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THE INTERACTIONS OF CENTRAL NEURONS IN CONDITIONING AND THE INFLUENCE OF SOME LIMBIC STRUCTURES UPON THEM

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Abstract. The interactions between the neurons in the cortex and the subcortical visual structures were studied with multimicroelectrode technique. Neurons revealing conditioned reactions were found at various levels of the brain. It is assumed that they are involved, by the conditioning, in a special cellular network: the microsystem, which is considered to be the definite functional level of brain processing mechanisms. The influence of some limbic structures on the formation of the microsystems of conditioning neurons was found. It was concluded that the elaboration or extinction of the temporary connection is performed through formation or destruction of a microsystem which takes part in the conditioning.

INTRODUCTION

We shall outline here two special problems of memory that we are interested in: interactions between neurons at different brain levels able to undergo conditioning, and the effect of electric stimulation of some limbic structures upon long-term conditioned responses of these neurons.

The data obtained during previous experiments concerned with changing the plastic functions of neurons by conditioning and prolonging these changes for some time in the intact neocortex (8, 10, 14), neuronally isolated cortex (9, 10, 14), and subcortical structures (11, 21), maintain that the intended functional changes are only found in some cortical and subcortical cells and not in all the studied units. A con-

clusion was made concerning the existence of some specificity in the connections between these cells, a specificity which enables them to fix the traces of joined stimuli (7). Therefore the integrative role of single neurons was repeatedly stressed in the last few years. But we have no doubt that the fixation of the temporary connection is accomplished by a rather great number of that type of neurons present in various structures participating in a general functional system of the conditioned reflex. Naturally, the question arises what interrelationships exist between the conditioning neurons of different level of the brain during the elaboration, realization and extinction of the conditioned reflex. To answer the question, some special series of experiments have been undertaken.

Experiment I: Neuronal interactions in the neuronally isolated cortex

The main purpose was to observe the formation of connections between single neurons registered in various cortical areas without ascending afferentation, which obscures purely cortical processes. Since the technique has been described in detail elsewhere (5), we only remind here of some basic stages of the operation. The main goal of the operation is to dissect all projections and commissural fibers of one or both hemispheres. As shown in Fig. 1, the incision separates all subcortical structures from the cortical ones. To achieve it, we made a small incision in the medial part of the lateral gyrus through grey and white matter to the lateral ventricle of the brain. Following that an incision was made through all the fibers in corona radiata surrounding the lateral margin of the hippocampus. It passed along the lateral margin of the hippocampus and more frontally the dissecting spatula advanced near the medial side of the nucleus caudatus.

A morphological study of the neuronally isolated cortex has led us to some conclusions about a possible structural substratum underlying the connective function of the neocortex. Brain sections stained by the Nissl technique demonstrated the survival of all cellular layers in the neuronally isolated cortex even 2 yr after the operation (16). But, on the other hand, investigations made with the Golgi technique revealed some changes of dendritic fields and dendritic synaptic structures. Only nerve cells in the 2nd and 6th layer remained unchanged after the dissection procedure (22). On the basis of the present data it may be supposed that pyramidal cells found in these two layers of the neuronally isolated cortex have mainly intercortical connections. Layers third to fifth of the visual isolated cortex belong to cortico-subcortical interaction mechanisms. The 2nd and 6th layer subserve predominantly the processes of intracortical nature.

Acute experiment was performed on 22 unanaesthetized immobilized adult cats, 2–3 weeks after surgery. Immidiately after the injection of immobilizing agent (Sol. Diplacini dichloridi, 2% — 5.0 ml, intraperitoneally) the animal underwent intubation and artificial respiration was switched on. Then a local anaesthesia of some points on animals was made with prolonged anaesthetic. The middle part of suprasylvian gyrus and the visual cortex (area 17) in the neuronally isolated cortex was stimulated through bipolar tungsten electrodes (diameter 100 μm, interelectrode distance 300–500 μm) with a single rectangular pulse (0.5 ms, 4–1.200 μA; 4/min).

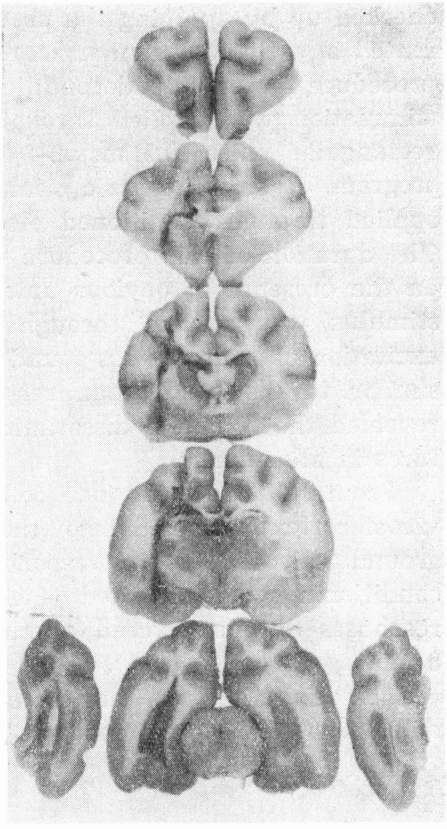

Fig. 1. Isolated cortex in the cat's brain at different frontal levels.

A conditioned electric stimulus was applied through one pair of electrodes to the medial part of the suprasylvian gyrus and an unconditioned stimulus to the visual cortex (area 17) through another pair of electrodes. The microelectrodes were placed near to and at an equal distance from the respective pair of stimulating electrodes. Two neurons, one in the visual cortex and another in the middle part of suprasylvian gyrus were recorded simultaneously. The interactions between neurons were studied by an analysis of both spontaneous and evoked activities. The experiment consisted of five stages.

1. Recording of spontaneous activity (5 min).
2. Recording of response to unconditioned stimulus (5 min).
3. Recording of response to conditioned stimulus (5 min).
4. Pairing of the conditioned and unconditioned stimuli: 35–40 pairings during 5 min. For each pair the conditioned stimulus preceded the unconditioned one (by 150–200 ms).
5. Examination of the conditioned response. The duration of this stage depended on the presence of obvious unit responses to conditioned stimuli (usually about 7 min). Thirty percent of all neuronal pairs were

checked up by applying the pseudoconditioning program, and none presented any changes similar to the conditioned responses. The following procedure for the pseudoconditional program was used (23). The tetanic stimulation was applied through unconditioned electrodes with single rectangular pulse (0.5 ms, 2–2,000 μA, 80–120/s) after the conditioned program. A single electric stimulus (0.05 ms, 2–2.000 μA, 4/min) was applied through conditioned electrodes during the tetanic stimulation. The duration of this procedure was from 5 to 10 min and it depended on the presence of obvious unit activity. Then only the single electric stimulus was applied through conditioned electrodes. No long-term excitation of neurons was found and none of them showed any changes similar to the conditioned responses. The morphological examination revealed the complete dissociation of neocortex from subcortical structures in all animals.

From 106 cell pairs (one cell in the associative and another in the visual cortex), which did not show any correlation between their background activities, 9 pairs responding to a conditioned stimulus after the conditioning were selected (Fig. 2). The duration of the conditioned responses did not exceed 5 min on the average (maximum duration 8 min).

The results suggest that trace reactions thus elaborated in the neuronally isolated cortex reflect the formation of some specialized neuronal

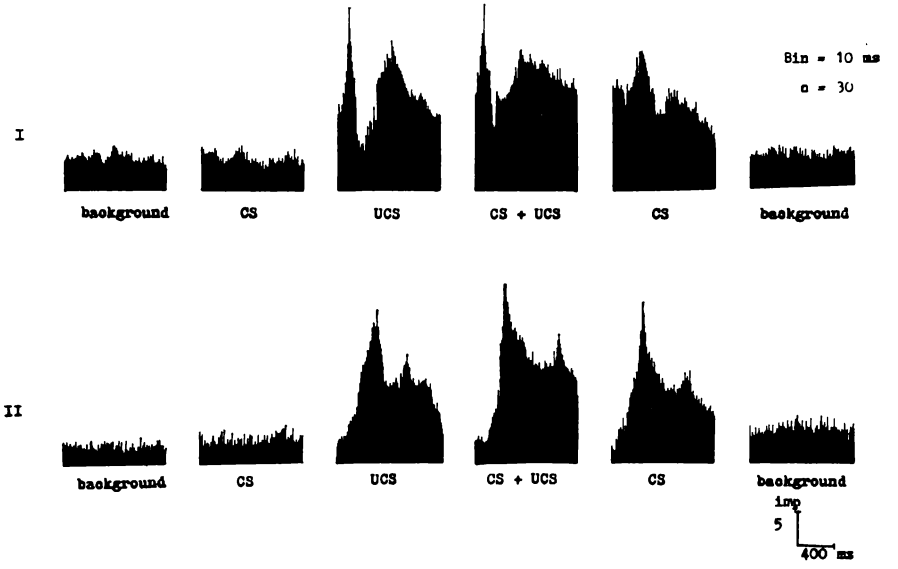

Fig. 2. Conditioning in the isolated cortex. I, unit "responses" in suprasylvian gyrus; II, visual cortex in different stages of experiment; CS, conditioning stimuli; UCS, unconditioned stimuli.

system that provides the establishment of a temporary connection under the conditioning procedure. The above experiment has shown reorganizations of interneuronal connections manifested by distinct changes of neuronal functional characteristics.

Experiment II: Interactions between cortical and subcortical structures in intact cats

Acute experiment was done on 26 unanaesthetized immobilized adult cats. A single unit activity was investigated in the visual cortex and superior colliculus. The electrical stimulation (single rectangular pulse, 0.5 ms, 4–1.000 μA, 4/min) of the middle part of the suprasylvian gyrus was a conditional stimulus.

Flashes of light spot (10 ms, 4 min, 0.5–2.0°, 50 lux, were used as unconditioned stimuli. The light spot was projected on a tangent white screen situated 1.5 m before the cat's eyes. Two neurons, one in the visual

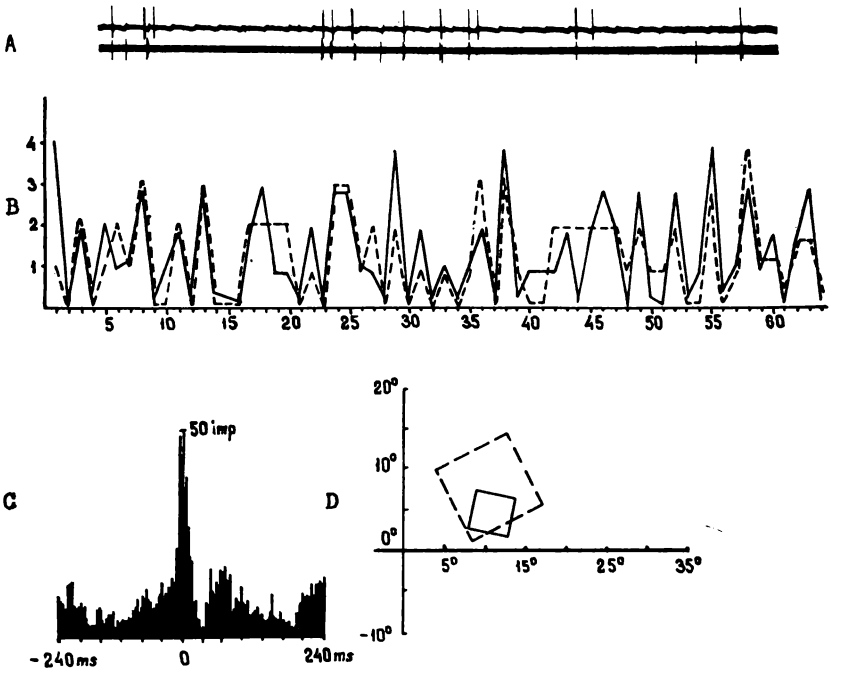

Fig. 3. Correlation between activities of two neurons during CS applied after conditioning (one from visual cortex, another from the superior colliculus). *A*, original neuronograms (upper line — visual, lower — collicular neuron); *B*, graphics of following rate (solid line — visual, interrupted — collicular cell); *C*, crosscorrelation histogram; *D*, schema of receptive field's interrelations (solid line, cortical cell, interrupted, collicular cell).

cortex and another in the superior colliculus were recorded simultaneously. The limits of the receptive fields were defined with moving spots of light, but exact investigation of neuronal functional properties (orientation, speed etc.) was not undertaken. The receptive fields of these units had similar localization and both were affected by the flash spot. The interactions between neurons were studied by an analysis of both spontaneous and evoked activities. All stages of the conditioning procedure were the same as in section I.

Of 137 neuronal pairs responding to unconditioned stimulus, 14 pairs showed clear conditioned response. Numerous correlations were detected in 6 pairs of neurons where a distinct correlation between the moments of spike generation of the collicular and cortical neurons may be observed (Fig. 3). The results correborate the functional significance of the long-axonal connections, since, as proved by the experimental results, strong correlations were found in some neuronal pairs.

The next experimental series was made on 63 unanaesthetized curare immobilized adult cats and showed interactions between the neurons of the visual cortex, the lateral geniculate body and the superior colliculus. Neuronal activity was recorded in these structures simultaneously during

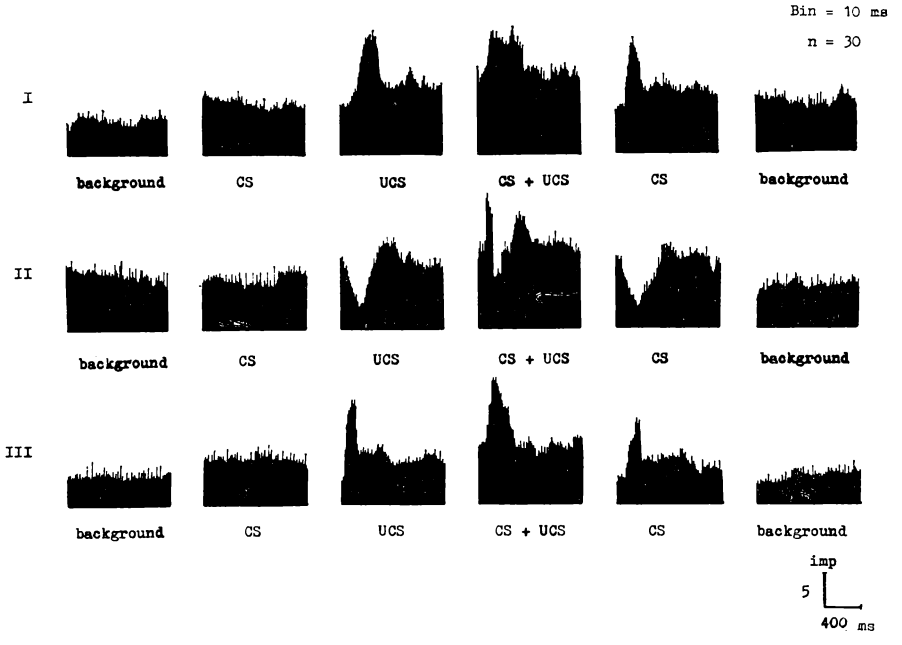

Fig. 4. Poststimulus histograms recovered simultaneously from three neurons in superior colliculus (I), lateral geniculate nucleus (II) and visual cortex (III) during conditioning. Oridante, stages of conditioning. CS, conditioning stimuli; UCS, unconditioned stimuli.

the conditioning. All stages of the conditioning were the same as before. Of 234 cell groups (three cells in each group), in 17 the change of responses was observed simultaneously in all three cells (Fig. 4). The responses lasted on the average 6 min (maximum 9 min). These data showed that some neurons form interconnections during conditioning. It would be of interest to single out two main points in the experimental materials presented here. Firstly, there exist distinct structures, and, secondly, the interactions occur not between all, but only between some of the neurons.

Experiment III: Influences of limbic structures upon neuronal interactions in the neocortex

Our previous experimental clinical investigations displayed the definite role of some limbic structures in the memory process in men and animals (15). Basing on these investigations, we studied the influence of the electric stimulation of some limbic structures upon the length of elaborated conditioned trace unit reactions in the neocortex. The comparison of duration of unit conditioned response (CR) was undertaken before and under limbic electrostimulation on unanaesthetized immobilized with curare intact adult cats in acute experiments.

The elaboration of unit CR was made on 26 cats under electrostimulation of the hippocampus in the series I, on 29 cats under electrostimulation of the amygdala in the series II and on 19 cats under the hypothalamic electrostimulation in the series III. The electrostimulation of the midbrain reticular formation was done in some units in all three series as a control of limbic effects.

The stimulating bipolar limbic electrodes were placed in one of the

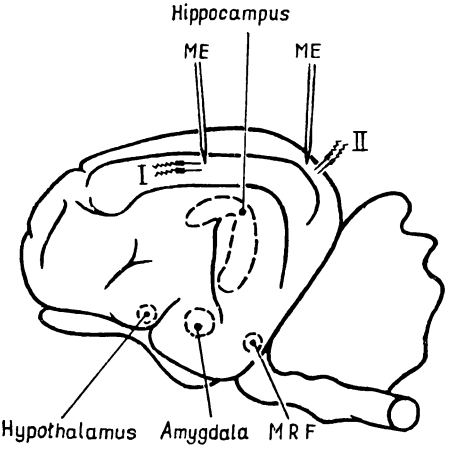

Fig. 5. Scheme of location of microelectrodes and electrodes for subcortex structures stimulation. ME, microelectrodes; I and II, electrodes for cortex stimulation; MRF, midbrain reticular formation.

TABLE I

he effects of limbic stimulation conditioned responses of cortical units. Symbols: VC, visual cortex (17 area): AC, the medial part of the suprasylvian gyrus; CR, conditioned response

Stimulated structure	Total number of units		Number of units with CR before subcortical structures stimulation		Number of units with CR under subcortical structures stimulation		Effect of limbic stimulation CR duration						Time of CR duration (in minutes)			
							increase		decrease		no change		VC		AC	
	VC	AC	VC	AC	VC	AC	VC	AC	VC	AC	VC	AC	mean	range	mean	range
Hippocampus	108	97	22	20	29	28	19	22	4	3	6	3	27.3	5–65	28.4	6–70
Amygdala	115	122	24	27	37	42	20	25	6	7	11	10	19.5	3–45	20.3	4–42
Hypothalamus	112	94	23	20	47	41	9	10	11	7	27	24	15.7	4–26	16.4	3–24
Reticular formation	68	54	15	11	17	14	5	3	8	6	4	5	14.2	3–19	14.7	4–20
Control (without stimulation)	403	367	84	78	—	—	—	—	—	—	—	—	13.5	3–20	15.1	4–21

limbic structures — areas CA_1 and CA_2 of the hippocampus (according to the classification of Lorente-de-No (13)), ventrolateral nucleus complex of the amygdala and the ventromedial nucleus of the hypothalamus. Groups of square-wave pulses were used for stimulation of subcortical structures (0.5 ms, 0.8 mA; 50–100 Hz). The single unit activity was recorded in the visual area 17 and in the medial part of the suprasylvian gyrus (Fig. 5). Under electrostimulation of hypothalamus two neurons were registered simultaneously. The first pair of bipolar cortex stimulating electrodes was placed at 2–3 mm from the microelectrode in the visual area and the second pair was put at 2–3 mm from the microelectrode in the medial part of the suprasylvian gyrus (associative area). Parameters of the cortex electrostimulation and all stages of the conditioning were the same as described in the first part of the paper. The results of conditioning and the influence of some subcortex structures upon them are shown in Table I.

In series I, under the hippocampal electrostimulation 29 units out of 108 units in the visual area (27% of the total number of registered units in the visual area) and 28 units out of 97 in the associative area) 29% of the total number of registered units in the associative area) showed a clear conditioned response (CR). In the visual area out of 29 units, 19 units revealed increasing, 4 decreasing and 6 no changes of CR duration. In the associative area out of 28 units, 22 showed increasing, 3 units decreasing and 3 units no changes of CR duration. 7 units of the visual area and 8 units of the associative area revealed a clear CR under the hippocampal electrostimulation only. Average time of CR duration was 27,3 min in units of the visual area and 28.4 min in units of the associative area. In three cases the duration of CR preservation was more than 60 min (Fig. 6). Average time of CR duration before subcortex electrostimulation was 13.5 min in units of the visual area and 15.1 min in units of the associative area.

In series II under the amygdala electrostimulation, out of the total number of 115 registered units of the visual area, 37 units (32%), and out of 122 units of the associative area, 42 units (34%) showed a clear CR. In the visual area 20 units out of these 37 revealed increasing, 6 units decreasing and 11 units no changes of CR duration. In the associative area out of 42 units with CR 25 units revealed increasing, 7 units decreasing and 10 no changes of CR duration. 13 units of the visual area and 15 units of the associative area showed a clear CR under the amygdala electrostimulation only. The average CR duration was 19.5 min in units of the visual area and 20.3 min in units of the associative area.

In series III under the hypothalamic electrostimulation, out of 112 units which were registered in the visual area, 47 units (42%), and, out

of 94 units in the associative area, 41 units (43%) showed a clear CR. In the visual area out of 47 units with CR, 9 units revealed increasing, 11 units decreasing and 27 units no changes of CR duration. In the associative area out of 94 units with CR 10 units showed increasing, 7 units decreasing and 24 no changes of CR duration. 24 units of the visual area and 21 of the associative area revealed a clear CR under the

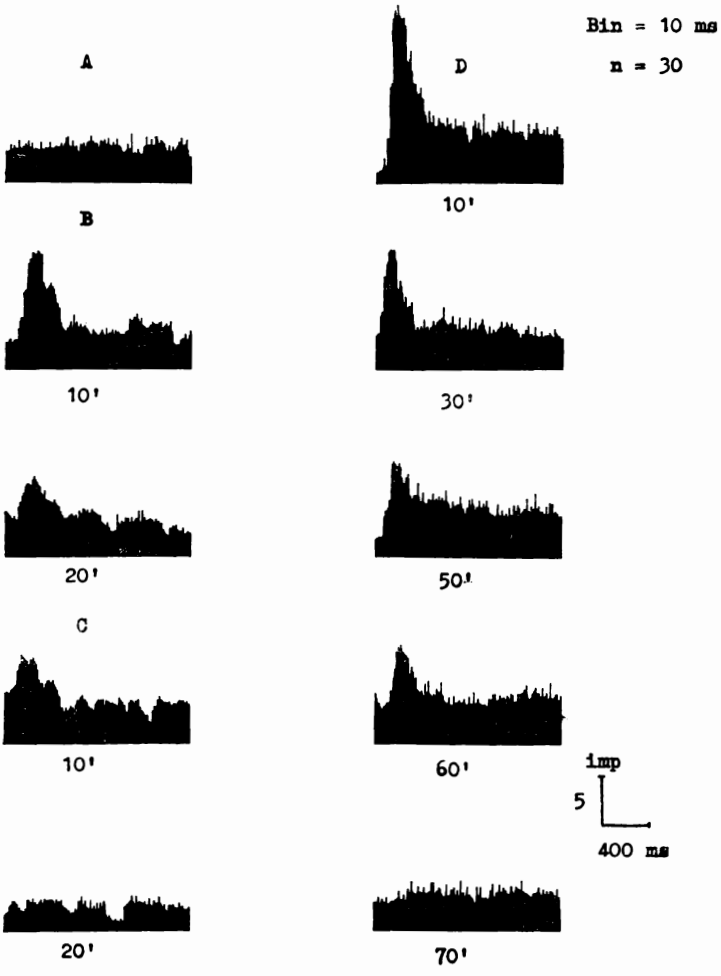

Fig. 6. The conditioning response dynamics of neuron from visual cortex to conditioning stimulus before and after direct electric stimulation of the hippocampus and of the reticular formation. *A*, spontaneous activity; *B*, conditioning response during some time after conditioning; *C*, conditioning response to conditioning stimulus during some time after conditioning under the stimulation of the reticular formation; *D*, conditioned response to conditioning stimulus during some time after conditioning under the hippocampus stimulation; time in minutes.

hypothalamic electrostimulation only. The average time of CR duration was 15.7 min in the visual area and 16.4 min in the associative area.

The average time of CR duration was 14.2 min in units of the visual area and 14.7 min in units of the associative area under the midbrain reticular formation electrostimulation in some units in all the three series. Data of unit reactions are shown in Table I.

DISCUSSION

The data obtained in the study of the neuronally isolated cortex indicate that the units showed CR. It may be suggested that this phenomenon seems to be caused by purely transcortical mechanisms and some neurons in different cortex areas form interconnections during conditioning.

In other experiments the authors have reported that they found distinct functional interconnections while simultaneously recording units in visual or non visual subcortical structures (1, 2). As the Experiment I has revealed, the interconnections are formed between neurons which are connected by direct long-axonal retinotopically organized pathways.

The experiments have suggested that neuronal interactions reflect the process of formation of some specialized neuronal system that establishes a temporary connection within the brain. Looking at strictly coordinated peripheral components of a conditioned act, a conclusion may be drawn that the activity of great neuronal masses, which perform the central control in the conditioned behavior, is coordinated with no lesser precision. Hence the neurons accomplishing some function in a specific conditioned act have to be united into a complex system. We call it a "microsystem".

Thus, the microsystem is an organized aggregation of specialized neurons and their connections which perform some function in a given conditioned act and exist as a separate unit with respect to other neuronal systems. Neuronal microsystems, in our opinion, may be considered as elementary units; some components of any microsystem can be found at various levels of the central nervous system. We suppose that the elements accomplishing some function during the conditioned activity should be composed into a specialized microsystem, the main predestination of which is to carry out the aforementioned function.

At the same time, the statement about microsystem emphasizes another idea the microsystem is primarily a system, so a number of rules should be used in its study which are applied to the investigation of any system. Hence the main task is to understand interactions between elements involved in the microsystems. According to our hypothesis the

microsystem includes neurons located not only within one structure, but cells of various brain levels.

The idea about special learning neurons and selforganizing neuronal systems subserving learning and memory brain processes was intensely discussed in the works of Hebb, Konorski (3, 12) and others. But no experimental data which could confirm the above theoretical speculations have been available until the fine electrode technique and multimicroelectrode methods began to be more or less widely used in experimental practice. For example, there were found distinct changes of interneuronal relations in various brain levels during the elaboration of some conditioned reflexes in freely moving awake animals (17, 18, 20). These results are in general agreement with the main stream of the microsystem conception.

When summarizing numerous experimentally found phenomena we must point out the most interesting fact. We recorded a number of neuronal pairs where both components responded simultaneously to the conditioned stimulus after the conditioning, which means that both components were involved in a microsystem performing an adequate reaction during the conditioning procedure. Some of them demonstrated interrelationships in their background or evoked activity.

But it was no surprise that synchronous activity was not found in other pairs of conditioning neurons. It is known that the elaboration of conditioned reflex excites all brain mechanisms, each of them being utilized during the appropriate conditioned reaction by a specialized neuronal system. For instance, during the elaboration and fixation of any temporary connection, and afferent system providing integration of the sensory image that reflects a conditioned or unconditioned stimulus, forms a special cell system containing neurons with plastic abilities, but none the less performing this sensory function. As some investigations (4, 19) have shown, the activity of sensory system including the visual one is determined not only by unconditioned, but also by conditioned mechanisms. The forming microsystem is composed of elements which are able to connect a specific reaction with a definite functional state of the other structure from the same sensory system. Keeping in mind that at the cortical level polymodal neurons play an important role, and influences of various modalities frequently converge upon the same cortical element, it is easy to suppose that in the process of conditioning, when recording from one neuron, we deal with the action of many components belonging to various microsystems.

The study of conditioning which was carried out with the aid of hippocampal electrostimulation has revealed that the duration of trace preservation increased 3–4 times (Fig. 6 and Table I). The comparison of duration of the conditioned trace under the amygdala electrostimulation

with that of the hippocampal stimulation, shows that this period was longer in the latter case. The data obtained under the hypothalamic stimulation make it clear that elaborated trace reactions of some neurons were prominent only during the stimulation period. After the stimulation of the hypothalamus the duration of the elaborated reactions did not differ from the duration of reactions without additional limbic stimulation.

As was established earlier by microelectrode investigations (8), about 25% of the intact neocortex cell and 23% cells of the neuronally isolated cortex (6) manifested the conditioning responses in different areas of the neocortex. Taking into consideration the number of cells showing elaborated conditioned reactions, it may be noted that most of them (43%) occur during hypothalamic stimulation. There are few cells showing CR duration during hippocampal electrostimulation. The stimulation of amygdaloid complex occupies the middle position among other investigated structures. On the basis of the fact that the electrostimulation of limbic structures involves conditioning of some new neuronal elements which did not show any learning effects before, it may be suggested that these limbic structures launch "reserve" cell elements to the memory mechanism. The following observations also draw our attention: first, there are some cells without any activity changes in the limbic stimulation period, and secondly, cell activity was increased with the limbic stimulation. Basing on these results, one may suggest that the limbic structure stimulation influences only the cell group, which has the property of fixing the elaborated trace processes. Limbic structures may produce some inhibitory influence on cell groups with some other functions.

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