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SIGNIFICANCE OF THE RETICULAR FORMATION FOR CONDITIONED REFLEX *

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The problem, I intend to discuss in this report, is especially urgent for the physiology of the central nervous system and, hence, for the physiology of the conditioned reflex.

The studies on the physiological significance of reticular formation have led, in the recent years, to some decisive results, which have greatly determined the neurohumoral aspect in the understanding of the activity of this central nervous system formation.

That is why I am particularly pleased to inform you about the progress our laboratory achieved at present in the study of brain stem reticular formation.

I suppose, the audience will understand my interest in the function of reticular formation, inasmuch as I am a disciple of *I. P. Pavlov* and a physiologist dealing with the higher nervous activity.

It becomes more and more evident, in the recent years, that the reticular formation physiology will constitute one of the cornerstones of the problem that was determined in *I. P. Pavlov's* laboratory, as the problem of cortico-subcortical relations. The remarkable provisions, *I. P. Pavlov* suggested long ago with such an amazing perspicacity, acquire a particular significance in this respect. He said, that the subcortex represents a „source of energy" for the cortical activity, and that without it a full value of cortical function is not possible.

This view is expressed still more definitely in his concept that the subcortex is the bearer of a „blind force", the last being directed and orderly used by the cortex.

It is hardly necessary to say that the discovery of the physiological properties of the reticular formation allows us, the disciples of the great *Pavlov*, to fill up the gap, which still existed in the problem of correlations between the cortex and the subcortical apparatus. This new research field gave us the possibility to put anew the problems both of the cortical closing function itself and of the energy potential of cortical activity.

The series of studies, I have the honour to present here, are most closely related with the remarkable work of Prof. *Dell* and *Bonvallet,* who have demonstrated the importance of adrenalin for the reticular formation activity (*Dell, Bonvallet, Hiebel,* 1956).

It became universally recognized, since Prof. *Dell's* studies, that adrenalin, injected into the bloodstream, caused a cortical „arousing reaction" by excitating the rostral part of the reticular formation and not by

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a direct action upon the cortical tissue. The reticular formation intensifies consequently its activating effect on the cortex and causes the desynchronization of cortical electrical activity. This concept of Prof. *Dell* has been lately confirmed by several investigators and particularly in the recent report of Prof. *Rothballer* (1956).

The main point of this problem is, as we see it, that the rostral part of the reticular formation is particularly sensitive to adrenalin and, therefore, must be functionally involved each time, when large amounts of adrenalin appear in the circulation. This concerns especially the „stress reaction" cases and all situations when the animal and man must urgently mobilize their energetical resources in order to fight a coming danger.

The studies of *M. Vogt*, performed by means of a biochemical method, have indicated a reliable basis for such an assumption, namely, there are large amounts of adrenalin and noradrenalin just in the hypothalamus area and in the reticular formation *(M. Vogt*, 1954).

Furthermore a special series of experiments made in our laboratory and reported in detail at the XX International Congress of Physiology in Brussels, have shown that this mobilization can be blocked by aminazine (chlorpromazine) at the sympathetic ganglia level as well as at the level of the brain stem reticular formation (*I. Anochina*, 1956; *V. Agafonov*, 1956). Morphological studies of the upper cervical sympathetic ganglion have indicated that chlorpromazine blocking action on sympathetic ganglia is connected with reversible destructive alternations in those „satellite" cells of the sympathetic ganglion, to which *De Castro* ascribed a certain receptive function in the synaptic mediation of excitation (*De Castro*, 1933).

It is of special interest that this chlorpromazine block can be removed by adrenalin, as well as the adrenal blood pressure effect is removed by chlorpromazine injection (*I. Anochina*, 1956). During recent years in search of some methods for the specific characteristic of the reticular formation chemical properties we used the Manoilov's reaction that is meant for detecting of sympathetic elements. Little pieces of sympathetic nerve or sympathetic ganglion exhibit definite blue staining while immersed in this reagent. Other tissues and, especially, parasympathetic substratum, do not stain this way *(Manoilov)*.

In our studies the brain stem was cut in the frontal plane into 2 mm slices. Each of these slices was immersed into a test-tube with Manoilov-reagent. Test-tubes containing slices of the rostral part of the brain stem stained, as a rule, blue; this made it possible to speak in a positive way about the chemical affinity of this region with sympathetic elements. In other words, this evidence confirmed our view that these regions of the reticular formation perform their function by means of adrenergic synaptic relay and are, at the same time, specific receptors of adrenergic influences mediated via the blood *(P. Anochin*, 1956).

Reciprocal relations between adrenalin and chlorpromazine displayed in their action on the rostral part of the brain stem recticular formation were quite definitely revealed in the experiments of our laboratory on the changes in cortical strychnine discharges *(V. Shelichow*, 1956).

The experiment was performed as follows: on a certain cortical point (sinciput) in one hemisphere was placed a piece of filter-paper soaked in strychnine solution (after the Baglioni-Amantea method). Several minutes later bursts of fast specific strychnine spikes could be recorded from several cortical points (fig. 1).

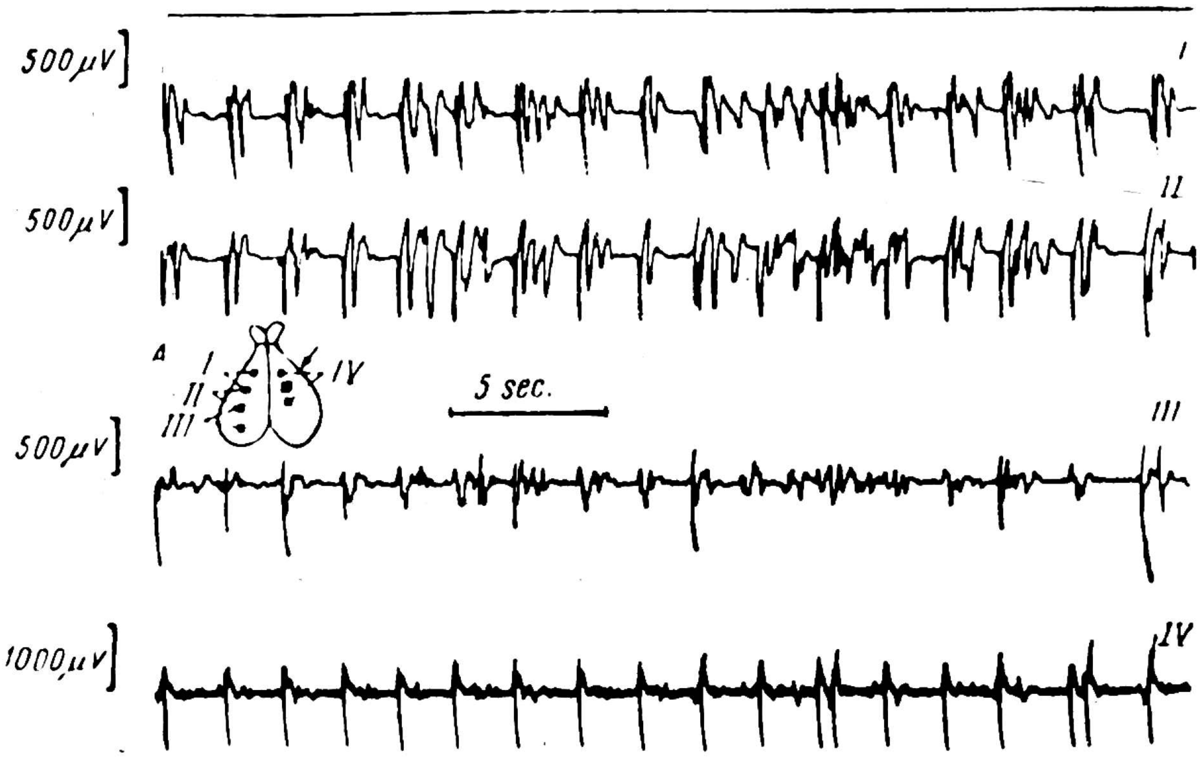

Fig. 1. The initial background of electric activity of cerebral cortex after 40 minutes of applying a piece of paper moistened with strychnine. Leads are marked on fig (A). Typical strychnine discharges are visible in all the points of the cerebral cortex. Arrow — the place of applying the paper moistened with 1% strychnine solution.

The presence of such synchronized discharges in all cortical areas suggested that they could be induced only by some primary discharges in a certain region of the brain effectuating the control over the whole cortex.

Multiple studies have indicated that this region is the thalamic and brain stem reticular formation exerting a simultaneous activating influence on all cortical areas. This assumption was confirmed in further experiments with cooling of the cortical point where strychnine was primarily applied (fig. 2).

The EEG shows that 10 sec. after ice was applied to the strychninized cortical point electric potential changes completely disappeared. Nevertheless in all the other cortical points synchronized discharges remained unchanged in intensity and rhythm (fig. 3).

These results demonstrate that the synchronized so-called „strychnine" cortical discharges are of a „strychnine nature" only by their genesis but not by their mechanism. At this stage, owing to the descending activating effect that strychnine exerts on the subcortical formations, the latter acquire the ability to give independent rhythmic generalized discharges.

It is most interesting, that during the recovery of the strychnine point from cooling, when it begins to give somewhat reduced discharges, these discharges have a completely different rhythm than those displayed in other cortical areas where they were induced from the same strychnine point.

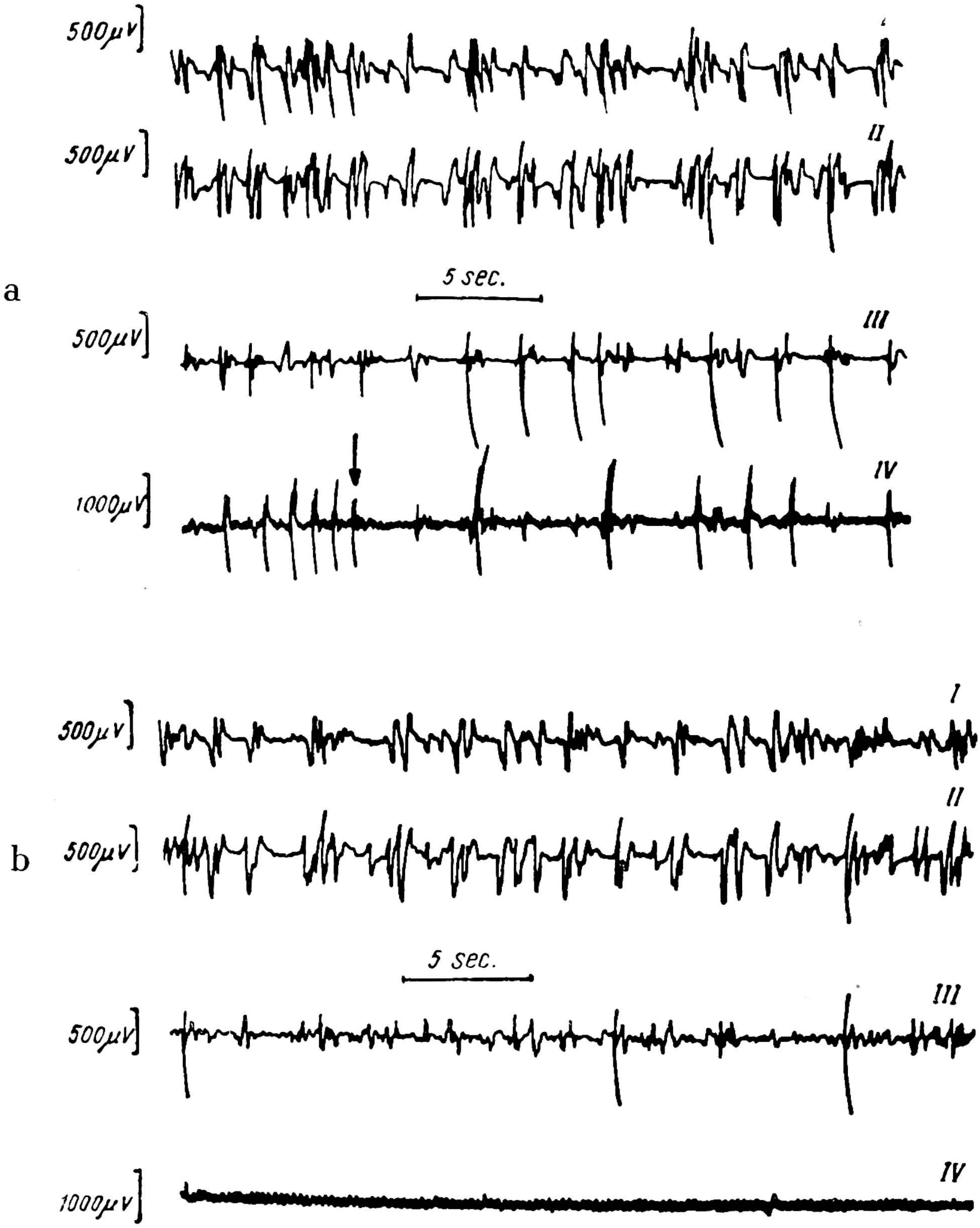

Fig. 2. Two stages of changes of electric activity of the cortex *a* — cat the moment of applying a piece of ice on the previously touched point of cortex by strychnine, *b* — 5 minutes after placing a piece fo ice. Complete disappearance of discharges is visible at the point of applying strychnine. On the contrary, in all the remaining points of the cortex the discharges are persisting at this tempo and with the same intensity. Designation as in fig. 1.

Thus, we have been convinced that simultaneous strychnine discharges in all cortical areas are probably, the result of rhythmic fluctuations of excitability in the brain stem region.

If these assumptions were right, then chlorpromazine, whose blocking effect on the rostral part of reticular formation was already investigated (*Agafonov*, 1956; *Anochin*, 1956) should suspend the generalized strychnine discharges in the cortex, The experiment shows that if aminazine is injected against a background of uninterrupted electrical discharges induced by strychnine, then in some these strychnine discharges are stopped *(Shelichov,* 1956, Unpublished) (fig. 4).

Fig. 3. Regeneration of electric activity in the point of applying a piece of ice. At the beginning arrhythmia is visible between this point and the remaining points of cortex. Designation as in fig. 1.

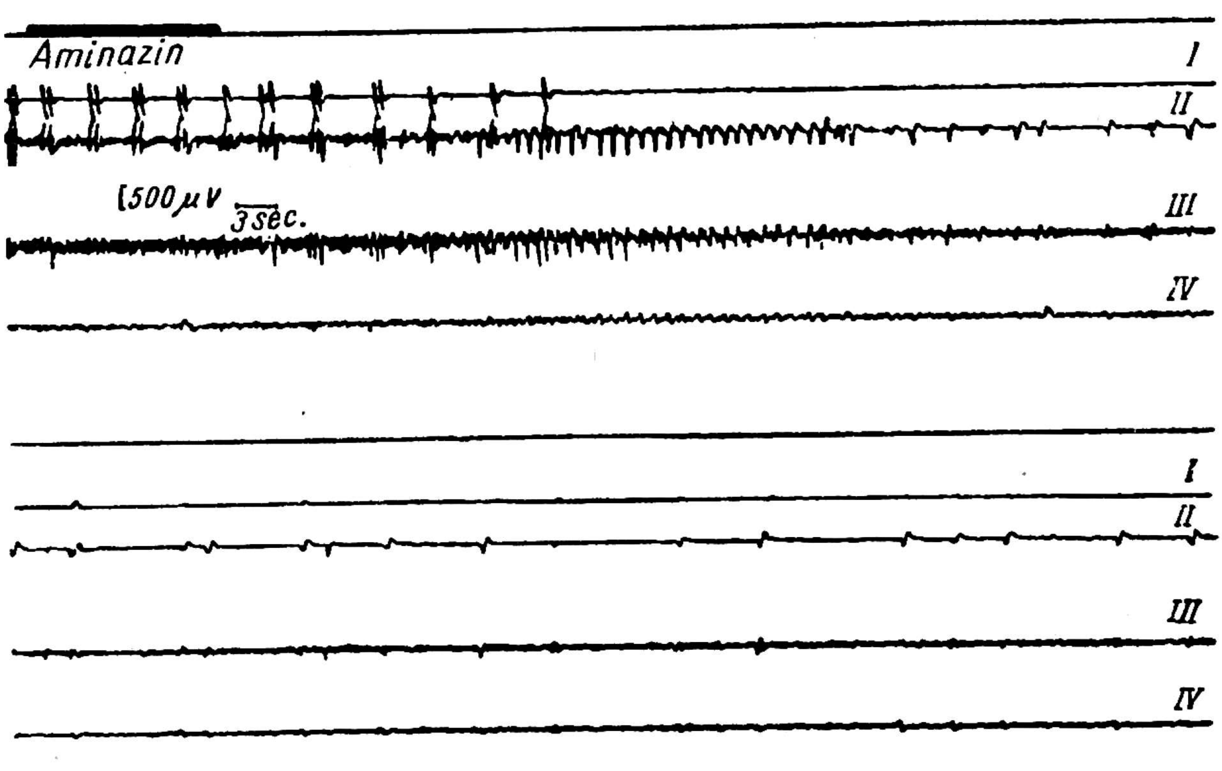

Fig. 4. Aminazine injections. Almost immediate disappearance of discharges of electric activity is visible in the remaining parts of the cortex — persisting after applying a piece of ice. Designation as in fig. 1.

If adrenalin is injected against this background of the aminazine inhibiton of strychnine discharges, then immediately these latter reappear all of a sudden (fig. 5).

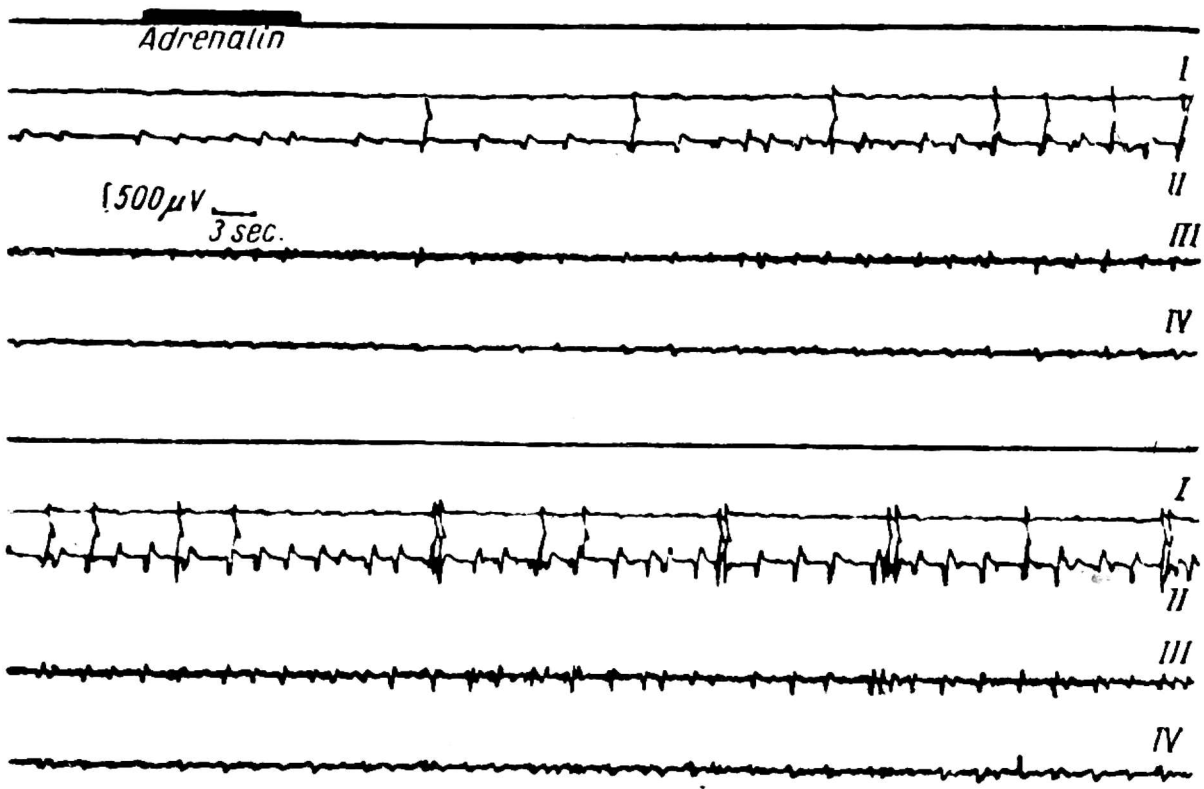

Fig. 5. Adrenaline injection on the background of aminazine action, regeneration of electric activity caused by strychnine is visible. Designation as in fig. 1.

The above series of experiments lead us to the following summary:

1. The rostral part of the brain stem reticular formation represents a region which is readily excited from the cortex and extremely sensitive to adrenalin.

2. Aminazine displays its adrenolytic effect exactly in this area, this effect beeing reversible and readily removed by injection of small amounts of adrenalin into the bloodstream. It may be assumed that the therapeutic effect of some neuroplegics is connected with this process.

3. All the above experiments lead to a general conclusion that adrenalin and aminazine are reticular substances considering their effect on the adrenergic mechanisms of the rostral part of brain stem reticular formation.

Since the mechanisms of cortical activation by means of a reticular formation apparatus has been established in multiple studies of the recent years, naturally an unavoidable question arises: what is the general significance for animal behaviour of the antagonism between the adrenergic mechanism and aminazine? What influence can be exerted by both substances on the qualitative aspect of animal behaviour?

In order to answer the above questions we conceived three series of experiments.

1. In chronic conditions three pairs of electrodes were implanted into the cortex, the thalamus and the stem reticular formation, respectively. In this case most important are systematic observations on the spreading of excitation both during the process of conditioned reflexes elaboration and the stage of their stabilization.

2. In an experiment on both food and defensive conditioned reflexes we performed observations on the state of the electric activity; in certain cases aminazine was used.

3. In conditioned reflex experiments performed by the classic Pavlovian method and the method of defensive conditioned reflexes a comparative evaluation was made of the effect of aminazine and adrenalin on both forms of conditioned reflexes. As the mechanism of relations between these substances at the reticular formation level was well-known from former studies, we expected to reveal their significance for the integral animal behaviour.

All these three series of experiments provided a rather important evidence. It is necessary to discuss in the first place the studies performed in our laboratory by *V. Gavlichek* (Prague).

A defensive conditioned reflex was elaborated by a rabbit on base of nociceptive unconditioned stimulation caused by an electro-cutaneous stimulus applied to the hind limb.

The rabbit was placed into an experimental „hammock", that allowed to combine experiments with electro-cutaneous stimulation and continuous EEG recording from 3—4 cortical points.

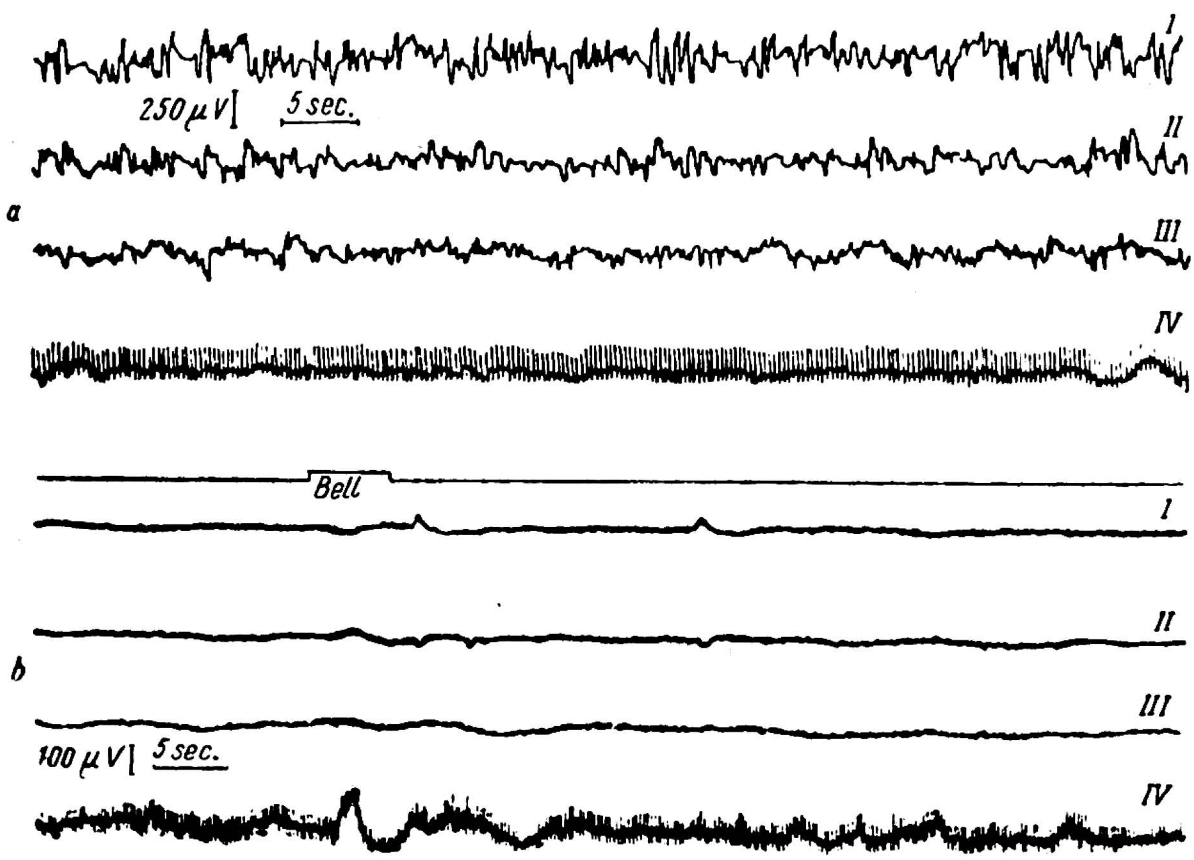

Fig. 6. EEG of a rabbit placed in an experimental „hammock" — *a* — before the action of the stimuli causing defensive reactions, *b* — after several strengthenings with unconditioned electric stimulus (acting on the skin). Visible desynchronization of electric activity of cortex IV EEG. The other designations as in fig. 1.

It was sufficient, as a rule, to put such a rabbit into the environment of the defensive conditioned reflex experiment, and immediately desynchronization of electric wave activity occurred in the cortex (fig. 6).

The EEG shows that the cortical electric activity of the rabbit was a usual one at the stage, when electro-cutaneous nociceptive stimulus had not been applied. It consisted mainly of slow, high-voltage potential changes, usually corresponding to the resting state of the animals (A).

If electro-cutaneous nociceptive stimulation was applied several times in this experimental environment, the electric cortical activity changed in a radical way. It underwent a marked desynchronization, which appeared immediately after the rabbit was placed in this experimental environment (B). From the point of view of physiology of higher nervous activity this represents the well-known conditioned-reflex effect of the experimental environment as a whole. If only food-conditioned reflexes are used, the experimental environment creates, usually, dominant food excitation in case of experiments using electro-cutaneous nociceptive stimuli; the same experimental environment causes a dominant defensive, protective condition.

This fact is quite clear. The most important problem is connected with the EEG correlation of both these biological antipode conditions.

The presence of different forms of cortical electric activity both in food and nociceptive-unconditioned reflexes may be well understood, if we will proceed from the generally recognized and well proved fact that any desynchronization (or activation) of cortical electric activity is a result of the activating effect on cerebral cortex by the rostral part of reticular formation.

This would mean, that nociceptive or protective excitation, arising in response to the signal action of the environment, as a rule, leads to a particularly intensive activation of electric cortical activity through the reticular formation apparatus.

On the contrary, food excitation induced by the same experimental environment is displayed with a rather low intensity in quite different neural subcortical structures, having different chemical specificity. It is necessary to note in this connection, that deliberate rising of excitability by means of preliminary fasting of the experimental rabbit, leads to desynchronization of cortical activity, which is, though, not so intense as in a case of a defensive reflex (*V. Gavlichek*, 1957, unpublished).

As it may be seen from the foregoing evidence, we were faced with two groups of phenomena. On the one hand, we have quite definitely demonstrated that defensive environment, as a whole, and also separate conditioning nociceptive stimuli create invariably a state of intensified activation of the cortical electric activity. On the other hand, data from the laboratory of *Dell*, recent studies of *Rothballer* and a series of investigations made in our laboratory (*V. Agafonov*, 1958; *I. Anochina*, 1956) (*A. Anochina, M. Serbinenko*, 1957; *V. Shelichov*, 1957) convinced us that the activating effect exerted by subcortical mechanism on the cerebral cortex is achieved by involving some adrenergic mechanisms of the brain stem reticular formation.

Confronting of these phenomena naturally leads us to the idea of using such a tried-out adrenolytic as aminazine, in order to follow the evolution of both groups of phenomena-electroencephalographic and behavioristic — in the conditions of defensive conditioned reflex experiments. We expected that such an experiment would provide some information about the biological and physiological nature of the activating effect of reticular formation on the electric cortical activity.

The results were rather interesting and had quite a definite physiological meaning. Several seconds after aminazine was injected intravenously at some distance (into the ear) single slow oscillations appeared against a background of primary defensive desynchronization; minutes later the electric cortical activity assumed the usual character peculiar for the environment of an experiment with food-conditioning stimuli (fig. 7).

a

b

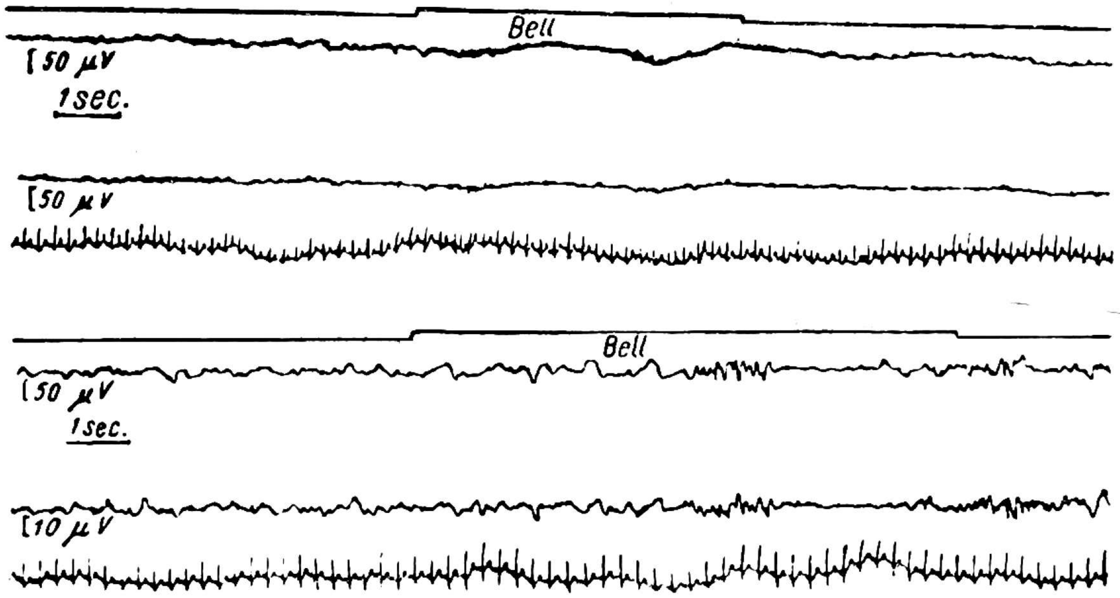

Fig. 7. EEG typical for experiments with defensive conditioned reflexes. Full desynchronization of electric activity (a) and EEG — 1½ hours after introducing aminazine. Well visible synchronization at the moment of conditioned action of the defensive stimulus (a bell).

The most remarkable phenomenon in this series of experiments was a radical change in the rabbits' behaviour, completely corresponding to the EEG evolution.

Any attempt to give the rabbit its favourite carrots, while in the environment of a defensive conditioned reflex experiment, resulted in intensification of the already dominant defensive reaction. The rabbit tried to jump aside and sometimes drew back the limb that had been electrostimulated in the past. But, as soon as desynchronization began to turn, after aminazine injection, into a definite synchronization; the rabbit's behaviour changed in a radical way: the animal grabbed the carrot rather willingly and ate it with a marked appetite.

Most interesting is the fact, that from now on the rabbit could eat its carrot even if the conditioning defensive stimulus signalizing the approach of a nociceptive electrostimulus was put in to action.

This correspondence between the radical behaviour and EEG changes was so demonstrative and constant, that we were able to show them to some of our guests.

Adrenalin injection contributed in the same experimental conditions to the intensification of defensive reflexes excitability and made their manifestation more marked.

This data coincided with the results of another experimental series performed in the conditions of a Pavlovian experiment with a special modification *(J. Makarov)*.

In a group of experimental dogs conditioned reflexes were elaborated as follows: defensive conditioned reflexes were formed in one chamber, and positive food-conditioned reflexes in another one.

Owing to such a spatial distribution of biological-positive and biological-negative conditioned reflexes the experimental animals, finally, elaborated a definite differentiated behaviour.

However, at a certain stage in the formation of this differentiation inadequate reaction in response to a food stimulus was met.

The opportunity presented by this stage of two latent dominant conditions of an antipode character, was used as follows: experimental animal received alternately one day adrenalin, and the next day-aminazine.

In the majority of experiments a complete correspondence with the previous findings was established: adrenalin injection favoured the manifestation of a defensive reaction, independently of the environment in which the conditioning stimulus was applied. On the contrary, aminazine completely eliminated defensive reaction (even in the „defensive" chamber and provided the preponderance of positive food reactions (*J. Makarov*, 1957, Unpublished).

The above studies of *Makarov correspond* to the previous data of our laboratory *(A. I. Shumilina)*. They have shown that aminazine eliminates specific vegetative effects proper to defensive conditioned reactions. This is especially obvious in the respiratory component of conditioned reaction (*A. I. Shumilina*, 1956).

All the above evidence lead us to the conclusion that the clue to the reticular formation activiting effect on the cortex lies in the chemical specificity of those integral reactions which are formed at the hypothalamus and brain stem level.

Most striking is one remarkable peculiarity of the facilitatory and inhibitory influences aminazine and adrenalin exert upon the area of the diencephalon.

While adrenalin provides chiefly the domination and facilitates the development of those reactions, which normally proceed with the prevailing participation of the sympatho-adrenal system, aminazine, on the contrary, blocks up the formation of exactly these reactions, and, hence, provides the domination of biological-positive reactions according to the principles of reciprocal relations.

Indeed, as we have seen, aminazine injection does not inhibit the activity of the subcortical mechanism, in general. Aminazine acts quite selectively on the systems of connections and centres related with the biological-negative reaction. On the contrary, the biological-positive activity is even somewhat emphasized. This is well illustrated by the above mentioned behaviour of the rabbit under aminazine. Furthermore, on the base of clinical observations on the aminazine treatment of mental patients, it is known, that during periods of alleviation of pathological emotional states the patients exhibit suddenly a voracious appetite. The experiments of my collaborator *V. Polyanzev* demonstrate the selective action of aminazine on the neural mechanisms, which are involved in patterning of 12 nociceptive and stress reactions.

He has shown that in decerebrated cats stimulation of the lingual nerve causes reflex secretion of the submaxillar salivary gland. Stimulation of the sciatic nerve invariably inhibits this salivation with great constancy. If aminazine is injected in such a preparation, the positive secretory reflex remains intact, while the inhibitory effect of sciatic stimulation on this secretion is completely eliminated.

It is evident that, in this case, aminazine acts selectively upon that connection system which patterns the nociceptive reaction, that usually inhibits all the other activities of the organism.

Thus, by comparing all the above mentioned variations of experiments performed in our laboratory, we may draw several conclusions which expand to some extent our notions on the patterning of the integral activities of the organism and especially on their significance in the study of higher nervous activity.

1. Experimental data have shown that all the external conditions which pattern defensive and nociceptive cortical reactions achieve this by excitation of adrenergic mechanisms in the hypothalamus and the brain stem reticular formation. This system of connections is specific by its chemical properties and probably, constitutes a base for the general formation of biological-negative reactions in the *Pavlovian* sense.

2. This system of adrenergic connections is reciprocally related with biological-positive reactions, and inhibits them already at the level of subcortical mechanisms.

3. The specific selective effect exerted by aminazine, as an adrenolytic substance, upon biological-negative reactions (defensive, nociceptive) strengthens the assumption that the pattern of biological-negative connections in the central nervous system has definite limits, is chemically specific and may be, therefore, subjected to specific chemotherapy. From the biological point of view this chemical specificity of nervous connections, patterning the biological-negative reactions of the organism, indicates that the neural defense mechanisms developed themselves in connection with and against a background of the development of the sympathoadrenal system. Herein may lie one of the causes contributing to the wide participation of the sympathetic nervous system in the cerebral activity (*L. A. Orbeli*, 1932).

The above evidence indicates that both planes of investigations — the central nervous system and the sympathetic nervous system-should be at present united in the fact, that desynchronization induced in cortical activity by subcortical formations is connected with the action of adrenalin on the subcortical adrenergic system.

4. The rather definite chemical outlining of biological-negative and biological-positive reactions confirms our former concept that although both these systems may serve, at the same extent, as bases of conditioned reflex elaboration, nevertheless they cannot be identified in the evaluation, of inhibitory conditioned reflexes (*Anochin*, 1935, 1949, 1958).

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