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FUNCTIONAL BLOCKING OF THE HIPPOCAMPUS BY TARGETED NEUROIMMUNIZATION

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To create models of disturbances in the activity of different parts of the brain, including the hippocampus, as a rule the methods used are quite crude and are associated with brain tissue destruction. In this respect the method of neuroimmunization (NI), which in some cases allows targeted functional inactivation of different parts of the brain, possessing antigenic specificity, to be achieved [1]. This method is widely used in the creation of models of autoimmune diseases of the nervous system [9, 10] and also in experiments involving procedures directed toward brain-specific proteins [2]. The method is based on induction of an immune response of the body to endogenous "barrier" antigens (AG), by immunizing animals with homologous AG-material. This paper gives physiological evidence of the applicability of NI for functional blocking of the hippocampus.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats of different ages. Adult animals (males weighing 200-250 g) were immunized with tissue cytosol of the hippocampus (HP) or the convex surface of the neocortex (NC), twice at weekly intervals with 2 mg of total protein (in 0.5 ml) mixed with 0.05 ml of Freund's complete adjuvant. Young rats aged between 1 and 7 days received injections of 0.13 mg of the same AG-material without the adjuvant, and maintenance immunizations were carried out on the 35th and 48th days (1 mg each time without adjuvant) and the 86th and 93rd days (2 mg with 0.05 ml of adjuvant each time) after birth. The physiological effects of NI in the adult animals were assessed 7 days after the second immunization, in the "open field" (OF) test, twice for 5 min each time, separated by an interval of 1 h, and during the formation of a conditioned active avoidance reflex (CAAR) on six consecutive days, with 30 combinations of acoustic and electrical stimuli each time. The same OF test was carried out on the young rats (a circular arena 95 cm in diameter) at the age of 23 days, and CAAR formation was carried out one week after the last reimmunization.

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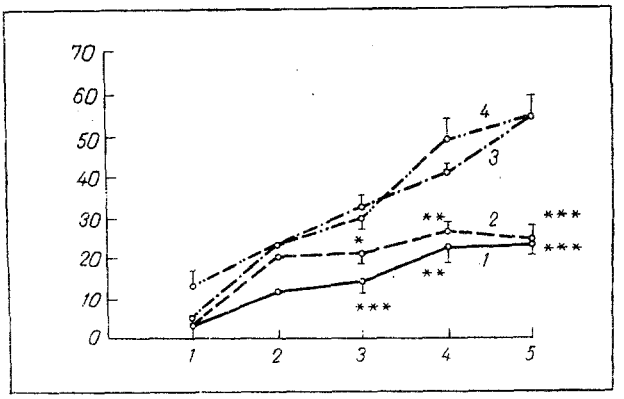

Fig. 1. Time course of formation of two-way active avoidance reflex in adult rats. Abscissa, days of experiment (30 combinations each day); ordinate, fraction of conditioned responses during experiment (in per cent). Groups of animals: 1) intact (n = 46); 2) immunized with neocortical AG (n = 32), and 3) with hippocampal AG (n = 44); 4) hippocampectomized (n = 13). Statistical significance of differences from group 3 (by Student's test): *p < 0.05, **p < 0.01, ***p < 0.001.

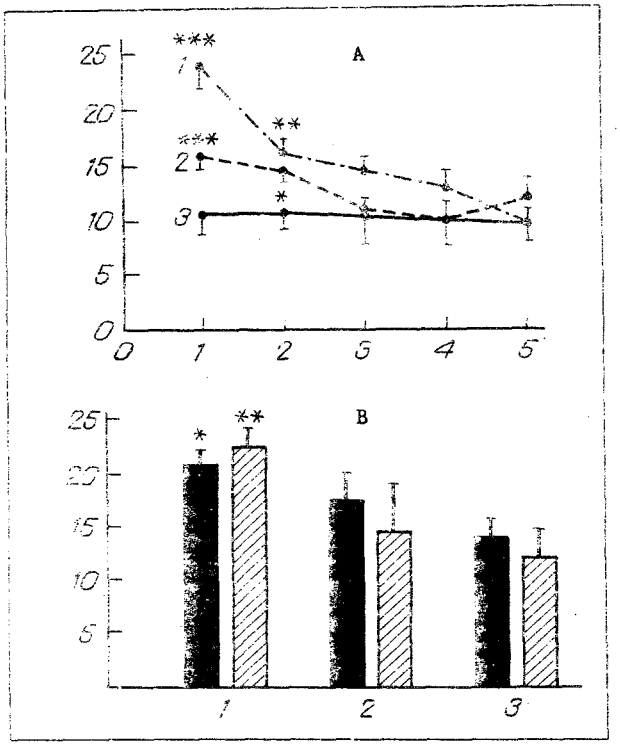

Fig. 2. Motor and investigative activity of 23-day-old rats in the "open field" test. A) First testing. Abscissa, time (in min); ordinate, number of radial lines crossed. B) Comparative total numbers of rears in 1st (black columns) and subsequent (obliquely shaded columns) tests. Groups of animals: 1) intact (n = 32), 2) immunized with neocortical AG (n= 36), and 3) with hippocampal AG (n = 35). Remainder of legend as to Fig. 1.

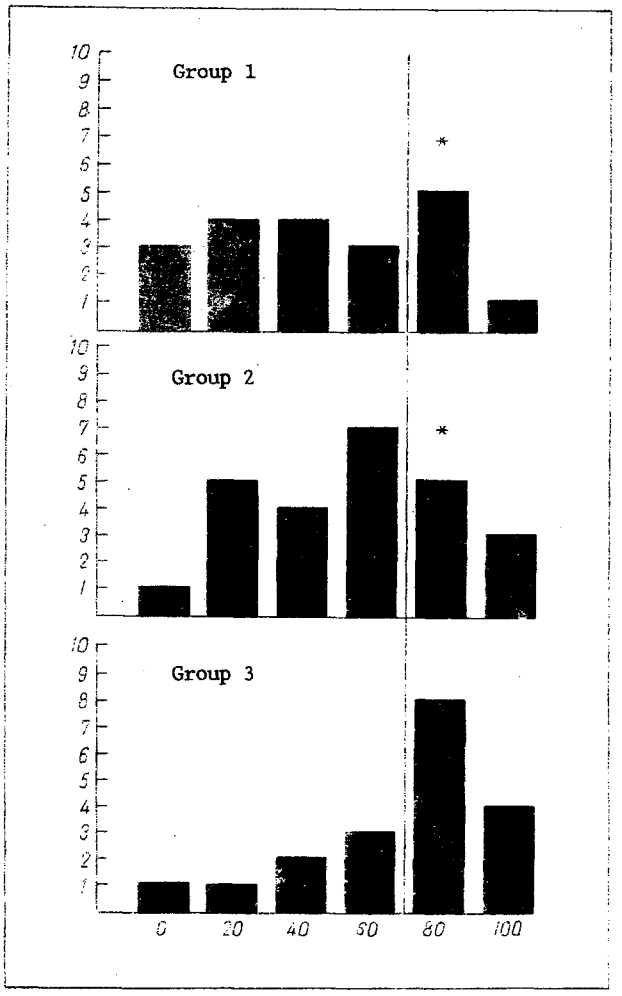

Fig. 3. Character of formation of active avoidance reaction in young rats aged 4 months, subjected to repeated immunizations after birth: distribution of animals of different groups with respect to level of learning (per cent of conditioned responses during experiment) achieved on the last experimental day. Abscissa, level of learning (60% level is indicated). Ordinate, number of animals. Groups of animals: 1) intact (n = 21), 2) immunized with neocortical AG (n = 27), and 3) with hippocampal AG (n = 20). Statistical significance of differences from group 3 (by chi-square test): *$p < 0.05$.

EXPERIMENTAL RESULTS

In order to establish that this experimental model of neuroimmune blocking of HP can be used in principle, several factors had to be analyzed. Among the aftereffects of hippocampectomy, the clearest are the high and irrepressible orienting-investigative activity and facilitation in CAAR training [3]. In the present case, similar patterns were observed in the adult animals. For instance, in the OF testing on rats immunized with hippocampal AG, at the first test a tendency toward higher values of motor activity was discovered. On the second application of OF, a statistically significant reduction of investigative activity, characteristic of the normal state, could not be demonstrated in these animals, just as was the case also in hippocampectomized rats. it will be clear from Fig. 1 that training in the CAAR was greatly facilitated in animals immunized with hippocampal AG, just as in hippocampectomized animals.

When newborn rats were immunized with the same AG-material the results also pointed to intervention in the working of HP. As other workers showed [8], the aftereffects of hippocampectomy in young rats have particular features connected with the different functional role of HP at different stages of ontogeny. A characteristic result of destruction of HP in young animals

is low motor and investigative activity of the young in OF, with disturbance of the time course of these parameters. In the present case, young rats immunized with hippocampal AG exhibited precisely these features (Fig. 2). We showed previously that definite difficulty in CAAR training is observed in such young animals at the age of one month [5], which can be compared with the aftereffects of disturbance of hippocampal function in young rats produced by other methods [11]. The results of experiments to form CAAR in animals aged 4 months, subjected to repeated immunization and demonstrating specific disturbances of behavior in OF at the age of 23 days, are shown in Fig. 3. Clearly when hippocampal AG was used, CAAR formation was facilitated, just as in animals of the homonymous group immunized only in the adult state. This state of affairs is a serious argument in support of the view that the changes described are due to factors acting on HP: the different trend of the changes in learning, connected with the different role of HP in the organization of behavior at different stages of ontogeny enables the predominance of nonspecific aftereffects of NI to be ruled out when the results are interpreted.

In the experimental and published data cited above in all tests the essential control group consisted of animals immunized with AG of a different brain formation, namely the convex lateral surface of NC. It must be particularly emphasized that virtually always the parameters of rats immunized with NC antigens had particular features of their own or closely resembled the unimmunized control and differed significantly from hippocampectomized and hippocampal-immunized animals.

The results of the immunologic control confirm the physiologically demonstrated structural trend of the NI method [4, 5]: as was shown in the complement fixation and precipitation tests, only as a result of immunization of animals of all ages with hippocampal AG was there an increase in the proportion of animals with a high (1:16 or higher) liter of antibrain antibodies.

In a parallel investigation of neuronal plasticity of HP in animals of different ages and under NI conditions [6], which we carried out, we found that action of this kind aimed at HP leads to disturbances of the properties of short-term plasticity of the system of connections of the dentate gyros with area CA_3 and to their pathological enhancement.

It can thus be concluded from the results of this physiological analysis of the aftereffects of iVI that immunologic activity can be targeted on HP of animals of different ages, leading to its functional blocking. Particular attention must be paid to the fact that this method proved to be more effective when used on young animals, thus confirming once again the increased sensitivity of the developing brain to the immune status of the body [7]. In adult animals, on the other hand, deviations of behaviour revealed by these experiments did not completely repeat the effect of hippocampectomy, but were identified more often as a similar tendency (for example, irrepressibility of orienting and investigative activity in OF). This approach may prove to be very useful when activity exerted on HP has to be "sparing," and also in experiments involving interference with the working of several brain formations at the same time, a situation calling for the simultaneous use of different methods.

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