Pharmacology Biochemistry & Behavior, Vol. 11, pp. 593–594. Printed in the U.S.A.

Autoradiographic Comparison of Neuronal and Glial Protein Metabolism in Rat Hippocampus after Food-Motivated or Footshock-Motivated Conditioning

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Received 12 February 1979

GLUSHCHENKO, T. S., L. Z. PEVZNER AND V. A. KLENIKOVA. *Autoradiographic comparison of neuronal and glial protein metabolism in rat hippocampus after food-motivated or footshock-motivated conditioning.* PHARMAC. BIOCHEM. BEHAV. **11**(5) 593–594, 1979.—Quantitative autoradiography has shown that initial food-motivated conditioning results in an increase in ^{3}H-phenylalanine incorporation into cytoplasmic proteins of rat hippocampal neurons. After 3 daily conditioning trials, the incorporation returned to an active control (pseudoconditioning) level while after 6 daily trials, the incorporation was decreased. No changes were revealed in the cells of hippocampal perineuronal glia. Four hours after a footshock-motivated passive avoidance trial, incorporation of ^{3}H-phenylalanine was increased both in the neurons and in their perineuronal glia of rat hippocampus. By the time of a consolidation of this conditioning, such increase still remained in the glia but disappeared in the neurons. Subsequently, no changes were found in the hippocampal neurons or in their perineuronal glia. An importance of the emotional background is outlined for a participation of glial cells in learning-induced metabolic changes in the nervous system.

Conditioning Motivation Proteins Neurons Glia Hippocampus

HIPPOCAMPAL neurons are particularly sensitive to a novelty factor of initial training [4]. This training can be either of a positive or of a negative emotional character. We have considered it interesting to compare protein metabolism in hippocampal neurons after conditioning based on an opposite character of motivation. Along with the neurons, their glial satellite cells were also analyzed because both the literature [4] and our earlier observations [6] indicated an involvement of neuroglial metabolism in a pattern of biochemical changes in the nervous system depending on particular conditions of the stimulation of neurons.

METHOD

Food-motivated conditioning was performed in adult male Wistar rats in a Y-maze according to Lashly's scheme [5]. The animals received food reinforcement for their runs to a lighted arm 10–20 sec after the light switched on. Left and right arms were alternated randomly. Initial trials lasted until 10 successive correct runs and consisted as a rule of about 120 combinations of the conditioned and unconditioned stimuli. Subsequent trials, with the same criteria, were repeated daily for 6 days. A pseudoconditioning group of rats was used as control, these animals getting the same amount of food and the same number of conditioned stimuli but the runs into either of the maze-arms were reinforced. The rats of both groups were sacrified after the first, third and sixth daily trial.

Footshock-motivated conditioning consisted in placing adult male Wistar rats into a large chamber neighboring a small and dark one [1]. Within about 3 min, all the animals entered the small compartment where they were stimulated for 1 min through the floor grid with 20 Hz frequency, 1 mA intensity electric current. Then the rats were returned to a vivarium where, as it was shown by Fedorov *et al.* [2], a consolidation of memory proceeds: on 6 hr-stay in the vivarium all the animals when placed again into the large chamber did not enter the small one. If the rats had been taken from the vivarium earlier than 6 hr after the trial only some of them entered the small chamber [2]. The animals were sacrificed 4, 6, 9, and 24 hr after the trial, simultaneously with the control (pseudoconditioning) group of rats. The latter were placed by force into the small chamber and received similar footshock.

All the rats were sacrificed by rapid decapitation without anesthesia. ^{3}H-2,3-DL-phenylalanine (Reanal, Hungary) with a specific activity of 64 mCi/ml was injected intraperitoneally in a dose of 8–10 mCi/g 1 hr before the

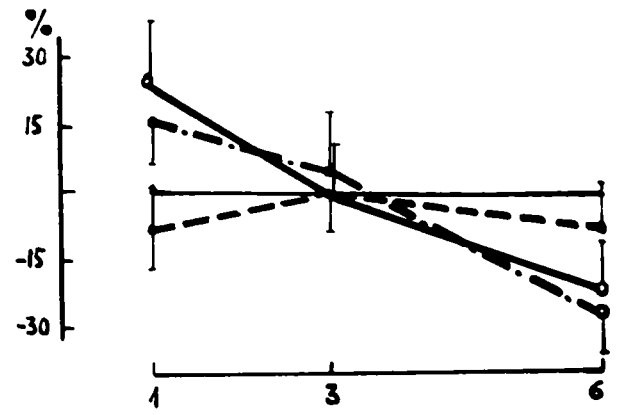

FIG. 1. Changes in ^{3}H-phenylalanine incorporation into rat CA_3 hippocampus neurons and perineuronal glia in the course of food-motivated conditioning. Ordinate shows percent deviation in Ag grain concentration (the number of the grains per area unit) in the rats submitted to conditioning as compared with the rats submitted to pseudoconditioning taken as 100%. Abscissa shows the number of daily conditioning trials. Solid line, the whole body of hippocampal neurons; dotted-broken line, nucleus of neurons; broken line, the whole body (in fact, nucleus) of perineuronal neuroglial cells. Vertical bars, standard error of the mean. Open circles, statistically significant ($p<0.05$) changes from the active control (pseudoconditioning) level; black circles, non-significant changes.

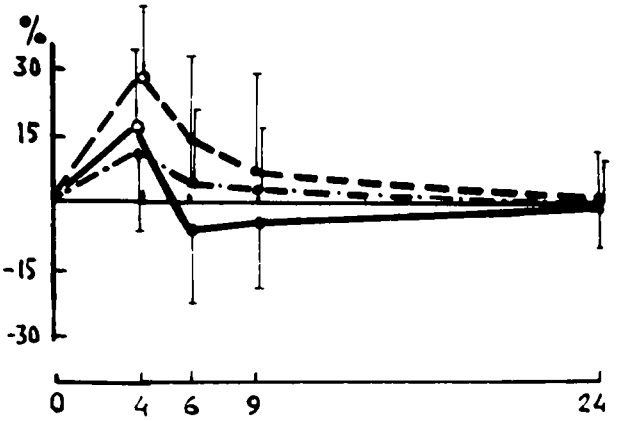

FIG. 2. Changes in ^{3}H-phenylalanine incorporation into rat CA_3 hippocampus neurons and perineuronal glia in the course of consolidation of one-trial footshock-motivated conditioning. Abscissa shows the time (hr) after footshock-motivated conditioning trial. All other designations are as those in Fig. 1.

sacrifice. The brain was quickly dissected, fixed in a chilled Carnoy mixture and embedded in paraffin. Five μm sections were coated with a liquid photographic emulsion and exposed for 40 days at 8°C. The developed autoradiographs were stained with hematoxylin. The number of silver grains per cell or per cell nucleus was determined by absorption method with the aid of the double-beam shearing microspectrophotometer MARSh. Details of the measurements and calculations were described earlier [3]

RESULTS AND DISCUSSION

As seen from Fig. 1, concentration of the label in the whole bodies of hippocampal neurons increased after the initial learning trial of the food-motivated conditioning but returned to the control (pseudoconditioning) level after 3 daily trials. After 6 such trials, a decrease in the labelling of proteins was revealed both in the body and in the nuclei of the neurons. No changes were observed in the glial cells throughout the experiment (Fig. 1).

A quite different pattern was found after footshock conditioning (Fig. 2). Four hours after the learning trial, concentration of the label was increased in the whole bodies of hippocampal neurons as well as in perineuronal neuroglia. By the moment of memory consolidation, this increase was preserved only in the glial cells. Nine and 24 hr after the trial, no differences from the pseudoconditioning level were seen in all cases (Fig. 2).

Pohle and Matthies [7] used an autoradiographic approach to compare neuronal and glial macromolecular metabolism in rat hippocampus after footshock-motivated discrimination learning. They found activation of RNA biosynthesis in hippocampal neurons without any changes in the cells of neuroglia. We obtained a similar pattern, as far as the protein metabolism is concerned, but in the case of food-motivated learning. Footshock-motivated learning in our experiments was characterized, on the contrary, by a number of metabolic changes in neuroglial cells, too. At present, it is only hypothetically that we think about some factors of an acute stress in the learning which provide for involvement of glial macromolecular metabolism.

In the present paper we did not describe the determinations of the label incorporation into the hippocampal cells of quiet (passive control) rats. All the changes revealed in the conditioning group of rats were compared only with those in the pseudoconditioning (active control) group. In this way, we tried to eliminate nonspecific changes resulting from stress, arousal, etc. The specific changes, i.e., the changes between conditioning and pseudoconditioning animals, were different in footshock-motivated and in food-motivated rats (cf., Fig. 1 and Fig. 2). Although the temporal dynamics of these two conditioning schemes is rather different, the other important factor which seems to result in the metabolic difference revealed is an emotional background of each particular kind of learning.

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