PHYSIOLOGY

Effect of a Short-Term Elaboration of a Local Food-Rewarded Instrumental Response on the Protein Content in Neurons of the Hippocampus

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UDC 616.8-009.81-02.613.2]-092-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 1, pp. 6-8, January, 1994
Original article submitted June 8, 1993

The protein content in neurons of areas CA1 and CA3 of the rat dorsal hippocampus is compared by cytointerferometry. The involvement of CA3 neurons is observed both during instrumental response conditioning and at delayed times after training, whereas neurons of the CA1 area are involved only at the earliest stages of conditioned response shaping: at the stage of formation of new motor coordination.

Key Words: *conditioning; hippocampal neurons; interhemispheric asymmetry; protein metabolism*

During the elaboration of instrumental food-rewarded conditioned responses (CR), protein metabolism in the neurons of the hippocampus is altered [1,7]. In previous studies the protein content has been found to be reduced following shaping of a local food-rewarded CR in the form of lever pressing with the preferred paw, a rather prolonged show of preference for one of the extremities (up to 23 correct movements out of 25) preceding the elaboration of the response [5]. Preference for one of the paws per se can markedly affect the protein content in neurons of the hippocampus and distort the effect of CR acquisition on this parameter.

The aim of the present study was to examine the CA1 and CA3 areas of the dorsal hippocampus of rats at the earliest stages of CR shaping, namely, training in retrieving food from a horizontal tube with the preferred paw on three successive takes.

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MATERIALS AND METHODS

The experiments were carried out on male nonpedigree rats weighing 150-200 g. Following a 48-h food deprivation, the preferred extremity was determined for retrieving seeds from a horizontal tube (13 mm in diameter) placed at a height of 5 cm from the floor. Just the rats (n=8) which used only one preferred paw were involved in the experiments [4]. Training was performed until seeds were retrieved from the tube with the same paw three times in a row. Eight control rats which picked up seeds from the floor (the group of passive controls - PC) were placed in the experimental chamber for the same period of time. (Elaboration of the CR of retrieving seeds from a horizontal tube with a preferred paw was described in detail elsewhere [4].) Four control and 4 experimental rats were decapitated immediately after the

0007-4888/94/0001-0001$12.50

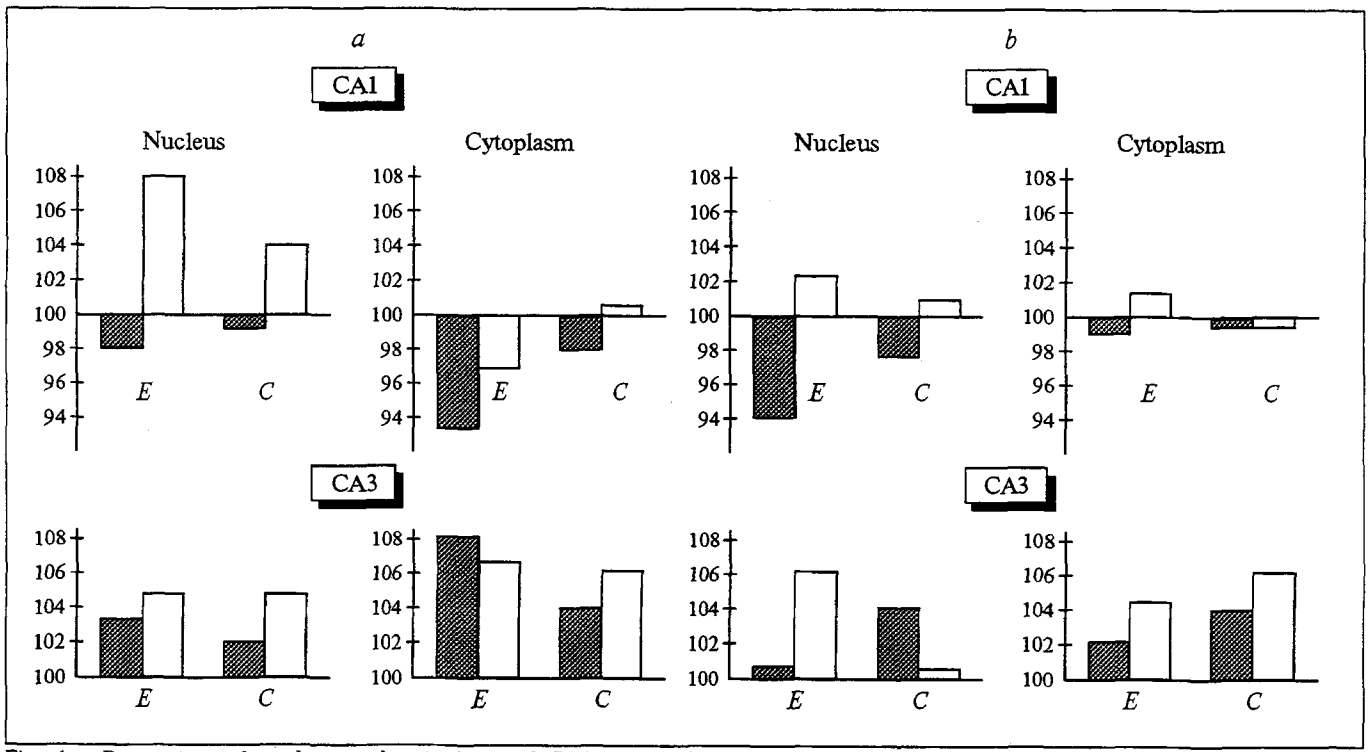

Fig. 1. Parameters of nucleus and cytoplasm of CA1 and CA3 neurons in hippocampus of rats decapitated immediately (*a*) and 2 weeks (*b*) after elaboration of a local food–rewarded instrumental CR in comparison with PC. Hatched bars: size of neuron nucleus and cytoplasm; light bars: dry weight of nucleus and cytoplasm. E: contralateral, experimental hemisphere; C: ipsilateral, control hemisphere (with respect to preferred paw). An asterisk denotes $p<0.05$ vs. PC.

session, and the remaining rats two weeks later, since it was previously established that, first, the preference can be definitely determined after 3 successful takes with the same paw and, second, such rats preferred using the same extremity even 2 weeks after the conditioning session [4]. The brain was fixed in Carnoy's fluid; the size of the nuclei and cytoplasm of the CA1 and CA3 neurons of the left and right hemispheres and the dry weight of the nuclei and cytoplasm of these neurons were determined in consecutive paraffin sections of the dorsal hippocampus with the aid of an MBIN-4 interference microscope [3]. A total of 3200 neurons were examined (16 rats). The results were statistically processed using Student's *t* test.

RESULTS

The results of cytointerferometric examination of neurons of the rat hippocampus are shown in Fig. 1. On the left (*a*) are the data obtained for immediate decapitation after the third retrieval of seeds; on the right (*b*) are the corresponding data obtained 2 weeks after the initial experiment. Here, the size of the nuclei and cytoplasm of neurons of the CA1 and CA3 areas and their dry weight (i.e., the protein content) are presented in percent of the values in the PC. A thrice-repeated retrieval of seeds with the preferred paw was attended by an increase of the dry weight of the nuclei of CA1 neurons in the ipsilateral hemisphere (for convenience defined by us, by analogy with the motor cortex, as control) and, especially, in the contralateral (experimental) hemisphere (with respect to the preferred paw) to 104.07 and 108.30%, respectively, as compared to that in the PC. At the same time, an interhemispheric asymmetry was observed, i.e., following a short-term performance of the motor habit, the changes of the dry weight of the nuclei of neurons of this area proved to be greater in the experimental hemisphere than in the control. The dry weight of the cytoplasm of the CA1 neurons was not reliably different from the PC, despite a markedly smaller size of the cytoplasm of neurons of the experimental hemisphere (92.62% of that in the PC). On the other hand, an interhemispheric asymmetry was discovered with respect to the dry weight of the cytoplasm of these neurons, which resulted from the nonuniformity of even these minor shifts. A slight increase of both the size and the dry weight of the nuclei was noted in the CA3 neurons of both the experimental ahd the control hemisphere (up to 104.18 and 104.12%, respectively, of the dry weight in the PC). In the experimental and control hemispheres an increase of the size (108.01 and 103.76%, re-

spectively) and dry weight (107.04 and 105.49%, respectively) of the cytoplasm of these neurons was observed as compared to those in the PC. The CA3 neurons in response to short-term shaping of the CR exhibited no interhemispheric asymmetry with respect to the main parameter (the dry weight), although a reliable asymmetry was observed with regard to the size of their nuclei and cytoplasm.

The dry weight of the CA1 and, notably, the CA3 neurons was shown to be reduced, vis-a-vis the PC, for multiple (and lasting for several sessions) lever pressings with the preferred paw. In addition, a reliable interhemispheric asymmetry was discovered in just the CA3 neurons of these animals [5]. It was also noted that in the large group of PC rats there were no differences between the right and left hemispheres with respect to the size and dry weight of neurons of the CA1 and CA3 areas. Similar results were obtained during examination of the hippocampal areas by Timm staining, microdensitometry, and quantitative image analysis, and in the CA1 and CA3 areas no right-left asymmetry was discovered with respect to these morphological parameters [8]. The presence of interhemispheric asymmetry with respect to the size or dry weight of the CA1 and CA3 neurons for the use of the preferred paw and its absence in the PC rats indicate that the above asymmetry (i.e., more pronounced shifts in the neurons of the experimental hippocampus as compared to the control) emerges as a response to elaboration of the new movement with the preferred paw. In addition, it should be mentioned that the findings on the increase in the dry weight of neurons of the two areas of the hippocampus after the third retrieval and the decrease in the dry weight of these neurons after multiple pressings of the lever [5] are in good agreement with the postulate that an increased rate of protein synthesis is characteristic of just the early stage of CR elaboration, whereas subsequent training which reinforces the already formed temporary connection is not accompanied by biosynthesis enhancement [2,6].

The results of studies of hippocampal neurons of rats decapitated 2 weeks after the thrice-repeated seed retrieval are presented in Fig. 1, *b*, which shows that by this time, following the elaboration of an instrumental CR, the dry weight of the nuclei and cytoplasm of the CA1 neurons in the experimental and control hemispheres differs little from the level in the PC (for the nuclei 102.20 and 100.56%, respectively). In contrast to the CA1 neurons, the CA3 neurons preserve the shifts in the dry weight at such delayed times: the dry weight of the nuclei and cytoplasm of neurons in the experimental hemisphere is 5.1 and 3.55%, respectively, higher than in the PC; in the control hemisphere only the dry weight of the cytoplasm is increased (by 4.52%). Comparison of the two parts of Fig. 1 (*a* and *b*) shows that the dry weight of the CA1 neurons, which increases by the third take, drops to the control level after 2 weeks. On the other hand, although 2 weeks after the session the dry weight of the CA3 neurons is reduced (especially for the nuclei of the control hemisphere neurons), it remains at a higher level than that in the PC. Following a long-term elaboration of the lever-pressing CR, the CA1 neurons also exhibit smaller shifts in the dry weight than do the CA3 neurons [5].

Hence, the CA1 neurons proved to be more functionally active at the earliest stages of acquisition of a local instrumental food response and only in the case where asymmetry with respect to the dry weight of the CA1 neurons was exhibited. The CA3 neurons exhibited asymmetry 2 weeks after a short-term elaboration of the instrumental response. The motor habits in the form of preference for one of the paws during seed retrieval from the tube were still preserved at this time.

Evidently, the neurons of the CA1 area (as well as the CA3 neurons) are involved in the formation of a new motor coordination, but, in contrast to the CA3 neurons, they make less of a contribution to the preservation of the motor habit.

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