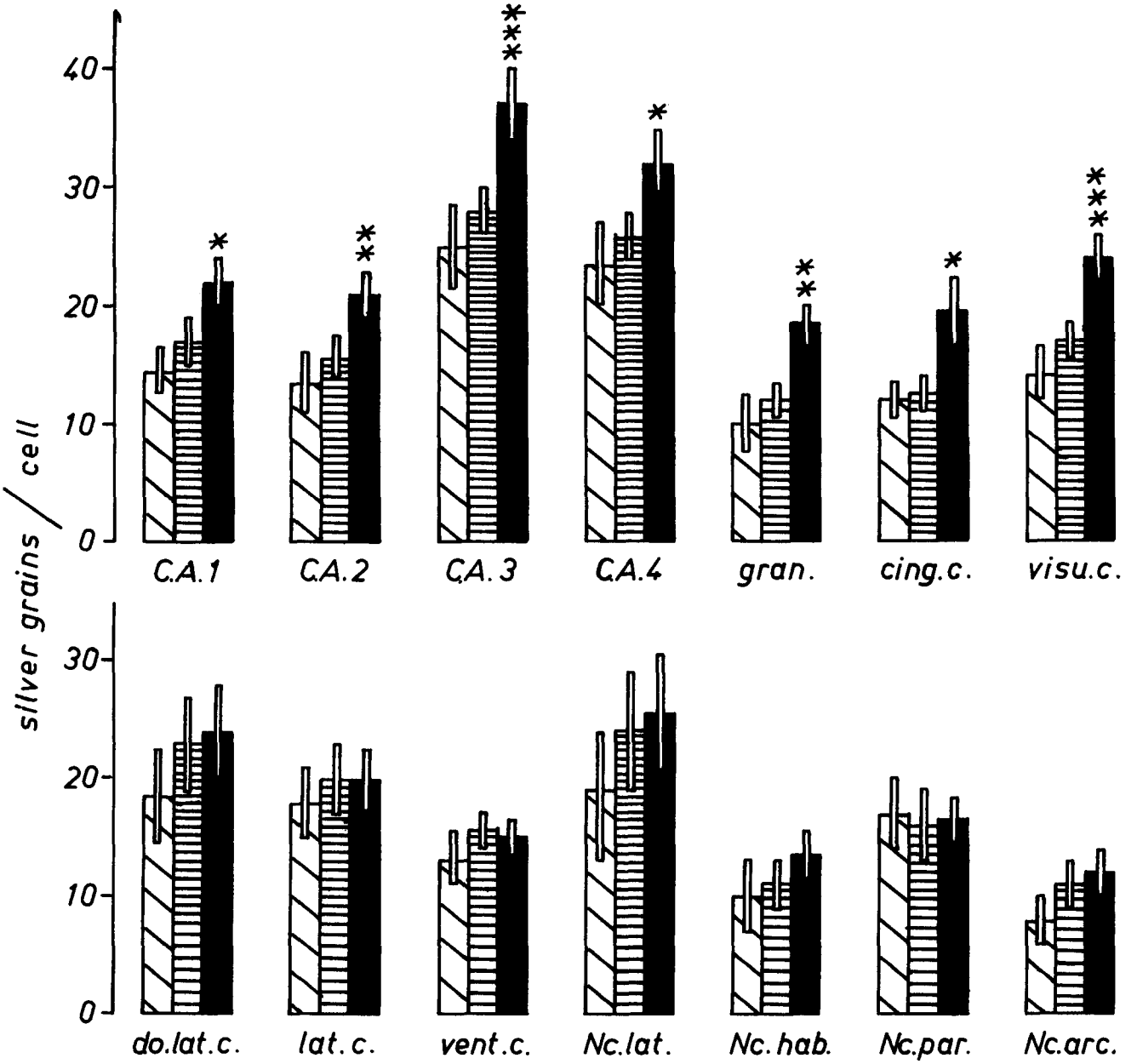

FIG. 1. Incorporation of ^{3}H-leucine into neurons of the rat brain immediately after a brightness discrimination. Leucine incorporation as silver grains per cell ±SEM ⁑ p 0.01; ⁂ p 0.02; * p 0.05 ■ = conditioned animals, ▤ = active controls, ▨ = passive controls, CA 1–4 = sectors of hippocampus, gran. = granular cells of area dentata, cing.c. = cingulate cortex, visu.c. = visual cortex, do.lat.c. = dorsolateral cortex, lat.c. = lateral cortex, vent.c. = ventral cortex, Nc.lat. = Nc. lateralis thalami, Nc.par. = Nc.paraventricularis, Nc. arc. = Nc.arcuatus.

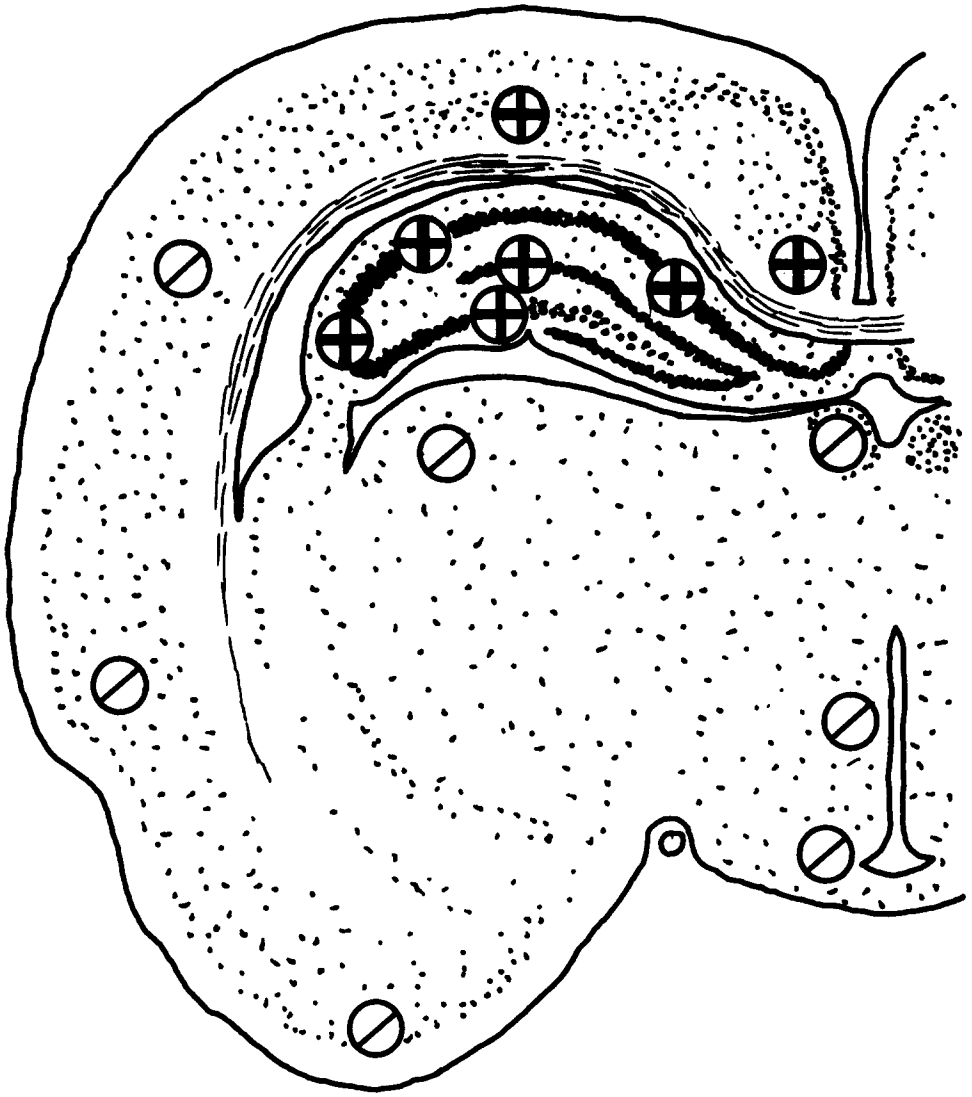

FIG. 2. Schematic representation of a rat brain slice showing the regions investigated in this paper.
⊕ = increased leucine incorporation due to the learning experiment
∅ = no increase in leucine incorporation

only.) No significant differences became evident in the dorsolateral, lateral and ventral cortices. Likewise, the labelling did not differ significantly in the nucleus lateralis thalami, nucleus paraventricularis and nucleus arcuatus. A small increase in the nucleus habenulae was not significant (Figs. 1 and 2).

DISCUSSION

Basically, the increase in the incorporation of ^{3}H-leucine after a learning experiment took place in the same neuronal cells of the rat brain, as the incorporation of labelled uridine monophosphate [20] or guanosine (own unpublished investigations) into the RNA increased under the same experimental conditions, only the differences being much more marked in the case of RNA incorporation. In our experiments we had proved the real incorporation of precursors into RNA by treatment of slices with RNAse. A similar proof is difficult to be performed in the experiments with incorporation of leucine. On the other hand, biochemical investigations into the incorporation of ^{14}C-leucine into the proteins of different brain regions showed significant increases in incorporation into both the hippocampus and the visual cortex. These results obtained with an other label and using biochemical methods, but under identical conditions of the learning experiment, are in reasonable agreement with the autoradiographic study and again emphasize the important role of the hippocampus

at least in this type of learning experiment [3, 4, 5, 7, 8, 10, 12, 13, 16, 18, 20, 22] and the particular participation of the visual cortex in our behavioral method.

An increase in the incorporation of amino acids into hippocampal neurons during a learning experiment was also found when using other models of learning or dissimilar species [2, 8, 16], even in the ganglion of insects [9].

During a learning experiment Kruglikov [15] observed a decrease in incorporation into the nuclei of hippocampal neurons in mice, but associated with their enlargement, and an increase in incorporation into the visual cortex. Labelling of the cytoplasm was not considered in this study. Some contradictions in the results of this kind of investigations appear to be quite understandable, as we have observed in our biochemical experiments phasic changes in the synthetic processes in the brain during consolidation after learning. Differences may also be due to the varying experimental conditions used by several workers.

In experiments with food reward [16] no marked differences of the specific activity of the precursor pool seem to occur. Even if in our investigations footshocks were applied to evoke the reaction as well as to punish incorrect responses, our active controls received the same number of footshocks as the trained animals. If the footshock would change the conditions determining the specific activity of the precursor, it should be relatively identical in both active controls and trained animals.

Moreover the determination of the specific activity of a precursor pool is a theoretical demand, which may be hardly accomplished in the most in vivo studies, because it makes necessary the measurement of both the intracellular concentration and radioactivity of the precursor. This general difficulty has to be overcome by a critical interpretation of the results and their completion by experiments with different methods. The determination of the specific activity in the soluble fraction of a homogenate seems not to be a satisfactory experimental solution, especially in neurochemistry, because the neuronal cell is an individual entity. The methods used in general in biochemistry, studying other tissues, should be regarded in this respect. We observed in trained animals neighbouring cells one type showing increased incorporation, the other type with unchanged labelling. This seems to indicate that changes in the functional properties of single neurons may be more likely than general changes in the extracellular environment of this small part of the brain.

The present results and those from previous papers [17,20] seem to confirm the assumption that the consolidation of a memory trace may be associated with the characteristic synthesis of RNA followed by formation of proteins in very distinct neuronal populations. This increase in macromolecular synthesis differs, at least quantitatively, from the changes occurring in the neurons of active controls receiving an equal number of stimuli and performing an identical number of runs in the Y-chamber, but without learning. Therefore, it is believed that the increased incorporation is not necessarily due to the stressful conditions of the experimental procedures. On the contrary, it seems more likely that in the course of learning approaching the criterion, the intensity of stress decreases as the number of punishments is reduced, thus mastering the environmental problem. If any effects of the stressful situation would become evident in terms of changes in incorporation, this should occur especially in the active controls. But even in these cell types exhibiting a marked increase of incorporation in trained animals, we found only small differences between active and passive controls. Further and more sophisticated investigations will be required to determine the properties of the proteins synthesized during the learning experiment.

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