terminant focus more often than in experiments on cats [2, 3]. This may be due to the fact that cortico-cortical connections in rabbits are less well developed than in cats [6, 7, 12, 13], and inhibitory mechanisms suppressing independent secondary epileptogenesis are less perfectly developed [14].

On the whole it can be concluded from the results of these investigations that species differences in the morphological and functional organization of the cerebral cortex are responsible only for some particular features of the realization of the determinant principle but do not modify it substantially. This fact, together with the observation that determinant relationships exist in different parts of the CNS [1], is evidence that the determinant principle is universal in character and is one of the essential principles governing activity of the CNS. As has been shown [1], it is a basic principle of intrasystemic relations.

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EFFECT OF THYMECTOMY AND THYMUS POLYPEPTIDE FACTOR ON INSTRUMENTAL REFLEX FORMATION TO FOOD IN RATS

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KEY WORDS: thymectomy; thymarin; instrumental reflex to food.

The writers showed previously [1, 3, 4] that low-molecular-weight polypeptides isolated by extraction with acetic acid from the thymus (thymarin) and from the cerebral cortex of calves (cortexin) are not only antigenically related [4], but also possess a common property of restoring the T-cell population in adult thymectomized mice by stimulating differentiation of T-cell precursors into mature T lymphocytes [3], normalizing immunologic reactivity to thymus-dependent antigen in the thymectomized animals [3] and stimulating it in normal animals [1]. It was decided to study whether this function of the thymus is limited to its effect on immunogenesis or, since it possesses common antigenic components with cells of the cerebral cortex [4], it can also influence processes of higher nervous activity.

The object of this investigation was to study the effect of thymectomy and of the thymus polypeptide factor thymarin on the effectiveness of instrumental reflex formation to food in adult rats.

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EXPERIMENTAL METHOD

Experiments were carried out on thymectomized noninbred male albino rats. The thymus was removed from animals aged 2-2.5 months under ether anesthesia, with local infiltration of the operative field with 0.5% lidocaine solution [2]. Experiments to study the effect of thymectomy on instrumental reflex formation to food were carried out 5-7 and 8-9 months after the operation. Rats of the same age undergoing mock operations, in which all stages of the operation except removal of the thymus were performed, served as the control. A course of injections of thymarin, which is a complex consisting of polypeptide fractions of the thymus with a molecular weight of under 10,000 daltons, was given to the thymectomized animals 9 months after the operation. Thymarin was injected intramuscularly daily for 10 days in a dose of 1.0 mg per rat, in 0.5 ml physiological saline. Completeness of removal of the thymus was verified by autopsy at the end of the experiments and by determination of the number of T lymphocytes in the spleen by the complement-dependent cytotoxic test [4] with antiserum against rat cerebral cortical antigens, absorbed twice for 1 h at 20°C by rat liver homogenate or by sheep and rat erythrocytes in the ratio of 0.1 ml of solid cell residue to 1.0 ml of whole serum. Fresh guinea pig serum (1 : 3) was used as complement, and in this dilution it has no direct toxic action on lymphoid cells. When the spleen was studied, 200 nucleated cells were counted in each animal and their viability assessed by means of 0.2% aqueous solutions of trypan blue. Antibrain serum, absorbed by the method indicated above, led to death of 98.5 ± 1.9% of thymocytes but of only 8.2 ± 1.9% of bone marrow cells of rats in the cytotoxicity test.

The experimental system used in the experiments for producing an instrumental reflex to food in the thymectomized animals consisted of a single box in which the rats could move about freely and press a pedal which automatically supplied food into a feeding bowl. After a high level of food motivation had been created in the experimental animal by keeping it for a short period (4-5 days) on a quantitatively deficient diet followed by deprivation for 24 h, it was placed in the experimental chamber where the number of times it pressed the pedal, which was accompanied by reception of food (pieces of cheese) was recorded. Responses followed by eating of the food between pressings on the pedal were classed as complete. The criterion of formation of an instrumental food reflex was five complete pressings on the pedal, not interrupted by any behavioral acts unassociated with the instrumental obtaining of food and its subsequent consumption. The time required to reach this criterion also was recorded. The animals were observed through a system of mirrors.

The character of motor activity and its level were studied in the rats by the "open field" method [5].

EXPERIMENTAL RESULTS

As Table 1 shows, 5-7 months after thymectomy a tendency was observed for values of the criterion of learning of the instrumental food reflex by the rats to fall: thymectomized animals were trained in 66.6 ± 21.1% of cases compared with 100% in the group of rats undergoing mock operations and the number of completed responses and the time taken for their performance were reduced about by half compared with the control. To reach the criterion of learning, 8-9 months after thymectomy three times more completed responses were needed; the time required for their performance also showed a similar increase compared with animals undergoing the mock operation; the number of trained thymectomized animals fell to 22.2 ± 14.7% compared with 85.7 ± 9.7% in the group of rats undergoing the mock operation ($P < 0.01$).

Injection of thymarin in a dose of 1.0 mg per animal intramuscularly for 10 days into the thymectomized rats 9 months after the operation increased the percentage of trained animals from 22.2 ± 14.7 to 85.7 ± 14.9%.

TABLE 1. Effect of Thymectomy and Thymus Polypeptide Factor on Performance of Rats Taught an Instrumental Food Reflex (M ± m)

Group of rats	Number of rats in group	Time after operation, months	Number of trained animals, %	No. of completed responses to reach criterion of learning	Time taken to reach criterion of learning, min
Mock operation	6	5—7	100	12,7±1.1	42,2±6.1
	14	8—9	85,7±9,7	10,2±0,7	37,1±5.5
Thymectomy	6	5—7	66,6±21,1	16,2±3,1	74,8±13.2*
	9	8—9	22,2±14,7**	31,5±0,8**	112,8±1,9**
Thymectomy + administration of thymarin	7	Over 9	85.7±14,9***	17,6±4,6***	55,6±9,9***

Legend. One and two asterisks indicate significant difference compared with corresponding values for rats of the same age undergoing mock operation, at $P < 0.05$ and $P < 0.01$ levels respectively; three asterisks indicate significant difference compared with corresponding values for thymectomized animals of the same age, at $P < 0.01$.

TABLE 2. Results of Study of Rats' Behavior by "Open Field" Test 5-7 Months (A) and 8-9 Months (B) after Operation (M ± m)

Parameter	Rats undergoing mock operation		Thymectomized rats	
	A	B	A	B
No. of standings on hind limbs	7,3±3,8	4,9±1,0	9,2±1,4	3,4±1,3
No. of crossings of center	1,0±0,8	0,75±0,2	1,53±0,3	0,6±0,4
No. of crossings of squares	75,3±28,7	46,2±6.0	74,0±9,7	41,8±12,7
No. of defecations	2,0±0,7	2,7±0,6	1,9±0,3	2,8±0,8

The character of motor activity, its intensity, and the level of emotional activity, measured as the number of defecations, were the same in animals undergoing thymectomy and the mock operation, both 5-7 months and also 8-9 months after the operation (Table 2).

The number of T cells in the spleen of rats before and 5-6 months after the mock operation was 29.8 ± 2.3 and 26.3 ± 3.1% respectively, and after 9 months their number had fallen to 19.0 ± 2.6%. The number of T lymphocytes in the spleen 5-7 months after the operation had fallen to 2.2 ± 0.7%, and after 8-9 months no T cells whatever could be found. Injection of thymarin into the rats 9 months after the operation restored the number of T lymphocytes from 0 to 25.0 ± 3.5%.

The results indicate that thymectomy not only disturbs the immunologic functions of the animal [3], but also affects higher nervous activity. In particular, it lowers the effectiveness of training in an instrumental food reflex: 5-7 months after thymectomy the percentage of animals trained in the instrumental food reflex was down to 66.6 ± 21.1, and 8-9 months after the operation it was only 22.2 ± 14.7 compared with 85.7 ± 9.7 in the group of rats undergoing the mock operation. The decrease in effectiveness of instrumental food reflex formation in thymectomized rats, it will be noted, was not accompanied by any change in the animals' motor activity – one of the important factors involved in instrumental behavior formation.

The antigenic kinship of thymarin and cortexin, but not of the preparation from the white matter of the brain [4], suggests that these polypeptides, even if not identical, at least must have common antigenic components with Thy-1 antigen, for the latter, like thymosin [11] (thymarin was used in the present experiments), is synthesized by the epithelial cells of the thymus [13], it has the same polypeptide molecular weight (12,500 daltons) [7, 10], and cortexin, like the Thy-1 brain antigen, is found only in the gray matter of the brain and is practically absent in the white matter [3, 4]. In turn, the Thy-1 antigens of thymus and brain are not only antigenically related, but they also have an absolutely identical amino-acid composition [6]. Meanwhile the Thy-1 antigens of thymus and brain do not possess quantitative correlations: congenital absence of the thymus in nude mice does not involve any quantitative changes in Thy-1 antigen in the brain [9]. Nevertheless, up to 20% of T lymphocytes can be found in these animals [8]. Partial restoration of this cell population is observed in adult thymectomized animals in the late stages after thymectomy [14]. It can be tentatively suggested that the presence of T lympohcytes in mice with congenital absence of the thymus, like the partial recovery of this cell population in adult thymectomized animals, is due to the regulating influence of brain Thy-1 antigen. Data on the discovery of a thymus factor (thymosin α_1) in the cerebrospinal fluid and its ability to penetrate into the brain [12] and to perform the function of a mediator [15] are particularly interesting from this point of view.

The fact that not only is the functional activity of the T lymphocytes of thymectomized rats restored quantitatively by thymarin, but ability to learn complex instrumental forms of behavior, when sharply depressed after thymectomy, also is restored, is evidence of close correlations between the immune and nervous systems.

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NONQUANTUM ACETYLCHOLINE RELEASE IN THE FROG MYONEURAL JUNCTION AFTER DISTURBANCE OF AXOPLASMIC TRANSPORT BY COLCHICINE

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Disturbance of axoplasmic transport (AT) by colchicine (Co) in a frog motor nerve lowers the resting membrane potential (MP) of muscle fibers, the potassium conductance of the membrane becomes greater than the chloride conductance, and extrasynaptic sensitivity to acetylcholine (ACh) appears, just as after denervation of the muscle [2-4]. However, in the first case transmission of excitation from nerve to muscle is preserved [1, 2]. Similar denervation-like changes in the membrane of skeletal muscles develop after AT blockage in mammals also [5, 11]. These observations suggest that the properties of the membrane of the innervated skeletal muscle fiber are determined by substances transported to the muscle by AT [2-5, 11].

However, the opinion also is held that synaptic ACh may also play the role of factor responsible for neurotrophic control of the muscle membrane [7]. A special role in this process, it will be noted, has recently been ascribed to ACh [7] liberated by nerve endings in nonquantum form [6, 10]. It was accordingly interesting to study the effect of AT blockade on nonquantum ACh release, and the investigation described below was carried out for that purpose.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscle of the frog Rana ridibunda in the fall and winter, using a standard microelectrode technique. AT in the nerve supplying the muscle was blocked with 10 mM solution of Co (from Merck, West Germany) by the method described previously [2]. During the experiment the muscle was kept in continuously flowing Ringer's solution of the following composition (in mM): NaCl 118, KCl 0,* $CaCl_2$ 1.8, in phosphate buffer, pH 7.3, at 10 ± 0.5°C. Before the experiment the muscle was kept for 30 min in Ringer's solution containing 5×10^{-6} M armin,† an irreversible acetylcholinesterase inhibitor, and was then rinsed for 20 min. MP was recorded alternately in the synaptic and extrasynaptic zones of several

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*As in Russian original – Publisher.

†Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

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