PROGRESS IN BRAIN RESEARCH

VOLUME 22

BRAIN REFLEXES

EDITED BY

E. A. ASRATYAN

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Proceedings of the International Conference dedicated to the centenary celebration of the publication of I. M. Sechenov's book *Brain Reflexes*

sponsored by the
Academy of Sciences of the USSR and the International Brain Research Organisation (IBRO)

EDITED BY

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ELSEVIER PUBLISHING COMPANY
AMSTERDAM / LONDON / NEW YORK
1968

ELSEVIER PUBLISHING COMPANY
335 JAN VAN GALENSTRAAT, P.O. BOX 211, AMSTERDAM

AMERICAN ELSEVIER PUBLISHING COMPANY, INC.
52 VANDERBILT AVENUE, NEW YORK, N.Y. 10017

ELSEVIER PUBLISHING COMPANY LIMITED
RIPPLESIDE COMMERCIAL ESTATE, BARKING, ESSEX

LIBRARY OF CONGRESS CATALOG CARD NUMBER 65-25214

WITH 356 ILLUSTRATIONS AND 15 TABLES

PRINTED IN THE NETHERLANDS

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Preface

This book contains research reports and review articles given at an interdisciplinary international meeting in Moscow. This book is a follow-up of the first volume of this series (titled *Brain Mechanisms*) and was sponsored by the International Brain Research Organization and the USSR Academy of Sciences.

The meeting was dedicated to the century celebration of Sechenov's book *Brain Reflexes*. It therefore illustrates the tremendous increase in our field since the publication of the famous book.

In order to give an overall review of the main aspects of the brain sciences the following three symposiums were being held:

(a) "Brain reflexes" and central inhibition;

(b) General principles of self-regulation in cortico-subcortical correlations;

(c) Evolutionary physiology of the nervous system and brain ontogenesis.

It was expected that this book will serve as a guide to classical and modern Soviet neurophysiologists.

The Editor

1st Symposium
"Brain Reflexes" and Central Inhibition

2nd Symposium
General Principles of Self-regulation in Cortico-subcortical Correlations

3rd Symposium
Evolutionary Physiology of the Nervous System and Brain Ontogenesis

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2. General Principles of Self-regulation in Cortico-subcortical Correlations

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'BRAIN REFLEXES' AND CENTRAL INHIBITION

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Central Inhibition of Reflexes and the Problem of the Coupled Activity of Cerebral Hemispheres

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The fact that the process of inhibition appears in living tissue was first discovered experimentally by the Weber brothers. These authors observed that when the vagus nerve is excited with electric current, the heart ceases contracting. It was not long after this discovery that I. M. Sechenov attempted to prove the presence of inhibition centres in the regio thalamica by his famous experiment of 1862. Further experiments have made it clear that there are no special inhibiting formations in the central nervous system, but that the process of inhibition that occurs within it is always associated with the process of stimulation.

Sechenov's experiment played a great role in the development of physiology of the central nervous system, since it was the initial phase of systematic studies of central inhibition.

A century has passed since versatile and intensive studies of this process started, involving prominent physiologists, and yet the nature of inhibition, and its relation to the stimulation are far from fully discovered. Inhibition, in the broad meaning of the term, is understood to be complete temporary cessation or temporary suppression of the functioning of an individual organ or the whole organism. Proceeding from this definition, it is easy to understand the process of inhibition when drugs or other toxic agents are introduced, when inhibition results from exhaustion of the energy-producing material in a working organ, with fatigue or when there is no stimulation; on the other hand, inhibition is difficult to understand when it appears with complex co-ordinated movements. This act is known to alternate rapidly between excitation and inhibition within one and the same group of muscles, or both of them proceed simultaneously in different groups of muscles, which ensures well-co-ordinated movement. It remains obscure how this co-ordinated correlation between excitation and inhibition is actually exercised. Almost every author believes that inhibition is an innate process and always appears alongside excitation of any afferent nerve, but this assumption does not solve the problem of their correlation, for instance, when excitation may partly or completely displace inhibition from some organ, and *vice versa*.

The great physiologist I. P. Pavlov introduced a new productive idea into the theory of inhibition by his experiments with conditioned reflexes. He demonstrated that

inhibition is not merely innate, but it always appears and again disappears in the central nervous system throughout the life of an organism. Pavlov thought that inhibition is the reverse process of excitation, though he believed that their intimate physiochemical processes are identical; both excitation and inhibition being active processes, they both irradiate, are concentrated and actively affect each other. Excitation may reduce or eliminate inhibition completely, and the reverse situation occurs as easily, but the nature of these relations has remained an enigma to this day: to the end of his life Pavlov considered it to be the 'accursed' problem. It is evident that a deeper study of the problem of inhibition will involve further accumulation of new facts, by means of diversified methods and from new angles.

It seems reasonable to plan experiments designed to study the problem of inhibition by analysing those processes which, according to Pavlov, are called inner inhibition. They appear in an animal's individual life. In experiments with conditioned reflexes this kind of inhibition is known to appear literally before the scientist's eyes and also to vanish under certain easily reproduced conditions.

We began our studies of inner inhibition which combined four types: differentiation, conditioned inhibition, delay and dying out. In our experiments we used the method of salivary conditioned reflexes. Our method of elaborating conditioned reflexes was modified by the operation of bringing out symmetrical strips of the back third of the tongue to the surface of the cheek which were implanted on to the skin of the dog's lower jaw separately from one another. When solutions of chemicals were applied to the mucous surface of one of them, regular salivation was observed with the ipsilateral gland; and it is on the basis of this unconditioned reflex that a conditioned reflex of salivation can be formed on the same side.

Further experiments demonstrated that, after application of the chemical is stopped and the resulting salivation ceases, excitation is retained in the respective area of the central nervous system during a period of two minutes to some hours, or even days. As this excitation cannot be detected through the outward effect of salivation, it was termed latent (concealed) stimulation, analogous to the long known latent excitation in experiments with subliminal excitation.

Special stress must be made of the fact that latent excitation remains in that part of the central nervous system where it appears and cannot be transferred to other areas: thus if it appears on one side of the central nervous system it stays there; if it appears in the food centre it is not transferred to the acid centre, etc. Yet every recurrent excitation on either side and at every point of the central nervous system is directed towards latent excitation, is added to it and produces an effect characteristic of a nervous centre where latent excitationp ersists. Such a summation effect was called a summation reflex. An obvious example of a summation reflex is observed in the experiment on a dog in which some areas of the tongue are brought out to the surface of the cheek. If we produce latent excitation in such a dog on the left side, for example, by applying acid to the mucous surface of a portion of the tongue on the same side, the positive conditioned contralateral stimulus (on the right side) will reveal its positive effect not on its own, right side, but on the left, that is, on the side of latent excitation. This finding demonstrates, beyond any doubt, that the presence of latent

excitation affects the right-side positive conditioned stimulus and switches excitation into its own direction. With a greater intensity of latent excitation the effect produced by the conditioned stimulus is completely transferred to its own side, with equal intensities, that is, the effect of the positive stimulus forks into two: part of it remains on its own side while the rest is directed towards latent excitation. In the third case, where latent excitation is present, there is no positive effect of the positive irritant on either side. Evidently, conditioned excitation is directed into the centre of latent excitation but it is not intensive enough to transform into the summation effect when added to the latter. The correlation of the conditioned stimulus and latent excitation is characterized by the above three cases.

Later, when correlations of the positive conditioned stimulus and latent excitation had been studied, experiments were planned to investigate correlations between the inhibitory stimulus and latent excitation. A differentiation stimulus and an extinguished stimulus, that is, a stimulus after a conditioned reflex to it had been extinguished, were used. When latent excitation was produced on one side and, after this, a differentiation or extinguished stimulus was used, they also produced a summation, *e.g.* a positive effect on the side of latent excitation as we have already seen in experiments with a positive conditioned stimulus. This observation appeared somewhat unexpectedly and was not perceived at first because we know that the summation effect appears provided new excitation is directed towards the centre of latent excitation. What, then, is the source of excitation with these inhibitory stimuli when we apply an extinguished or differentiation stimulus and obtain a positive summation reflex? One could imagine that if a latent situation appears on one side and an inhibitory stimulus is applied to the other, the latter is disinhibited and thus produces a positive effect. Yet this is impossible as the inhibitory stimulus causes a positive effect, not on its own side where disinhibition should take place, but on the opposite side. One could still imagine that if an inhibitory stimulus is applied to one side, excitation is induced on the other side and, added to latent excitation, it produces a positive effect. This is also improbable because if a differentiation stimulus has been applied to one side, and a positive stimulus follows on the other, the latter does not cause a great positive effect, but, on the contrary, diminishes it, which testifies to the view that absolutely no positive induction from the inhibitory stimulus could have taken place here. One could resort to still another though strained interpretation, namely, admit that an inhibitory stimulus is produced by both excitation and inhibition to the same extent, and that with a centre of latent excitation on one side, it is only excitation that is transferred to it from the inhibitory stimulus of the other side, which is added to the former and produces a positive effect. This suggestion also attracts criticism, as, when an inhibitory stimulus is applied to one side, and a positive one to the other, the latter is almost always inhibited. What are the grounds then, for thinking that an inhibitory stimulus spreads only excitation towards latent excitation, while it sends out inhibition alone in the direction of excitation from a positive stimulus?

The above phenomena having been mentioned there remains nothing else, at least at this initial phase of study, but to state that the inhibitory stimulus alone is the source of

excitation, and thus inhibition and excitation represent one and the same process of excitation.

If we admit that the two processes are identical, further objections arise: how are we to understand the obvious difference which distinguishes an animal's behaviour under inhibition from that under excitation, and how are we to interpret successive inhibition when a positive stimulus ceases to be effective partly or wholly, after an inhibitory stimulus has been applied? These objections are so sound and difficult to disprove that the student of this problem must either reject identity of inhibition and excitation or admit that our notion of inner inhibition and of how it differs from excitation is the result of erroneous perception of facts, similar to our wrong idea of the sun revolving around the earth when it is observed from the latter.

The key to the understanding of the nature of inhibition is probably to be found in the very mechanism of its formation. This mechanism consists in that the selected future inhibitory stimulus is applied, and always left, without being reinforced with an unconditioned reflex. There can be no doubt that, while affecting the receptive surface of an animal, this stimulus causes excitation at the respective point of the central nervous system through centripetal nerves, but in accordance with the conditions of our experiments, it is not reinforced with an unconditioned reflex, namely the unconditioned salivary reflex, which means that this excitation is not manifested through the activity of the salivary gland (the outward sign), but remains within the central part. With repeated applications of an inhibitory stimulus, the respective excitation is concentrated in the centre of its emergence to a still greater extent. This real central excitatory process cannot be observed in routine experiments with conditioned reflexes through outward signs of the activities of the outer organ (the salivary gland), and consequently, it appears as an inhibitory stimulus. As we have already seen, this central process of excitation is made manifest through latent excitation.

If identity of excitation and inhibition is admitted, successive inhibition is also easy to explain. Since an inhibitory stimulus induces excitation in a certain part of the central nervous system, it leaves behind latent excitation in that part, and every new excitation applied after this inhibition, including that from a conditioned stimulus, is directed towards it. Consequently, the effect of the positive conditioned stimulus, as we have seen above in the correlation between a conditioned stimulus and latent excitation, is reduced or vanishes altogether, which stimulates inhibition from an applied inhibitory stimulus.

The above arguments lead to the conclusion that inner inhibition and excitation are identical phenomena, that they represent one and the same process of excitation, and that all the diversified appearances of correlation between excitatory and inhibitory processes may be classified as different degrees of transition from nonlatent excitation* (be it conditioned or unconditioned) to the centre of latent excitation or to the working organ.

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* While giving an account of our materials, we had to find a specific term to denote excitation which exists when its agent is acting. For the sake of simplicity, it was termed nonlatent excitation as distinct from latent excitation which remains after the stimulus ceases to act.

SUMMARY

In our experiments Pavlov's classic method of salivary conditioned reflexes has been used. Recently it has been modified by an operation of bringing out symmetrical strips of the back third of the tongue to the cheek surface. Treatment of the surface of one of these strips with solutions of chemical substances causes unconditioned salivation from the ipsilateral gland and on the basis of this reflex a conditioned reaction on the same side may be elaborated. When the cortex of one of the hemispheres is removed, conditioned salivary reflexes disappear and unconditioned reflexes decrease only ipsilaterally. Thus unilateral conditioned salivary reflexes are formed with the participation of the cortex of the corresponding hemisphere.

In intact dogs after cessation of surface stimulation of the exposed strip of the tongue, and after cessation of the reflex salivation, latent excitation of the ipsilateral hemisphere still persists from two minutes to several hours or even days.

Any newly evoked excitation in either hemisphere is directed to latent excitation and, being summated with the latter, evokes a summation reflex.

When latent excitation arises in one hemisphere and the other receives a unilateral positive conditioned stimulus, then this stimulus gives a positive effect not on its side, as usual, but on the opposite one, *i.e.* on the side of latent excitation.

When one of the hemispheres is in the state of latent excitation and the other one receives either a differentiation stimulus or an extinguished conditioned stimulus, then these inhibitory stimuli also produce a positive effect on the side of latent excitation.

From these results it may be assumed that in our experiments the central effect caused by a positive stimulus and that caused by inhibitory stimuli are evoked by one and the same excitatory process, with the only difference that excitation elicited by the positive stimulus manifests itself in the form of external reactions, whereas excitation produced by inhibitory stimuli evokes only central excitation without any external manifestation. Therefore inhibitory stimuli should be only 'formally' considered as inhibitory.

Some Peculiarities of Formation, Functioning and Inhibition of Conditioned Reflexes with two-way Connections

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The bold and profound ideas of Sechenov on the reflex origin and nature of psychic activity and his interpretation of the cerebral or psychic reflex as the product of association of elementary reflexes are justly considered as the prelude to Pavlov's theory of the conditioned reflex and to his teaching on higher nervous activity. Therefore every step directed to expansion and deepening of our knowledge of the conditioned reflex, this central phenomenon in cerebral activity, ought to be considered not only as a furthering of Pavlov's basic teaching, but also as a furthering of the main idea of that great work of Sechenov, to the centenary of whose publication the present Conference is dedicated.

My collaborators and I are happy to report some results of our experiments concerned with the study of one of the most important but little-studied aspects of conditioned reflexes, *i.e.* two-way conditioned connections. In this report four sides of this problem will be discussed: (1) the very existence of conditioned reflexes with two-way conditioned connection; (2) the nature of this connection; (3) certain functional peculiarities of these connections; and (4) their physiological importance.

1. Conditioned reflexes with two-way conditioned connection

The idea of two-way conditioned connections, as well as the idea of association in general, came into the physiology of brain from psychology. In its time this problem attracted the attention of Pavlov, the founder of the teaching on higher nervous activity, and of a number of his associates. This question has been studied for a long time by Beritashvili (1932) and his collaborators; recently we too have joined the army of scientists studying this problem; and a number of Russian and foreign physiologists have followed.

The problem of two-way conditioned connection or two-way conductivity of conditioned connections still makes its way into physiology rather slowly and with some difficulty; the majority of investigators in the field of physiology of conditioned reflexes do not pay it its due. Moreover, there are discrepancies in the opinions of even

those few who admit the existence of such conditioned connections. Beritashvili was the first among our physiologists to suggest the existence of these connections that consist, according to his terminology, in a forward connection that transmits excitation from the nervous elements pertaining to the conditioned stimulus to the nervous elements pertaining to the unconditioned stimulus, and of a backward connection that transmits excitation from the latter nervous structures to the former. Beritov's interpretation of the role of these connections was rather peculiar. He believed that excitation of both the connections leads to diametrically opposite results, namely, excitation of forward conditioned connection brings out the conditioned reflex, whereas excitation of backward connection finds its external expression in the phenomena of extinction, differentiation, delay, *i.e.* in the phenomena which Pavlov and his disciples considered as a manifestation of internal or conditioned cortical inhibition. It should be remembered that this interpretation of the role of backward connection was suggested by Beritashvili in that period of his scientific activity when he denied the ability of cortical cells to develop inhibition. Pavlov, in whose laboratories the most precise and valuable experimental data on this problem were obtained, suggested the existence in motor conditioned reflexes of two-way conditioned connection, along which 'the process of excitation moves to and fro, *i.e.* in the opposite directions'. He believed these connections to constitute the physiological basis of voluntary movements. 'Thus', Pavlov wrote 'kinesthetic cells of the cortex get connected with all the cortical cells, representatives of both the external influences and various internal processes. This is the physiological basis of the so-called voluntarism of movements, *i.e.* of their being determined by the total activity of the cortex' (Pavlov, 1938, p. 703).

As far as I can judge, this point of view is shared in principle by the overwhelming majority of Pavlov's disciples, if not by all.

Dedicating our efforts, among other disciples of Pavlov such as Konorsky, Kupalov, Fyodorov, Skipin, to experimental and theoretical furthering of this problem, which is the central part of Pavlov's teaching, a few years ago we suggested a hypothesis that coupling of two-way conditioned connection is not an individual phenomenon, characteristic of only a certain category of conditioned reflexes, but a universal phenomenon for all the vast and multiform class of conditioned reflexes. This point of view seems to be shared at present by other scientists, among whom Dostalek from Czechoslovakia should be mentioned as one who, in collaboration with his associates, has of late carried out interesting researches in this field.

In this report I would like to discuss some old and new facts concerning the question of two-way conditioned connection obtained by our collaborators Varga, Lian Chi An, Pressman, Struchkov and Khachaturian on the peculiarities of these connections and dynamics of their formation, functioning and inhibition.

Here I would like to draw your attention to one methodological particularity of our experiments. As distinct from other investigations performed in our country and abroad, which mainly employ the so-called indifferent stimuli for elaboration of conditioned reflexes with two-way connections, as a rule we pair such stimuli, each of which, taken separately, evokes easily observable and graphically registrable

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reactions. Besides routinely used stimuli — food and electrocutaneous stimulation — we also employ such stimuli as a puff of air into the eye (to evoke the reaction of blinking), passive lifting of the leg (which is accompanied by lessening of discharges in the electromyogram of extensor muscles of the given leg), cooling of a limited skin area (to evoke local vasomotor reflex), local electrical stimulation of certain areas of the motor cortex (to evoke the movement of the respective peripheral organ), etc. Pairing of such stimuli allows us not only objectively to observe and record the expected two-way conditioned reflexes directly, without employing other stimuli and manipulations, but also to observe and record the inborn reflexes to both signal and reinforcing stimuli and to follow up their evolution. From time to time in our experiments we use the so-called indifferent light and sound stimuli, but not in order to combine them with each other, as many scientists have done and are doing, but for pairing either of them with some stimulus mentioned above in this or that sequence. This gives us the possibility of evaluating the interesting phenomena directly, without the help of irrelevant stimuli and additional manipulations that are needed when indifferent stimuli are paired. At present the fact of existence of conditioned reflexes with two-way connection may be considered as established not only in the sphere of skeletal motor activity, but also in the field of secretive, vasomotor and other reflex reactions. There is every reason to believe that formation of such reflexes enters the sphere of general regularities of cerebral activity, at any rate in higher animals. This is evidenced by a number of old and fresh data: by the results from animal training, by the results obtained in special laboratory experiments by Kalisher, Konorsky, Miller and others and used by Pavlov for formulating his hypothesis on the question here discussed; by the results obtained later by P. S. Kupalov, G. V. Skipin and others; by the data of Zelyony, Podkopaev and many others in experiments on the so-called associative reflexes; by the old and somewhat contradictory results of Pimenov, Krestovnikov and by the fresh data on conditioning with the method of 'covering', and finally, by the new facts obtained in the laboratories of Kupalov, Beritashvili, in our laboratories, and in the laboratory of the Czechoslovakian physiologists Antal, Dostalek and others.

In this report I would like to dwell in detail on some of the fresh data obtained in our laboratory.

From the published data of our collaborators Varga, Lian Chi An, Pressman, Struchkov and Khachaturian it is known that conditioned reflexes with two-way connection are elaborated not only when the stimuli are presented in a variable sequence, but also to any stereotype of their combination. In principle this applies to the stimuli of approximately equal as well as different intensities. In our experiments such results were obtained upon the stereotyped combination of the following pairs of stimuli: sound and passive lifting of the leg; sound and a puff of air into the eye; passive lifting of the leg and a puff of air into the eye; food and electrocutaneous stimulation; food and passive lifting of the leg; food and local cooling of the skin. It is true that the connections thus elaborated largely varied in the degree of their establishment, regularity, stability, and other functional properties, dependent on a number of circumstances (which will be discussed below), but in each given example this dependence has no principal bearing. The important fact here is that any sequence

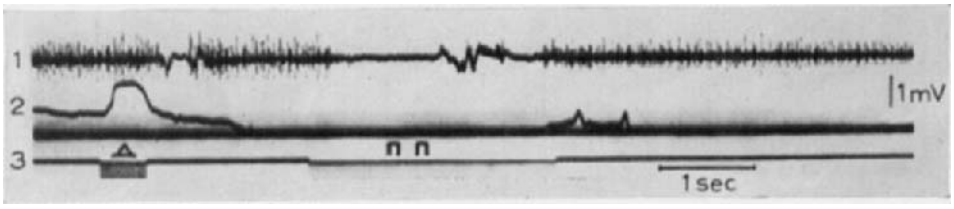

Fig. 1. Conditioned reflex with two-way conditioned connection upon stereotyped sequence of stimuli presentation: blowing of air and passive lifting of the limb. Blowing of air, apart from unconditioned blinking, evokes a conditioned reaction in the form of falling out of potential in EMG (direct conditioned connection). Passive lifting of the limb (PL), apart from unconditioned reaction of falling out of potential in EMG, evokes a conditioned reflex reaction of blinking (backward conditioned connection). (1) electromyogram of the calf muscle of the leg; (2) eye lid movement; (3) stimulation.

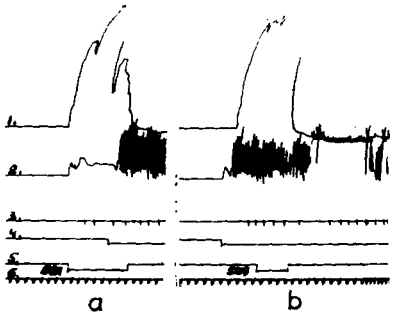

Fig. 2. Conditioned reflex with two-way connection upon alternating sequence of stimuli presentation: (a) electric current — food; (b) food — electric current. (a) Electric current apart from unconditioned defence motor reaction evokes conditioned alimentary reflex. (b) Food, apart from unconditioned alimentary reaction, evokes conditioned alimentary reaction. (1) movement of the leg; (2) chewing; (3) salivation; (4) food presentation; (5) electrocutaneous stimulation; (6) time in sec.

and pairing of stimuli result in elaboration of conditioned reflexes with two-way connections. This is illustrated in Figs. 1, 2 and 3 by kymograms of the reflexes from new experiments of Varga, Pressman, Struchkov and Khachaturian. Apart from the data or our laboratory, fresh and valuable material on the question discussed herein has been obtained in other laboratories. From the experiments of Kupalov's laboratory (1955) performed in conditions of unrestricted behaviour of animals the following interesting facts were obtained: in the intervals between presentations of positive alimentary sound conditioned stimuli (metronome), the majority of experimental animals were standing near the origin of the stimulus. In other experiments the metronome stimulation was timed to an occasional shaking reflex and reinforced by food; the result was that the animals started shaking more often and looking in the direction of the metronome. In the opinion of Kupalov, this is evidence that the process of excitation goes from the centres of alimentary reflex to the centres of reflexes evoked by conditioned stimuli. In the experiments of Antal (1959), out of many similar signs the animals chose the one that was turned into the alimentary conditioned signal. Interesting observations were made in this respect by Dostalek (1961). In the experi-

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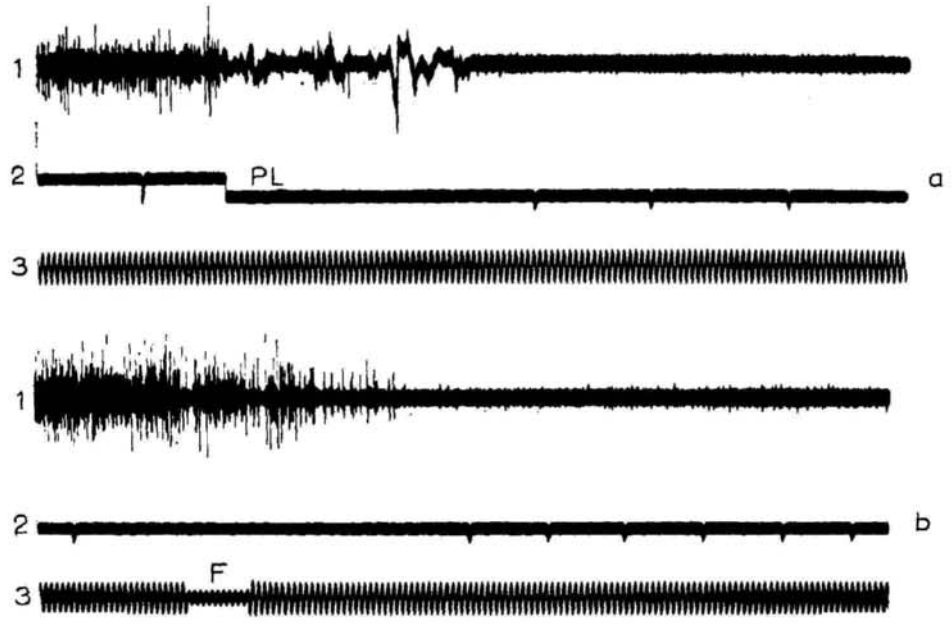

Fig. 3. Conditioned reflex with two-way connection upon stereotyped sequence of stimuli presentation: passive lifting of the leg and food; (a) passive lifting of the leg (PL), apart from unconditioned reflex of falling out of potentials in EMG, evokes conditioned salivation (forward conditioned connection); (b) food (F), apart from unconditioned salivation, evokes conditioned falling out of potentials in EMG (backward conditioned connection).

ments performed on 5-year-old children and on adults he found a regular formation of conditioned reflexes with two-way connections (which he termed 'double-sided conditioned reflexes') upon application of stimuli in the stereotyped sequence. For instance, upon application of flickering light and electrocutaneous stimulation in any stereotyped sequence, each of these stimuli acquired, with time, the ability to elicit the effect of the partner stimulus. Dostalek and his collaborators also showed that this principle of elaboration of two-way connections in the sphere of secondary signal conditioned reflexes is even better manifested than in the primary signal reflexes.

2. Nature of the two-way conditioned connection

A question arises: what is this two-way conditioned connection between two cerebral points? Is it a one-track connection with two-way conductivity, or is it two-way traffic conducting excitation in opposite directions?

Pavlov seems to have thought it to be the latter. He wrote: 'The acceptance of the hypothesis of one-way conduction of nervous processes in all the points of the nervous system necessitates our assuming an additional connection between these points in the reverse direction[9] (Pavlov, 1938, p. 569). The same point of view is shared by Beritashvili. The results obtained in our laboratory also favour the hypothesis on the coupling of two separate and reverse-conducting conditioned connections. This relates to two-way conditioned reflexes elaborated to stimuli combined in the alter-

nating sequence as well as to stimuli presented in stereotyped sequence. The correctness of this concept seems to be corroborated by the following observations. First, coupled conditioned reflexes are elaborated, as a rule, not simultaneously, but mostly one after the other, regardless of whether the sequence of stimuli presentation is alternating or stereotyped. Secondly, application of one of the stimuli is often accompanied not by two, but by a succesion of three and even more reflexes. For example, if the two-way conditioned reflex is elaborated to stereotyped combination of blowing of air into the eye with passive lifting of the leg (experiments of Varga and Pressman), a puff of air into the eye results in blinking reflex (unconditioned reflex), often followed by lessening of the potentials of the leg's extensor muscles (conditioned reflex), and then, almost immediately after, the blinking reflex reappears (conditioned reflex). The kymograms of these experiments (fig. 4) cleary show circulation of excitation between two nervous foci along contrarily directed conditioned connections.

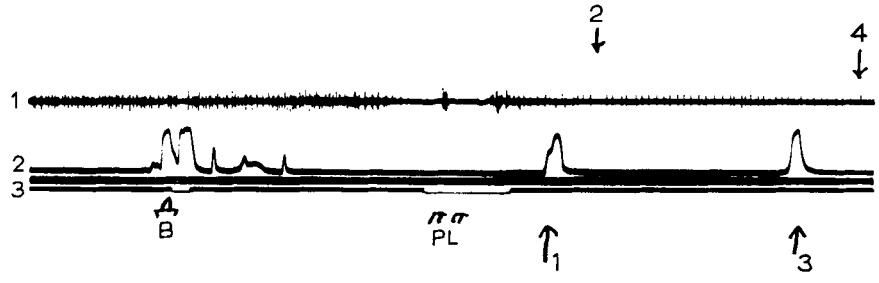

Fig. 4. Circulation of excitation between the nervous centres of blinking and motor reactions in the conditioned reflex elaborated to the combination of stimuli: blowing of air and passive lifting of the leg. As usual, blowing of air (B) evokes unconditioned blinking reaction and conditioned lessening of potentials in EMG. Passive lifting (PL) evokes unconditioned lessening of potentials in EMG, and through backward conditioned connection, conditioned blinking reaction (arrow 1). It should be noted that this is followed by another lessening of potentials (arrow 2), which again entails blinking reaction (arrow 3), and the latter entails new lessening of potentials (arrow 4).

The existence of two separate connections is also confirmed by the dynamic peculiarities of the process of inhibition in them. Inhibition of each of the paired conditioned reflexes may take a separate course, at least for some time after its appearance. When, for instance, one of the reflexes is fully extinguished, the other persists for a while, often even intensified. This is illustrated by the kymograms of the experiments of Struchkov and Khachaturian (Figs. 5 and 6). The degree and the time of appearance of inhibition in paired conditioned reflexes may be different even when it originates from the action of extra stimuli or other external or internal factors.

These and other relevant and absolutely authentic observations obtained in our laboratory support our hypothesis on separateness of the conditioned connections of two paired conditioned reflexes; moreover they corroborate another assumption of ours, made many years ago, that inhibition initially appears and localizes in the structures of the conditioned connection itself.

In the dynamics of inhibition of two-way conditioned reflexes there are two pecu-

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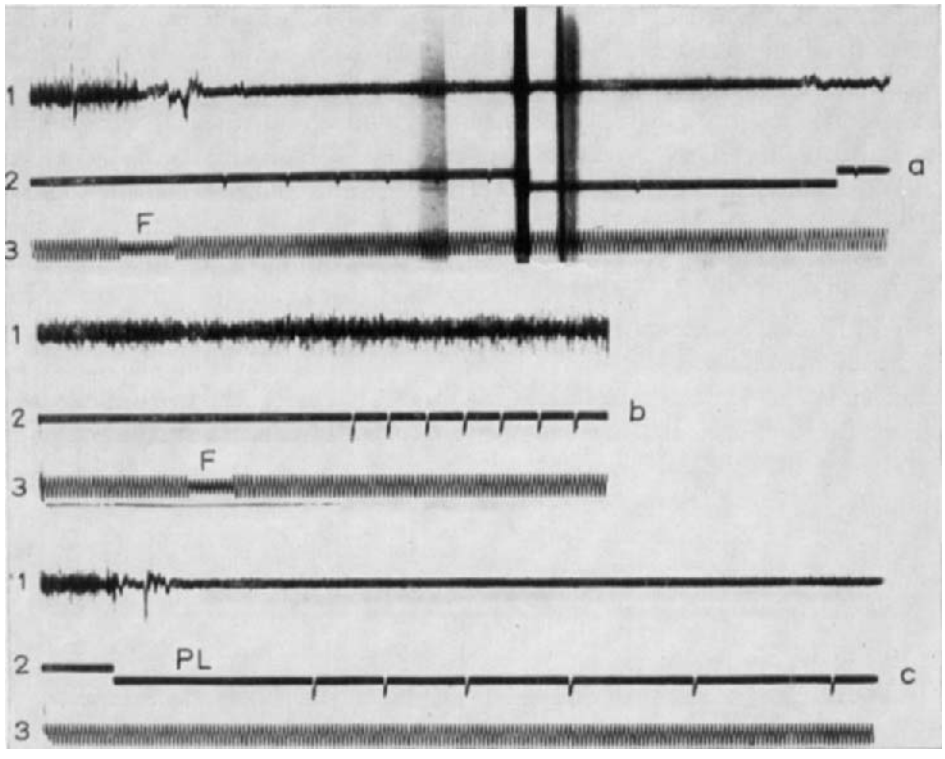

Fig. 5. Manifestation of forward conditioned connection (c) after extinction of backward conditioned connection (a, b). Stimuli are applied in the stereotyped sequence: passive lifting of the leg and food. (a) during the first trial of food presentation (F), the effect of backward connection, *i.e.* falling out of potentials in EMG, is observed. (b) during the fourth trial of food effect, backward connection turns out to be extinct. (c) subsequent passive lifting of the leg (PL) preserves forward conditioned connection (salivation).

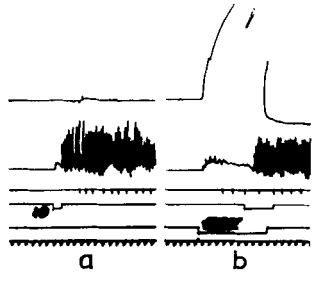

Fig. 6. Manifestation of alimentary conditioned connection to electric current (b) after extinction of defence motor reaction to food (a) in conditioned reflex with two-way connection elaborated to alternating sequence of stimuli presentation: electric current — food; food — electric current.

liarities which indicate that, although conditioned connections of these reflexes exist separately and may even be inhibited separately, nevertheless they are closely bound with each other and interact continually. When, after a full extinction of one of the paired reflexes, a trial of the partner stimulus is performed (*i.e.* that stimulus, pres-

entation of which was temporarily cancelled to obtain the desired extinction), it results in restoration of the extinct reflex, as occurs with reinforcement of any extinct conditioned reflex. For example, after extinction of a motor conditioned reflex to food, even a single eliciting of the movement cancelled in order to extinguish this reflex leads to restoration of the extinct conditioned reflex (experiments of Struchkov, Fig. 7). Another interesting phenomenon is observed in those two-way conditioned

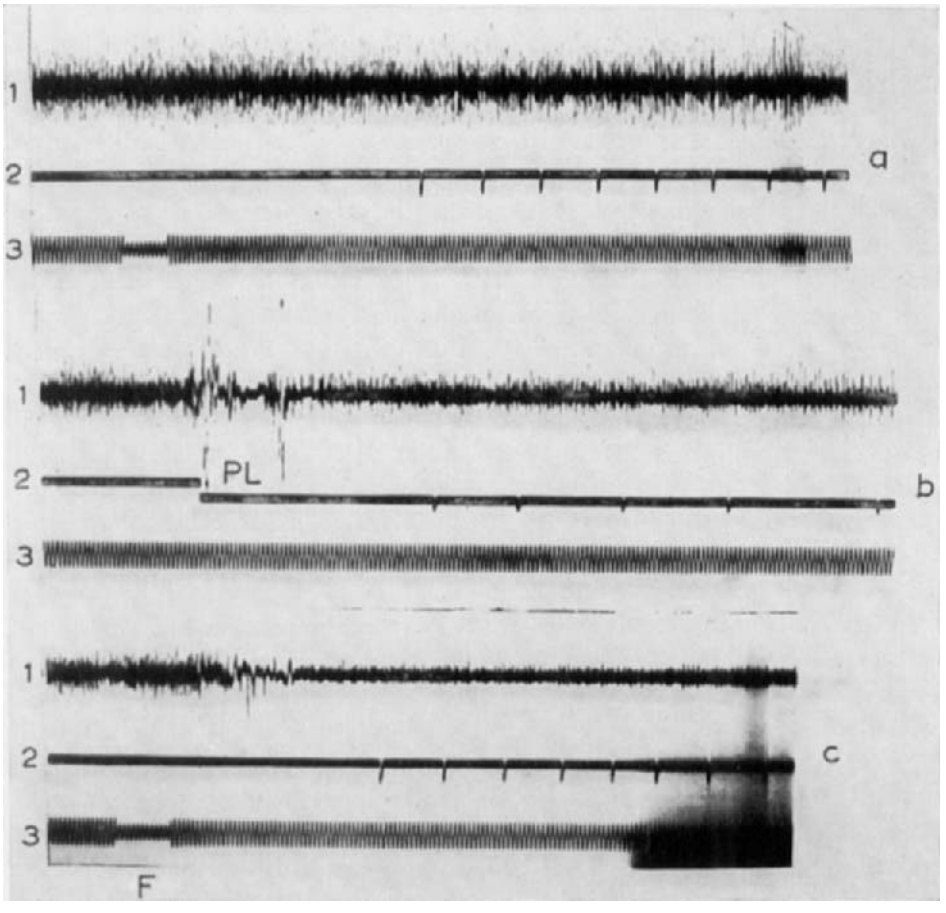

Fig. 7. Restoration of extinct backward conditioned connection (falling out of potentials in EMG to food presentation) after single passive lifting of the leg (forward conditioned connections). (a) after five isolated presentations of food backward connection is extinct; (b) application of passive lifting of the leg (forward conditioned connection); (c) backward conditioned connection to food is restored after a single application of passive lifting of the leg (b). The symbols are the same as in Fig. 3.

reflexes that have alimentary reflex as one of their components. Namely, when, in a two-way conditioned reflex food → leg movement (passive or active, no matter which), extinction of motor conditioned reflex to food is performed, *i.e.* presentation of food is not accompanied by the leg movement, then at a certain phase of the extinction process, when presentation of food stops eliciting the leg movement, the alimentary

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unconditioned reflex lessens too, and the latency of unconditioned salivation considerably increases (experiments of Khachaturian). Further evolution of this process results in the dog's refusal to take food at all, unless its leg is raised; then the dog starts eating.

From these observations it follows that the leg movement so-to-speak mingles with the alimentary reflex, and becomes a necessary part of its implementation.

3. Functional peculiarities of two-way conditioned connection

Certain functional peculiarities of forward and backward conditioned connections may be regarded as a new proof of the separateness of conditioned connections of two-way conditioned reflexes. The properties of these connections and of the respective conditioned reflexes are determined by the sequence of the stimuli, by the latters' absolute and relative intensity, by the degree of excitability of the corresponding nervous structures, by the functional state of the entire central nervous system, etc. Both the elaborated reflexes are, naturally, dependent on the above-mentioned and other external and internal factors which spur them into being and form their properties. But, as shown by the experiments of Varga and Pressman, Lian Chi An, Struchkov and Khachaturian, the degree of this dependence for each of the two reflexes is different. For example, presentation of stimuli, approximately equal in their intensity, in alternating sequence (which means that as regards the sequence, the stimuli are also given an equal chance) leads to elaboration of two-way conditioned reflexes which are approximately equal in intensity, stability, regularity of occurrence etc. If, however, the stimuli are presented in a stereotyped sequence, the reflexes thus elaborated prove to be of unequal functional properties: the reflex elicited by the first stimulus, *i.e.* the forward one, is much stronger, more stable and regular, than the backward reflex, *i.e.* the one provoked by the second stimulus. These results on the importance of the sequence of stimuli presentation refer not only to stimuli of big intensities but also to stimuli of moderate and small intensities. A decrease in the physiological power of the paired stimuli results in reduction of both the reflexes; and here also the changes in the backward conditioned reflex are much more pronounced than in the forward one. For example, the conditioned reflexes elaborated to very weak stimuli presented in a stereotyped sequence are both unstable, irregular and easily inhibitable, but in the backward reflex these features are more manifest, and it disappears earlier. The properties of forward and backward conditioned connections change considerably when the stimuli of unequal physiological intensity are combined in a stereotyped or alternating sequence. Without going into much detail we may note that, judging from the results obtained in our laboratory, when stimuli of unequal intensities are paired, the backward conditioned reflex thus elaborated is stronger when the stronger of the two stimuli is the first in the sequence. If it is the second, the difference between the reflexes is very sharp. Relative weakness, brittleness and irregularity of backward conditioned reflexes, and consequently, of backward connections evoke a justified doubt: is the backward reflex really conditioned, and the backward connection a real conditioned connection? On this important principal question

there are sharp discrepancies in the opinions of different physiologists. Even in our small laboratory there is no unanimous opinion about it. Many physiologists do not consider the backward reflex and the backward connection to be conditioned, and refer them to the category of those phenomena in the central nervous system that are known under the names of dominant, summation reflex, Bahnung, pseudo-conditional reflex, sensibilization etc.

For want of time I cannot here analyse in detail this very important problem. My point of view boils down to the following. To solve this complicated problem will help an evolutionary approach to it. Pavlov and many other Russian and foreign physiologists believed that at the basis of conditioning lie phenomena of the type of summation reflex, Bahnung etc. We agree that the phenomena observed and described under the name of conditioned reflexes in elementary multicellular animals with primitive nervous systems, in unicellular organisms, in bacteria and even in plants in reality are not conditioned reflexes, but phenomena of the type of summation reflex, dominant, Bahnung etc. These fleeting, unstable phenomena, constituting the prelude to conditioned reflex and akin to it, play a very important role at this stage of development of living beings, and provide their individual adjustment to environment. Only at later stages of evolution of organisms and their nervous systems does the actual conditioned reflex appear as relatively stable and chronical phenomena. Our supposition is that in the evolutionary plane this dialectical transition from 'the phenomena akin' to actual conditioned reflexes — and in our discussion to forward and backward conditioned reflexes, or rather conditioned connections — does not occur simultaneously: for forward conditioned reflexes and connections it comes more quickly than for backward. According to our concept, at certain stages of fauna evolution, the forward connection becomes really conditioned, while the backward one still remains within the limits of 'phenomena akin'. In higher animals and humans the backward connection also becomes, with time, really conditioned. It is possible, however, that even with these the backward connection of primitive two-way conditioned reflexes preserves certain features of 'phenomena akin'.

This is, of course, just a hypothesis, which should stand its test to reality, should be proved experimentally, but which allows us to orient ourselves in this complicated problem and to a certain extent to understand the essence of many related phenomena.

4. Physiological importance of backward connection

In conclusion I would like to say a few words about the physiological importance of backward connection. This question, undoubtedly, is very complex, and at present may be answered only in general terms and very cautiously. First it should be said that acceptance of the assumption of the existence of backward connection, side by side with the forward one, between two nervous points paired by a conditioned reflex — *i.e.* the acceptance of the assumption of formation of a nervous circle as the central part of the conditioned reflex arc — makes it possible to extend to the field of conditioned reflex activity one of the main principles of modern neurophysiology, *i.e.* the principle of circular interaction of central nervous structures, as an important

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moment of their reflex activity (Lorente de Nó, 1938; Burns, 1958; Bremer, 1953; Roitbak, 1962 and others). Further, this hypothesis fundamentally supports one of the old concepts in the theories on conditioned reflex activity, namely, the assumption of constant interaction between paired nervous foci of conditioned and unconditioned reflexes. Evidently there is great variety in the forms of interaction through forward and backward connections between these foci, and a number of ways to actuate this interaction. Sometimes, through backward connections, by way of impulse excitation of sufficient intensity, actual backward conditioned reflexes may be elaborated. That is what Pavlov believed about motor conditioned reflexes, and what we assume (on the basis of our experiments) as regards various motor and a number of vegetative reflexes. In such reflexes, dependent on circumstances, the role of a stimulus of signal meaning is played now by the first and now by the second of the paired stimuli. In other reflexes, thanks to the existence of backward connection (apart from the forward) between two nervous foci coupled by a conditioned reflex, repetitive circulation of impulse excitation of moderate intensity becomes possible between them, which may lead to a considerable protraction of the conditioned reflex reaction in one or both directions, and even to its intensification. There is no doubt that this interpretation, better than any other, elucidates the physiological mechanism of the long-known phenomenon of protraction of a conditioned reflex under a brief action of conditioned stimulus, the phenomenon studied by Kupalov and his laboratory. It should be noted that it was Kupalov who suggested the above-mentioned interpretation of this observation. We are only pointing out the role of backward connection in it.

Interaction through forward and backward connection between the nervous foci coupled by a conditioned reflex, is possible, however, not only by means of non-impulse excitation but also through the mechanism of electrotone. In other words, there may be such ways of interaction which entail changes in the functional state of corresponding central nervous structures and make them ready for activity. At any rate, this hypothesis helps us to understand the intimate mechanisms of many complex phenomena in the conditioned reflex activity, among them the phenomenon of switch, environmental conditioned reflex, dynamic stereotype and other varieties of complex tonic conditioned reflexes.

SUMMARY

The problem of the conditioned reflexes with 'two-way' connection has long been raised in the physiology of higher nervous activity and psychology. Taking into consideration its importance and at the same time its insufficient clarity, we have been studying this problem for many years. Our old and new data and views related to this question are summarized in the report.

In order to elaborate conditioned reflexes we, with collaborators (Varga, Lian Chi An, Struchkov, Pressman, Pakovich, Khachaturian and Daurova), combined two such stimuli, both of which evoke easily observable and precisely recordable innate or unconditioned reflexes. In particular, pairs of the following stimuli were presented in stereotyped or altered sequence: food, electrical cutaneous stimulation,

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air-puff into the eye, passive lifting of a limb, local skin cooling. Sometimes we coupled one of these stimuli with so-called indifferent stimuli (light, sound, touch).

The conditioned reflexes with the two-way connection appear regularly after combining any pair of mentioned stimuli. The rate of formation of the connections conducting the excitation in opposite directions, the degree of their stability and also some other functional features to a great extent depend on the absolute and relative strength of the coupled stimuli, on the sequence of their application, particularly on whether they are coupled in stereotyped or altered sequence, whether their action coincides in time or they act successively with definite intervals. It seems that typological features of the experimental animals also play a role.

When two stimuli approximately equal in strength are paired in altered time sequence, two conditioned connections conducting in opposite directions but practically equivalent in their functional peculiarities are formed between corresponding cerebral nervous foci. If stimuli of different strength are paired in this altered sequence, then the produced conditioned connections are unequal, namely, the connection from the nervous focus of the weak stimulus to the nervous site of the strong stimulus is much stronger and more stable in comparison with the opposite connection (Varga, Khachaturian).

When the stimuli are coupled in a stereotyped sequence then the conditioned connections which are linked between the corresponding nervous foci, are unequal in their functional peculiarities, independent of the absolute and relative strength of the stimuli. The conditioned connection from the focus of the preceding stimulus to the focus of subsequent stimulus (the forward connection) is, as a rule, much stronger and more stable than the reverse one (backward connection). While having some common features with the phenomena of the Bahnung-dominant type, the backward connection possesses all basic features of a real conditioned reflex, at least in higher animals.

When one of the conditioned connections elaborated to stimuli is used in altered or stereotyped sequence in the state of extinctive inhibition, then the reverse one remains for some time free from inhibition; moreover, it even shows some signs of functional exultation. It is noteworthy that activation of the reverse conditioned connection eliminates the developed extinctive inhibition in the first conditioned connection. With deepening of the extinctive inhibition in the first conditioned connection, the reverse connection is also gradually involved into inhibition. At this phase the unconditioned reflexes are considerably weakened, especially in response to the first stimulus. For example, after a profound extinction of the conditioned connection 'food–electric current', not only is the connection 'electric current–food' inhibited but the animal refuses to take food, if the presentation of food is not followed by the electrical stimulation of the paw.

On the basis of the data obtained we develop our concept on the regularities of closing of a two-way conditioned connection in the form of separate oppositely conducting connections, with any sequence of stimuli.

Further we believe that the circulation of excitation between the coupled nervous foci along these opposite connections is an indispensable term of normal conditioned

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activity which ensures the multiform interaction between these foci and which, in particular, provides for a considerable duration and strength of the conditioned reaction.

Some of these data corroborate our concept that conditioned inhibition first appears in the elements of the conditioned reflex arc and only subsequently, with its considerable deepening, extends to the structures of the coupled nervous foci of conditioned and unconditioned stimuli.

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Central Inhibition according to I. M. Sechenov's Experiments and Concepts, and its Modern Interpretation

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CENTRAL INHIBITION ACCORDING TO I. M. SECHENOV

I. M. Sechenov's main concepts of central inhibition were formulated when reflexs activity of the frog was studied between 1862 and 1868, that is, a century ago. The basic principles of this theory consist in the following (we cite them from article. incorporated in the Collected Works by I. M. Sechenov, I. P. Pavlov and N. E Vvedenski, Issue 3, 1952; in Russian):

(1) I. M. Sechenov discovered that if middle regions of the brain and the medulla oblongata were stimulated, depression or inhibition of reflex activity generally resulted. For instance, when the brain was excited, it was much more difficult to provoke defensive reflexes on hind extremities in response to stimulation of the skin. The brain was stimulated by chemical agents — with a crystal of salt — or by an electric current. Inhibition was particularly intense with excitation of the thalamus and somewhat weaker when the medulla oblongata was excited (pp. 25, 42, 86).

(2) This inhibition was general in nature, and in Sechenov's experiments it was spread over the whole of the skeletal muscles as suppression of reflex activities on all extremities and in the body, as well as over the autonomous nervous system such as suppression of automatic regulation of blood and lymph flow (pp. 25, 26).

(3) With stimulation of the thalamus general inhibition appeared without motor reactions; when the medulla oblongata was excited reflex movements caused by stimulation of the skin surface appeared first, followed by their suppression. This replacement was the quicker the more intense the stimulus (pp. 91, 95).

(4) Sechenov showed that general inhibition of reflex movements with excitation of the brain stem is produced through stimulation of specific inhibitory mechanisms in the middle regions of the brain and in the medulla oblongata. 'The retarding nerve centres and nerve cells', Sechenov writes, 'are spread across the whole of the thalamus in the corpora quadrigemina of the midbrain and in the upper portion of the medulla oblongata' (p. 49). Judging by the anatomy chart supplied by Sechenov (Fig. 21 on p. 50) these inhibitory mechanisms occupy the part of the brain stem where the reticular formation is now known to be localized. Sechenov discovered that their efferent nerve pathway passes through the frontal parts of the spinal cord and produces

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effects similar to those produced by the vagus nerve as regards the heart. According to Sechenov, the ensuing inhibition of reflex activity is underlain with suppressed excitability of the nerve substratum of the skin and muscular reflexes along the entire spinal axis (p. 140).

(5) Further Sechenov found that physiological activation of mechanisms inhibiting the reflexes is exercised through stimulation of sensory nerves, as it is when chemical or electric agents act on the sciatic nerve. Concurrently, inhibition of reflex activity takes place, which is as general as with stimulation of the brain stem. It is manifested both in desensitization of the skin over the entire body and in inhibition of all reflex movements caused by stimulation of the skin (pp. 120, 121).

(6) In addition, the inhibitory effect of stimulation of sensory nerves affected preparations devoid of the cerebral hemispheres to a much greater extent than decapitated ones. I. M. Sechenov concluded that, in frogs whose cerebral hemispheres had been removed, suppression of reflex activity occurred, mainly, through stimulation of inhibitory mechanisms in the brain stem (pp. 121, 122).

(7) Sechenov also studied phenomena of galvanic electricity in the brain under central inhibition. With tetanization of the sensory nerves he observed in the medulla oblongata a long negative electrical oscillation intensified as excitation increased. When stimulation was strong rhythmic oscillations appeared against the background of a negative deviation, alongside emergence of motor effects. With still stronger stimuli these rhythmic discharges became weaker and then disappeared together with a dying out of motor effects and desensitization of the skin. This suppression of galvanic discharges was regarded by Sechenov as a manifestation of inhibition of reflex motor mechanisms in the medulla oblongata. Hence, in preparations with the medulla oblongata under stimulation of sensory nerves, inhibitory mechanisms affect not merely the spinal cord but the medulla oblongata as well, which means that this action has an over-all effect.

(8) Having analysed the observed facts Sechenov arrived at the conclusion that during stimulation two antagonistic reflex mechanisms are excited, some of which cause reflex movement, while the others inhibit them. Generally, with less intense stimulation the motor effect predominates, while with stronger stimuli the inhibitory reflex mechanism takes the upper hand. Sechenov noticed that under certain conditions inhibition of reflexes occurs, primarily, with weaker stimulation.

(9) It was again Sechenov who discovered a phenomenon of great importance: dozens of years before Sherrington, the famous British scientist, he observed that upon cessation of inhibitory stimulation the organism not merely revives after the state of inhibition, but the latter is soon replaced by a short period of intensive excitement in the reflex apparatus and increase of the skin excitability. The same was observed upon transecting a nerve stimulation with NaCl: chemically caused general inhibition was replaced with general excitation.

A similar result was observed when galvanic effects were studied: upon cessation of stimulation provoking inhibition of galvanic discharges, the latter appeared anew and were more intense. Later on this phenomenon was termed 'rebound contraction' — a recoil following inhibition — by Sherrington.

(10) Central inhibition was regarded by Sechenov as the reverse process of excitation, which does not appear as a result of fatigue, exhaustion or overstimulation of the nerve centres, as it may start at the very beginning of excitation, before the first reflex movements, and is generally replaced with excitation when the inhibiting agent ceases to act.

LATER STUDIES OF GENERAL INHIBITION BY OTHER AUTHORS

All the above concepts concerning general inhibition discovered by I. M. Sechenov a century ago are absolutely correct and provide a true picture of central nervous activity.

Sechenov's theory was not immediately studied in detail. Only two years after him Herzen (1864) was the first to confirm the appearance of general inhibition following stimulation of the central nervous system, but he observed it not with stimulation of the brain alone, but of the spinal cord as well. In his new studies Sechenov confirmed that Herzen's observation was correct under certain conditions. Later Richet observed general inhibition with chemical stimulation of spinal nerves. But when the first works of Sherrington on reciprocal inhibition of antagonistic muscles were published (1906) all neurophysiologists focused their attention on this phenomenon, and Sechenov's general inhibition was almost abandoned till the thirties of this century.

In all the above mentioned studies general inhibition was caused by inadequate stimuli including chemical, electrical and mechanical agents acting on the brain and sensory nerves. Only a few publications on general inhibition with adequate stimulation were published during the long period since I. M. Sechenov's first works reached the public. Thus Heubel (1877) observed less mobility in an animal forcibly placed on its back and fixed in this position by the body and head. All reactions to stimulation of the skin on the limbs were considerably suppressed. Some years later Danilevski (1891) caused such loss of mobility and suppression of reflex activities by pressing the skin and muscles on limbs with tight bandages. Meltzer described general inhibition with swallowing movements in cats (1882); Jerks and Merzbacher, under photic and acoustic stimuli in frogs (Meltzer, 1882; Merzbacher, 1900); Ukhtomski, with defaecation and swallowing movements in cats (Ukhtomski, 1910); and Anne Tonkikh discussed the role played by the synaptic system in general inhibition (Tonkikh, 1927).

OUR STUDIES OF GENERAL INHIBITION

A systematic study of general inhibition was started by us in 1927 (Beritov, 1928a, b, 1929). It was studied both with adequate stimulation of receptors — cutaneous, muscular, optical, auditory, vegetative — (Bakuradze, 1943; Beburishvili, 1937; Dzidzishvili, 1937, 1940b; Kadjaia, 1959, 1960; Tevzadze, 1951; Chichinadze, 1946) and with electrical excitation of the dorsal roots and sensory nerves of the limbs (Beritov and Bakuradze, 1939, 1940b, 1941; Beritov *et al.*, 1937a, b, c; Gedevani, 1938a, 1939b). Craniocerebral nerves (the trigeminal nerve and the vagus nerve) were also stimulated

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(Gedevanishvili, 1941; Tevzadze, 1951) and then all the vegetative nerves (Beritov and Bakuradze, 1943a, c; Roitbak, 1950a, b), in particular those which pass through the frontal roots (Beritov and Bakuradze, 1940d). General inhibition was also studied with electrical excitation of different trunks of the spinal cord (Beritov and Bakuradze, 1940c, d, 1943b; Kvirkvelia, 1962a, b), with chemical excitation of the spinal cord (Beritov and Bakuradze, 1940c; Kashakashvili, 1963; Oniani, 1961), of the brain stem (Beritov and Nivinskaia, 1941; Bolotov, 1919; Gotsiridze, 1929; Narikashvili, 1937) and of the cerebral cortex (Beritashvili and Gedevanishvili, 1941; Dzidzishvili, 1937).

Inhibition was estimated by general movements of the extremities, as well as through myographic records of antagonistic muscles, and by electrical effects in the brain (Bakuradze *et al.*, 1947; Beritov, 1946, 1961; Beritov *et al.*, 1943; Beritov and Gedevanishvili, 1945; Beritov and Roitbak, 1955; Gedevanishvili, 1943a; Roitbak, 1950b, 1955). Experiments were conducted on several animals — frogs, pigeons, rabbits, dogs, monkeys — throughout many years between 1927 and 1943.

Several scientists participated in these studies, though Bakuradze, Dzidzishvili, Gedevanishvili, Gogava, Narikashvili and Roitbak were major contributors.

We confirmed, and in many aspects developed, the above theory by I. M. Sechenov. First, we proved that central inhibition caused by stimulation of sensory nerves embraces the entire central nervous system, that is both the spinal cord and the brain, the cerebral cortex inclusive. At the same time it was found that such general inhibition is similarly provoked by adequate stimulation of the optical, auditory, skin-surface and muscular receptors. It can be caused by mechanical (pressure), thermal or chemical stimuli acting on a small portion of the skin, but the immediate effect may be so insignificant that it does not manifest itself outwardly (Beritov and Govaga, 1936, 1937; Dzidzishvili, 1939, 1940a). Moreover, general inhibition may be caused by single electrical stimuli acting on the skin or a sensory nerve even with intensities so small that they do not result in an observed reflex motor effect (Beritov and Bakuradze, 1939, 1940a; Gedevani, 1938b, c, 1939a).

We further demonstrated that with intense stimulation of the skin or sensory nerves general inhibition also develops and increases with stimulation, when some co-ordinated motor effect of reciprocal excitation and inhibition of skeletal muscles appears (Beritov and Bakuradze, 1940b; Beritov *et al.*, 1937a, c).

A characteristic observation is that enhancement of general inhibition appears not merely in muscles which fail to contract but also in contracted ones; that is, general inhibition involves all motor neurons, even those which are stimulated reflexly. This is well seen with somewhat longer stimulation: in fatigue of reflex contraction, the accompanying inhibition appears well as the latter is less subject to fatigue (Beritov, 1941, 1946; Beritashvili, 1941; Beritov and Bakuradze, 1940b). This is also evident from the fact that whenever a strong stimulus ceases to act after contraction has been reduced, the latter is intensified for a while and thus a 'rebound' takes place, which testifies to the presence of inhibition during contraction (Beritov and Bakuradze, 1939; Beritov and Bakuradze, 1943c; Beritov *et al.*, 1937a, b, c).

Thus we adduced experimental proof of Sechenov's suggestion that excitation and

inhibition start simultaneously, and that the course of every reaction depends on the predominance of the former or the latter process.

We repeatedly studied general inhibition with stimulation of the cortex in cats (Beritov *et al.*, 1943; Beritashvili and Gedevanishvili, 1941; Gedevanishvili, 1943a, b), as well as monkeys (Beritov and Gedevanishvili, 1945). In our experiments not only was electrical cortical activity inhibited but also motor reactions caused by stimulation of the cortex and sensory nerves. We also studied general inhibition with conditioned reflexes and its role in the genesis of external and internal inhibition (Beritashvili, 1956a, b; Beritov, 1961).

Further we found that in man general inhibition also occurs in his deliberate psychic activities, such as doing sums, as suppression of conditioned and unconditioned reflex activity (Beritov and Dzidzishvili, 1934). It was found that when the skin on the head or limbs was pressed, general inhibition spread also to those nerve substrates which perform subjective emotion of pain such as toothache, headache, neuritic pain, etc. (Tskipuridze and Chichinadze, 1944a, b, c; Chichinadze, 1946).

We also confirmed Sechenov's statement that whenever a relatively strong stimulus ceases to act on sensory nerves or skin and muscular receptors, excitability is enhanced in inhibited nerve elements, and general facilitation results. We observed this phenomenon both with artificial stimulation of any sensory nerve and with adequate stimulation of receptors (Beritov, 1941; Beritashvili, 1941; Beritov *et al.*, 1937b). This increased sensitization following inhibition also appeared with stimulation of the skin or a sensory nerve with a single electric shock (Gedevani, 1938a, b, 1939c).

And last, it was at our Institute that, for the first time after I. M. Sechenov, general inhibition was studied on the basis of electrical phenomena in the medulla oblongata. Using an amplifier with a large time constant, Roitbak (1950c) recorded on a cathode oscillograph electrical phenomena emerging in response to tetanic stimulation of the sciatic nerve which Sechenov studied with a galvanometer. Among other things, Roitbak observed in the medulla oblongata long negative oscillations increasing with stimulation. With more intense and longer tetanic stimulation of the sciatic nerve these electrical phenomena became weaker, and upon its cessation they were immediately enhanced, as in Sechenov's experiments.

Outside the Soviet Union, general inhibition was observed with adequate stimuli by Gerebtzoff (1949) in Bremer's laboratory, and was widely recognized only in connection with studies of the reticular formation, as such areas were discovered here where stimulation causes general inhibition of the brain and spinal cord.

Especially spectacular general inhibition is produced by stimulation of the paleocortex — as well as calling forth a certain type of behaviour, all the other somatoautonomic reactions are inhibited to a greater or lesser extent (Kaada, 1951).

In addition, our studies enabled us to demonstrate the biological significance of general inhibition in animal behaviour. We found that general inhibition appears with every reflex or behavioural reaction, as a rule before any outward manifestation, and accompanies this effect throughout its existence. This peculiarity of the central nervous system provides for localization of stimulating impulses within certain motor nerve complexes, as general inhibition prevents spreading of excitation to the other

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intermediate and motor neurons. Further, when some behavioural act is present, general inhibition of the central nervous system prevents emergence of other behavioural acts by excitation in some other nerve complexes under the influence of some less significant external or internal stimuli. Consequently, precision and direction of the dominant motor reaction are preserved. Lastly, with relatively weak stimuli, the resulting general inhibition unaccompanied by manifest motor reactions serves to safeguard the organism from waste of energy with various insignificant environmental effects. This means that general inhibition is the basic factor providing integrity of every behavioural reaction, as alongside excitation of a certain complex of intermediary and motor neurons, inhibition of all the other parts of the brain takes place.

THE STRUCTURAL BASIS OF GENERAL INHIBITION

At present we can not merely present an exhaustive and comprehensive account of general inhibition discovered by I. M. Sechenov, define the prerequisites of its emergence and spreading and define its biological significance in human and animal reflex and behavioural activities. We are now able to outline the structural basis which underlies general inhibition. As early as 1937 we voiced the suggestion that general inhibition spread over the entire central nervous system is the primary function of the reticular formation of the brain stem (Beritov, 1937a, b, 1938). At that time we proceeded primarily from Herrick's well-known anatomical information on the neuron-neuropil structure of the reticular formation, and suggested that brain neuropils consisting mainly of dendritic ramifications with synaptic terminations of collateral nerve paths on them, comprise the nervous substrate which engenders both general inhibition and general facilitation of the brain. During the last twenty years this suggestion was accepted *ipso facto* in studies by Magoun, Moruzzi, Jasper, Bremer, Narikashvili and some others.

We also found out exactly how stimulation of the middle regions of the brain causes general inhibition in the spinal cord. Sechenov postulated that impulses of excitation are sent from the inhibitory centres of the brain to the spinal cord, and it is here that these impulses cause general inhibition. We have discovered the structural basis of this phenomenon.

In the course of detailed and thorough studies of spinal activities we arrived at the conclusion that Rolando's gelatinous substance causes inhibition of intermediate neurons in the cornu posterius (Beritov, 1961; Kadjaia, 1960; Kashakashvili, 1963; Kvirkvelia, 1962a, b). It is activated with almost every active state of the spinal cord as a response to stimulation of the skin and muscular receptors and nerve tracts descending from the brain.

Recently, as a result of new histological studies carried out at our Institute, it became clear that all nervous tracts in the spinal cord, both afferent from skin and muscular receptors and efferent from various regions of the brain, also, in some way or other, terminate in the gelatinous substance (Mikeladze, 1965; Totibadze, 1963).

Thus, it was not until almost a century after Sechenov discovered general inhibition that the nervous substrate was found which generates it and thus provides for integ-

rity of nervous activities with every reflex and behavioural reaction.

SUMMARY

The main concepts of central inhibition, put forth by I. M. Sechenov a hundred years ago, have played an important part in the physiological analysis of the reflex and psychic activity.

One of the main concepts says that stimulation of cross-sections of the midbrain and medulla oblongata in frogs causes a depression of the spinal cord reflex activity. Under certain conditions this inhibition is preceded by reflex movements. This central inhibition is a general one embracing the whole skeletal musculature of extremities, as well as the vegetative system in the form of a depression of automatic activity of blood and lymphatic hearts.

This general inhibition, according to Sechenov's ideas, is effected through the excitation of depressing mechanisms of the nerve cells distributed through the middle parts of the brain and upper parts of the medulla oblongata. These mechanisms act on the spinal cord through their descending paths in the same manner as the vagal nerve acts on the heart.

I. M. Sechenov also found that a general inhibition is observed after initial reflex movements upon stimulation of sensory nerves of extremities, and that this inhibition is based on a decrease in the excitability of nervous centres of cutaneous-muscular reflexes along the whole spinal cord axis, as in the preceding example.

I. M. Sechenov also showed that inhibition might occur simultaneously with excitation. After the cessation of inhibiting stimulation of sensory nerves, the state of depression is usually rapidly replaced for a short time by an increased excitation of the reflex apparatus.

Subsequent investigations of many scientists have shown that general inhibition appears not only after direct stimulation of various parts of the brain but also after an adequate threshold stimulation of receptors that does not bring about external motor responses (Danilevski, Beritov, Bakuradze, Dzidzishvili, Gogava).

General inhibition was found to be an integral part of any reflex and behavioural response. It provides the integrity of the central nervous activity: along with excitation of definite nervous complexes effecting the adaptation of the organism to the change in its environment, an excitability decrease takes place in the rest of the nervous system (Meltzer, Ukhtomski, Beritov). In this way excitation processes are focused in certain neuron chains, and a simultaneous excitation of other neuron chains in response to different external stimuli becomes impossible.

This general inhibition also involves excited nervous chains. If inhibition is strong it can depress excited chains. Excitation and inhibition are then antagonistic, and proceed in the same elements. As a result of their algebraic summation, it is sometimes excitation and sometimes inhibition that prevails.

Later investigations have also shown nervous formations of the brain stem and of the spinal cord to participate in the appearance of general inhibition in both intact and decerebrated animals. Certain areas of the reticular formation in the brain stem

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effect depressing function, inhibiting both the brain and the spinal cord when being excited (Magoun, 1958).

It is the gelatinous substance which plays the role of such inhibitory mechanism in the spinal cord. The brain inhibits the spinal cord through this substance (Beritov, Ioseliani). According to recent data, general inhibition caused by the gelatinous substance seems to be based on the generation of slow potentials, arising in dendrites of gelatinous neurons under the effect of collaterals from dorsal and dorsolateral tracts and from different kinds of descending tracts, and also in the dendrites of proprial and pericorneal intermediate neurons under the effect of the same collaterals of ascending and descending fibres. An electrotonic action of currents corresponding to these potentials upon axosomatic synapses apparently blocks inflowing peripheral impulses, while the electric field arising around activated dendrites causes anelectrotonic block of nervous fibres passing through this field (Beritov, Roitbak, Kvirkvelia).

The total increase in the reflex excitability taking place after inhibition when the stimulation ceases, occurs in the same elements that have been inhibited, as suggested by Sechenov. This increase in the total reflex excitability seems to arise as a result of the cessation of the above anelectrotonic action, as in the case of nerve stem polarization in the region of switching off the polarizing current.

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Resting Saliva of Men as a Reflex

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The first report from Pavlov's Institute dealing with the then newly discovered acquired reflexes was published in 1899 (Pavlov, 1927). Pavlov continued to the time of his death to carry out research on conditioned reflexes in dogs, almost exclusively with salivation to food or acid placed in their mouth.

Since Pavlov's work was made available to the American psychologists, the salivary method has sometimes been called a classical one, in comparison with the researches of the conditioning of other small animals or of other reflexes. The method of conditioning could not be viewed as the standard from the physiological point of view, for it consisted of rather ambiguous, secondary indicators though favoured by psychologists.

In 1909–1913, Krasnogorski (1909, 1913) a student of Pavlov, performed some conditioning experiments on children, and they came to the attention of American psychologists. In 1918 Mateer (1918) published her experiments on children. She used the method of placing a bandage over the eyes of the child just before food was placed in his mouth. The bandage came to serve as the conditioned stimulus before the food was presented. In 1916, Lashley (1916a,b) began but abandoned an attempt to condition the salivary response in man. After Lashley, many experiments of conditioning in man have been reported, but they have not been done with the salivary method.

Hayashi has continued his study of conditioned reflexes after his return to Japan from Pavlov's Institute, but he has been silent till 1954 (Hayashi, 1959; Hayashi and Ararei, 1963; Hayashi, 1956, a, b) and there still remains much unpublished work including the following, carried out on human beings.

Salivary measurement

A suction cup after Lashley (1916a) was made first with light metal and afterwards with glass as shown in Fig. 1. The furrowed circle around a hole served for negative pressure which could be adapted to the orifice of a parotid gland, from which a tube led to the measuring apparatus. The saliva was measured by noting the distance within the tube in millimeters. A young adult was used as experimental subject sitting as

shown in Fig. 2, and the measurement was carried out in the outside chamber as in Fig. 3.

The experiments described below were carried out on 20 university students between 18–25 years old, who were all healthy volunteers.

Resting saliva

At first, when we attached a suction cup to a parotid gland of a subject, we found the

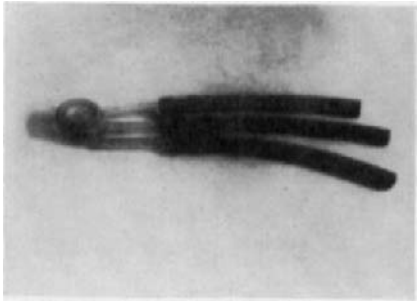

Fig. 1. Suction cup after Lashley (1916a).

saliva flowing continuously, as shown in Table I, in which each 3-min amount was measured. The amount was different with each man, but it was almost the same in the same subject. At first we called this 'proper saliva', for we believed that this was not reflex saliva, but one which every man and woman would have from birth. On the contrary, we have no experience of resting saliva in dogs with salivary fistulas (Table I).

Fig. 2. See text.

Babkin (1950) discussed and denied the resting saliva from the physiological standpoint, but according to our experience it existed in every subject without difference of sex. There are 3 groups of men and women according to the amount of their resting saliva. The first group produced during 3 min under 2–5 mm saliva in the

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TABLE I

RESTING SALIVA OF THE AVERAGE JAPANESE ADULT

Subject	*Sex*	*Age*	*Amount of resting saliva in mm in each 3 min (200 mm makes 1.0 ml)*																				*Average (mm)*
1	Male	20	3	1	0	1	2	5	2	0	3	3	2	0	3	0	0	15	35	2	1	5	2.1
2	Male	21	1.5	2.5	1	1	1	9	3	0	2	1	2	4	1	4	1	4	6	3.4	9	1	2.7
3	Male	19	15	17	13	25	50	21	16	9	10	12	40	10	30	12	12	40	79	20	27	23	23.9
4	Male	25	12	38	20	10	55	85	55	55	60	40	71	35	10	38	17	40	10	10	17	10	30.7
5	Male	19	150	140	120	130	150	250	110	190	280	150	140	150	250	130	100	60	120	80	70	60	161.5
6	Male	16	85	200	170	220	150	230	160	180	250	200	250	220	140	120	140	160	150	170	190	150	176.7

TABLE II

RESTING SALIVA OF DOGS

Dog	*Sex*	*Amount of resting saliva in mm in each 3 min (190 mm makes 1.0 ml)*																	*Average (mm)*
1	Female	3	4	6	4	19	13	8	3	2	5	15	20	30	22	8	8	3	10.3
2	Female	35	63	60	50	40	60	41	46	68	38	24	28	45	48	48	35		45.6
3	Male	140	120	220	160	137	37	210	240	110	210	190	30	140	136	220	160		153.8

tube, of which the diameter was calculated to make 180 ~ 200 mm to be 1.0 ml. The above lowest group covered 50% of Japanese. The second group produced 20–40 mm per 3 min and comprised 35%; and the third group, 80–160 mm per 3 min, was calculated to represent 15%.

The significance of the resting saliva was obscure, till eventually we suspected the suction cup attachment might provide the stimulus to start reflex secretion, and we

Fig. 3. See text.

tried to attach a suction cup to the parotid gland of dogs. Table II shows the result with the suction cup, namely that dogs also had resting saliva which was similar to that in man. The permanent fistula of the parotid gland of dogs, of course, had shown no resting saliva (Table II).

Thus it has been made clear that it was not proper saliva but it proved to be a reflex one. At any rate, in man as well as in dog, when a suction cup was applied, we observed the resting saliva.

Resting saliva as a reflex

The observation that a dog had no flow of resting saliva from a fistula opened outside his cheek, but on the contrary, he had a distinct flow when a suction cup was attached to the inner side of his cheek, meant that the mechanism of the flow must be a reflex.

The first of the three components of the reflex, the receptor stimulation, must be a mechanical stimulation due to the suction cup. The ascending tract must be a nerve from the inner side of the mucous membrane of the cheek to the thalamus through the trigeminal nerve, of which a branch in the brain stem might reach the salivary centre. The supposed centre of reflex of resting saliva would be situated at the salivary centre of the medulla which ordinarily acts in the reflex secretion of the saliva due to taste stimuli.

Alternatively, the centre might be situated in the thalamus or at the same level. A third possibility is that the reflex centre could be situated in the cortex. To test these hypotheses, we tried first the investigation at a higher level (Fig. 4).

As early as 1875 Lepine and Bochefontaine (cited in Babkin, 1950) pointed out that electrical stimulation at the coronary gyrus of the dog's cortex produced salivary secretion, and Hayashi (1954) confirmed it by chemical stimulation.

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First we took up the last problem and experimented to discover whether or not the dog, of which the gyrus coronarius and its vicinity was extirpated as shown as the shaded area in Fig. 4, had a resting saliva. After such extirpation of the shaded gyri

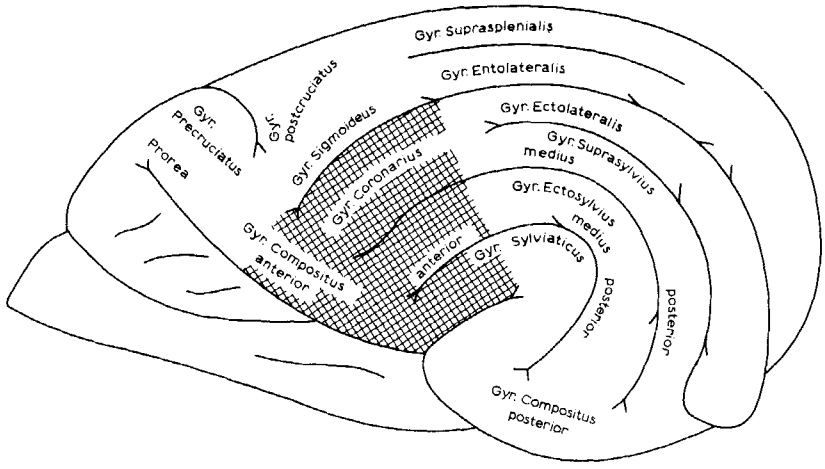

Fig. 4. Face area for saliva secretion of dog's brain. (See text.)

from the cortex in dogs, the resting saliva completely disappeared, although the reflex saliva stimulated by acid existed.

This result showed that the resting saliva was a reflex one, of which the centre must

Subjects	Resting saliva		
1	27.0 mm	31.0 mm	51.0 mm
2	13.5 mm	7.5 mm	3.5 mm

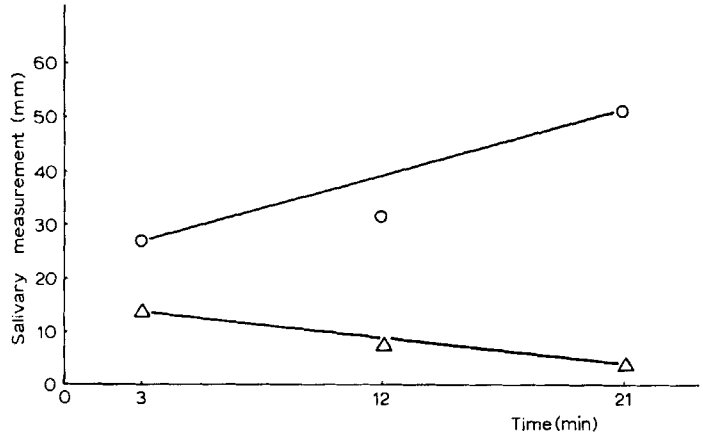

Fig. 5. Standard line of changes of the resting saliva calculated from the first and the last values

be in the cortex. Are there any reflexes besides the above, of which the reflex centres are situated on the cortex? Known examples include the righting reflex in the gyrus sigmoideus of a dog (Fulton, 1949); and the placing reflex as well as the hopping and grasping reflexes are recognized as having their centres situated in the cortex. All these reflexes we class as postural reflexes, among which we do not recognize the allied reflex in autonomic secretion as resting saliva.

Measurement of conditioned saliva

Now, in using the suction cup, we cannot entirely avoid meeting resting saliva. If the resting saliva should be stationary, even though at different levels in different individuals, the measurement of conditioned saliva would be obtained by simple subtraction of the resting one. But in reality, the amount of resting saliva might be variable, fluctuating over 15–60 min. Fig. 5 shows the value of the resting saliva at the beginning and at the end of two experiments; after 21 min it gradually decreased in one subject and increased in another. Therefore, we draw a line joining the initial and final values, and determine the real saliva from this standard line of resting saliva as shown in Fig. 6.

Establishment of positive conditioned reflexes

Conditioned stimuli were metronome at 120/sec (abbreviated M), and an electric

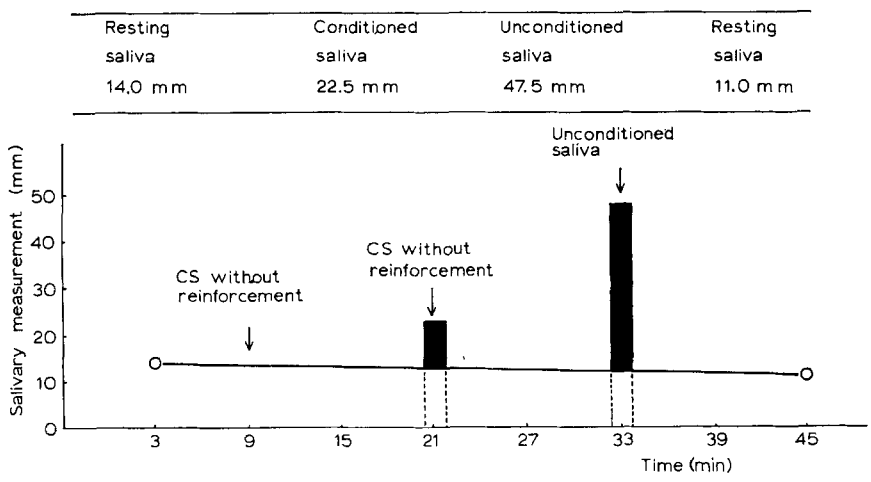

Fig. 6. Establishment of a positive conditioned reflex in man. (Conditioned stimulus was a 60 W lamp, unconditioned stimulus 1.0 ml of 1/16 *M* tartaric acid.)

lamp of 60 W 1.5 m in front of the experimental subject. The unconditioned stimulus was 1.0 ml of 1/16 *M* tartaric acid solution poured into the mouth, on the tongue on the same side as the attached suction cup. Fig. 6 presents the results on the 31st experimental day. The resting saliva was 14 mm just before the experiment and 11 mm

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immediately after it. The conditioned salivary amount for the lamp was 35 mm which was corrected graphically as shown in Fig. 6 by subtracting the standard resting amount, and we obtained 22.5 mm of saliva for the established positive conditioned reflex. The other example is represented in Fig. 7, showing that the positive conditioned

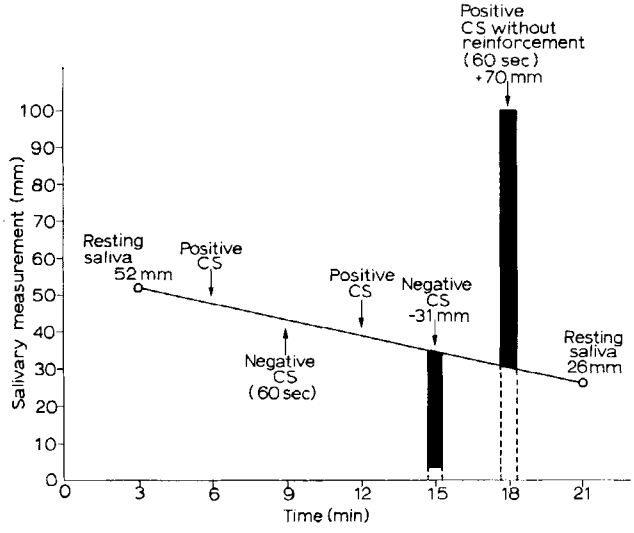

Fig. 7. Establishment of positive and negative salivary conditioned reflexes (CS) in man. (Conditioned stimulus was a 60 W lamp, unconditioned stimulus 1.0 ml of 1/16 *M* tartaric acid.)

stimulus produced 100 mm saliva, from which 30 mm was subtracted according to the standard line leaving 70 mm of established positive conditioned reflex.

When other results not shown here were included, the positive conditioned salivary reflex in man was established in the same way as that of dogs.

Natural conditioned salivary reflex in man

In addition to the artificially conditioned salivary reflexes as above, we also found naturally conditioned saliva in men by the suction cup method: namely (a) drawings elicited no naturally conditioned reflex either in adults or children between 9 and 10 years old; (b) the amount of naturally conditioned saliva was fixed according to the strength of the unconditioned stimulus; and (c) a more noticeable findings was that, if one used a natural conditioned stimulus, for instance the sight of a pickled plum, conditioned saliva was observed in every Japanese, but he had produced more when he saw someone eat the plum. The last phenomenon was called by Hayashi 'external induction of natural conditioned reflex of man' (Hayashi and Ararei, 1963).

Establishment of negative conditioned reflexes and their measurement

A positive conditioned reflex once established, the negative one was easily made by

the method of alternating stimulation as Pavlov taught. Fig. 7 shows the results when the positive reflex was established upon the electric lamp of 60 W and the negative upon the metronome (120/sec).

The standard resting saliva was from 52 mm to 26 mm during the lapse of 21 min. The positive conditioned saliva was 70 mm, reduced by the amount which was below the standard line. As for the negativity of the negative conditioned reflex, it was simply stated as —31 mm which was the remainder from 35 mm below the line reduced by 4 mm of secretion. In other words, the negative conditioned stimuli inhibited the resting saliva. The ordinary measurement of the negativity of the negative conditioned reflex must be the difference between the established positive conditioned reflex and the negative conditioned one as shown in Fig. 8. Thus we have two ways of knowing the negativity in the experiment in man.

The above results show that the negative conditioned reflex is established in men

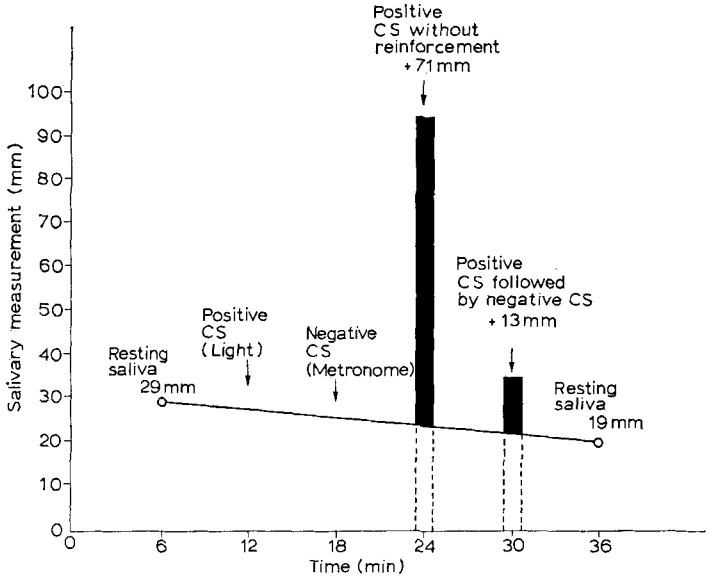

Fig. 8. Measurement of the positive and negative conditioned reflexes.

as in dogs. The positive one was measured as 71 mm. When both stimuli, positive and negative, were applied at the same time, the conditioned saliva was 13 mm, which must mean that the negativity of the negative conditioned reflex was 71 — 13 = 58 (—58 mm) as shown in Fig. 8.

Influence of the tentative substance of cortical excitatory and inhibitory transmitter on conditioned salivary reflex

The mechanism of the establishment of conditioned reflex has been the most important problem in Pavlovian disciplines. Pavlov made no attempt to interpret the conditioned reflex in terms of relationships between cortical neurons. He merely

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postulated the connection between analysers and effectors. But if his postulation is foreseen to be solved in terms of synaptic activities, there must have been some advancement in his theory which might be based upon chemical transmitters.

Since 1956, Hayashi tried to solve this problem, and he suggested a tentative inhibitory transmitter substance of mammalian brain, namely γ-amino-β-hydroxybutyric acid (GABOB). He has confirmed that (a) GABOB exists in the mammalian brain, (b) it has an inhibitory action at the cortical level (Hayashi, 1960a,b; 1961a,b; Hayashi *et al.*, 1959a,b); (c) its mother substance is γ-aminobutyric acid (GABA), which is also the mother substance of cortical excitatory transmitter. Evidence for this last hypothesis is as follows (Hayashi, 1960a,b; 1961a,b; Hayashi *et al.*, 1959a,b):

(1) GABA was converted to the excitatory substance by cerebral tissue *in vivo* when it was introduced into cerebrospinal fluid (Hayashi, 1959). The substance is produced from GABA as the substrate through a reaction by coenzyme vitamin B_{12} (or folic acid), having been driven by the energy of ATP, for which vitamin B_1 could be substituted.

(2) It was confirmed that cortical excitation occurs by introducing an appropriate dose of vitamin B_1 into cerebrospinal fluid of dogs owing to the excessive genesis of the excitatory transmitter (Hayashi, 1961a).

(3) Hayshi and Nagai (1961a,b) have extracted a substance, presumed to be excitatory transmitter, from cerebrospinal fluid of dogs during seizure induced by electrical stimulation. They named it 'substance K'. It was obtained from gray matter of the cortex, from white matter in small amount, and from brain stem in negligible amount.

(4) On the other hand, it was proved that GABA introduced into cerebrospinal fluid was converted to GABOB through a reaction in which vitamin B_6 acted as a coenzyme.

According to the above deductions, we experimented on the actions of vitamin B_1

TABLE III

ENHANCEMENT OF INHIBITION IN CONDITIONED SALIVA OF A SUBJECT WHEN GABOB WAS INTRAVENOUSLY APPLIED

Positive stimulus (light, 40 W)	*Negative stimulus (metronome, 120/min)*	*Light + metronome*	*Calculation*	
			Excitatory process	*Inhibitory process*
Measurements of established conditioned saliva (mm after which resting saliva had been subtracted)				
+16.5	—4.5	+3.5	+25.0	—4.5
5–10 min after intravenous injection of 1.0 ml of 5.0% GABOB				
+9.5	—6.5	—3.5	+9.5 (62%) decrease	—13.0 (188%) increase

expecting to have an excitatory effect and GABOB or vitamin B_6 to have inhibitory effects on the conditioned salivary reflex in man.

Table III shows one of the examples. A positive reflex was established by an electric lamp in adult men, and a conditioned inhibitory reflex by the sound of the metronome. The 40-W lamp stimulated a secretion of saliva +16.5 mm and the metronome —4.5 mm. To calculate the excitatory process we solve the equation:

light (+16.5) + metronome (—4.5) + K = +3.5

$\therefore$ K = +8.5.

When this K value is added to +16.5 we obtain the measure of the excitatory process as +25.0. Here the inhibitory process need not be corrected, for K was positive.

5–10 min after 1.0 ml of GABOB in an appropriate concentration was given, the positive reflex was reduced to +9.5 as shown in the table, and the negative one was augmented to —6.5. The inhibitory process is calculated by solving the equation:

light (+9.5) + metronome (—6.5) + K = —3.5

$\therefore$ K = —6.5.

This K value should be added to —6.5 and we obtain the inhibitory process as —13.0. The excitatory process need not be corrected, for K was negative (Table III). The results were the same after applying 1 ml of pyridoxine preparation (vitamin B_6).

TABLE IV

EFFECTS OF VITAMIN B_1 ON CONDITIONED SALIVARY REFLEX OF AN ADULT SUBJECT

Positive stimulus (light, 40 W)	*Negative stimulus (metronome, 120/min)*	*Light + metronome*	*Calculation*	
			Excitatory process	*Inhibitory process*
Measurements of established conditioned saliva (mm after which resting saliva had been subtracted)				
+37	—24	+19	+43	—24
5–10 min after intravenous injection of 1.0 ml containing 10 mg thiamine hydrochloride				
+45.5	—21.5	+25.5	+47 9.4% (increase)	—21.5 10.4% (decrease)

Another experiment is summarized in Table IV, in which 5–10 min after 10 mg of thiamine hydrochloride (vitamin B_1) had been given, the positive conditioned reflex was augmented from +43 to +47 and the negative one was changed from —24 to —21.5. Thus we have come to the conclusion that GABOB, the physiological inhibitory transmitter, has the action of deepening the inhibitory process of the conditioned salivary reflex, and on the other hand vitamin B_1, the coenzyme that promotes the genesis of excitatory transmitter, enhances the excitatory processes (Table IV).

Among many substances which we tried on the conditioned reflex in dogs, as shown in Table V, there were several that augmented the excitatory conditioned reflex, (Table V) but there were none that enhanced the inhibitory effect of the conditioned reflex except GABOB and vitamin B_6.

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TABLE V

EFFECTS OF VARIOUS SUBSTANCES UPON THE EXCITATORY AND INHIBITORY CONDITIONED REFLEX IN DOGS

Substance	*Excitatory process*	*Inhibitory process*	*Unconditioned reflex*
Ethylurethane	decreased	decreased	decreased
Chloral hydrate	decreased	decreased	decreased
Barbital	decreased	decreased	decreased
Sulphonethylmethane	decreased	decreased	decreased
Phenobarbital	decreased	decreased	decreased
Morphine	decreased	decreased	decreased
Strychnine	decreased	decreased	decreased
Picrotoxine	decreased	decreased	decreased
Metrazol	decreased	decreased	decreased
Nicotine	increased	decreased	increased
Pilocarpine	increased	decreased	increased
Atropine	decreased	decreased	decreased
Eserine	increased	decreased	increased
Acetylcholine	increased	decreased	increased
Histamine	decreased	decreased	decreased
Adrenaline	decreased	decreased	decreased
Amphetamine	decreased	decreased	decreased
Insulin	decreased	no change	decreased
Sodium glutamate	increased	no change	no change
Sodium aspartate	increased	no change	no change
Potassium bromide	decreased	no change	no change
Diphenyldantoin	decreased	no change	no change
Potassium bromide (chronic application)	increased	no change	no change
Amphetamine (chronic application)	increased	no change	no change

SUMMARY

Parotid saliva of man can be measured exactly by a suction cup method. Experiments on conditioned reflexes in man can be carried out during 21–60 min daily or on alternate days.

Artificial conditioned reflexes, positive as well as negative, can be established by Pavlov's method in man in the same way as in dogs, even though man has resting saliva.

Resting saliva could not be observed in a permanent fistula opened outside a dog's cheek; on the contrary, dogs had resting saliva, when a suction cup was attached to the orifice of the parotid gland.

Resting saliva must be a reflex saliva, of which the receptor stimulation is a special mechanical stimulus due to the attachment of a suction cup, and of which the reflex

centre was situated at the gyrus coronarius in the cerebral cortex of dogs. Presumably the incidental location in man would be the face area of the anterior central gyrus.

Calculation of the amount of established conditioned saliva can be obtained by subtracting the resting one measured before and after the experiments.

The positive as well as negative conditioned salivary reflex in men could be established in the same way as in dogs.

Intravenously injected vitamin B_1 augmented the positive conditioned saliva and reduced the negative one; on the contrary, GABOB and vitamin B_6 reduced the positivity and enhanced the negativity in the conditioned salivary reflex.

It is concluded that the inhibition in the conditioned reflexes is due to the increasing inhibitory chemical transmitter in the synapse of nerve cells concerned in the formation of the conditioned reflexes. The increase in the chemical transmitters should be due to the enhanced activities of enzyme systems at the site of synapses.

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On the Central Inhibiting Mechanism of the Thalamic Suppression of Spinal Motor Reflexes

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The problem of central inhibition and plans of its experimental investigation were directly connected in Sechenov's studies with the development of basic concepts of his work 'Reflexes of the Brain'. Sechenov came to the conclusion that the reflex theory of the behaviour of animals and man requires a premise that the central nervous system has mechanisms of active inhibition or suppression of the reflex activity of one centre by the effects of other centres. With his usual keen penetration into the essence of things he guessed that, in the vagus inhibition of the heart (Weber and Weber, 1846) and in the sympathetic inhibition of the intestines (Pflüger, 1857) there were physiological processes functionally similar to the phenomenon of the inhibition or suppression of reflexes in the central nervous system. The activity of the central nervous system that conditions these phenomena of the suppression of reflex activity was called by Sechenov 'central inhibition'.

The above-mentioned general concept led him to the plan of the experimental investigation and proof of this phenomenon. This plan was based on the well-known prevalence of nervous apparatus of cerebral segments of animals over the reflex activity of spinal segments. The methods of Sechenov's experiments were as follows. A frog's cranium was opened to expose the dorsal surface of its brain. Then successive (in downward direction) cross-sections of the cerebral axis were obtained at different levels — in the middle part of the hemispheres, in the thalami optici and at the level of the lobi optici and medulla oblongata (Fig. 1). The animal was hung by the lower jaw and its freely-hanging hind legs were irritated by dipping its toes into a weak (0.25%) solution of H_2SO_4. The period from the moment of dipping till the reflex withdrawing of the legs from the solution was fixed as a criterion of the reflex irritability — the threshold time. This method for determining the quantitative characteristic of the reflex irritability was first suggested by Türk (1851), and when used with caution it is a sufficiently accurate test of the reflex irritability. After each determination of the threshold the leg is thoroughly washed to remove the remains of the acid, and after 3–5 min the test is repeated.

Sechenov's experiments were aimed at determining thresholds of the reflex excitability before the excitation and during the excitation of different cerebral sites. To excitate cross-sections of the brain he used NaCl crystals; he thought that the use of a chemical agent in a crystalline form would provide the highest localization of

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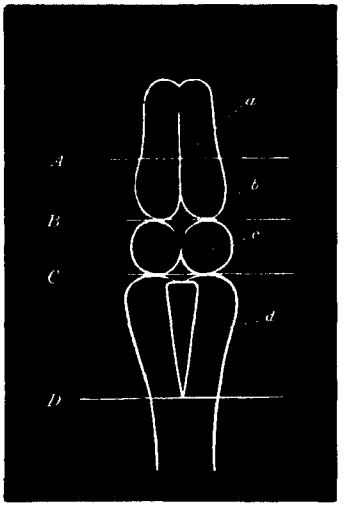

Fig. 1. The scheme of cross-sections of the frog's brain in Sechenov's experiments. A, section of hemispheres (a); B, section of thalami optici (b); C, section of medulla oblongata (c) along its upper edge; D, section of medulla oblongata (d) along its lower edge. The brain above the cut was removed and a NaCl crystal put on the cut (Sechenov, 1956, p. 389).

action, at least in the initial period. But later Sechenov also confirmed all his results by the electrical rhythmic irritation of cerebral cross-sections with inductive currents. These experiments of Sechenov showed that the excitation of the thalami optici results in a sharp increase in the threshold time — the inhibition of reflexes. He considered this inhibition to be a result of the inhibiting effect of neurons of the thalamic area, excited with NaCl on the motoneurons of the spinal centres of the hind legs. The excitation of other sites in the brain with NaCl crystals either did not greatly affect the reflex excitability or increased it to a certain extent. In some of his studies devoted to the detailed analysis of the phenomenon of the thalamic inhibition of spinal motor reflexes, Sechenov (1956) developed the concept of the central inhibition as a specific active nerve process.

We find in his work an experimental criticism of the concept of Schiff and his disciple Herzen (1864) who tried to explain the results of Sechenov's experiments by the phenomena of fatigue and overirritation.

Sechenov's works on the thalamic inhibition of spinal reflexes were published in Russian, French and German. Later he carried out investigations which demonstrated the phenomenon of inhibition in motor centres of the spinal cord (1956, pp. 554–607), in the respiratory centre of the medulla oblongata (1956, pp. 661–662), provoked by the reflex method, namely by the excitation of afferent nerves.

The problem of central inhibition, formulated in Sechenov's work in 1863–1865, became a universally recognized concept by the beginning of the 80's; later it was accepted as one of the fundamentals of neurophysiology. But the functional role of the thalamic suppression of spinal reflexes remained for a long time an object of discus-

sion. It is natural to ask if the thalamic suppression of spinal reflexes conforms to the concept of central inhibition in its present interpretation and to what type of inhibiting central reactions does it belong?

According to up-to-date notions central inhibition can manifest itself either by a delay in the reflex reaction or by the suppression of the developing reaction. The dynamics of the central inhibiting process, characterized by the duration of the latent period and the rate of the suppression and restoration of the inhibited reaction, fully conforms to the dynamics of the central excitement process (Creed *et al.*, 1935).

The inhibiting effect of the thalami optici on motor centres of the spinal cord was studied myographically, by registration of reflex contractions of antagonistic muscles — m. semitendinosus and m. triceps (Tonkich, 1927; Golikov and Kiselyev, 1937; Kiselyev, 1948).

The reflex tetanus of the antagonistic muscles lasting 2–3 sec was the result of the repeated tetanization of the n. peronei with intervals of constant duration. The excitation of the thalami optici with NaCl crystals or with electric stimuli in the interval between the tests of the afferent irritation resulted in the full suppression of following reflex responses to this excitation (Fig. 2). After the withdrawal of the

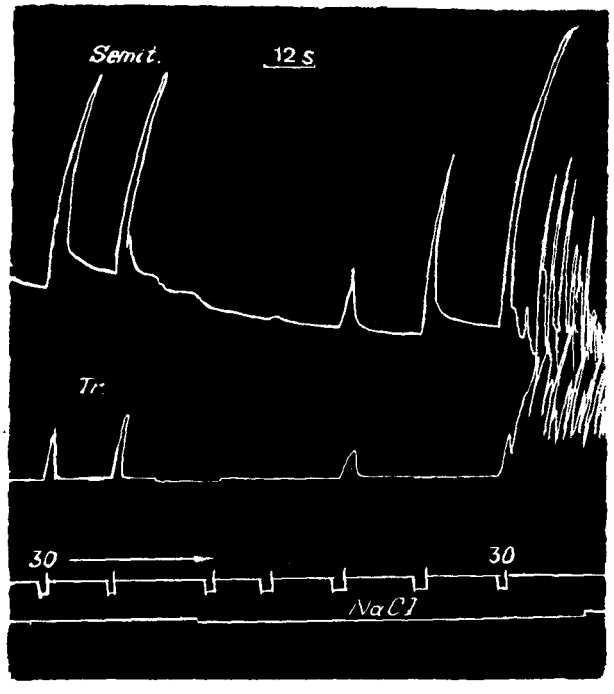

Fig. 2. The inhibition of reflex responses of antagonists with later renewal transformed into convulsions as a result of the irritation of the thalami optici with a NaCl crystal; lower reading of the register, irritation of the thalami optici; upper reading, that of the n. peronei ips. The intensity of irritation on all myographic tracings, in cm of induction coil.

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thalamic irritation the reflex contractions of antagonistic muscles returned. In this experiment the thalamic irritation preceded the afferent excitation, the inhibition being observed as the delay of responses to following tests. Then, in another experiment, the thalamic irritation was accompanied by a strong reflex tetanus, provoked by the earlier lasting afferent excitation. It resulted in full inhibition, that is suppression of the reflex response (Fig. 3). When the thalamic area is excited by rhythmic electrical

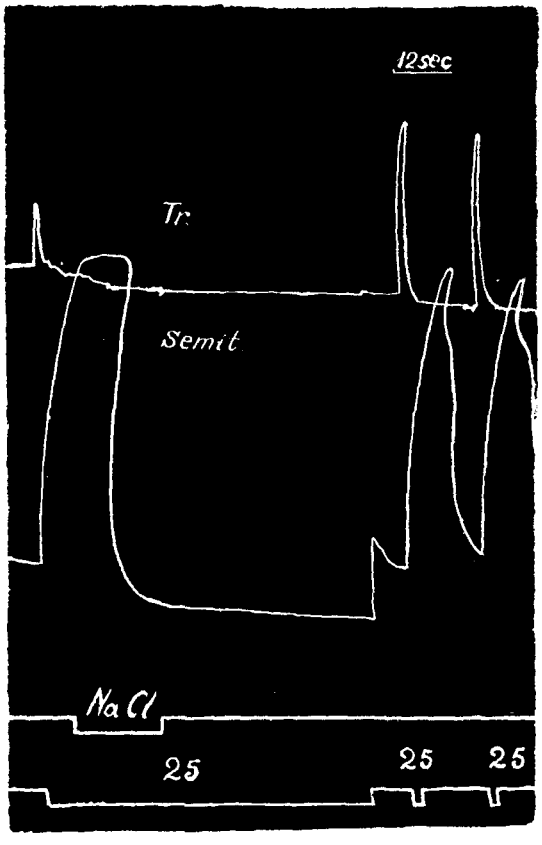

Fig. 3. The inhibition of the reflex tetanus on irritation of the thalami optici with a NaCl crystal; lower reading, the irritation of the n. peronei ips.; upper reading, that of the thalami optici.

stimuli, one can many times provoke the suppression and the restoration of the same reflex response by applying or removing the thalamic irritation (Fig. 4).

The simultaneous suppression of reflex reactions of both antagonists is a characteristic feature of thalamic inhibition. This fact excludes the possibility of interpreting the thalamic suppression as a reaction of the reciprocal inhibition (Bolotov, 1918).

Data on the duration of the latent period of the thalamic suppression of spinal motor reactions were obtained by the methods of electrophysiology when reflex responses were recorded by measuring the action potentials either from muscles (Rudashevski, 1954) or from nerves innervating these muscles (Kiselyev, 1957). When the thalami optici were irritated with NaCl crystals the latent period of the thalamic

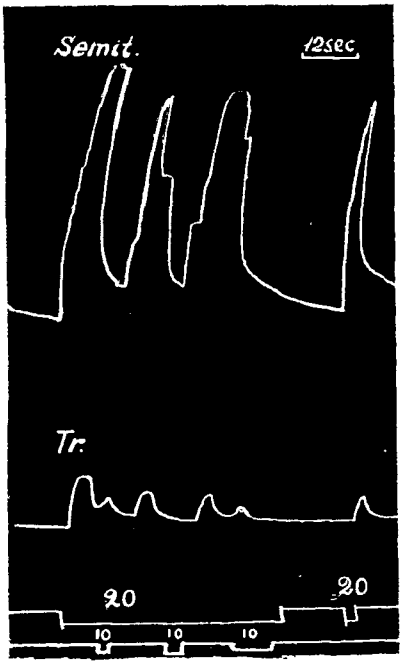

Fig. 4. The inhibition of the reflex tetanus with repeated tests of the rhythmic electrical irritation of the thalami optici. Lower reading, irritation of the thalami optici; upper reading, irritation of the n. peronei ips.

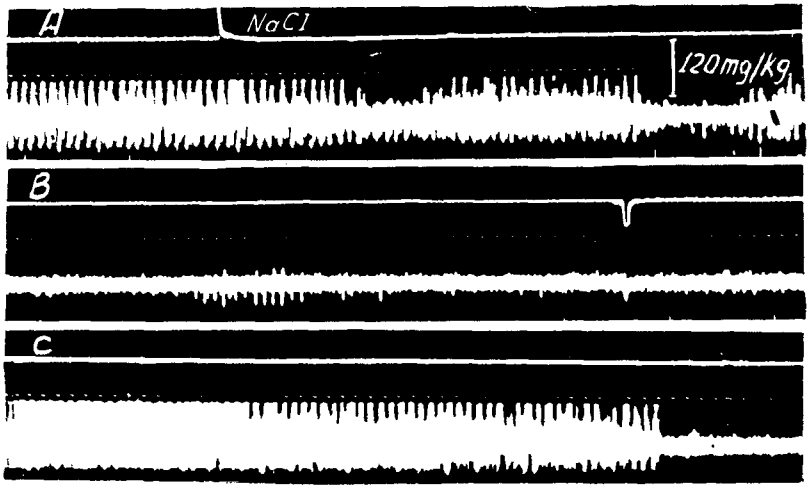

Fig. 5. The effect of irritation of the thalami optici with a NaCl crystal on the reflex impulses of the flexory centre. A, the application of NaCl; B, the continuation of A; C, after the removal of NaCl and washing the brain with Ringer solution. Upper line, irritation of the thalami optici; lower line, action potentials of the n. semitendinosi provoked by the irritation of the n. peronei ips. Time, 0.05 sec.

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suppression in different experiments averaged from 0.4 to 2 sec (Fig. 5). A comparatively long duration of the latent period of the salt excitation of the thalamic area may be attributed to the time taken for a salt crystal to dissolve, and to the increase in its concentration to the threshold level of the excitation of cells of the thalamic area. The thalamic suppression of the reflex response displays itself as a decrease in the amplitude and the rhythm of the action potentials — up to the entire blocking of the efferent impulses of the motor reflex centres of the spinal cord. The complete suppression of the reflex impulses of spinal centres observed in a number of experiments was achieved 50–75 msec after its beginning.

A more accurate determination of the true duration of the latent period of the thalamic inhibition of reflexes was obtained as a result of the electrical irritation of the thalamic area. It was achieved with the help of the polarization of a cross-section of the thalamic area with a continuous current cathode during 0.5–1 sec. It is known that the cathode polarization with a current of moderate intensity and short duration excites nerve cells — but does not suppress them. The use of continuous current for the electrography of reflex reactions excludes difficulties connected with an artefact produced by rhythmic irritation with the impulse current. The electrical

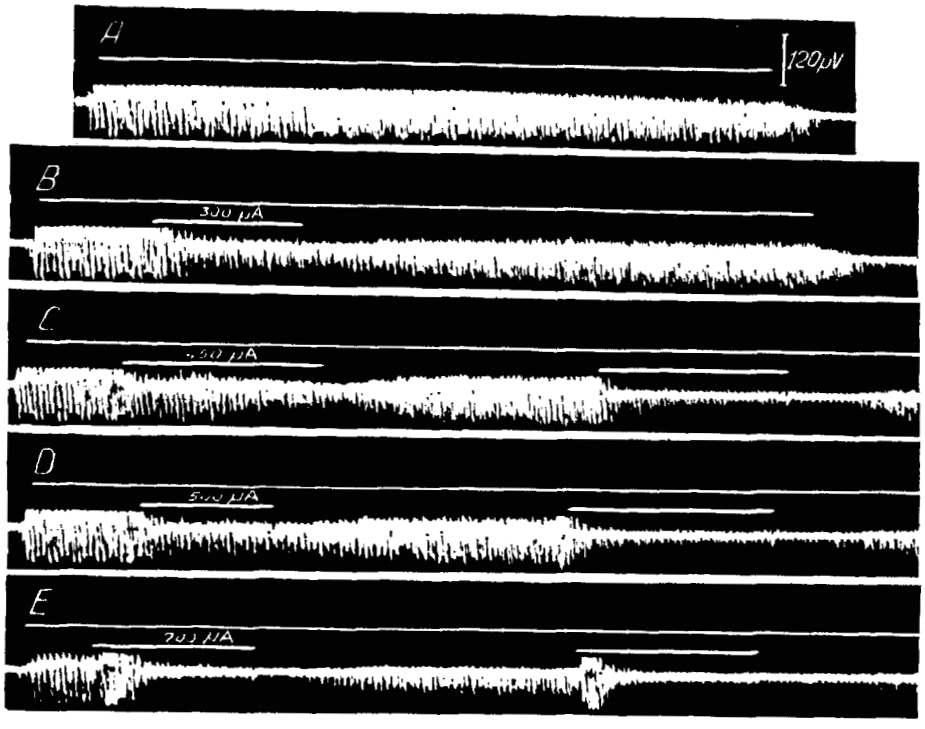

Fig. 6. The effect of the irritation of the thalami optici with a continuous current cathode on the reflex impulse of the flexory centre. A, action potentials of the n. semitendinosi at the irritation of the n. peronei ips. with inductive stimuli at a frequency of 40 per sec with an intensity of 18 cm; B, C, D, E, irritation of thalami optici with a continuous current cathode at different intensities accompanying the reflex impulse. Time, 0.05 sec.

excitation of the thalamic area with the continuous current cathode results in the shortening of the latent period of the thalamic suppression of spinal motor reflexes to 30–50 msec which correlates closely with latent periods of different reactions of the central inhibition (Fig. 6).

The absence of the specificity and localization of action of Sechenov's thalamic inhibition is its peculiar feature. It spreads simultaneously over the whole area of spinal motor innervations. The suppression of strychnine tetanus and of strychnine convulsions by thalamic excitation are the most convincing manifestations of this peculiarity. This phenomenon was visually observed for the first time by Sechenov's disciple Matkevich (1865) and Kiselyev (1957) confirmed by electrophysiological procedures. An intensive efferent impulse of motor spinal centres, resulting from strychnine intoxication, was fully suppressed by thalamic irritation (Fig. 7). After the NaCl

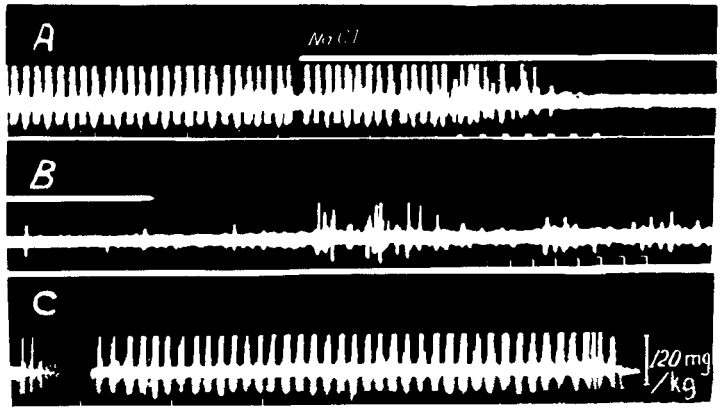

Fig. 7. The effect of the irritation of the thalami optici with a NaCl crystal on the convulsive impulses resulting from strychnine intoxication and registered in the n. semitendinosi. A, the irritation of the thalami optici. B, the continuation of A; C, after the removal of NaCl and washing the thalami optici with Ringer solution. Upper line, irritation of the thalami optici.

crystals had been removed from thalamic cross-sections and the thalami optici had been washed with Ringer solution, the efferent impulse of motor centres acquired its initial intensity.

Numerous authors showed that there is a spontaneous electrical activity in the spinal cord. Ten Cate (1950) investigated in detail the origin of this activity. He came to the conclusion that the activity reflects the tonic excitement of spinal motor centres, supported by reflex-afferent effects on skin and muscle receptors. In Ten Cate's opinion, the Brondgeest tonus is a motor manifestation of this excitation (Brondgeest, 1860). Hence one may assume that the excitation of the thalamic area with a NaCl crystal would influence in a certain way the spontaneous electrical activity of the spinal cord. This assumption was verified experimentally (Fig. 8). With a thin concentric electrode penetrating the spinal cord to a depth of 2–3 mm from the caudal end along the axis were recorded the spontaneous electrical rhythmics consisting of slow oscillations of different forms.

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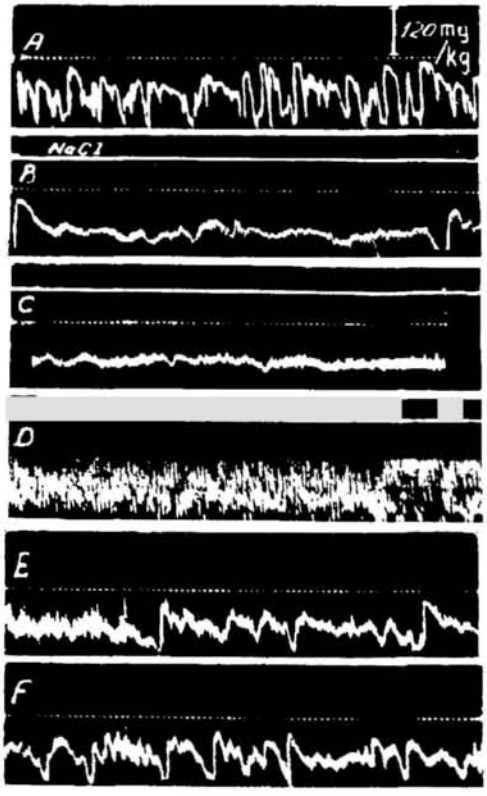

Fig. 8. The effect of the irritation of the thalami optici with a NaCl crystal on the spontaneous biolectrical activity of the spinal cord. A, readings from the VII–IX segments of the spinal cord; B, after 3 sec; C, 20 sec and D, 35 sec, of NaCl action on the thalami optici cut; E and F, after the removal of NaCl and washing the thalamic cut with Ringer solution. The line above oscillograms B, C and D shows the thalamic irritation. Time, 0.05 sec.

The thalamic irritation fully suppressed these rhythmics. At the end of the action of salt irritation of the thalamic area the suppression resulted in the intensive development of peak potentials. This phenomenon reflects the convulsive excitation conditioned by the 'overcome' localization of the thalamic excitation because of the penetration of salt from the surface of a cross-section into depth. Sechenov frequently pointed out that the suppression caused by the salt thalamic irritation is transformed after several dozens of seconds into convulsive excitation, because of the penetration of salt into deeper layers, to the lobus opticus, in particular.

The above-cited results prove that the thalamic delay of spinal motor reflexes is connected with the mechanism of the central inhibition. Sechenov thought that there are cellular elements in the optic area which exert the inhibiting influence on motor centres of the spinal cord. A convincing proof of the effect of the thalamic irritation in Sechenov's experiment on cells and not on the conduction paths from the above-located cerebral sections (hemispheres, smell area of the brain) has been provided by Stefanzov (1939). He showed that the thalamic inhibition in frogs with a chronic

thalamic cut is observed after periods that are quite enough for the transformation of all fibres of descending paths from cerebral areas located above the cut.

The thalamic inhibition of Sechenov has the following principal features: (1) cellular structures responsible for this reaction are located in subcortical formations of the brain; (2) the thalamic inhibiting influences are of sub-segmentary, unspecific nature; (3) their action leads to the inhibition of reflex responses and the suppression of the spontaneous electrical activity of the spinal cord; and (4) the thalamic inhibition is not weakened by strychnine but suppressed the strychnine excitement. These peculiarities lead to the conclusion that the thalamic inhibition discovered by Sechenov in 1863 belongs to that type of descending inhibiting effect of the brain on the spinal cord which, since Magoun's studies (1950), is usually associated with the function of the reticular structures of the subcortical cerebral departments. According to Eccles (1957), the inhibition of the strychnine excitement is a principal and important feature of inhibiting reactions of this type. It is well known that strychnine affects reflex inhibiting reactions of the spinal cord (for example, the reciprocal inhibition of antagonists) in an opposite way: it weakens and, if the intoxication is severe, fully suppresses, the reflex inhibition.

Sechenov put forward the following important suggestion with regard to the mechanism of the central inhibition. He thought that inhibiting neurons differ only as to terminal formations of their axons. As to cells themselves and their axons, they respond to the irritation by a simple process of excitement. The process of excitement, reaching terminal axon apparatus, results either in the inhibiting or in the exciting, effect on cells that are in contact with the terminal apparatus, depending on their properties. This concept fully conforms to the universally-recognized concept in modern neurophysiology on the mechanism and interrelations of irritation and inhibition processes in the central nervous system.

SUMMARY

The thalamic depression of reflexes observed upon stimulation of the thalamus of the frog is characterized by a relatively short latency, a high rate of development of the maximal inhibition and quick restoration of the reflex after the cessation of stimulation. The thalamic inhibition may manifest itself both in delay of the response when thalamic stimulation precedes the afferent one, and in inhibition of the following reflex when afferent stimulation precedes the thalamic one. The time course of thalamic inhibition corresponds with that of the reflex one, for instance, of the reciprocal inhibition of antagonists. However, thalamic inhibition significantly differs from reflex inhibition in two respects. Firstly, thalamic inhibition is not local, and spreads over the motor centres of all the skeletal muscles. Secondly, it is not reduced by strychnine; however, it inhibits strychnine excitation.

These peculiarities form the basis for our belief that Sechenov thalamic inhibition is related to that type of descending inhibitory influence of the brain which, according to Magoun's investigations (1950), has been associated with the functions of the reticular formation of the brain stem.

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Inhibition of Nerve Cells and Nerve Centres

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The discovery of the central inhibition phenomenon made by Sechenov was a major step on the way to natural and scientific analyses of the higher functions of the cerebrum. As a result, general contours of the nature of psychical activity were outlined. The mechanism has a double nature: it consists of excitation and inhibition. The successes marked by further development of physiology of the nervous system, and especially of the studies of the higher nerve activity carried out by Pavlov, were to a considerable extent based on the concept of interaction of excitative and inhibitory processes, as a principle of nerve centre operation. At present, *i.e.*, one hundred years after Sechenov's discovery, neurophysiology makes another step into depth of the nature of the nerve activity and attempts to analyse the central processes of excitation and inhibition in terms of functioning of the nerve units (neurones), of which the centres are made.

In recent years, physiology has rapidly accumulated information on two lines. Firstly, release from neurones of various types of response (reaction) (Jung, 1953; Li and Jasper, 1953; Baumgarten, 1956; Lettvin *et al.*, 1959; Smirnov, 1963; Sokolov, 1963). Secondly, determination of the interdependence of the responsiveness of neurones and impulse activity (Mountcastle, 1957; Li, 1959; Amassian *et al.*, 1959; Verzeano and Negishi, 1960; Leman and Murata, 1962; Livanov, 1963), in particular, the neurones of the cortex in the conditioned reflex activity (Jasper *et al.*, 1958). We have made attempts to investigate the neurone structure of the central nervous processes by simultaneously studying electrophysiological, cytochemical and morphological data which specify the condition of several neurones incorporated into a special functional system (Kogan, 1962b, 1963).

In what way do the excitation and the inhibition of a neurone manifest themselves? The excitative or inhibitory state of an individual neurone is usually specified by the onset of the impulse activity or by its suppression. The inhibition of a neurone, if judged by the absence of the pulse discharge, does not differ from its state at rest. The opportunity does not often offer itself to solve this problem by way of investigating the membrane potential which requires, for its observation, intracellular abduction. The metabolic characteristics of the neurone under test can contribute considerably to the solution of this problem. The successes gained by functional chemistry (Hydèn, 1955; Palladin, 1956; Vladimirov, 1957; Portugalov, 1957;

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Shabadash, 1957; Kreps, 1958; McIlwain, 1955; Vinnikov, 1959) make it possible to specify the metabolic and chemical changes, characteristic of various functional states of the neurone.

Distinct cytochemical and structural changes in the course of excitation and inhibition of an individual nerve cell were observed by S. Zaguskin in our laboratory who carried out tests on a simple object, *i.e.*, on the receptory neurone of strained muscle of a crawfish (Fig. 1).

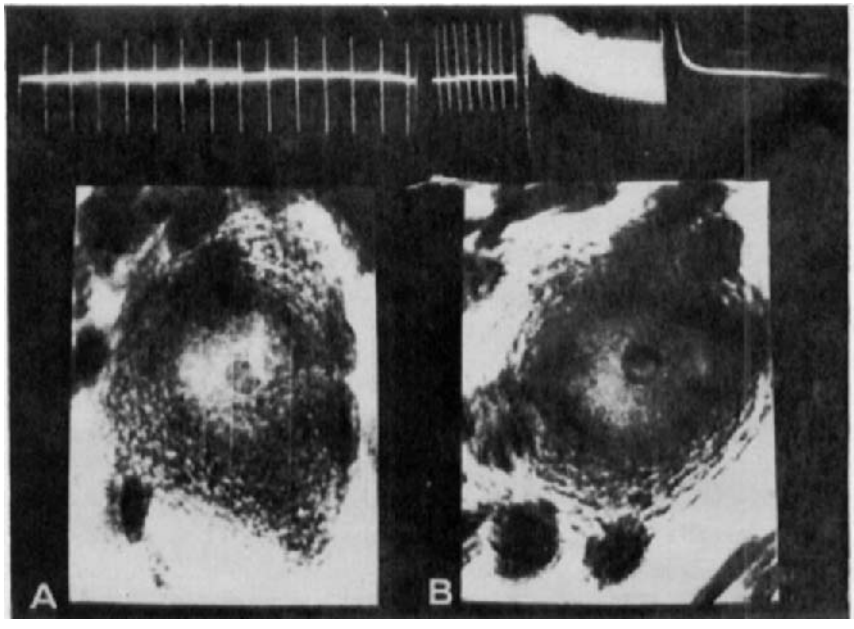

Fig. 1. Impulse activity and distribution of the ribonucleic acid (RNA) in the neurones of strain after ten-minutes' adequate excitation. A, superimposed on the background of the impulse discharge (8 per sec), shown above the microphotograph. B, superimposed on the background of the impulse discharge stoppage (6 per sec), caused by excitation of the inhibitory nerve during 2 sec (artefact on the electrogram). To indicate the discharge, the period of excitation of the inhibitory nerve and the subsequent inhibition of the impulses, the recording of the potentials was deliberately slowed down.

In the resting nerve cell, whose condition was checked functionally by relaxation of the muscle and electrophysiologically by the absence of discharge of nerve impulses, the ribonucleic acid (RNA) may be seen in the form of spindle-shaped and stem-shaped structures located in the cytoplasm concentrically relative to the nucleus on the background of the fine grain scattered in diffusion (Fig. 1, A). The adequate excitation of the neurone, that occurs owing to muscle strain, causes its impulse activity and is accompanied by disintegration of the spindle-shaped structures of the ribonucleic acid (RNA); as a result, coarse grains of irregular shape are formed (Fig. 1, B).

The restoration of the initial cytochemical state of rest usually occurs after the excitation process has ceased, in 10 min. However, if on the background of the existing strain of the muscle, the neurone is inhibited by the action of the inhibitory

nerve, then even until several seconds after the excitation of the latter it is possible to observe ever more abundantly and distinctly (as compared with that at rest) the concentric arrangement of the ribonucleic acid (RNA) structures in the cytoplasm of the perikaryon and a natural increase of RNA in the nucleus (Fig. 1, B).

Such a simple material, whose functional state can be exactly determined, has presented a graphic demonstration of the active nature of the inhibitory structural and chemical rearrangement of the neurocyte. Further it offers the opportunity, through a graduation of the intensity of its excitation and inhibition, to determine their metabolic equivalents. Electrophysiological and cytochemical manifestations of the excited and inhibited state of the neurone testify to the distinct and direct bonds with its functional state.

When, however, the neurones enter the interacting systems of the nerve centres, the relations become more complicated. The complication occurs together with the evolution of the impulse activity. The discharges of the neurones have a comparatively regular rhythm only in relatively simply-organized nerve centres of invertebrate animals and at the spinal level of the central nervous system of vertebrates.

Occurring at the nerve formations of the cerebrum is the so-called spontaneous or main continuous activity of the neurones, which is arhythmic in nature (Fig. 2).

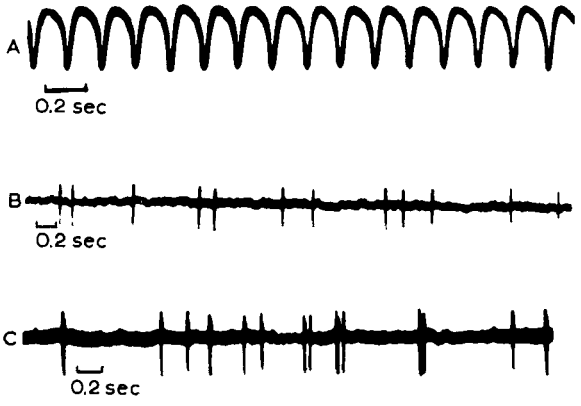

Fig. 2. The main activity of the neurones: A, gullet ganglia of the snail (experiment carried out by I. Karpenko). B, cover of the frog midbrain, the layer III according to Huber and Cross (experiments made by O. Tchorayan). C, crusts of the large hemispheres of the cat brain, the 4th layer of the superciliary convolution (experiments carried out by I. Chasabov).

What is the arhythmia of the neurone discharges caused by and in what it may result? A comparison of the morphological and electrophysiological data indicates that the arhythmia of the main activity is connected with the complication of the intracentral bonds. The neurone subjected to ever increasing excitations lacks the regularity of its own rhythm of impulse activity: the pulse discharge is being either quickened or slowed. However, the quick or slow discharge produces oscillations of the functional state of the neurocyte between times of excitation and inhibition. Hence, it is clear that, as distinct from individual nerve cells or from those which form primitive switching stations, the neurones of the higher sections of the cerebrum

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are (even in the absence of the central excitation or inhibition) in a state of continual oscillating excitation. It may be that this is the mechanism that maintains functional ability at the definite level of the highly-organized systems.

How are the neurones organized into the processes of the central excitation or inhibition? In the primitive nerve centres, the central excitation or inhibition represents a simple sum of the synchronously excited or inhibited neurones of the very centre. These neurones, if not excited, are in a relatively neutral state of rest. A more complicated problem pertains to the formation of the central nervous processes in the higher sections of the cerebrum due to the activity of the neurones of oscillating excitation. Some data for consideration of the problem are provided by the results of studying the interdependence of their arhythmic impulse activity. To this end, one of our scientific workers, O. Tchorayan, carried out some tests in which he recorded up to six neurones on three channels under the conditions of their main and induced activity. As Fig. 3 shows, there is (at a glance) no link between the discharges

Fig. 3. Simultaneous recording of the main activity of several neurones (by two microelectrodes) spaced at a distance of, approximately, 0.5 mm from each other in the surface layers of the optic lobe of the frog brain.

of the six recorded neurones related to the cover of the frog midbrain. Nevertheless, there is good reason to believe that every three neurones recorded by one microelectrode, *i.e.*, located in immediate proximity, function under conditions which make them somewhat interdependent. With a view to revealing this interdependence,

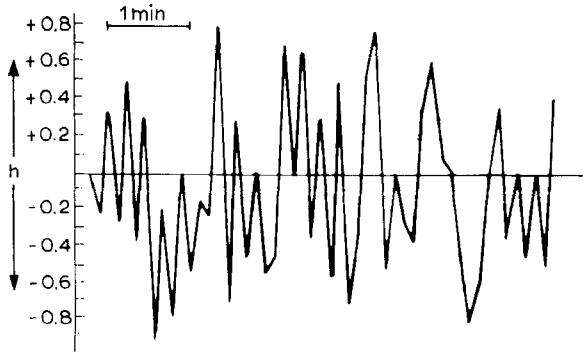

Fig. 4. Dynamics of the correlation coefficient of two neurones, the layers I–II of the cover of the frog midbrain.

the correlation coefficient of the impulse time of neurone pairs was calculated. This correlation of the main activity, as shown in Fig. 4, undergoes considerable changes. The period of conjugated changes of the neurone discharges (*i.e.*, positive correlation) is superseded by a period of reciprocating relations (negative correlation) and then by correlated changes. Sometimes it is possible to observe more prolonged periods of correlation increase or decrease. Consequently, even in the relatively resting centre, mosaic rearrangement of the more excited or more inhibited neurones takes place continually. The cyclic recurrence revealed by the graph does not reflect the inherent periodicity of the structural nature of the nerve centre; it is determined by the time interval selected for calculating the correlation coefficient. In our opinion, the changes in the correlation coefficient that occur as a result of excitation which causes functioning of the nerve centre (Table I) are essential.

TABLE I

CHANGES IN CORRELATION COEFFICIENT OF NEURONE PULSE ACTIVITY WHEN THE NERVE CENTRE EXCITATION IS ADEQUATE

	Positive correlation		*Negative correlation*	
	Basic activity	*Response to excitation*	*Basic activity*	*Response to excitation*
Mr	0.14	0.28	0.24	0.42
τ	0.15	0.16	0.18	0.19
$t\triangle$	2.5		2.3	
$P\triangle$	0.02–0.05		0.02–0.05	

Table I presents mean calculations of the correlation of the activity of twenty-three neurone pairs (frog brain optic lobes). As shown in the table, the correlation of the neurone discharges in the optic nerve centre, when the eye receives sunlight, is increased. If the positive correlation is characteristic of the main activity, then, when the nerve centre functions, it becomes ever more positive; but if the correlation is negative, it becomes ever more negative. Consequently, the superficial chaotic state of the arhythmic discharges (proceeding asynchronously) of the neurone units conceals a certain interdependence which manifests itself more distinctly during central activity. Thus, the excitation of the primary nerve centre only increases the degree of displaying those patterns of the mosaic structure of the excited and inhibited neurones which has already taken place in their basic activity. Hence, the above-mentioned results suggest that there is a specific predetermined way for the various ensembles of neurones to participate in performing the central nervous processes.

To discover the possible space distribution of the neurone ensembles, some information may be obtained as a result of studying the correlation of the neurone pulse activity, the neurones being spaced at different distances from one another.

The space dependence does not manifest itself notably during the main activity, but it is quite obvious during the period of response to excitation.

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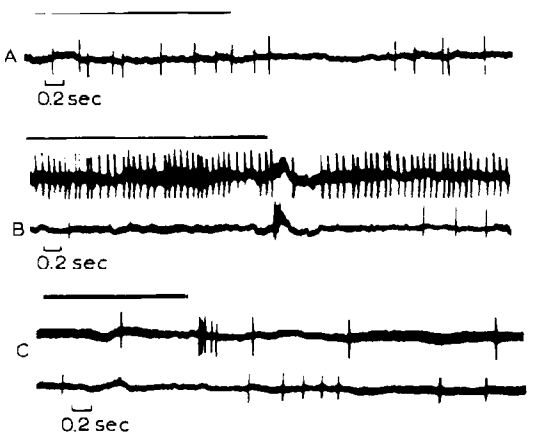

Fig. 5. Simultaneous recording of the responses displayed by the neurones of the frog brain optic lobe (spaced at different distances from one another) when the illumination of the eye is stopped (10 sec; 500 lux). The line over the record indicates the light availability. A, immediate proximity (recording is performed by one microelectrode). B, distance up to 0.5 mm. C, distance over 0.5 mm.

Presented in Fig. 5 are typical interrelations of the response of variously spaced neurones. When the neurones are spaced close to each other and recorded by one microelectrode, they often respond in the same way, in this particular case 'off' by the inhibition process (Fig. 5, A); whereas those spaced at a distance from tens of microns to approximately 0.5 mm respond most frequently reciprocally. For instance, one 'off' with inhibition, and the other 'off' with excitation (Fig. 5, B). Lastly, if the distance between them exceeds 0.5 mm, it is, as a rule, impossible to note any specific interdependence of their response (Fig. 5, C).

When the relations are reciprocal, the response of the inhibitory neurones is usually followed by the response of the excited neurone (Fig. 6). This phenomenon manifests

Fig. 6. Simultaneous recording of the responses of two neurones of the frog brain optic lobe to switching-on of the light. The neurones are spaced at a distance of 150 μ from each other. The line above the record indicates the light.

itself most vividly during the conjugated reactions of the two neurones of the frog brain optic lobes to light excitation, which is shown in Fig. 6. The first to act was the inhibitrope neurone, and after 0.18 sec it was joined by the excitoneurone. The process of the central excitation starts with the activity of the inhibitory neurones. Probably

such a sequence is of physiological importance for the preliminary creation of the excitation frames, *i.e.*, a sort of matrix-mould, to form the complicated mosaic (tesselation) of the excitative centre.

Thus, the investigation of the pulse activity of the neurone makes it possible to outline two stages of evolution in their arrangement. In more primitive nerve structures, excitation or inhibition of the centre is arranged by way of mass excitation or inhibition, or inhibition of the neurones forming this centre, whereas in the highly-organized sections of the brain, both the excitation and the inhibition are formed by the mosaic distribution of the excited and inhibited cells.

The availability of the two methods of functional arrangement of the neurones into the nerve centres is corroborated by the metabolic findings, which can be seen in the dynamics of the ribonucleic acid (RNA) in the nerve cells of the motor cortical centres of the segmented reflexes of the spinal cord and the systems of high analysis in the cortex of the large hemispheres of the cerebrum (Fig. 7).

The investigation of the dynamics of the ribonucleic acid (RNA) distribution in the spinal motor neurones, during the performance of the one-sided flexor reflex, accomplished in our laboratory by T. V. Ivannikova, has shown that the coming changes are of synonymous nature for the main bulk of the cells of the motor nucleus of the respective muscle. Fig. 7 illustrates the decrease in ribonucleic acid (RNA) in the motor neurones of the nucleus of the semi-tendon muscles, on the side of contraction (Fig. 7, A), as compared with the reference ones on the other side (Fig. 7, B) after 5 min of inducing the reflex. The content of ribonucleic acid is simultaneously increased in the motor neurones of the nucleus of the inhibited antagonist, *i.e.*, the four-part muscle. Quite a different picture is presented by the distribution of the ribonucleic acid in the pyramidal cells of the superficial layer of the optic motor analyser after the adequate excitation of 5 min by a flashing light under conditions of the chronic experiment with the local congelation of the electrical response zone through the internal capsule provided with electrodes. The mosaic arrangement of the cells is clearly visible with relatively increased or decreased content of ribonucleic acid even in the check area (Fig. 7, D), especially in the area of the primary response (Fig. 7, C).

It may be expected that in the first type of nerve organization, the total metabolic characteristic of the central excitation or inhibition will correspond to the metabolic characteristic of the neurone excitation or inhibition, whereas in the second type of organization the mosaic of the inhibited and excited neurones may present the most different resultant metabolic characteristics of the cerebrospinal nerve processes. Maybe this is the explanation of some contrasts in the results of biochemical investigations of the higher sections of the cerebrum, in which the shifts, having the exact opposite direction in different cells, are averaged.

The problem of the functional significance of the alterations in the ribonucleic acid content in the nerve cell is very complicated; it requires further study, and it would be a premature conclusion to connect any change in the ribonucleic acid in the neurone directly with its excitation or inhibition. It can be shown graphically, for example, that depending upon the duration and intensity of excitation and inhibition,

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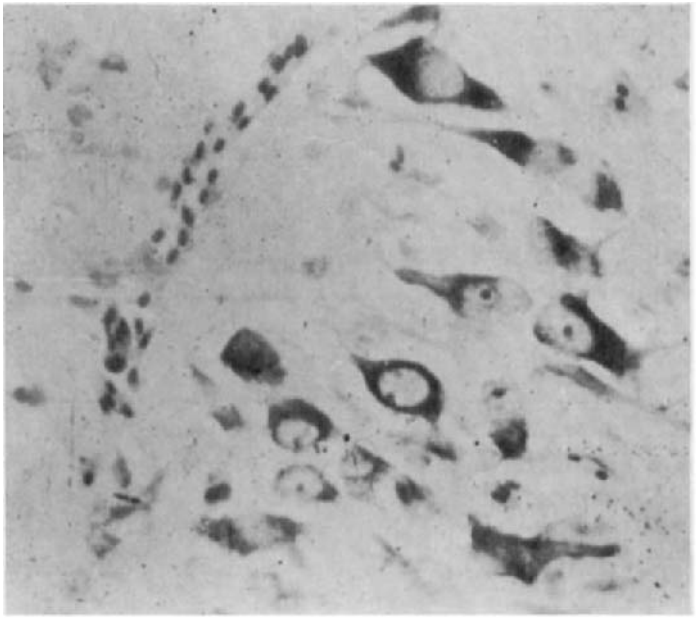
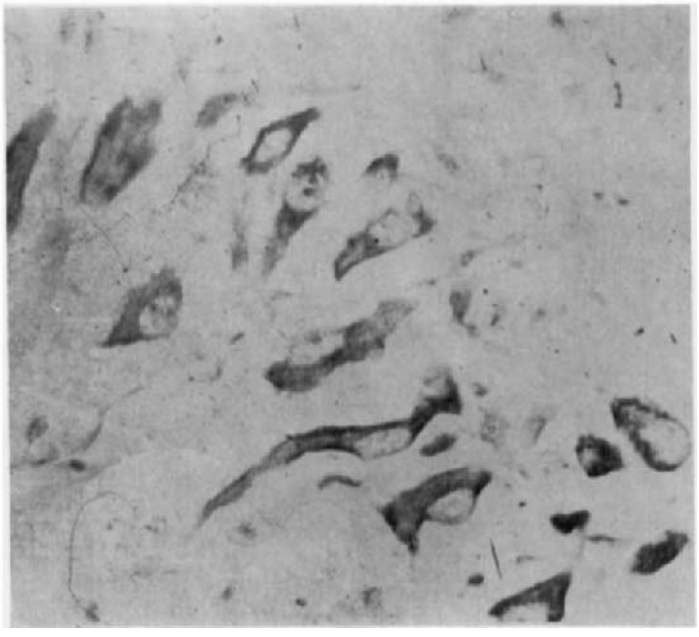


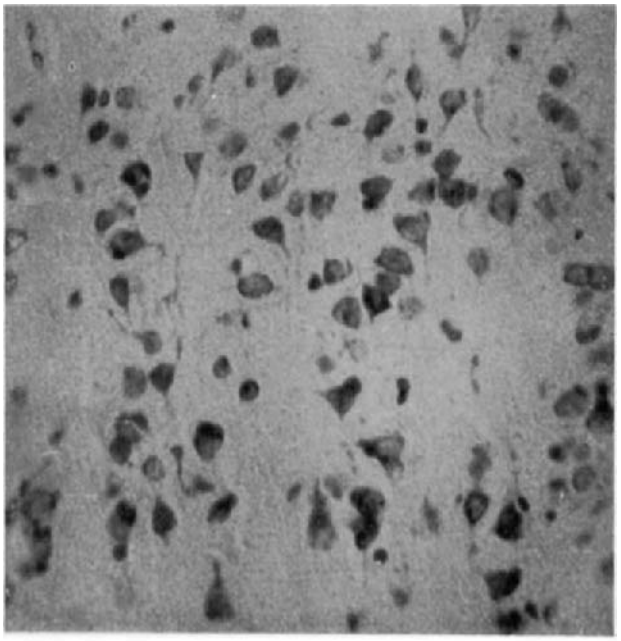
A



B

the metabolic shifts caused by excitation can proceed in the manner typical for the inhibitory state of the neurone or *vice versa* (Table II).

Table II shows the results of the microphotometric determination of the ribonucleic acid in motor neurones of the innervation of the muscle, *i.e.*, antagonists (semitendoneous and quadriceps), during different periods of the flexor reflex performance. Even in 15 sec, it is proved statistically that the content of ribonucleic acid in the



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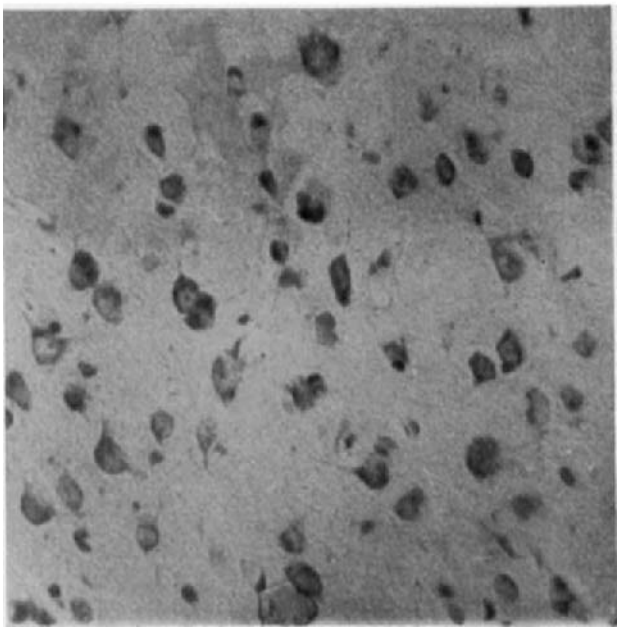

D

Fig. 7. Distribution of the ribonucleic acid (RNA) in the nerve cells of the nerve centres of various type of organization at relative rest and in excitation (magnification 6.3; 40). Motor neurones of the nucleus of the frog spinal semi-tendon muscle during central excitation (A) and at relative rest (B). The pyramidal cells of the cat brain crust in the excitation centre indicated by the data of the electrical responses (C) and beyond the centre (D).

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TABLE II

RELATIVE PHOTOMETRIC DATA, INDICATING THE CHANGES IN THE RIBONUCLEIC ACID IN THE RAT SPINAL MOTOR NEURONES DURING ANTAGONISTIC REFLEX

Duration of reflex activity	*Nucleus of semitendoneous muscle (excitation)*	*Nucleus of quadriceps muscle (inhibition)*
15 sec	—45.0 ($t_A = 6.8, P < 0.01$)	+185.5 ($t_A = 23.4, P < 0.01$)
1 min	—0.5 ($t_A = 0.07, P > 0.9$)	+11.0 ($t_A = 1.69, P = 0.1$)
5 min	+221.5 ($t_A = 109.4, P < 0.01$)	—59 ($t_A = 9.7, P < 0.01$)

excited neurones decreases, while in the inhibited neurones it increases. In 1 min, however, relatively small oscillations occur that are statistically not trustworthy. In 5 min, another phase of the metabolic changes takes place, *i.e.*, accumulation of ribonucleic acid in the excited neurones and its expenditure in the inhibited neurones. Clearly, the metabolic characteristic of the cerebrospinal nerve processes in the mosaically arranged higher sections of the cerebrum will become complicated.

On the basis of what principles are the complicated dynamic correlations created, owing to their electrical and metabolic indices, in the neurones of the mosaically-organized higher nerve centres during their activity?

At one time an attempt was made to analyse, by a statistical method, the properties of the high plasticity and large compensating abilities of the higher sections of the cerebrum on the hypothesis that the neurone elements are being organized into functional systems (Kogan, 1962a, 1964). In accordance with such a hypothesis, the state of the central excitation or inhibition is determined statistically as a result of multiple interaction of the neurones which form the functional mosaic according to the laws of probability distribution. As viewed in this manner, the inhibition of the mosaically-organized nerve centre can differ from its excitation only by the nature of the space–time distribution (in the main portion) of, probably, one and the same neurone being in the oscillating functional state. Specialization of the neurones and their synapse meters imparts an especially complicated character to such a distribution.

The hypothesis of the statistical probability organization of the neurone into the functional systems of the higher sections of the cerebrum are similar to numerous ideas put forward lately by physiologists on the stochastic nature of the high nerve activity (Fessard, 1960; Grey Walter, 1962; Livanov, 1962).

Exceptional flexibility, reliability and integrity, which are characteristic of this activity, cannot be confined to formal logic schemes or simply-determined intra-neurone combinations. And they can be understood only from the point of view of the probability logic of the neurones' recurrently determined interactions. The discovery of the physiological laws according to which such an interaction of the nerve cells

form excitation or inhibition of the nerve centres would be one of the many steps on the way outlined by Sechenov in his thesis of 'Cerebrum machinery'.

SUMMARY

Functional heterogeneity of neurones participating in formation of central nervous processes was studied with the help of simultaneous recordings of activity of several neurones whose topographic relations were determined by marking.

The experiments on the frog midbrain tectum with adequate photic stimulation (Tchorayan), and the experiments on the suboesophageal ganglion of the snail *Helix pomatia* with stimulation of commissures (Karpenko) revealed certain features of the structural dynamic organization of the nerve centre from excited and inhibited nerve cells.

The comparison of electrophysiological and cytochemical manifestations of central excitation and inhibition showed chemical heterogeneity of excited and inhibited neurones. However, such heterogeneity may be levelled in total characteristics of chemical shifts in the central nervous formations. In reciprocally inhibited spinal motoneurons the same changes in ribonucleic acid content take place as at the initial stage of excitation of neurone antagonists (Ivannikova). This may point to the existence of common physico-chemical links in excitation and inhibition of the neurone, as well as to the importance of the time factor in the formation of excitation or inhibition of the nerve centre.

Thus central inhibition is not purely diffuse inhibition of the neurones of a given centre, but is a complex process of reorganization of the dynamic system of excited and inhibited cells constituting the nerve centre. Formation of central excitation or inhibition may be based on a stochastic organization of the functional systems, the latter consisting of the totality of excited and inhibited neurones (Kogan).

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Synaptic Mechanism of Central Inhibition

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The celebration at this Conference of the 100th anniversary of Sechenov's book 'Reflexes of the brain' is also a celebration of the 100th anniversary of Sechenov's discovery of central inhibition. This discovery has played an extreme role in the development of the whole of neurophysiology.

Central inhibition was studied during years and years by many prominent physiologists and many scientific groups; its mechanism was the subject of long-lasting discussions. But the recent time is especially important for the development of our knowledge about central inhibition. Because of the rapid progress of modern electrophysiological methods of investigation of the nervous system we have now wide possibilities for direct studies of cellular mechanism that forms the basis of central inhibition. Such studies have given us a vast amount of experimental data, which disclose many aspects of the nature of inhibition.

The main conclusion that we can draw in this direction from recent electrophysiological investigations is the following.

The central inhibition which is externally manifested by depression of nervous excitation by another excitation can be connected to cellular processes of different nature and different localization. But all such processes are similar in their incapability of propagating actively along the nerve cell and its processes. Inhibition arises in certain structures under the influence of an excitatory volley (which alone can propagate) and exerts its inhibitory influence in connection with another excitatory volley. Of course, this conclusion is justified only when we consider central inhibition at a cellular level. If we think about such systemic nervous processes as, for example, Pavlov's internal inhibition in the cerebral cortex, we can imagine their movement over its surfaces; but this does not mean that during such movement inhibition as such is conducted from one neurone to another through synaptic or some other connections. So what happens in a single nerve cell and its synaptic apparatus during inhibition? At this level the excitatory process is converted into its antagonist — the inhibitory one.

The physiological processes in a single nerve cell during inhibition can now be studied very exactly by intracellular potential recording. The data that have been accumulated about cellular processes in neurones of spinal cord and brain of higher animals and in peripheral and ganglionic neurones of invertebrates show us clearly that there are at least two different modes of inhibitory action.

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In one the inhibition of cell activity is a primary process for this cell; it is not connected to its previous excitation. Such primary inhibition is a result of activity of special inhibitory structures that make connections with the cell; the excitatory influences are exerted through other structures, and their status is changed during inhibition.

In the second the inhibition of nerve cell activity is produced without special inhibitory structures. Inhibition arises in the cell secondarily after its previous synaptic excitation; the excitation of the cell itself depresses the subsequent excitation.

Let us first consider the primary inhibition that is generated by special inhibitory structures. As is well known, Sechenov was the first to express the idea that special inhibitory structures in the brain produce depression of reflex activity when stimulated. This hypothesis of Sechenov is confirmed by recent investigations at a cellular level.

The main experimental data about the mechanisms of primary synaptic inhibition have been obtained from spinal motoneurones, mainly by Eccles and his collaborators (Brock *et al.*, 1952; Coombs *et al.*, 1955, etc.). The inhibition here is produced in this way: the nerve impulse that reaches the cell *via* special afferent pathways produces in it a particular type of postsynaptic change which is qualitatively different from changes during an excitatory synaptic action of an impulse in other afferent pathways. During the inhibitory synaptic action the postsynaptic membrane is hyperpolarized (the inhibitory postsynaptic potential) and its ionic conductance is simultaneously increased. Examples of such inhibitory postsynaptic potentials (IPSP) in motoneurones are shown in Fig. 1.

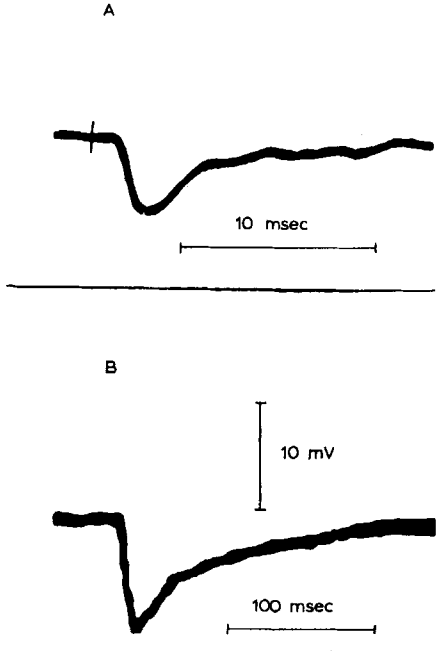

Fig. 1. Examples of inhibitory postsynaptic potentials in motoneurones during Lloyd's 'direct inhibition' (A) and polysynaptic inhibition in a flexor reflex (B). Note the slower sweep speed in (B). (Kostyuk, 1958b)

The depolarization of the postsynaptic membrane necessary for cell excitation is decreased when it coincides with IPSP (because of decreased membrane resistance and algebraic summation with hyperpolarization).

If we want to understand the mechanism of the creation of the inhibitory postsynaptic potential, we have to answer several questions. What ionic processes form the basis for changes in conductance and transmembrane potential in the postsynaptic membrane? What is the nature of the transsynaptic action that produces this change? What is the structure and function of synaptic endings that produce the specific inhibitory action?

Ionic processes in motoneurones during primary inhibition are now investigated very precisely. Two experimental approaches have been especially effective: the study of the dependence of the inhibitory postsynaptic potential (IPSP) from the transmembrane potential difference and from the transmembrane ionic gradients. It has been shown that the amplitude of the IPSP due to the same synaptic action changes in relation to transmembrane potential difference. The IPSP decreases to zero if the cell membrane is hyperpolarized from the resting level to approximately —80 mV. Stronger hyperpolarization brings an inversion of the IPSP to a depolarization. This shows that the electric polarization of the postsynaptic membrane during inhibition is fixed at a certain equilibrium potential. The equilibrium is defined by the concentration (activity) gradients of ions to which the membrane becomes permeable. The direction of changes in the transmembrane potential difference is not defined by the synaptic action: it depends entirely on the relation between the ionic gradients and transmembrane potential difference.

The correctness of this conclusion is clearly shown by experiments with artificial changes in ionic gradients during fixed transmembrane potential difference. If we produce in the cell (by iontophoresis from the microelectrode) an excess of certain

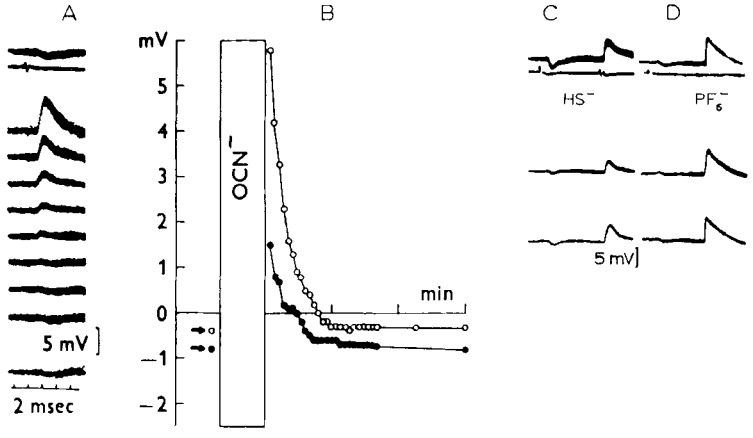

Fig. 2. Influence of intracellular injection of three different types of anions on IPSP of motoneurones. (A) and (B) injection of OCN^-; (C) injection of HS^-; (D) injection of PF_6^-. Control IPSP's are shown in the top row, in (C) and (D) also EPSP's from the same motoneurone. IPSP's during different time intervals after injection, in the others. (B) presents the relation between the peak value of IPSP (ordinate) and time after two injections (abscissa) in the same motoneurone. (Ito *et al.*, 1962)

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ions, then the changes in the electric polarization are reversed. But if we inject another type of ion, the IPSP remains unchanged. Examples of such action are shown in Fig. 2.

An extensive series of experiments with injections into motoneurones of 32 different species of anions is now completed (Araki *et al.*, 1961; Ito *et al.*, 1962). They are divisible sharply into 2 categories; those that change and those that do not change the IPSP. No transition types of behavior are observed. If we arrange all these anions in order of increasing hydrated size (Fig. 3), we can see a clear relation between the ionic size and influence on IPSP. All ions whose hydrated size exceeds that of potassium ions not more than 1.24 times penetrate through the postsynaptic membrane during inhibition; all larger ions do not. There are two exceptions from this rule: the HS^- and HCO_2^- ions. The first probably cannot exist in a free state in the protoplasm and forms a big complex with proteins. The reason for the second exception is not clear; maybe the calculation of the hydrated size in this occasion was not correct.

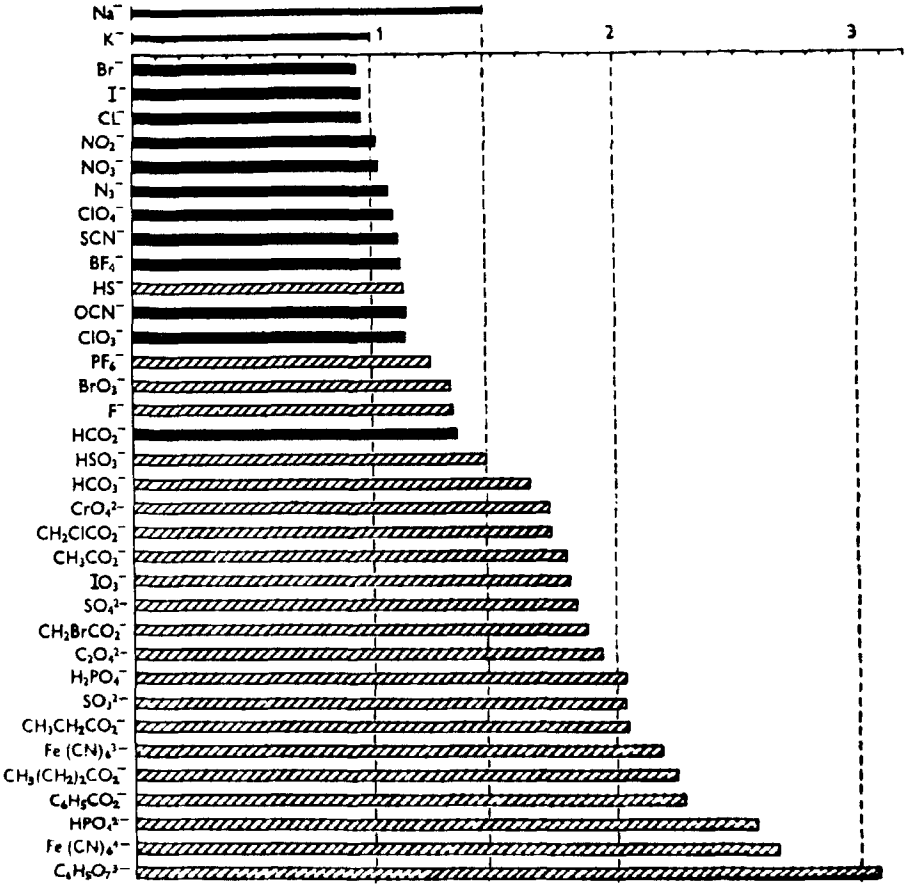

Fig. 3. Diagrammatic illustration of the correlation between size of ions in aqueous solution and the effect of their injection into motoneurones upon IPSP. The length of bands indicates the ionic size (relative to the size of potassium ion). Black bands are for ions that reverse the hyperpolarizing IPSP into depolarizing direction; hatched bands for ions not effective. (Ito *et al.*, 1962)

If we neglect the two exceptions, than we have good reason to suggest that the basis for postsynaptic changes during primary inhibition is formed by opening in the membrane of channels of a certain size which pass all ions that fit into them. The movement of such ions in one or other direction is defined, as already mentioned, by relations between transmembrane potential difference and ionic gradient.

Probably the postsynaptic membrane during inhibition also becomes more permeable to potassium cations (but not to sodium cations whose hydrated size is 1.5 times as large as that of potassium). But such permeability increase is difficult to prove by the above mentioned method, because the internal potassium concentration is very high and we cannot change it significantly by iontophoresis from the microelectrode.

If we want to know the nature of the transsynaptic action that creates the IPSP, we must first of all measure the ionic currents that flow through the postsynaptic membrane during its development. These currents can be calculated approximately from the values of membrane resistance, capacity and time course of the changes in transmembrane potential. Such calculations made by Coombs *et al.* (1955) led to the conclusion that the duration of subsynaptic current after a single inhibitory volley does not exceed 1.5 msec and reaches a maximum 0.5 msec after beginning. During this period the ionic conductance of the postsynaptic membrane is increased, as was shown by several methods of conductance measurement. The part of IPSP that follows after this period is due only to passive redistribution of charges on the membrane occurring in accordance with the membrane electrical time constant. The mean value of the time constant of the decay of IPSP in motoneurones (3.0 msec) is only slightly higher than the mean value of the time constant of their membrane (2.5 msec), but the time constant of the decay of the excitatory postsynaptic potential (EPSP) is much longer.

Recently this calculation was confirmed by direct measurements of transmembrane currents with the voltage-clamp technique. Araki and Terzuolo (1962), using 2 single microelectrodes with an interelectrode distance of 3–6 microns inserted into the same motoneurone, showed that during a single IPSP there is an inward current through the postsynaptic membrane about 1 msec in duration (Fig. 4). If the membrane of the cell is artificially hyperpolarized to a level at which the hyperpolarizing responses are changed into depolarizing, then the inward transmembrane current is also changed into an outward one — in complete accord with the theory that considers the ionic current during primary inhibition as a purely passive one defined by the relation between concentration gradients of ions and electric polarization of the membrane.

So we conclude that the transsynaptic inhibitory action on motoneurones, which is most likely confined to liberation of some physiologically highly active substance, is very brief. This can be connected with its rapid diffusion from the synaptic cleft or rapid destruction by enzymatic systems. Unfortunately, we know nothing about its chemical structure. The single observation here is the specific effect of strychnine which easily blocks the inhibitory synaptic action without influencing the properties of the postsynaptic membrane or the excitatory synaptic action (Curtis, 1962). But now it is even difficult to say if this effect of strychnine is connected with 'competition' for receptor sites or its action on the liberation of the 'inhibitory' substance.

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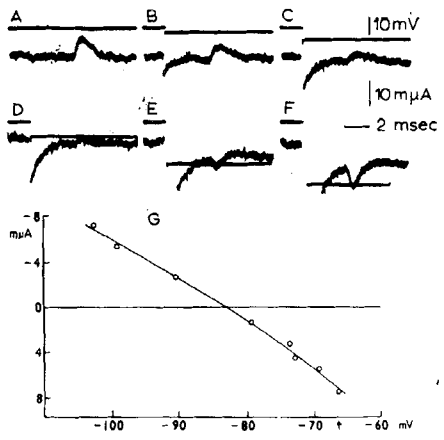

Fig. 4. Transmembrane current in a motoneurone during IPSP measured with voltage-clamp technique. (A) current during normal membrane potential of the cell, (B)–(F), during its hyperpolarization in steps before inhibitory synaptic activity. Inward current corresponds to upward deflection of the beam. (G) relation between the inhibitory transmembrane current (ordinate) and membrane potential (abscissa). (Araki and Terzuolo, 1962).

The origin and structure of these synaptic endings that produce primary inhibition of motoneurones also remains unknown. Recently very precise measurements of the central latency of IPSP were carried out (Araki *et al.*, 1960). They have confirmed the existence of a certain additional delay comparing with the monosynaptic excitatory action; this is in accord with the hypothesis about the existence of special inhibitory interneurones with synaptic endings producing the inhibitory action upon motoneurones. Experiments of Eide *et al.* (1961) also conform with this: during direct stimulation of the intermediate nucleus of Cajal (that means during direct stimulation of interneurones) they have recorded from motoneurones IPSP's with exactly the same central latency as by EPSP's. But Szentágothai (1958) has carried out experiments with isolation of the ventral horn of the spinal cord; after such isolation all synaptic endings on motoneurones must degenerate except the inhibitory endings from Renshaw's cells. But in fact no typical synaptic endings on motoneurones have been found.

The question arises how universal is this mode of inhibition? Experiments of recent years carried out in different laboratories have shown its presence in different types of neurones in all parts of the central nervous system of higher and lower animals, including invertebrates. The inhibitory postsynaptic potentials are well marked in giant neurones of molluscs (Tauc, 1957; Arvanitaki and Chalazonitis, 1961), in neuromuscular synapses (Fatt and Katz, 1953) and receptor nerve cells (Kuffler and Eyzaguirre, 1955) of crustaceans, in smooth muscle fibers (Orlov, 1963) and heart-muscle fibers during parasympathetic inhibition. Nice IPSP's were found in frog motoneurones (Kubota and Brookhart, 1963); in mammals they appear in certain spinal interneurones (Curtis *et al.*, 1958; Kostyuk, 1961), neurones of the brain stem reticular formation (Limansky, 1962), thalamic nuclei (Purpura and Cohen, 1962), hippocampus (Andersen *et al.*, 1963) and cerebral cortex (Li and Chou, 1962). Examples of IPSP's from different neurones are shown in Fig. 5.

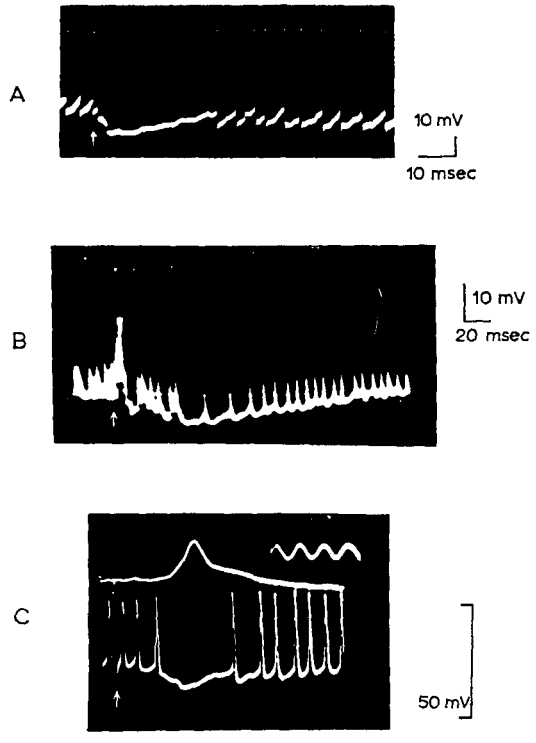

Fig. 5. Examples of IPSP in different neurones of cat's brain. (A) an interneurone of the dorsal horn of lumbar spinal cord; stimulation of n. femoralis (Kostyuk, 1961). (B) a neurone of nucleus gigantocellularis of the medullary reticular formation, stimulation of n. ischiadicus (Limansky, 1962). (C) a neurone of thalamic nucleus ventralis anterior, stimulation of centrum medianum (Purpura and Cohen, 1962).

In most cases the IPSP is in a form of hyperpolarization of the cell membrane; sometimes the hyperpolarization is absent or replaced by depolarization, but it is a result, probably, only of peculiarities in ionic distribution between cell protoplasm and the external medium. The equilibrium potential for ions that become permeable during inhibition is then equal to the transmembrane potential or below it. In the absence of membrane hyperpolarization the inhibitory action is connected only with increased conductance of the membrane that decreases the amplitude of depolarization produced by excitatory synaptic endings. Of course, such inhibition is shorter than that in the presence of hyperpolarization.

Among all these manifestations of primary synaptic inhibition at least one (apart from motoneurones) is studied in detail in the direction of ionic mechanisms. IPSP's in giant neurones of the snail are formed by ionic processes that are identical with ionic processes during inhibition in cat motoneurones. Experiments with injections of many different types of anions in the giant neurones have shown that the changes in permeability of their postsynaptic membrane during inhibition are similar to such changes in motoneurones (Kerkut and Thomas, 1963). Preliminary experiments allow

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us to suggest that the same processes occur also in other examples of primary inhibition.

Experiments on giant neurones of molluscs offer another approach to the problem of the nature of the transsynaptic inhibitory action. A direct iontophoretic application of acetylcholine to such neurones has shown clearly that postsynaptic changes characteristic of primary inhibition can be produced by acetylcholine (Tauc and Gerschenfeld, 1962). Ionic changes in cells inhibited by acetylcholine proved to be identical with ionic changes during natural synaptic inhibition (Kerkut and Thomas, 1963). But acetylcholine in other neurones of the same animal produced excitatory postsynaptic changes. Even more, we have reason to assume that endings of the same interneurone can excite one neurone and inhibit another neurone by producing the same transmitter (Tauc and Gerschenfeld, 1961). These results (as well as data about the possibility of obtaining excitatory or inhibitory changes under the influence of the same transmitter in different parts of the autonomic nervous system) show clearly that the specificity of the inhibitory synaptic action is determined not only by the nature of the transmitter; properties of the postsynaptic membrane and the ionic processes in it play a decisive role here.

Obviously the inhibition in the central nervous system of higher animals can be produced also by different transmitters; at least in neurones of the olfactory bulb, in contrast to other neurones, it is not changed by strychnine (Green *et al.*, 1962). A specific inhibitory substance is identified now in inhibitory axons of crustaceans, and after some controversy it seems to be proved by Kravitz *et al.* (1963) that this substance is GABA. But on the other hand, GABA has nothing to do with synaptic inhibition in other cases (Curtis *et al.*, 1959).

Therefore we are justified in speaking about an 'inhibitory' postsynaptic membrane as a special apparatus different from the 'excitatory' postsynaptic membrane. When we distinguish this membrane as a special functional structure, we do not claim that it can be clearly distinguished morphologically. As in the differentiation between 'electrically-excitable' and 'electrically-inexcitable' membranes (Grundfest, 1957), these are usually not spatially different parts of the cell membrane, but different submicroscopic or even molecular parts that can be closely intermingled with one another. This is especially obvious on the soma of molluscs' giant neurones which have no synaptic endings, are wholly electrically excitable and despite this can produce typical excitatory and inhibitory postsynaptic reactions during direct application of a transmitter.

The study of primary inhibition in some special types of neurones also gives promising results for the morphological identification of the inhibitory synaptic endings. Pyramidal cells of the hippocampus are subjected to primary inhibitory action after stimulation of the commissural, septal and local pathways. Recording of the extracellular potential field and intracellular recording show that intensive hyperpolarization is located at the cell soma (Andersen *et al.*, 1963). The synaptic endings at the soma of these cells originate from the axons of basket cells and belong to the electronmicroscopic type II after Grey (Blackstad and Flood, 1963; Fig. 6). Preliminary data indicate that similar polarization occurs in Purkinje's cells of the cerebellum.

Fig. 6. Electron-microscopic pictures of assumed inhibitory synaptic endings on soma of a hippocampal pyramidal cell. C = soma of a pyramidal cell; g = glial processes; t = synaptic knobs with vesicles. Arrows indicate somatic invaginations into knobs (× 15 000) (Blackstad and Flood, 1963)

Of course, such results cannot be considered as an indication that all Grey type II synaptic endings are inhibitory or that inhibitory endings must be located only at cell soma; but they are certainly very stimulating in our search for the morphological structure of inhibitory synapses.

There is a special question about the possibility of primary inhibition of synaptic transmission of excitation of quite another type, not connected with ionic changes in the postsynaptic membrane. This inhibition could be connected with cessation or weakening of the activity of the presynaptic endings themselves under the influence of some factors external to them. It was suggested in 1955 by Howland *et al.* on the basis of precise studies of electric fields in the spinal cord during the interaction of two afferent volleys, that the inhibitory influence of one volley on another can be connected with conduction blocking in presynaptic elements. Later Frank and Fuortes (1957) showed, with intracellular recording from motoneurones, that in certain cases there can be a diminution of the excitatory synaptic action without special inhibitory changes in the postsynaptic membrane. Extensive measurements of the time course of such 'presynaptic' inhibition have shown that it coincides with long-lasting depolarization of central afferent fiber terminals which is well known as the 'electrotonic potential of the dorsal root' (Eccles *et al.*, 1961; Eccles *et al.*, 1962a). An example of diminution of IPSP in a first-order interneurone during presynaptic inhibition is shown in Fig. 7. The important thing is that such depolarization arises in afferent fiber terminals primarily; a passage of an afferent impulse through them is not necessary for its origin. A certain specificity in appearance of presynaptic inhibition after stimulation of different reflex arcs indicates that it is based on specific central pathways which can exert a direct influence on presynaptic endings, maybe through inhibitory synaptic

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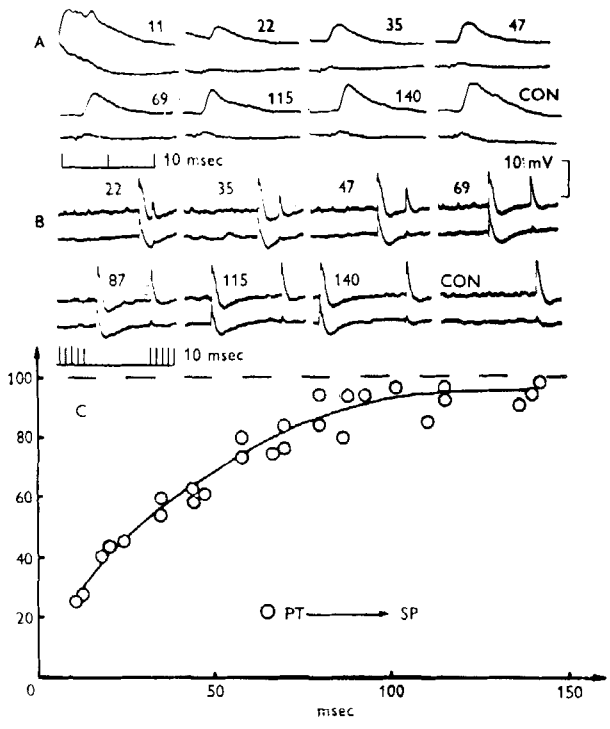

Fig. 7. Diminution of monosynaptic EPSP of an interneurone during presynaptic inhibition. (B) intracellular EPSP of the cell (upper beam) when testing stimulation of n. peroneus superficialis (SP) was conditioned with different intervals by stimulation of n. tibialis posterior (PT). The intervals between stimuli are shown near each oscillogram. The lower beam shows potential of cord's dorsal surface; CON = the control value of EPSP (without conditioning stimulus). (A) the same testing EPSP recorded with a faster sweep. (C) dependence of EPSP size in % of control value (ordinate) from interval between conditioning and testing stimuli (abscissa). (Eccles *et al.*, 1962a)

endings on other synaptic endings (Eccles *et al.*, 1962b; Eccles *et al.*, 1963a).

There are several indications that a steady depolarization of the membrane of presynaptic endings really decreases the transsynaptic action of a nerve impulse travelling through such endings. This was shown at a giant synapse by Hagiwara and Tasaki (1958) who inserted two microelectrodes in the presynaptic and postsynaptic fibers and changed at will the transmembrane potential of both. Similar but less descriptive results were also obtained during electric polarization of afferent fiber terminals in the spinal cord (Eccles *et al.*, 1962c). Here a depolarization of afferent fiber terminals was produced by an electric polarization of the whole spinal cord in a dorsoventral direction (Fig. 8) and its effectiveness was controlled by changes in excitability of the terminals to direct stimulation through a microelectrode.

It is suggested, in analogy with primary postsynaptic inhibition, that the depolarization of presynaptic terminals is produced by special interneurones ('D-neurones'). The inaccessibility of terminal central endings of afferent fibers for microelectrode penetration now makes a precise study of equilibrium potentials for presynaptic depolarization of transmembrane ionic currents etc. impossible. A peculiar feature

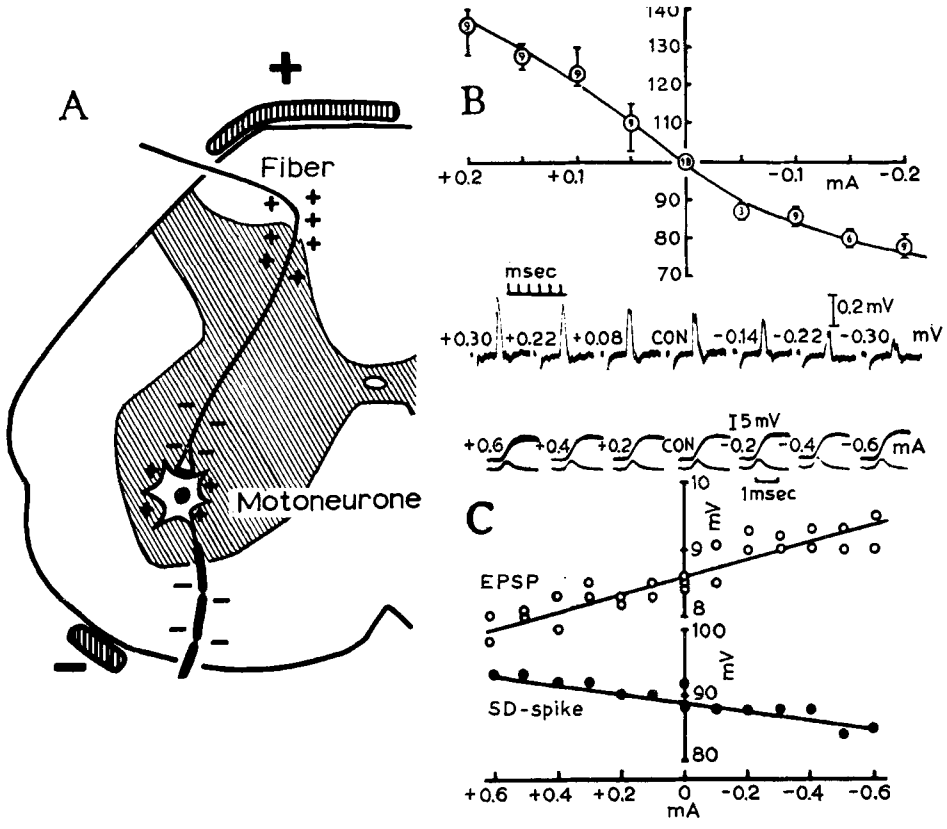

Fig. 8. Influence of electric polarization of the spinal cord on transsynaptic activity of central afferent terminals. (A) schematic diagram illustrating the method of electric polarization and the assumed electrotonic changes in different parts of afferent fibers and motoneurones. (B) changes in excitability of afferent terminals in the gastrocnemius motor nucleus during such polarization. Stimulation was performed through a microelectrode inserted in the motor nucleus and antidromic action potentials from n. gastrocnemius were recorded. Changes in excitability in % of resting level (ordinate) are plotted against the strength of polarizing current (abscissa). Currents that depolarize the terminals are plotted on the left side. Examples of oscillograms used for calculation of excitability changes are shown below. (C) changes in size of monosynaptic EPSP (ordinate) during the same polarization (abscissa) together with changes in the antidromic SD-spike of the motoneurone. Examples of EPSP during different polarization are shown above (Eccles *et al.*, 1962c).

is the extremely long time-constant of the presynaptic depolarization; its duration after one synchronous afferent volley amounts to hundreds of msec. This makes an impression that such duration is produced by repeated reverberation of nerve impulses in neurones that act depolarizingly upon afferent terminals. A possible apparatus for such reverberation is a gelatinous substance, and some evidence indicates that it really is connected with inhibition of reflex activity (Ioseliani, 1961) and presynaptic depolarization (Wall, 1962).

Normally the presynaptic depolarization exerts its inhibitory influence on synaptic transmission without any excitatory influence on afferent fibers; but an excitatory effect is very prominent during hypothermia, producing the 'dorsal-root reflex'.

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The long-lasting presynaptic inhibition connected with depolarization of presynaptic terminals is present in different afferent systems of the brain of higher animals (Eccles *et al.*, 1963b; Schmidt and Willis, 1963; Andersen *et al.*, 1962; and others). A very intensive inhibition of this kind takes place in the central nervous system of lower vertebrates, where it plays an important role also in descending regulation of afferent inflow and where it can be activated by feedback systems not only from interneurones, but also from motoneurones (Kostyuk and Timchenko, 1963).

It is quite clear that these two types of synaptic inhibition differ greatly one from another. The postsynaptic inhibition exerts its action by combination of numerous synaptic influences at the nerve cell; nothing hinders the origin of each of them, and the result is defined by algebraic summation of all influences (taking also in account their spatial position in relation to site of impulse generation). The presynaptic inhibition switches the neurone off from a certain part of the afferent inflow and makes it free for dealing with that impulsation which comes through the region of presynaptic inhibition.

All the data mentioned above have been analyzed from the viewpoint of chemical theory of synaptic transmission of excitatory and inhibitory influences. The microelectrode studies of extracellular electric fields and of changes in transmembrane potential differences all clearly show the impossibility of the generation of postsynaptic electric changes by an electric action from presynaptic structures.

However, in certain types of synaptic transmission the electrical mechanism of synaptic excitation and inhibition is effective; the synaptic junctions here have special morphological features that provide a very high density of current near the postsynaptic membrane and a possibility for them to produce strong electrotonic changes in the postsynaptic membrane. The electric synaptic primary inhibition was beautifully shown in the Mautner cell of the goldfish by Furukawa and Furshpan (1963). This cell has the inhibitory endings on the axon hillock, and a special structure (axon cup) densely covers them from outside. This structure probably prevents the extracellular currents generated by inhibitory synaptic endings from shunting, and therefore they are sufficient to produce strong electrotonic changes in the postsynaptic membrane of the axon hillock. The electrical mechanism of synaptic inhibition here is undoubted, although the question how the synaptic endings can produce just anelectrotonic and not catelectrotonic changes at the subsynaptic membrane remains obscure.

Beside the inhibition connected to activation of special inhibitory structures, nervous activity can certainly be inhibited without such structures; the inhibition appears as a secondary process in the same structures that produce excitation of the cell. Many investigators prefer not to classify such changes as 'inhibition' and preserve this term only for the primary synaptic inhibition already described. But such an approach is probably not correct, because from the point of view of the final functional meaning there is not much difference between the two types of change. The term 'inhibition' for description of transition from excitation to its depression in the same structures was widely used, for instance, by Vvedensky and Pavlov, and is firmly established in physiology.

The secondary inhibition is not equally prominent in different neurones; this depends especially on intensity and stability of the excitatory synaptic influences, on peculiarities of interaction between synaptic processes and propagated impulse, etc.

First of all it is necessary to find out if a single excitatory postsynaptic potential (EPSP), produced by a synchronous afferent volley, is capable of depressing the activity of the nerve cell.

In spinal motoneurones the monosynaptic EPSP always produces a propagated impulse when it reaches the critical level of depolarization. There is no inactivation of the impulse-generating mechanism that could take place because of extensive synaptic depolarization of the motoneuronal membrane (cathodal depression of Verigo). The first action potential generated in the motoneurone greatly decreases the EPSP and removes the possibility of cathodal inactivation (Brock *et al.*, 1952).

Somewhat different are the events during excitation of a motoneurone by a single afferent volley through polysynaptic pathways. The EPSP has a long undulating time course, and the origin of a propagated impulse does not remove a significant part of the EPSP. It is, probably, steadily supported by delayed synaptic bombardment from interneurones. Therefore the subsequent action potentials which originate on a polysynaptic EPSP are often partly depressed. Sometimes they have only the IS-component, and this indicates that the spike-generating mechanism of the somatic membrane is inactivated (Kostyuk, 1960). Sometimes the generation of spikes is completely depressed.

A much stronger inhibition of action potential generation of this kind is observed in spinal interneurones (Kostyuk, 1961), in reticular neurones of the brain stem (Limansky, 1962), in Purkinje's cells (Granit and Philipps, 1956) and so on. Examples of such inhibition are shown in Fig. 9. The synaptic depolarization in these neurones is stable, and the generation of action potentials has some characteristic features such as absence of marked afterhyperpolarization. Therefore there is always not a single impulse superimposed on the EPSP but a rhythmic discharge, sometimes of a very high frequency. During intense synaptic excitation an inhibition of action potential generation occurs always when the depolarization is especially deep. The inhibition can be partial (producing abortive, gradual spikes) or complete.

What is the reason for such marked differences between responses of spinal motoneurones and of other central neurones is not clear. Probably, there are important differences in the mechanism of generation of propagated impulses between the somatic membranes of these neurones, especially in the time course of increased potassium conductance during the descending part of the action potential and after its end. On the other hand, the stability of the synaptic excitatory action may be connected with some morphological and functional properties of the synaptic apparatus that make the inactivation of the transmitter or its diffusion out of the synaptic cleft more difficult. These properties together with the bombardment of the cell by delayed impulses can fix the postsynaptic membrane for a longer period in an activated state.

When rhythmic excitatory impulses reach the cell, the possibilities for inhibition of its activity are greatly increased. We then meet the classical 'pessimum inhibition'

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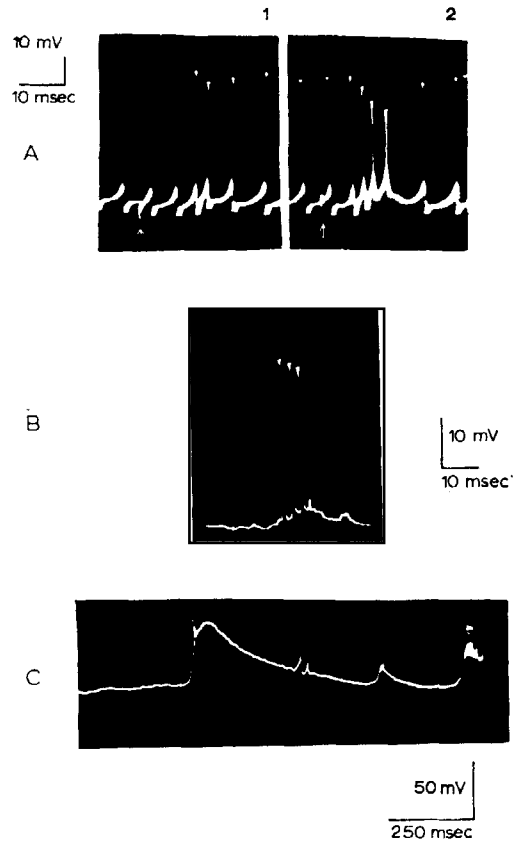

Fig. 9. Inhibition of spike generation during intense EPSP in different neurones. (A) an interneurone of the dorsal horn of the lumbar spinal cord. Different strength of stimulation of n. peroneus (Kostyuk, 1961). (B) a neurone of nucleus gigantocellularis of the medullary reticular formation. Stimulation of n. ischiadicus (Limansky, 1962). (C) a neurone of the cerebral cortex. Strychnine poisoning (Li, 1959).

of Vvedensky. The Vvedensky inhibition is in its nature a very complicated process and includes changes in the presynaptic as well as in the postsynaptic part of synaptic junctions.

First, when repeated excitatory influences reach the cell, there is a cumulation of depolarization at the postsynaptic membrane. This cumulation during monosynaptic excitation is never very great. The size of a single EPSP is decreased during low rates of excitation (1–10 per sec). The EPSP only increases when the frequency rises to 50 per sec (Curtis and Eccles, 1960). The highest stable depolarization of the postsynaptic membrane develops with frequencies above 100 per sec (Vartanyan, 1964). The subsequent EPSP's could be decreased because of decrease in effectiveness of the synaptic endings during rhythmic excitation and of desensitization of the postsynaptic membrane during the long-lasting action of a transmitter. An evaluation of the relative values of these two mechanisms in diminishing the EPSP's in central neurones is very difficult. Their relationships can be analyzed much more easily at a neuromuscu-

lar junction, where it is possible to measure directly the chemical sensitivity of the postsynaptic membrane (end-plate membrane) by iontophoretic application of acetylcholine from a microelectrode. But the results from different laboratories are controversial. According to Thesleff (1959), rhythmic excitation of neuromuscular synapses in rat diaphragm produces a marked desensitization of the end-plate membrane. In experiments of Otsuka and Endo (1960) no changes in sensitivity of end-plates of the same preparation or of frog sartorius have been found during Vvedensky inhibition.

In our laboratory Vladimirova (1964b) was also unable to find any desensitization of sartorius end-plates in conditions where the end-plate potentials had been greatly diminished due to rhythmic stimulation. An example from this investigation is shown in Fig. 10.

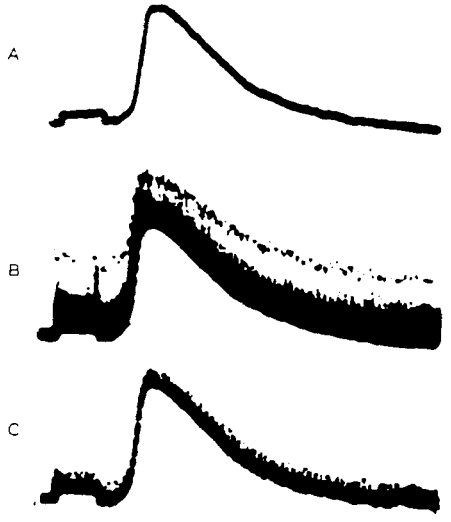

Fig. 10. Absence of desensitization of an end-plate of frog sartorius muscle fiber during nerve stimulation with frequency 100 per sec. (A) depolarization of the end-plate due to iontophoretic application of acetylcholine from a micropipette. (B) and (C) the same depolarization immediately after the beginning of tetanic stimulation and 5 sec later, when single EPSP's were already greatly reduced. At the left end of each oscillogram there is a calibration pulse (5 mV, 200 msec). (Vladimirova, 1964b.)

During rhythmic polysynaptic activation the steady depolarization of the postsynaptic membrane is much more prominent, and it can markedly exceed the critical level necessary for action-potential generation (Shapovalov, 1963). In many motoneurones this produces a decrease in the amplitudes of action potentials generated during the steady depolarization; this decrease grows with time despite a constant level of the depolarization. The same happens during steady synaptic depolarization of amphibian motoneurones (Araki and Otani, 1959). It seems that this also takes place in cortical and spinal neurones during epileptogenic discharges produced by cooling or strychnine; the intense synaptic depolarization produced by high-frequency discharges leads to a strong reduction in spikes and to their complete inhibition (Li, 1959; Shapovalov, 1962; Goldensohn and Purpura, 1963).

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It is clear from all these examples that the inhibition of the activity of nerve cells during excitatory synaptic influences develops usually when there is a massive synchronous excitation of many afferent channels produced by stimulation of nerve trunks, action of convulsant drugs, etc. There is no decisive answer to the question about the possible role of this mechanism in natural reflex activity based on impulses from adequate stimulation of receptors; we do not know examples of inhibition of central neurones by excessive synaptic depolarization during such activity.

But in peripheral receptor nerve cells of the crayfish the possibility of complete inhibition of spike generation during excessive depolarization from adequate stimulation (strong stretch of the muscle fiber) was shown by Eyzaguirre and Kuffler (1955).

Beside changes in the postsynaptic part of the synapse, special changes also occur during rhythmic stimulation in its presynaptic part. A conduction blocking often develops in the presynaptic endings which brings their transsynaptic activity to a complete cessation. In usual conditions it is difficult to study such changes in the presynaptic part of the central synaptic junctions. Each afferent volley activates a large amount of parallel terminals. Since the intracellular recording allows one to study only the joint effect of their activity, the presynaptic blocking can be observed only if it occurs synchronously in many terminals. But usually this does not happen, and therefore such changes are masked. They appear only as a certain diminution of the EPSP's similar to the changes during other processes.

The intervention of presynaptic conduction blocking into Vvedensky's inhibition can be seen very well when it is possible to investigate a single synaptic junction or to reduce artificially the number of the excited presynaptic elements. In the neuromuscular junction this blocking occurs with frequencies above 100 per sec and is manifested by complete absence of single end-plate potentials (Kostyuk, 1958a, 1959; Krnjevic and Miledi, 1958). The presynaptic blocking develops in the most terminal parts of the presynaptic fibers (Krnjevic and Miledi, 1959) and depends upon the level of electric polarization of the terminals. A depolarization of terminals by electric current or an increase in external potassium greatly accelerates the onset of presynaptic blocking and decreases the necessary frequency of stimulation. A hyperpolarization of presynaptic terminals immediately removes the presynaptic blocking (Vladimirova, 1963, 1964a). So it is probable that the presynaptic blocking of impulse conduction itself is based on a steady depolarization of terminals which appears when impulses pass through them with high frequency.

All this shows the complexity of the phenomena that develop in a synaptic junction during Vvedensky's inhibition. In the past they were all described by Vvedensky as steady focus of excitation in the synaptic junction ('parabiosis'). The modern progress in our possibilities of analyzing the synaptic processes allows us to separate this complicated process into several parts and to start with investigations of their intrinsic mechanisms and their interrelations during different patterns of incoming impulsation.

This presentation was specially devoted to synaptic mechanisms of inhibition, and therefore I did not touch the problems of neuronal architectonic of the inhibitory processes in the central nervous system.

In recent investigations a vast amount of material has been collected about the peculiarities of synaptic processes in different central structures. These data show an extreme variability in intensity and functional meaning of different inhibitory mechanisms, and it is hard to find some order in them. But in general we can say that it is determined as much by morphological types of cells and their synaptic connections as by the functional structure of reflex activity in which these cells take part. It is necessary to know both if we want to integrate our knowledge about a single synaptic process into a knowledge about the function of a system.

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Inhibition in Neural Systems of the Cerebral Cortex

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It is well known that different neurons respond to one and the same stimulus in different ways. Accordingly Jung and Baumgartner (1955) classified cortical nerve cells into five groups.

The data obtained in our laboratory by Kaburneyeva, Kondratyeva and Shulgina substantially confirmed this classification on a large number of neurons. We have seen: 'A' type neurons that are active in the background and do not change their impulsation in response to stimulation; 'B' type neurons responding to stimulation with quickened impulsation; 'D' and 'C' types responding either with slower impulsation or not responding at all to flashes and activated by darkness; and 'E' neurons whose reaction has a phasic character, beginning with a brief inhibition followed by activation in response to switching both on or off of the stimulation.

Our analysis began with a determination of the latencies of appearances of, or changes in, impulsation of separate cortical neurons to a stimulation. Formerly a similar analysis had been done by Mountcastle and his co-authors (1957), Patton and Amassian (1960) etc. We worked on unanaesthetized rabbits. In an attempt to collect sufficient statistic data, we recorded latencies of neurons in a great number of tests on different species under standard experimental conditions. One and the same stimulus was presented, the electrical activity of the nerve cells was led off from the same cortical points, and the insertion of electrodes and other experimental conditions were comparable.

In Table I are presented the latencies in reactions of different neurons of the sensory-motor cortical area of the rabbit caused by standard electrical shocks, applied to the hip of the contralateral hind limb. Repeated analyses of the latencies of responses in one and the same cell with an interval of about 3 min showed them to be relatively stable with a tendency towards dwindling upon repetition of the stimulation. Relative stability of latencies of certain neurons points to the view that each of them occupies, so to say, one and the same place in the system of reaction to repeated presentations of one and the same stimulus. In other words, it gives ground for us to assume the existence of more or less stable neuronal pathways of propagation of excitation in the brain cortex upon application of one and the same stimulus, the stability of the 'excitation system' in the cortex.

TABLE I

LATENCIES OF REACTIONS OF SINGLE NEURONS OF THE SENSORYMOTOR CORTICAL AREA IN RESPONSE TO BRIEF REPEATED ELECTROCUTANEOUS STIMULATION

No.	*Depth from pia mater (μ)*	*Latency period (μsec)*
1	947	50; 45; 45
2	964	55; 50; 45; 30
3	1086	20; 20
4	1172	50; 40; 35; 30; 40; 40; 30; 30
5	1344	50; 40; 60
6	1586	90; 60
7	1620	35; 20; 20
8	1672	20; 20; 25
9	1672	40; 40; 35; 25; 25; 25
10	1760	190; 190; 170
11	1844	40; 40
12	2138	440; 480

The relative constancy of the latencies of neuronal reactions enabled us to calculate their averages from the number of their successive values and to present them in a graph (Fig. 1). On the abscissa are marked latencies, on the ordinate the percentage of neurons having those latencies. The graph is drawn from the data of 65 neurons of the sensorymotor cortical area responding to standard electrocutaneous stimulation of the hip. However, this curve shows something more than a mere distribution of neurons according to their latencies. To evaluate it properly we must bear in mind the elementary notion that, while being inserted an electrode will meet on its way the neurons with most often-met latencies. In other words, the probability of such meetings will be proportionate to the number of neurons possessing each given latency. The graph in Fig. 1 demonstrates in terms of volume the propagation of excitation evoked by electrocutaneous stimulation in the sensorymotor cortical area. It shows that the process begins in 10 msec with recruiting of a small number of initial neurons. It

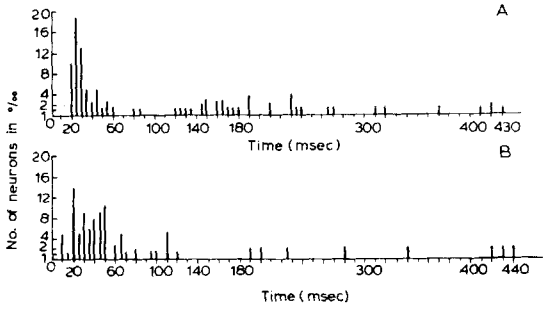

Fig. 1. Distribution of latencies of reactions of different cortical neurons. A, optical cortex (light flash), $n = 91$. B, sensorymotor area of cortex (electrical skin stimulation of leg), $n = 65$.

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develops quickly and soon recruits 14.5% of neurons. Then the process fluctuates, now waning, now waxing, and then, 50–60 msec after the beginning, declines sharply. Thus it may be clearly seen that there are 2 periods of neuron recruiting into the reaction. The first stage is that of intensive development of the process during 50–60 msec, whereas the second one is that of its gradual extinction. The latter takes rather a long time, about 1 sec, and is maintained by the inflow into the reaction of a very small number of neurons; in short, volumetrically it is quite limited. A question arises: what is the mechanism of this process?

The sequence of latencies of single neurons first of all prompts the idea that propagation of excitation from neuron to neuron goes chain-like, each link in this chain being repeated regularly. However, it is hardly correct to think that the whole process develops entirely within the cortex. It must evidently include afferentation undergoing a temporary dispersion and arriving in the cortex during later moments of stimulation or even after its discontinuance. But without going into much detail, we may suppose that this process in the cortex proceeds in a chain-like manner regardless of how its separate links are formed.

Fig. 2A gives a scheme of a typical chain process for which there is a mathematical equation. However, if in the brain cortex the reaction were to go this way, it would inevitably lead to a widespread excitation equivalent to a burst. In fact this does not occur. This means that the reaction is continually restricted, inhibited. If a scheme of the process already demonstrated in Fig. 1 were made, it will look approximately

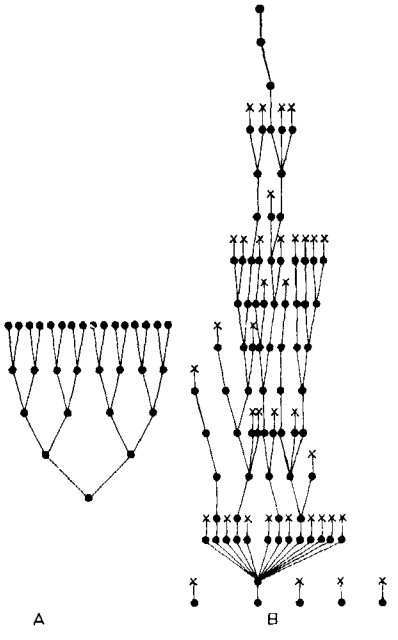

Fig. 2. Typical scheme of chain reaction (A). $N = n \sum_{k=1}^{m} \varepsilon^k$ The scheme of the probable course of the process of excitation in the sensorymotor cortical area of rabbit in response to brief electrocutaneous stimulation (B).

like that in Fig. 2B. As may be seen, the majority of 'branches' of the process are continually being cut off and only some of them continue the propagation. We all know Pavlov's concept that excitation in the cortex exists side by side with inhibition and is restricted by it.

Such is our hypothesis, but what are the observations to prove it? First of all let us be sure that the development of the reaction to electrocutaneous stimulation is really connected with the appearance of inhibition and restricted by it. For this purpose let us analyze the graph in Fig. 3 that demonstrates the time course of neuronal reactions to a given stimulus. On the abscissa we mark time intervals, 100 msec each, beginning from the moment of stimulation and on the ordinate mean values of impulsation frequency, for example of 62 neuronal reactions.

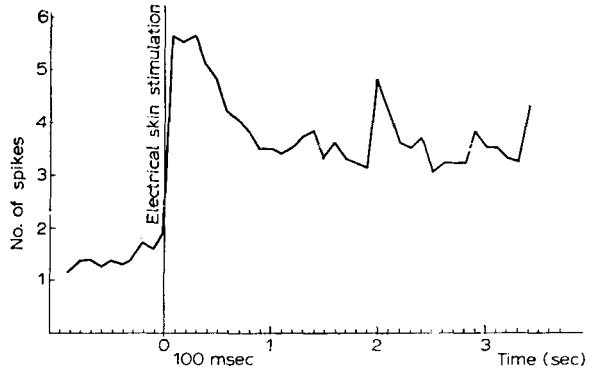

Fig. 3. Reaction of neurons of sensorymotor cortical area to electrocutaneous stimulation (the graph is composed from the results of 62 reactions).

The graph shows that a brief electrocutaneous stimulation evokes in single neurons a prolonged discharge. For different cells this discharge is of different duration, ranging from 1–6 sec and even more. The pattern of discharges may also vary. Sometimes it is of even character and is extinguished gradually; more often, however, its frequency gives several repeated peaks.

If we now compare this graph of duration of reactions for single neurons of the sensorymotor cortical area with the curve of their latencies analyzed above (Fig. 1), in other words, with the process of their recruiting into the reaction, we see that recruiting of new neurons into the response reaction is complete in a maximum of 1 sec after the beginning of electrocutaneous stimulation, whereas the increased activity of the recruited neurons continues much longer (up to 6 sec). From this it follows that with the development of the reaction the long lasting intensive impulsation of the recruited neurons loses its ability to activate new nerve cells. This process of restricting inhibition at the beginning quantitatively lags behind the excitation. At first the excitation increases but after 50 msec begins to be extinguished, and by the end of the 1st sec is completely inhibited, despite the continuing high activity of the earlier recruited neurons.

Is it possible to observe this restricting inhibition in separate cortical neurons?

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Jung and Baumgartner (1955) observed that impulsation in 'B' group neurons (in their classification) developed in parallel and reciprocally with suppression of impulsation, *i.e.* with inhibition in 'D' group neurons. In our laboratory these relations have been proved quantitatively. On the basis of numerous records obtained from 70 neurons responding to flashes with enhanced impulsation and records obtained from 49 nerve cells responding with inhibition, I. N. Kondratyeva has drawn the graph presented in Fig. 4. On the abscissa there are time periods of 100 msec before

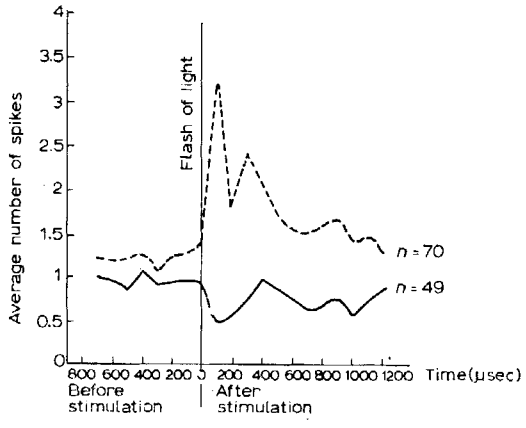

Fig. 4. Modification of the mean impulsation frequency of neurons of the visual cortex to a flash.

and after the photic stimulation. On the ordinate, mean values of impulsation changes of activated and inhibited neurons. The graph shows that intensification of the activity of nerve cells evoked by light is accompanied by a parallel decrease in impulsation in inhibitory neurons. On the contrary, reduction of excitation is connected with a corresponding reduction of inhibition. Thus, neuronal inhibitory reactions are indeed closely bound up with the intensity of excitation and in the main follow its changes.

We deem this concept to agree fully with the experimental data of Granit (1962), Hartline *et al.* (1953) and others who, using recurrent spinal inhibition or lateral inhibition in the retina, showed quantitative interrelations of these processes. According to our hypothesis we believe that classification of neurons into excitatory and inhibitory ones depends on the topography and strength of the excitatory process. The nerve elements directly recruited into the excitatory process respond with increased impulsation, whereas others, localized in the vicinity, restrict its development, cut off the chains and prevent the cortex from global excitation. Naturally the stronger the first process, the stronger must be the second and *vice versa.* This is illustrated by the quantitative relations mentioned above. In the light of these facts the spatial distribution of the inhibitory and excitatory neurons in the cortex is of interest. Widen and Ajmone-Marsan, 1960; Patton and Amassian, 1960; have attempted to study the distribution of latencies of single neuron responses depending on the depth of their localization in the brain cortex. In our laboratory we have also studied such a distribution.

Fig. 5 illustrates the localization of neurons responding with excitation and inhibition to flashes along the whole depth of the visual cortex of rabbit. On the abscissa two groups of neurons (inhibitable and excitable) are marked; on the ordinate, the

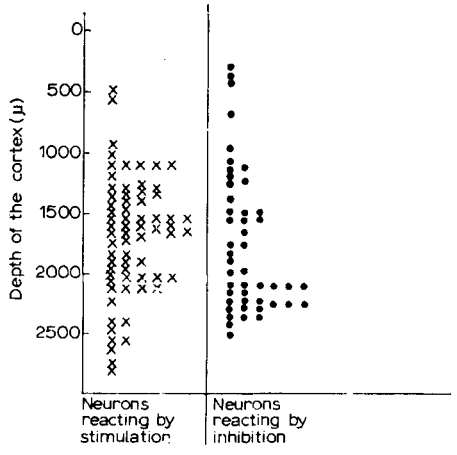

Fig. 5. Distribution of the analyzed visual cortex neurons according to the depth of their localization and the type of reaction.

depth of the cortex in microns from the surface. The figure shows that inhibitable neurons are distributed about the cortex like the excitable, as though they accompany them. But at the same time the inhibitable neurons are largely concentrated in the lower layers of the cerebral cortex.

So the excitation, having recruited a number of neurons and persisting in them for a long time, cannot spread over to new neurons, evidently due to inhibition. But what is this prolonged impulsation for? We think, it is necessary for formation and fixation of working neuronal systems connected possibly with the phenomenon of retention. In other words we believe that the prolonged impulsation of excited neurons is necessary for their interaction, that it is not a temporary chain of excitation that is formed and vanishes, but a system of persisting interactions. The chain process, once provoked, can maintain itself for a long time within the system of recruited neurons. The maintenance of this process and its configuration cannot be imagined without inhibition which forms it spatially.

We have as yet no observations that reveal the intimate mechanism of this inhibition. Judging by its external manifestations, it may be classified as reciprocal or inductive inhibition.

When studying the phenomena taking place in neuronal cortical systems, we come across other manifestations of inhibitory states. To elucidate them let us return to the graph of distribution of latencies of neuronal reactions, but this time in response not to electrocutaneous, but to photic stimulation (of 1 msec duration). This graph is given in Fig. 1 (top). It is based on the material obtained from the recording of 91 neurons. The comparison of the process flow caused by electrocutaneous and photic

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stimulations at a first glance shows their resemblance. Indeed, in photic stimulation the process also has two distinctly pronounced phases: the initial stage of intensive activation, followed by the prolonged phase of extinction. However, a more detailed comparison of the graphs reveals marked differences. Firstly, the initial stage of the response to photic stimulus develops more rapidly and intensively, so that with latencies of 25 msec the recruiting of neurons reaches its maximum. Secondly, it is over much sooner. To electrocutaneous stimulation it lasts 50–60 msec, whereas to photic stimulation it lasts only 35–45 msec. Thirdly, and this is very important, the second prolonged phase of extinction in response to photic stimulus has a few stages itself. Here one may see alternation of periods of reduction of the neuronal recruiting reaction, up to its full extinction, with periods of considerable and repetitive enhancement. There are two such periods of inhibition and excitation. So the development of excitation from these two types of excitation displays a number of essential differences. They may depend on different modalities of stimuli, on their unequal intensity, and on structural peculiarities of different cortical areas as well as on different degrees of involvement of the reticular formation in the organization of different reactions. At present there are insufficient data to differentiate these reasons. It may be assumed that the recruitment of cortical neurons into the reaction provoked by a flash reveals a certain periodicity. There are two stages in this periodicity during which the recruitment of new neurons in the excitation state is sharply inhibited.

It should be emphasized that since the recruitment relates to the whole cortical area analyzed, this periodic inhibition is of 'systemic' character, simultaneously embracing large groups of neurons.

We analyzed these phenomena from the standpoint of regularity of individual responses of single neurons of the visual cortex to the same flashes of 1 msec duration. We found that the reaction of single neurons usually also bore a periodic character. Fig. 6 shows that this periodicity manifests itself in the appearance of groups of spike discharges separated by spells of inhibition.

In our experiments primary activation began 20–30 msec after the moment of stimulation, the secondary one 120–140 msec later and the tertiary 300–400 msec after stimulation. One could usually see all three periods of activation and the reaction seldom began with the second, or even with the third period of impulsation. It is pertinent to mention that Jung, Baumgartner, Kornmüller and Defoneska observed the existence of cortical neurons responding to flashes with short and long latencies.

Grüsser and Grutzner (1958) recorded a similar periodicity in the reaction of single retina neurons of the cat to flashes. They associated the repeated periods of activation with the appearance of trace images.

If such a cyclic phenomenon occurs in the periphery, it would appear reasonable to associate the periodic inhibition of the cortex with influences from receptor systems. However, Creutzfeldt and co-authors (1956) showed that inadequate electric stimulation of the cortex evokes in cortical neurons absolutely similar periodic reactions of excitation and inhibition. This points to the possibility that they might be formed locally, in the cortex itself. Kondratyeva made some observations essential

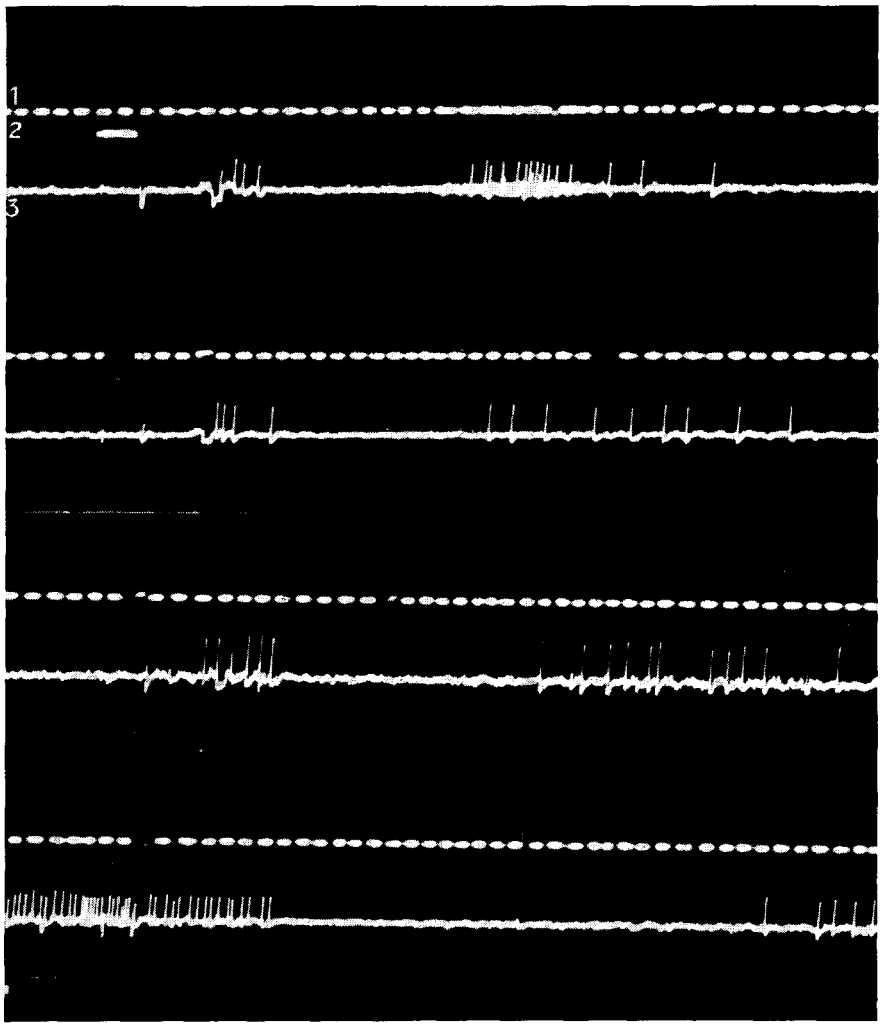

Fig. 6. Dynamics of reactions of the visual cortex neurons to a flash of 1 msec duration. Marks from the top downwards: (1) Time, 10 msec. (2) Stimulation, a flash coincides with the end of the white line. (3) Impulsation activity of neuron.

for elucidation of the periodicity mechanism of those reactions. According to her data, the time of commencement and duration of the inhibitory periods in single neuronal responses do not depend on the time of commencement, intensity and duration of their initial reactions. This is illustrated in Fig. 6, which shows that time variations in the beginning, and the duration and intensity of the initial activation do not usually affect the time of commencement of the inhibition period of single neurons. This inhibition occurs approximately at one and the same time and consequently does not depend on individual variations in the initial excitation of single neurons. All this leads us to the assumption that the periods of inhibition, running parallel in many neurons, must reflect some process which develops in the whole system and embraces large groups of cortical neurons. This process evidently does not directly

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depend on the phenomena taking place in single neurons but subordinates them.

What is the nature of this process and how is it formed? To answer this question one must proceed from the following experimental results. There must be some connection between the first positive phase of the primary response and the initial impulsation discharges in cortical neurons (Li *et al.*, 1956; and others). In our laboratory Kondratyeva and Lebedev showed the existence of a connection between the first period of inhibition of neuronal discharges and the negative phase of the evoked potential (Fig. 7). This agrees with the data of Widen and Ajmone-Marsan (1960) and Stohr *et al.* (1963) and others. In Fig. 7 on the abscissa are marked time periods, each of 10 msec duration, and on the ordinate the total number of impulses generated by

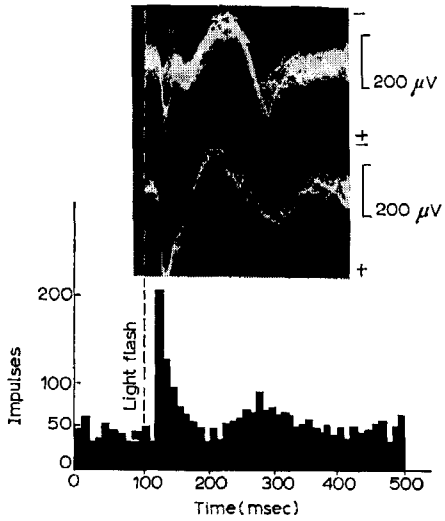

Fig. 7. Correlations between surface slow potentials and the activity of single visual cortex neurons in response to a flash. Top, evoked potential before the puncture of the cortex with an electrode (overlapping of 200 responses). Bottom, evoked potential after the puncture (overlapping of 50 responses). On the graph: total frequency of neuronal discharges before and after a flash (300 reactions of 53 neurons).

50 neurons in each of the time spells. The curve thus obtained is similar in its form and time course to the typical curve of the evoked potential to a flash. This similarity doubtless points to the existence of close interrelations between the impulsation changes of cortical neurons and the phases of the evoked potential.

The surface negative phase of the evoked potential is evidently due to depolarization of apical dendrites, which develops under the influence of stimulation. Thus we have an indication that inhibition of the impulsation activity of neurons is really connected with depolarization of apical dendrites. These notions are in full agreement with the concept of Beritov and Roitbak, according to whom depolarization of dendrites conditions the emergence of inhibition in cellular bodies. Thus the periods of inhibition appearing in the cortical neuronal systems and manifesting themselves both in periodic suppression of discharges of single neurons and in impeding of the

recruitment of new neurons into a prolonged reaction, are evidently connected with the periods of widespread depolarization of apical dendrites, and consequently with simultaneous inhibition of the majority of the nerve elements in the cortex.

In conclusion let us summarize the results obtained. Microelectrode investigations open wide possibilities for studying the processes of excitation and inhibition in neuronal systems of the cerebral cortex. Inhibitory processes normally always accompany excitatory ones, and, in the opinion of a number of authors, restrict them. However, the role of neuronal inhibition is much wider. It not only restricts, but also forms systems of neuronal excitation. This formation effect of inhibition develops both in space and in time. Due to inhibition in the cortex there appear spatially restricted areas of excitation in which a long-lasting circulation of the latter is possible. The inhibitory processes accompanying it evidently bear the character of reciprocal, inductive, synchronous inhibition.

In the nerve cell activity of the brain cortex one may see other forms of external inhibition, namely successive inhibition. It is a process that simultaneously involves a great number of nerve elements and which spreads over the whole system. This kind of external inhibition may be connected with depolarization of apical dendrites of cortical neurons. It develops during the negative phase of the primary response and is manifested in the inhibition of impulsation of excited neurons. We believe that at the basis of this kind of inhibition lies the mechanism of electrotopic influences suggested by Beritov.

SUMMARY

Recent microelectrode research (Jung and Baumgartner, Mountcastle, Mkrticheva, Vinogradova and Lindsley, Kondratyeva, Kaburneeva and Shulgina *et al.*) has revealed cortical neurons which react selectively and characteristically to different repetitive stimuli.

Observing the latencies of responses of different cortical neurons (Mountcastle, Grüsser and Grutzner, Patton and Amassian *et al.*) one may conclude that their recruiting in the reaction is not a simultaneous process. Research carried out in our laboratory has revealed a great variability in the latencies of responses of single cortical neurons (from 10 msec to 1 sec). On the other hand, the latencies of reaction of one and the same neuron when stimulated repeatedly and in a standard way, though fluctuating to a certain extent, do not undergo, as a rule, any marked changes. Apparently each neuron takes its own more or less constant place in the process of a repeated reaction.

Using statistical data on the distribution of different cortical neuron latencies one can draw a graph which reflects quantitatively the process of their recruiting evoked by some stimulus. In response to an electrical cutaneous stimulation this process flows identically in different regions of the cortex. On the contrary, the recruiting of neurons in reaction to stimulations of different intensity and modality is not identical.

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The experimental results lead one to assume that the cortical reaction to exteroceptive stimulations bears a chain character.

If neurons recruit in the evoked reaction the process is much shorter than the period of their activity. Evidently, the process persists in a system of excited neurons over a long period and, being at the same time restricted by inhibition, does not spread further.

The comparison of impulsation changes of activated and inhibitable neurons shows a similar time course of neuron excitation and inhibition. They seem to reflect each other. This points to their inductive nature.

The graphs of neuron distribution responding to stimulation either by excitation or inhibition, depending on their localization in the depth of the cortex, shows that everywhere the inhibitable neurons are situated in accordance with the localization of excitable ones. One sometimes observes a predominant grouping of inhibitable neurons on a definite level.

Besides inductive inhibition other kinds of inhibition can be seen in the reactions of cortical neurons. Grüsser and Grutzner showed that impulsation from the retina, induced by a flash, has a periodic character. Discharge periods alternate with periods of inhibition. A similar periodicity is observed in cortical neuronal reactions evoked by flashes or direct electrical stimulation (Creutzfeldt, Baumgartner and Sholl).

Kondratyeva showed that the appearance of an inhibitory phase of a neuron discharge is independent of latency changes in the initial discharge, its duration and its intensity. This presupposes the existence of an external, more general cause inhibiting the evoked neuronal discharges.

It is concluded that the classical concept of constant co-existence of excitatory and inhibitory processes and their mutual restricting influence is confirmed by interneuronal interrelations.

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On the Structural Mechanisms of Functional States in Neurons of the Cerebral Cortex

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A generalization of the accumulated material concerning the structural organization of the central nervous system and its development in the course of phylogenesis and ontogenesis makes it possible to elaborate a preliminary scheme of a rational classification of neurons and interneuronal connections. The complication of the central nervous system of vertebrates in the course of evolution is expressed in a progressive differentiation of neuronal groups, which vary in their functional significance and in the character of their interconnections. On the basis of the results obtained in the field of morphological and physiological investigations it is possible to single out 2 basic types of neurons, which participate in definite forms of 'switching over' impulses in the central nervous system: (a) efferent neurons which form the principal links of the switching of impulses along the reflex arcs and the analyzers, and (b) intermediate or internuncial neurons which enter these links as additional elements. The latter along with the recurrent axon collaterals of the efferent neurons, play an important role in the coordination of the functional interactions between the efferent neurons and in the closing in of the loops of circulation of impulses within the switching key-points.

The most characteristic structural peculiarity of all the efferent neurons as elements which are specialized for a distant transmission of impulses, is a long axon with its lateral and recurrent collaterals which are especially numerous in the cerebral cortex and in the adjacent subcortical formations. Depending on their localization in the central nervous system, the intermediate neurons have axons of various lengths and differ in the number of their longer or shorter collaterals. In the reflex arcs of the spinal cord and in the brain stem these elements are represented by reticular neurons with a more or less elongated axon (Fig. 1, I); as a rule, the latter is divided into ascending and descending branches oriented along the axis of the central nervous system and originating collaterals on different levels. Investigations carried out in our laboratory established the presence of neurons of a reticular type in the lower links of the analyzers. However, the analyzers, on their higher cortical and subcortical levels, are particularly characterized by a different kind of intermediate neurons which are represented by elements with a short, more or less ramified axon (Fig. 1, II, III). In the cerebral cortex neurons with short axons, known as star cells, are specialized for accomplishing the most complex and finely differentiated forms of intracortical connections, namely, of a functional unification and division of the efferent neurons into groups. The star

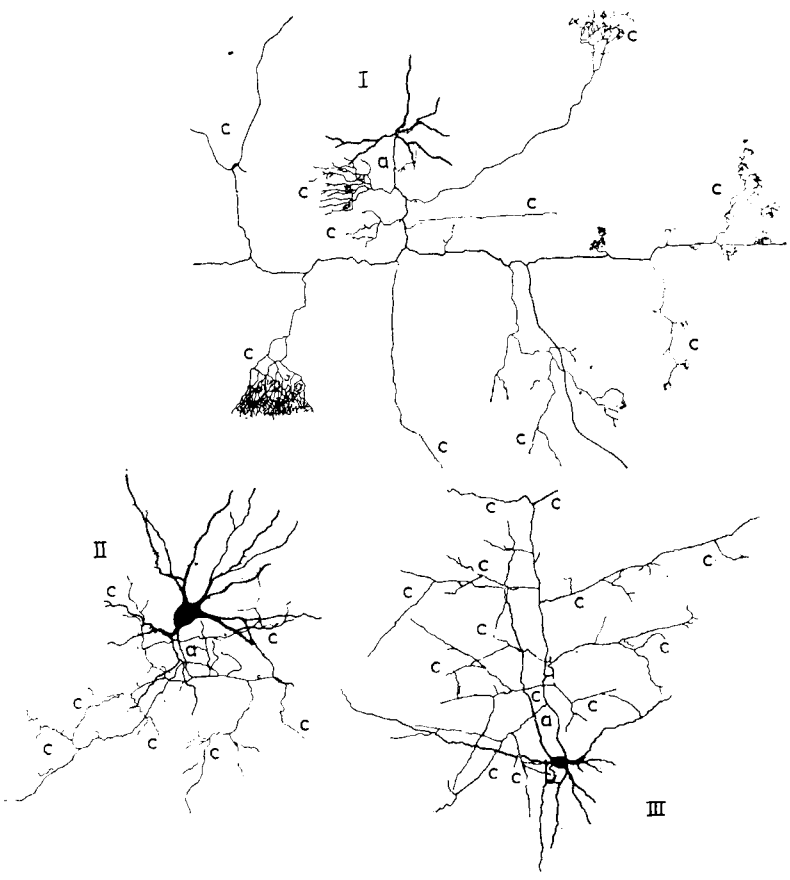

Fig. 1. Different forms of intermediate neurons in the central nervous system. I, Reticular neuron from the medulla oblongata (M. and A. Scheibel, 1958); II, neuron with a short axon from the subcortical ganglia (Leontovich, 1954); III, star cell with a short axon from the cerebral cortex (Poliakov, 1960). a, Axon; c, axon collaterals.

cells are characterized by a great variety of their contours, as well as of their size and peculiar features of the axon ramifications (Fig. 2).

Apart from the afore-mentioned differences in the structure of the axon, neurons of various types are distinguished also by the pattern of their dendrite ramifications. One of the most substantial morphological indices of the neuron is the presence or absence of special protoplasmatic outgrowths, appendages or 'thorns', on the dendrites. These structures differ in their shape, number and distribution along the dendrites of neurons of diverse functional significance (Fig. 3). In efferent neurons, entering the higher cortical and subcortical parts of the analyzers, the appendages on the dendrites are especially numerous and densely situated (Fig. 3, a–c). The appendages in these neurons are characterized by a short stalk and a spherical head. In the intermediate neurons the appendages on the dendrites are either sparse or fully absent; they have a long stalk and an elongated head. Such appendages which are spaced at definite intervals along the dendrites are typical for the reticular neurons (Fig. 3, g). The appendages are

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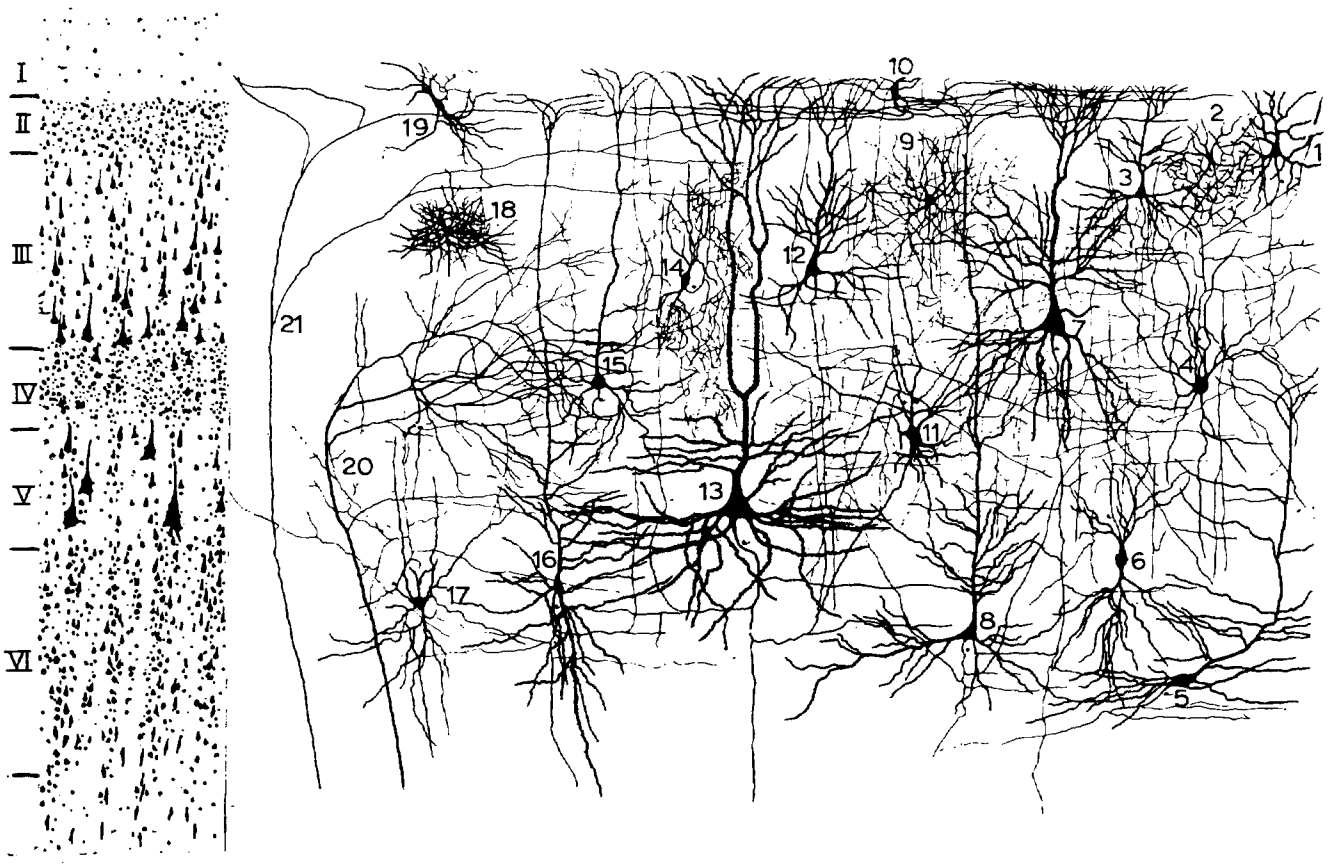

Fig. 2. Chart illustrating the neuronal structure of the cerebral cortex (Poliakov, 1949). 1, 3, 5, 7, 8, 12, 13, 15, 16, Efferent (pyramidal and fusiform) neurons with a long axon 2, 4, 6, 9, 10, 11, 14, 17, 18, 19, intermediate neurons (star cells) with a short axon; 20, 21, projectional and associative afferents of the cortex. Roman figures, cytoarchitectonic layers of the cortex.

sparse (Fig. 3, e) or absent (Fig. 3, d) on the dendrites of the cortical star cells and on the dendrites of cells with a short axon in the subcortical formations. The effector (motor) neurons are devoid of appendages on the dendrites.

In the course of a progressive differentiation of the central nervous system various complexes of neurons are formed in a definite sequence. The effector (motor) neurons, as well as the internuncial neurons of the reticular type which intermediate the functional relationships between them, prove to appear earliest. Both of them jointly participate in the accomplishment of the coordinated activity of the reflex arcs which are situated along the entire extent of the axial part of the central nervous system. At later stages of evolution are formed complexes of efferent and intermediate neurons intended for the building up of analyzers, whose higher brain ends are connected with the most finely and complexly differentiated forms of analysis and synthesis of stimulations.

Of all highly diverse forms of interneuronal connections within the central nervous system it is possible to single out 2 basic types of contact (synaptic) connections which are represented by formations differing in origin and structure. The endings of axon ramifications of diverse forms on the bodies and dendrites of the nerve cells constitute the principal means of interneuronal connection (Fig. 4). According to our observations, such contacts appear in the cerebral cortex at earlier stages of ontogenesis. At later stages, along with the further development and complication of the terminal contact axon apparatus, contacts of a different type are formed in the higher brain representations of the analyzers in connection with a progressive growth of the dendrite ramifications; these contacts are effectuated with the participation of the appendages on the dendrites. The heads of the appendages, just as the axon endings, present peculiarly specialized synaptic surfaces, since they establish contacts not only with axon ramifications of other neurons which come up to them, but also with those which extend nearby (Fig. 5). Such a contact between the appendages and axon branchings which extend near them, proves to be the most typical one. Unlike the terminal contacts, this type of interneuronal connections may be characterized as a tangential type of contacts. The existence of such contacts was first established by us in the human cerebral cortex; subsequently they were discovered by the workers of our laboratory also in the subcortical formations. The tangential contacts, which appear in ontogenesis (and possibly in phylogenesis too) later than the terminal contacts, may be regarded as a supplementary form of interneuronal connections within the central nervous system. The emergence of a system of tangential contacts, along with the terminal contacts, was, apparently, determined by a further complication in phylogenesis and ontogenesis of the entire sphere of axon and dendrite ramifications in the most highly organized divisions of the central nervous system.

Proceeding from the structural distinctions of both afore-mentioned types of contacts, we may assume that their functional significance in the mechanisms of interaction between the neurons is different. Apparently the terminal contacts are in the main adjusted to effecting a direct action of certain neurons on others, as a result of which there takes place a transition of the neurons which are subjected to such action from one basic state into another. On the contrary, it may be assumed that contacts of the

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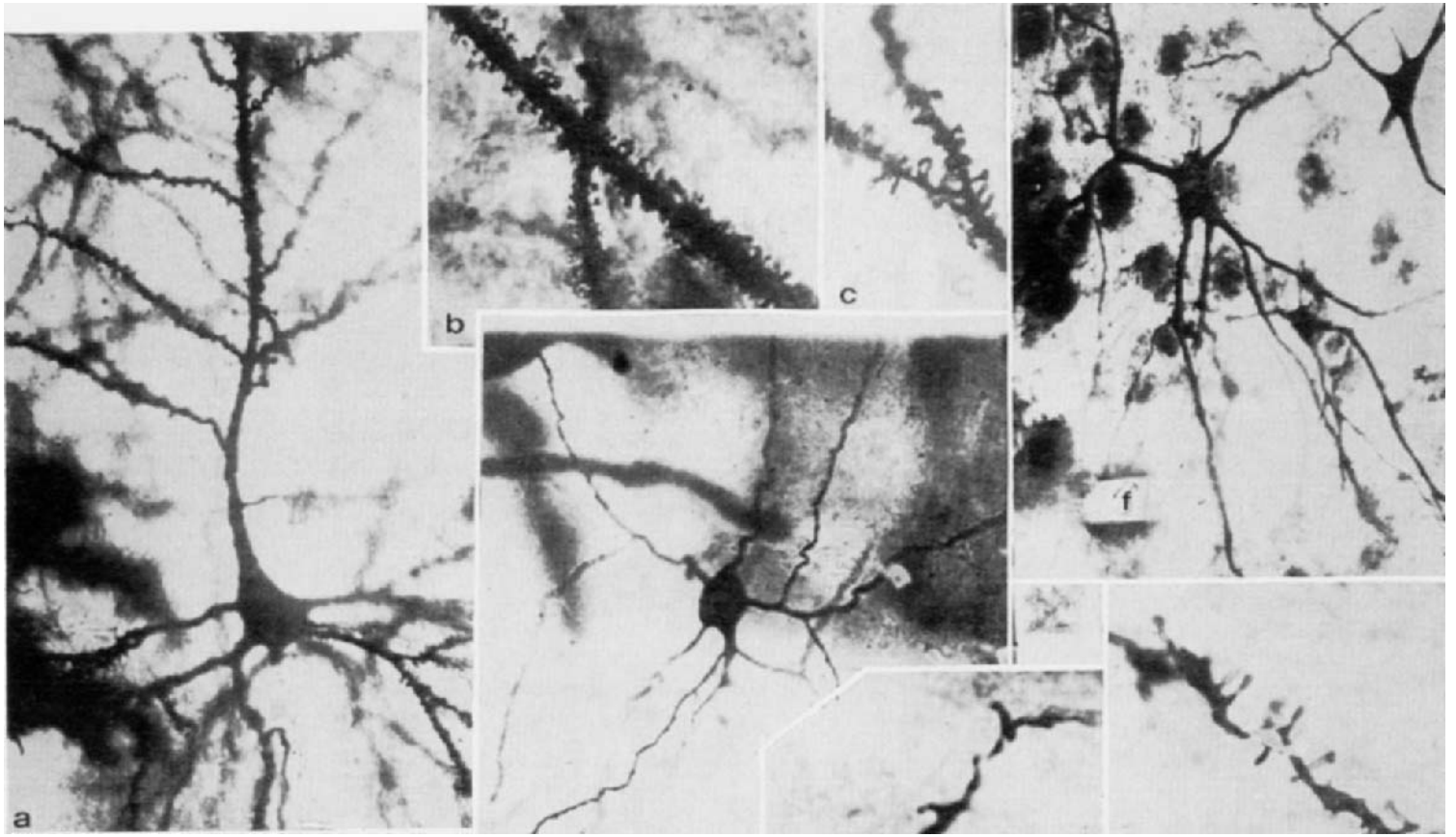

Fig. 3. a, Pyramidal cell of the cortex; b, section of the dendrite of a pyramidal cell; c, ramification of the dendrite of an efferent neuron from the subcortical ganglia with a long axon; d, star cell of the cortex; e, section of the dendrite of a star cell; f, reticular neuron from the medulla oblongata; g, section of the dendrite of a reticular neuron. (a, b, d, e, After Poliakov (1961); c, g, after Leontovich (1954); f, after Zhukova (1959).)

tangential type ensure indirect influences of the neurons upon one another. Thus, the general state of each neuron at any given moment of a reflex act may be regarded as a result of interaction of direct and indirect influences exerted upon it by numerous other neurons. The dendrite apparatus, which takes upon itself the bulk of the tangential contacts (Fig. 5) is, probably, a fine modulator of the functional state of the given neuron. With the development of the dendrite ramifications, there is observed a corresponding increase of the functional plasticity and reactivity of the neuron, *i.e.* its 'responsiveness' to changes taking place in the states of other neurons.

In order to understand the character of the influences which are exerted by the neurons upon one another in the course of their joint activity, it is important to bear in mind that the correlation of the distribution of terminal and tangential contacts along the surface of the cell body and of its dendrites is not the same in neurons of different functional significance. In the efferent neurons of the cortex (pyramidal and fusiform cells with long axons) the body of the cell proper and the initial sections of the dendrite

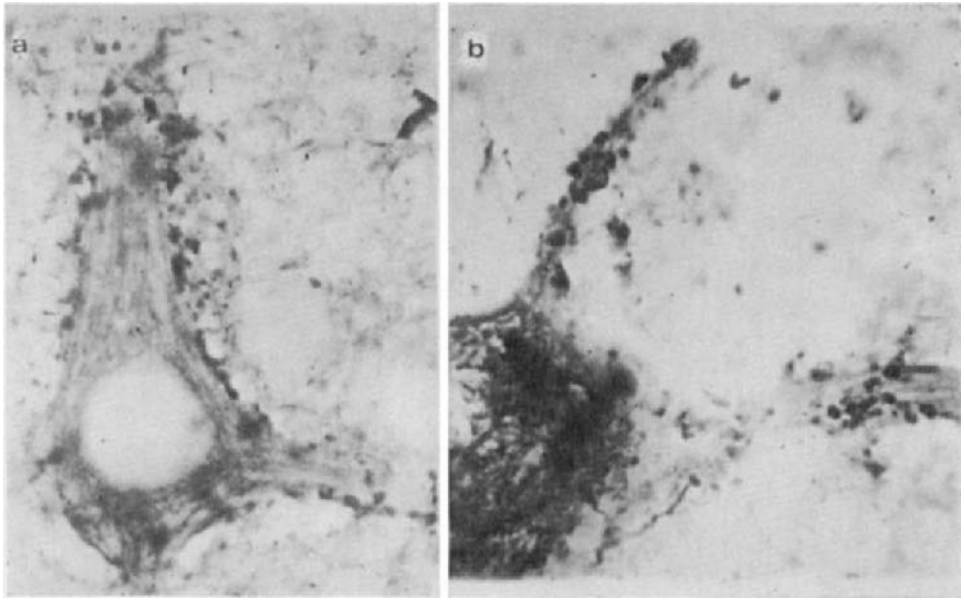

Fig. 4. Terminal synapses on pyramidal (a) and star (b) cells of the cortex (preparations of K. K Blinova, 1961).

trunks which emerge from it, as a rule, have no appendages. Neurohistological investigations showed that this part of the entire perceptive surface of the efferent neuron is occupied by terminal contact apparatuses (Fig. 6). Therefore, it may be assumed that the basic functional state (excitation or inhibition) of the efferent neuron is determined by impulsation which exerts influence precisely on its central part. The highly developed dendrite ramifications of the efferent neuron provided with numerous appendages, being predominantly an additional area of tangential contacts, are apparently, adapted mainly to the perception of indirect influences. Consequently, the efferent cortical neuron may be regarded as a collector of various impulses which

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reach the cortex from different closer or more remote sources and which circulate in all directions within the gray matter.

From the above-described structural features of the nerve elements, which we relate

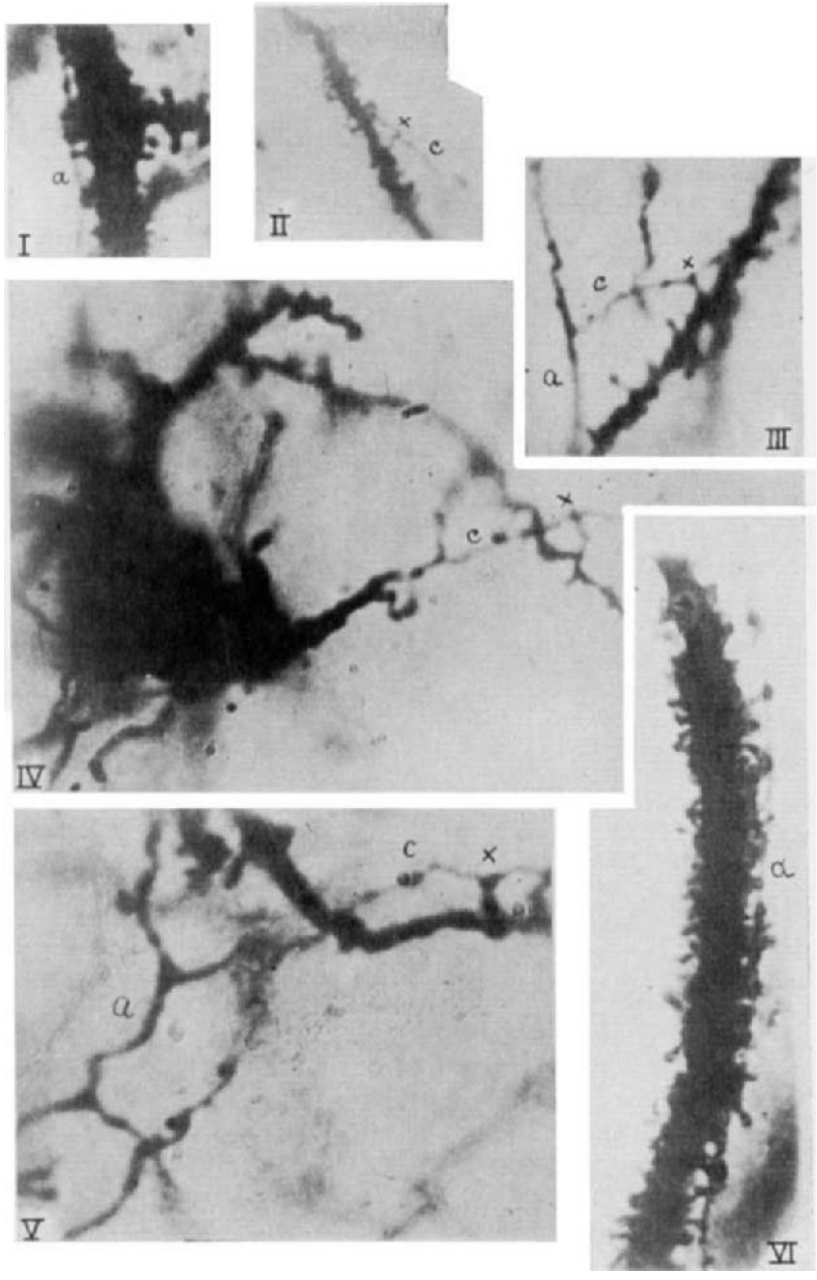

Fig. 5. Tangential contacts (x) between the appendages on the dendrites and the axon ramifications (a). I, II, VI, tangential contacts with dendrites of pyramidal cells; III, IV, V, tangential contacts with dendrites of star cells (Poliakov, 1955, 1961).

to one common group of intermediate neurons, it follows that the latter are specialized for another type of interneuronal connections essentially differing from those observed

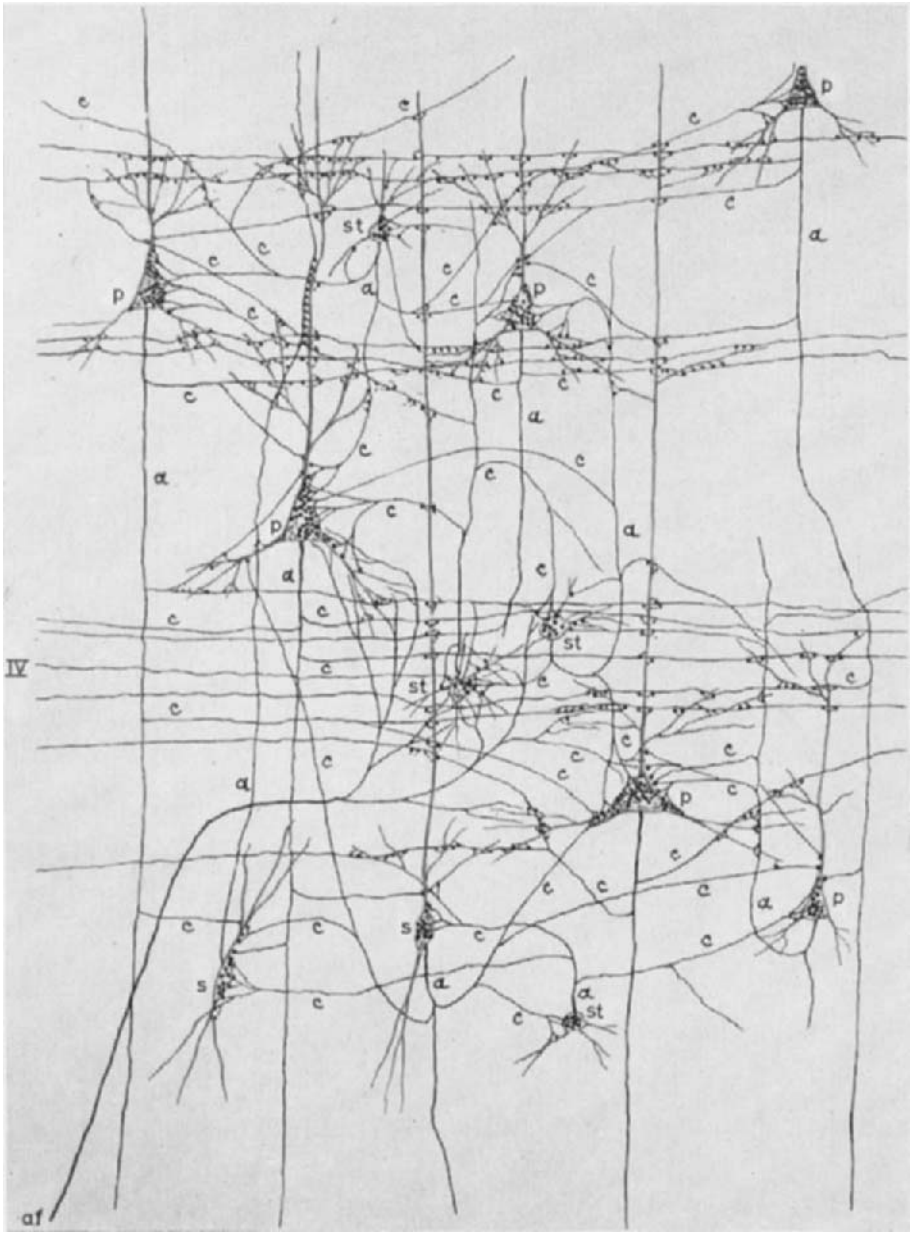

Fig. 6. Scheme illustrating various forms of interneuronal connections in the cerebral cortex. p, s, Pyramidal and fusiform cells with a long axon; st, star cells with a short axon; a, axon; c, axon collaterals; af, projectional afferent fibre ramifying on the level of layer IV. The scheme shows terminal synapses formed by ramifications of the short axons of the star cells and by the collaterals of long axons of efferent neurons on the bodies and dendrites of the nerve cells, as well as tangential contacts with appendages on the dendrites which are particularly perceptible along the horizontal nerve fibres (Poliakov, 1961).

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in the efferent neurons. The presence of scarce appendages on the dendrites or even their full absence indicates that the reticular neurons, just as the star cells with short axons in the cerebral cortex, are in the main adapted to establishing terminal contacts with other neurons. In particular, this assumption was confirmed with the help of corresponding neurohistological methods, with regard to the reticular neurons: it proved possible to observe numerous terminal synapses which cover their dendrites along a considerable extent. According to these structural features, the entire perceptive surface of an intermediate neuron may be regarded as similar to the central part of an efferent neuron. The elongated form of the sparse appendages on the dendrites is, apparently, due to the fact that the intermediate neurons, along with many terminal contacts, form relatively scarce contacts of the tangential type.

The pattern of interconnections between the efferent and intermediate neurons which jointly switch over the afferent impulses may be pictured in the cerebral cortex as follows.

The star cells, which, as stated above, concentrate mainly the direct influences coming from other neurons through the terminal contacts in their turn exert similar influences upon other neurons. This assumption is corroborated by the fact that, according to our observations, the terminal ramifications of the short axons of the star cells form interlacings in the shape of nests or baskets on the bodies and initial sections of the dendrites of the efferent neurons (pyramidal and fusiform cells with long axons). This leads to the conclusion that the star neurons are specialized to establish electively differentiated connections with definite groups of efferent neurons, and are, apparently of particular importance for determining the functional state (excitation or inhibition) of the efferent neurons. This assumption fully accords with the idea of the physiological role of the cortical star cells as elements which ensure fine coordination of simultaneous and consecutive 'switching on' of certain groups of pyramidal (and fusiform) cells and 'switching off' of other groups.

The afore-mentioned structural mechanisms, which govern the interaction of the efferent and intermediate neurons, constitute a highly important integral part of the single complex organization of interneuronal connections in the cerebral cortex.

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Role of Synaptic Inhibition in Synchronization of Thalamocortical Activity

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INTRODUCTION

Since Sechenov's discovery of bulbospinal inhibition (Sechenov, 1963), inhibitory processes have been considered important components of all behavioral activities ranging from relatively simple spinal reflexes to integrative functions of the brain. Although an adequate picture has been emerging of the various inhibitory mechanisms in spinal reflexes (Eccles, 1961), few data are available concerning the nature and contribution of inhibitory processes to higher nervous activity. Consequently the notion that inhibition is involved in these activities is generally accepted more as an article of faith than of proven fact. Clearly, what is required is a more comprehensive description than has heretofore been possible of the role of inhibition in higher nervous functions.

The present report summarizes recent studies which have permitted some progress along these lines. Attention has been focused here on several types of electrocortical phenomena generally recognized as prominent features of neural events underlying sleep and wakefulness and specific and nonspecific activation of cortex.

In these investigations the intracellular recording technique has been applied to problems relating to: *(1)* the synaptic mechanisms involved in the thalamic and cortical stage of evoked recruiting responses; *(2)* the subcortical events in the transition from recruitment to reticular activation of thalamic neuronal discharge; *(3)* the mode of engagement of specific and nonspecific pathways in cortical organizations; and *(4)* the origin and nature of evoked cortical potentials.

Each aspect of this study has provided important clues to the neurophysiological mechanisms of EEG-synchronization and of the contribution of inhibitory synaptic activities to integrative functions of thalamocortical projection systems.

THALAMIC STAGE OF EVOKED EEG-SYNCHRONIZATION

Analysis of the synaptic events underlying the thalamic stage of EEG-synchronization evoked by low-frequency (7/sec) stimulation of midline thalamic nuclear structures (CM) was achieved by intracellular recording from neurons located in various nuclear

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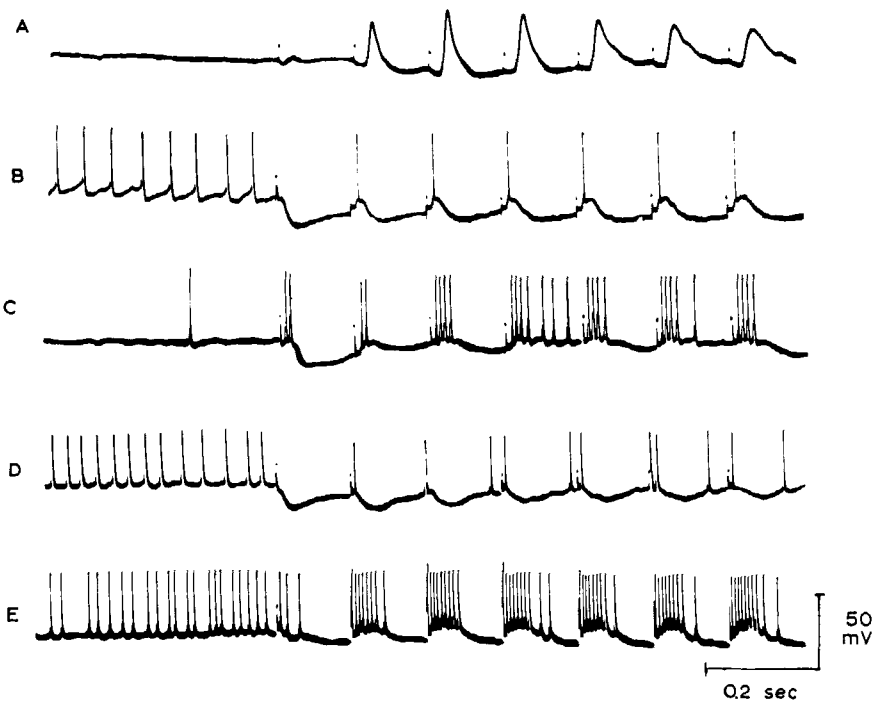

Fig. 1. Patterns of intracellularly recorded activities of thalamic neurons during cortical recruiting responses evoked by 7/sec midline (CM) thalamic stimulation. (A) characteristics of surface-negative responses (motor cortex) elicited throughout the experiment from which the intracellular records (B-E) were obtained. (B) neuron in ventral anterior region of thalamus exhibiting prolonged IPSP following first stimulus then EPSP–IPSP sequences with successive stimuli. (C) relatively quiescent ventrolateral neuron develops double discharge with first stimulus. The ensuing IPSP is succeeded by another evoked EPSP and cell discharge. Note alternation of IPSP. (D) neuron with discharge characteristics similar to that shown in (B). (E) neuron in intralaminar region exhibiting an initial prolonged IPSP that interrupts spontaneous discharges. The second and all successive stimuli evoked prolonged EPSP's with repetitive discharges that are terminated by IPSP's. Negativity upwards in cortical surface records and downwards in intracellular records in this and all subsequent figures. From Purpura and Shofer (1963).

groups of the exposed thalamus (Purpura and Cohen, 1962; Purpura and Shofer, 1963). Intracellular recordings have disclosed several patterns of evoked synaptic activities during long-latency recruiting responses elicited in motor cortex by 7/sec CM stimulation (Fig. 1).

In approximately 70% of impaled neurons, prolonged (80–100 msec) inhibitory postsynaptic potentials (IPSP's) were observed during recruitment. The latencies of IPSP's varied from 15–40 msec in most of the responding neurons, although latencies as short as 2–5 msec and as long as 60–70 msec were also observed. In some instances, IPSP's persisted for nearly 200 msec (Fig. 2, H). Excitatory postsynaptic potentials (EPSP's) were elicited in over 50% of thalamic neurons. Their latencies varied from 4–15 msec and their duration ranged from 15–80 msec. For present purposes particular attention is directed to the temporal sequence of evoked PSP's in thalamic neurons during recruitment, as summarized in Fig. 1.

The first stimulus in CM, which produced little in the way of an evoked cortical potential, exerted profound effects on thalamic neuronal activity. In a large proportion of neurons, the initial stimulus evoked prolonged IPSP's which abruptly blocked ongoing spontaneous discharges (Fig. 1,B,D). Successive stimuli at low frequency (7/sec) evoked EPSP's and cell discharges which were followed by additional prolonged IPSP's.

The initial stimulus may be considered the 'get set' stimulus since the predominant synaptic activity generated in intrathalamic neurons is prolonged inhibition asso-

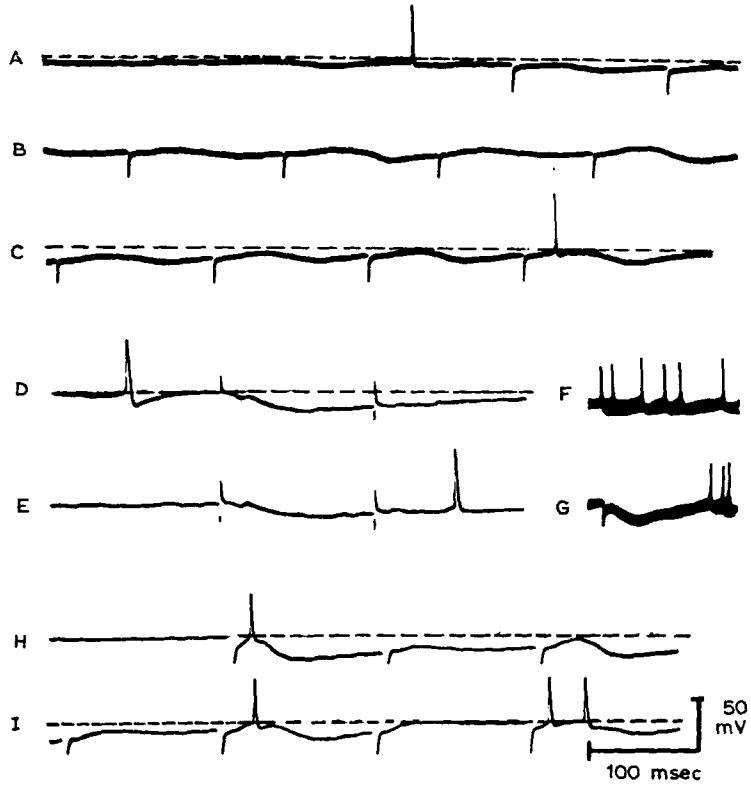

Fig. 2. Examples of evoked IPSP characteristics of four different neurons from VM and VA regions of the thalamus. Broken lines in (A), (C), (D), (H), and (I) are drawn through base of spikes to show relationship of assumed firing level to synaptically induced membrane potential oscillations. (A), (B) and (C) continuous record. Neuron exhibited intermittent discharges prior to 7/sec CM stimulation. Stimulation elicits low-amplitude EPSP–IPSP sequences of long duration, but these membrane potential oscillations are below the firing level. (D) and (E) records from another neuron show the first two stimuli in successive trains with several seconds between periods of stimulation. (D) shows development of short-latency IPSP's that are not preceded by EPSP's. The stimulus is preceded by a spontaneous discharge with a small 'undershoot'. Stimulation evokes an IPSP that persists throughout the second stimulus. (E) 30 sec later, after a period during which the element was silent, the first CM stimulus elicits a smaller IPSP. A prominent EPSP elicited after the second stimulus evokes a spike that exhibits no 'undershoot'. (F) superposed traces of spontaneous activity of a partially traumatized neuron. (G) blockade of spontaneous discharges during short-latency prolonged IPSP's evoked by 7/sec CM stimulation. (H) and (I) continuous record of activity evoked in another neuron silent before stimulation. Note prolonged IPSP initiated by first stimulus and alternation in magnitude of succeeding evoked PSP's. From Purpura and Cohen (1962).

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ciated with elimination of prior spontaneous activity. The same stimulus also sets into operation long-latency facilitation of EPSP's as evidenced by build-up of excitatory synaptic activity between periods of inhibition. The second and additional 'go' stimuli of the repetitive 7/sec train effectively trigger EPSP–IPSP sequences which contribute to the overall synchronization of thalamic neural discharge.

The magnitude of EPSP's or IPSP's in a particular temporal pattern varies considerably in different neurons in the same and different nuclear groups. In several instances elements have been encountered in which EPSP–IPSP's were not associated with cell discharge (Fig. 2).

Powerful excitatory synaptic drives were observed in cells located in intralaminar regions of the thalamus during recruiting responses (Fig. 1, E). Irrespective of the specific pattern of activation noted in these elements, the development of IPSP's in response to the first stimulus of the 7/sec CM train was characteristically observed. The patterns of intracellularly recorded synaptic activities of thalamic neurons observed during recruitment have their counterpart in extracellular recordings from different thalamic nuclei. The temporal relations and overt effects of extracellularly and intracellularly recorded activities during low-frequency CM stimulation suggest that the prominent long-latency focal positivity recorded in the thalamus during recruitment represents the prolonged IPSP's synchronously generated in a large population of thalamic neurons. Thus transmembrane recordings from thalamic neurons indicate that IPSP's are initiated in the majority of thalamic neurons for over 60–70% of the period of low-frequency midline stimulation.

The long-latency prolonged IPSP's effectively block activation of neurons from other sources and render these elements unresponsive to excitatory synaptic drives except those initiated by subsequent CM stimulation.

Since inhibition is the dominant synaptic event elicited in thalamic neurons during recruitment, it follows that synchronization of thalamic neural activity is as much a consequence of restricting cell discharge to periods prior to and after prolonged IPSP's as it is a result of simultaneous activation of large numbers of elements *via* excitatory pathways (Purpura and Cohen, 1962). Prolonged IPSP's have also been considered of importance in synchronizing activity of neurons in the ventro-basal complex (Anderson and Eccles, 1962).

INTRATHALAMIC SYNAPTIC EVENTS IN TRANSITION FROM EVOKED EEG-SYNCHRONIZATION TO RETICULOCORTICAL ACTIVATION

Observations on the patterns of evoked PSP's in thalamic neurons during changes in frequency of stimulation of the thalamic reticular system have provided additional information on subcortical synaptic mechanisms involved in the transition from EEG-synchronization to reticulocortical activation (Purpura and Shofer, 1963). The major events disclosed in these studies are summarized in Figs. 3 and 4.

Cells exhibiting EPSP–IPSP sequences characterized by prominent IPSP's during recruitment (Fig. 3, A) also exhibited IPSP's during the first stimulus of a high-

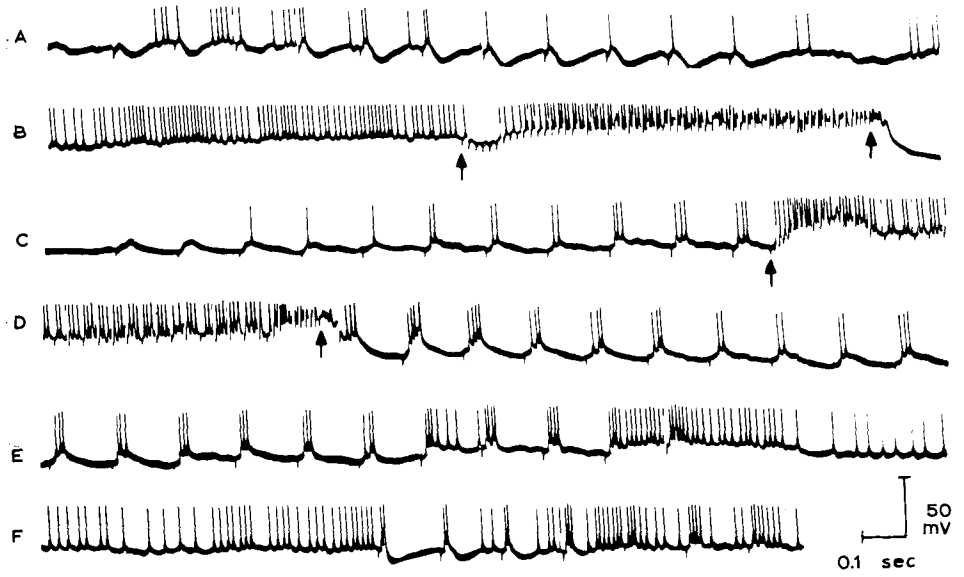

Fig. 3. Effects of repeated high-frequency CM stimulation on a ventromedial neuron exhibiting preactivation recruiting pattern characterized by short-latency EPSP's and prolonged prominent IPSP's. (A–E) continuous record. (A) 7/sec CM stimulation succeeded by a phase of hyperexcitability (B). At first arrow in (B) a prolonged IPSP is initiated by the first stimulus of the high-frequency (60/sec) repetitive train. Successive stimuli after the IPSP evoke summating EPSP's associated with high-frequency spike attenuation. (D) second period of 60/sec CM stimulation after repolarization initiates only prolonged, slowly augmenting EPSP's. Changes in stimulus frequency between arrows, in (C) and (D) induce high-frequency repetitive discharges superimposed on depolarization, whose magnitude is related to stimulus frequency. (F) several seconds later. Note reappearance of IPSP's during 7/sec CM stimulation. From Purpura and Shofer (1963).

frequency repetitive train in CM (Fig. 3, B). All subsequent stimuli failed to elicit IPSP's, but EPSP summation was pronounced. The excitatory synaptic bombardment resulted in sustained soma depolarization during which partial spikes or soma-spike inactivation was observed. After-effects of short periods of high-frequency CM stimulation were equally impressive.

Whereas low-frequency stimulation evoked prominent long-latency IPSP's prior to the change in stimulus frequency, IPSP's were not observed in the post-activation period. Low-frequency CM stimulation elicited instead only EPSP's (Fig. 3, C). In the absence of IPSP's additional periods of high-frequency CM stimulation produced immediate summation of EPSP's.

The magnitude of soma depolarization depended on the frequency of stimulation and consequently the intensity of the excitatory synaptic drive. After dissipation of the excitatory after-effects of high-frequency CM stimulation IPSP's were again elicited (Fig. 3, F).

The marked alterations in evoked PSP patterns produced by changes in CM stimulus frequency were particularly dramatic in thalamic neurons located in intralaminar regions. As noted above, such elements frequently exhibited IPSP's during initial stages of CM stimulation and powerful excitatory synaptic drives during recruitment. A cell showing this pattern of evoked synaptic activity is illustrated in

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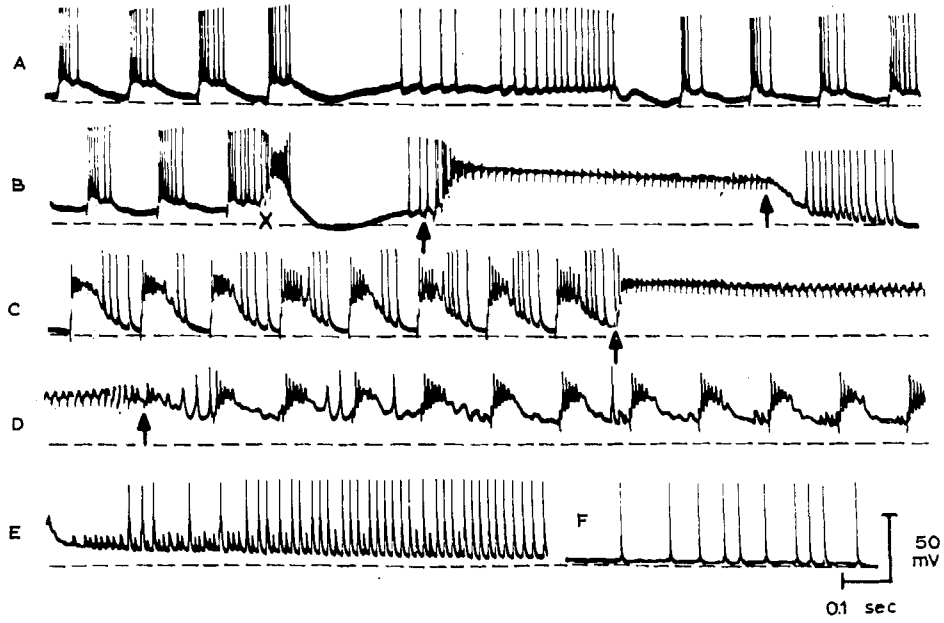

Fig. 4. Persisting effects of repeated high-frequency CM stimulation on an intralaminar neuron exhibiting rapid EPSP summation during recruitment. A–E, continuous record. A, terminal phase of a period of 7/sec CM stimulation followed by spontaneous discharge and initiation of a second period of 7/sec stimulation. First stimulus after spontaneous discharge elicits a prolonged IPSP–EPSP complex. B, short burst of high-frequency stimulation at X. Enhancement of high-frequency evoked repetitive discharges on 80 msec wave of depolarization is succeeded by repolarization, then beginning of spontaneous activity. High-frequency (75/sec) CM stimulation indicated by arrows. C, 7/sec CM stimulation initiated powerful excitatory synaptic drives that are associated with brief activation phases. A second period high-frequency CM stimulation occurs in C. D, depolarization persists for several seconds. E, gradual repolarization associated with reappearance of partial spikes, then full spikes. F, 20 sec after E. From Purpura and Shofer (1963).

Fig. 4. It should be noted that high-frequency stimulation produced sustained soma depolarization and that after recovery of membrane potential low-frequency stimulation elicited powerful excitatory synaptic drives resulting in 'inactivation responses' (Fig. 4, C). A second period of high-frequency stimulation elicited a prolonged phase of post-activation facilitation characterized by sustained membrane depolarization and repetitive, partial or complete spikes (Fig. 4, E).

The foregoing brief survey of intracellular activities of thalamic neurons indicates that thalamic neurons participating in recruiting responses are also involved in the EEG-desynchronizing effects of high-frequency midline thalamic stimulation. Thus the electrocortical effects of low- and high-frequency thalamic reticular system stimulation are largely determined by patterns of synaptic activities evoked in thalamic neurons by intrathalamic and possibly extrathalamic pathways, rather than activation of distinct and mutually antagonistic systems of thalamic neurons. The major alterations in the patterns of synaptic activities observed in thalamic neurons during the transition from low- to high-frequency midline stimulation is reflected in blockade of pathways generating IPSP's during low-frequency stimulation and enhancement of

excitatory synaptic drives. The blockade of IPSP's or inhibition of inhibition (Purpura and Grundfest, 1957) appears to be of importance in converting phasic activity during recruiting responses to sustained discharges during and after TRS-activation (Purpura and Shofer, 1963).

Implicit in this assumption is the recognition that the overall effects of high-frequency CM stimulation must include activation of inhibitory neurons capable of blocking intrathalamic inhibitory pathways involved in recruitment. Although the pathways responsible for inhibition of inhibition are not known, it is clear that even under conditions in which powerful excitatory activities predominate in the overall synaptic effects on thalamic neurons, inhibitory mechanisms must be invoked to account for blockade of some components of pathways ordinarily set into operation during synchronization of thalamic neuronal discharge (Purpura and Shofer, 1963).

POSTSYNAPTIC POTENTIALS OF CORTICAL NEURONS DURING THALAMOCORTICAL EVOKED ACTIVITIES

The temporal patterns of EPSP–IPSP's generated in thalamic neurons by intrathalamic synaptic pathways originating in the midline nuclear complex are reflected, in part, in corticipetal volleys which are distributed to cortical neuronal organizations involved in the production of recruiting responses. The latter, together with primary and augmenting responses to ventrolateral (VL) stimulation, represent the major types of activity evoked by localized thalamic stimulation. Intracellular recording combined with current-injection techniques have disclosed inhibitory as well as excitatory postsynaptic potentials in different organization of cortical neurons during

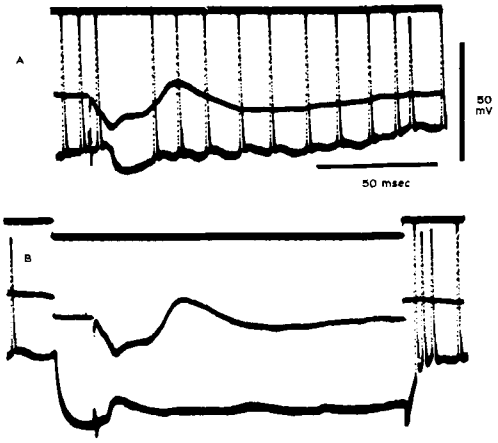

Fig. 5. (A) IPSP evoked in a cortical neuron by 6/sec stimulation in n. ventralis lateralis (VL) and its inversion (B) during injection of a hyperpolarizing current of 0.2 μA. In this and all subsequent tri-beam records upper trace signals magnitude of injected inward (hyperpolarizing) current; middle trace, the monopolarly recorded cortical surface activity (negativity upwards); and the lower trace, the intracellular record. Responses recorded during stabilization phases of augmenting responses. The synaptically induced hyperpolarization (A) was evoked on a background of injury discharge. Injected current increased membrane potential 10–15 mV above level at which first anode-break response was elicited. From Purpura and Shofer (1964).

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different phases of primary, augmenting and recruiting responses (Purpura and Shofer, 1964; Purpura *et al.*, 1964).

Short-latency hyperpolarizations of 15–20 msec duration are the least complex types of evoked synaptic activity recorded in cortical neurons during augmenting responses elicited by low-frequency (7/sec) VL stimulation. The IPSP-nature of this response is revealed by the finding that membrane hyperpolarization produced by injection of inward current through the recording microelectrode results in inversion of the synaptically evoked hyperpolarization (Fig. 5) (Eccles, 1961; Grundfest, 1959).

More complex patterns of EPSP's and IPSP's have been recorded in the majority of cortical neurons during augmenting and recruiting responses. Several of these are

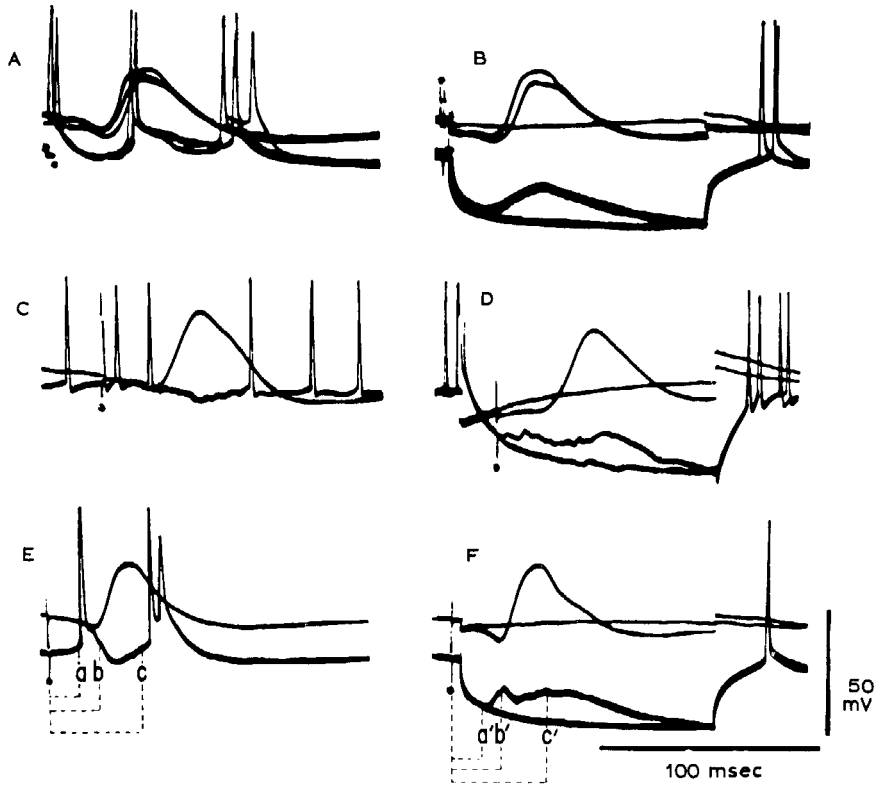

Fig. 6. Different patterns of PSP's evoked in cortical neurons during recruiting responses elicited by 6/sec stimulation in thalamic midline nuclear complex (CM). (A), (C) and (E) examples of relationship of cortical surface-negative recruiting responses to intracellular activities of three neurons impaled during the same experiment. (B), (D) and (F) corresponding superimposed traces of effects of injected hyperpolarizing currents before and after CM stimulation. (A) neuron responding with two phases of discharge during peak and near termination of surface-negativity exhibits a prolonged, slowly developing EPSP during membrane hyperpolarization (B). (C) neuron in which peak of recruiting negativity is associated with membrane hyperpolarization exhibits an inverted (depolarizing) potential during inward current injection. Analysis of temporal relations of early EPSP (a, a'), onset of repolarization potential (b, b') and late EPSP (c, c') is shown in (E) and (F) before and after induced membrane hyperpolarization, respectively. In (B) and (F) thalamic stimulation preceded start of current injection by several msec. Note in (F) superimposed anode-break responses following periods of current injection before and during thalamic stimulation. From Purpura and Shofer (1964).

illustrated in Fig. 6 with respect to the effects of injected hyperpolarizing currents on synaptic activities evoked during recruiting responses. Discharge patterns characterized by phases of grouped spiking (Fig. 6, A, E) were generally attributable to prolonged EPSP's (Fig. 6, B) or EPSP–IPSP–EPSP sequences (Fig. 6, F). A number of motor cortex neurons were impaled exhibited membrane repolarization of hyperpolarization during recruiting negativities (Fig. 6, C). Inversion of these hyperpolarizations was also readily achieved during injection of hyperpolarizing currents (Fig. 6, D).

The relationship between EPSP–IPSP sequences to discharge patterns observed during latency variations in recruiting responses evoked by stimulation of different midline thalamic sites is shown in Fig. 7. In both cases, repetitive discharges asso-

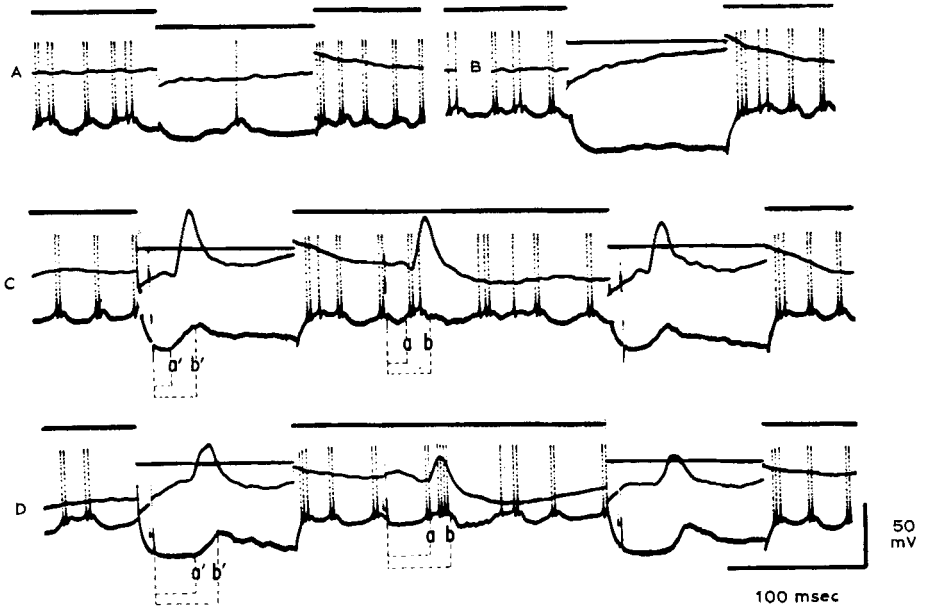

Fig. 7. Evoked synaptic activities associated with recruiting responses of different latency elicited by stimulation in rostral (C) and caudal (D) regions of the midline thalamus. Fifth, sixth and seventh responses in the repetitive sequences are illustrated in (C) and (D). Strong hyperpolarizing currents were injected just prior to the fifth and seventh responses. Effects of injected currents of different intensity are shown in A (0.2 μA) and B (0.45 μA) in the absence of thalamic stimulation. Note relationship of ripples of depolarizing waves in (B) during current injection to rhythmicity of double discharges. Analyses in (C) and (D) illustrate temporal relations of EPSP's (a, a') and onset of IPSP's (b, b') for different types of recruiting responses before and during injection of strong hyperpolarizing currents. From Purpura and Shofer (1964).

ciated with recruiting negativity were succeeded by hyperpolarizations. Injection of strong hyperpolarizing currents revealed prominent and complex depolarizing potentials in relation to these activities. Early phases were EPSP's temporally related to periods of increased firing, whereas later phases represented long-latency inverted IPSP's.

A number of differences were observed in the characteristics of PSP's evoked in cortical neurons during augmenting and recruiting responses. Apart from differences

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in latency, EPSP's and IPSP's associated with recruiting responses exhibited slower onset and less prominent peaks than those evoked during augmenting responses. No cells were found in these studies in which injected hyperpolarizing currents failed to attenuate or invert hyperpolarizations or accentuate depolarizations related to different components of thalamically evoked cortical surface potentials (Purpura and Shofer, 1964).

Elimination of spike discharges and their sequelae by injection of hyperpolarizing currents into cortical neurons thus reveals only one general class of transmembrane potentials capable of accounting for different slow wave components of evoked cortical potentials, *i.e.* postsynaptic potentials (Purpura, 1959; Purpura and Grundfest, 1956).

Analysis of the time course of PSP's evoked during augmentation and recruitment, as well as the effects of membrane hyperpolarization on these PSP's suggest that spatial and numerical differences in the distribution of synapses on cortical neurons are important factors in determining the overt effects of specific and nonspecific activation of cortical organizations. These functional differences in the mode of activation of cortical neurons by specific and nonspecific thalamocortical projections are perhaps best illustrated in the effects observed on corticospinal neurons during augmentation and recruitment (Fig. 8).

Low-frequency stimulation in VL elicits short-latency EPSP's which are associated with repetitive discharges and 'inactivation responses'. These EPSP's are initiated

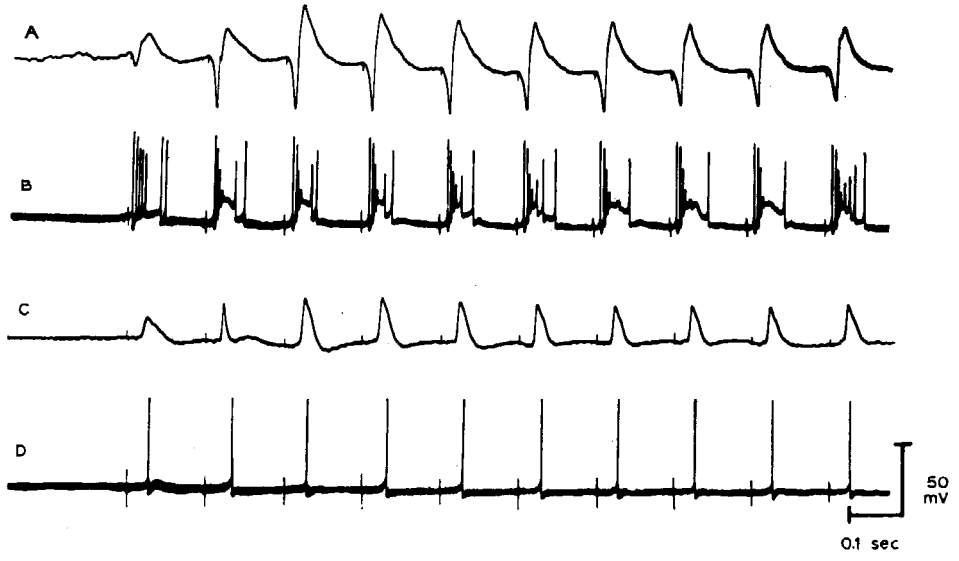

Fig. 8. Comparison of synaptic activities evoked in a Betz cell by low-frequency (7/sec) repetitive stimulation in n. ventralis lateralis (VL) and the midline thalamic nuclear complex (CM). (A) cortical surface augmenting response to VL stimulation; (B) intracellular activities associated with augmenting sequence. (C) and (D) cortical surface recruiting response and related intracellular activities, respectively. Build-up of augmenting sequence is reflected in enhancement of EPSP's, repetitive firing and 'inactivation responses'. Long-latency single discharges superimposed on low-amplitude short-duration EPSP's are observed during recruitment. From Purpura *et al.* (1964).

during the surface-positivity of cortical augmenting sequences and persist throughout succeeding surface-negative components.

In marked contrast to the powerful excitatory synaptic drives observed during augmentation, midline thalamic stimulation elicits single discharges which develop from low-amplitude EPSP's. The discharges tend to coincide with the peak negativity of the cortical surface recruiting waves. Differences in the potencies of the synaptic drives initiated in Betz cells during augmenting and recruiting responses are also seen in the relationship between slow variations in membrane potential and the effectiveness of EPSP's in securing cell discharge. In the case of augmenting responses such slow variations have little or no significant influence on the ability of EPSP's to trigger repetitive high-frequency cell discharges. An entirely different situation is en-

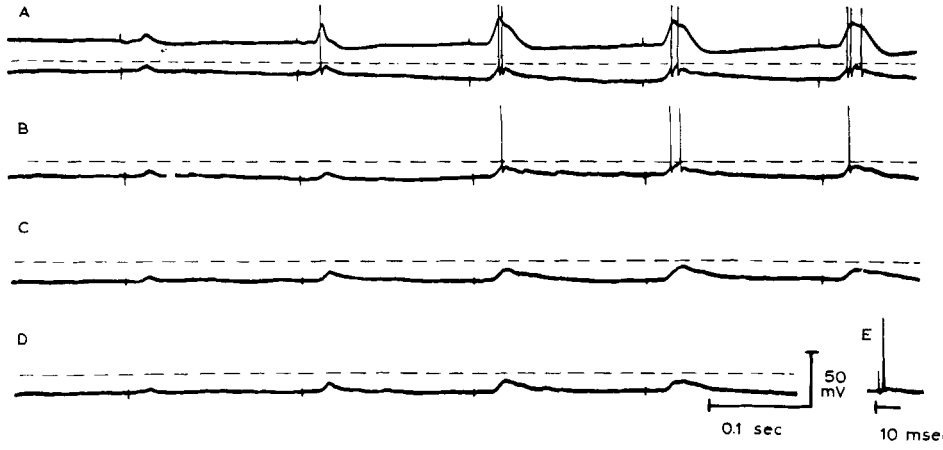

Fig. 9. Effects of slow variations in membrane potential of a Betz cell on EPSP's and spike discharges associated with long-latency recruiting responses. (A–D) four series of responses during initial phase of recruitment elicited by 7/sec CM stimulation. Several seconds elapsed between periods of stimulation. Simultaneously recorded cortical surface responses and intracellular potentials are illustrated in (A). Firing level indicated by dashed lines in all records. (A) first stimulus evokes a small EPSP and minimal cortical surface-negativity, whereas subsequent stimuli elicit larger EPSP's and 2–3 spikes. (B) effectiveness of EPSP's is reduced. (C) and (D) EPSP's fail to attain firing level during phases of increased membrane polarization as indicated by displacement of baseline from firing level. (E) antidromic response of the Betz cell. From Purpura *et al.* (1964).

countered with respect to recruiting responses. EPSP's evoked on a background of slow increases in cell polarization may fail to attain firing level, despite the fact that no detectable change has occurred in the magnitude or duration of such EPSP's (Fig. 9). These and other differences related to the influence of spontaneous discharges on the effectiveness of PSP's evoked during recruitment indicate that the major determinants of specific and nonspecific activation of cortical neurons are referable to quantitative as well as qualitative differences in PSP's evoked by the two pathways (Purpura and Housepian, 1961; Purpura and Shofer, 1963, 1964).

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CONTRIBUTION OF INHIBITORY SYNAPTIC ACTIVITIES TO COMPONENTS OF EVOKED POTENTIALS

Previous pharmacological (Purpura and Grundfest, 1956, 1957; Purpura *et al.*, 1959, 1960) and ontogenetic studies (Purpura, 1961) of evoked potentials have indicated that different components of focal or surface evoked activities represent composites of excitatory and inhibitory postsynaptic potentials generated at different sites in the soma-dendritic membrane of neurons in different synaptic organizations (Purpura, 1959).

It has already been noted above that PSP's represent the major transmembrane potential change observed in cortical neurons during evoked potentials. The extent to which IPSP's may contribute to the production of different components of electrocortical activities especially those elicited by medial and lateral thalamic stimulation remains to determine.

Several examples have been cited in Figs. 5–7 of the inclusion of IPSP's in patterns of synaptic drives in cortical neurons during augmentation and recruitment.

It is apparent that temporal sequences of EPSP's and IPSP's elicited in a particular

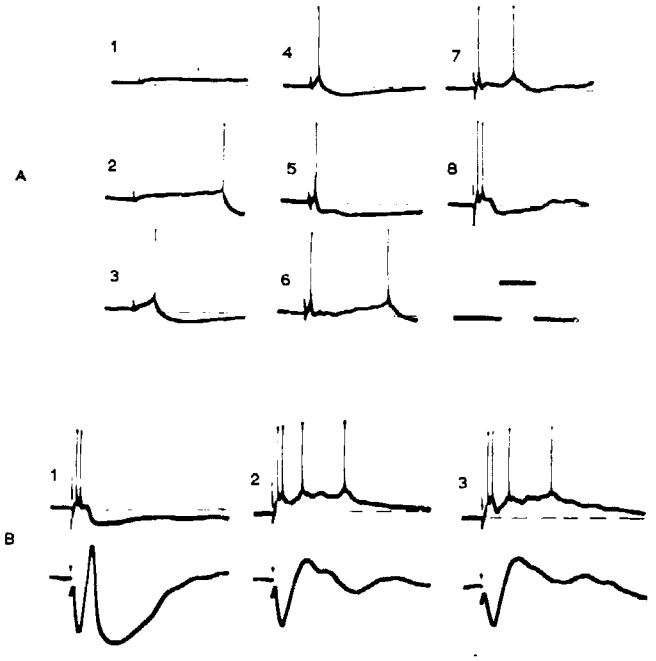

Fig. 10. (A, 1–8) Increase in complexity of PSP's evoked in a non-PT cell during stimulation in VL with single shocks (0.5/sec) of progressively increasing strength. (1) Weakest stimulus elicits a short-latency prolonged EPSP. (2–4) Progressive enhancement of EPSP and decrease in latency of initial discharge. (5–7) Stronger stimulation elicits additional long-latency EPSP and spike discharge. (8) Maximal stimulation initiates a long-latency IPSP after the EPSP and associated double discharge. (B, 1–3) First three intracellular responses and cortical surface activity (below) during 7/sec VL stimulation at strength similar to that for response shown in (A, 8). Note transition in PSP-patterns between first and second stimulus of the repetitive train and relationship of these changes to alterations in surface evoked potential. Calibrations: 20 msec, 20 mV. From Purpura *et al.* (1964).

neuron during stimulation of an afferent pathway are attributable to synaptic bombardment by inhibitory and excitatory neurons interposed between the thalamocortical afferents and the element under examination. Some evidence of the extraordinary complexity of the patterns of these excitatory and inhibitory synaptic drives on a cortical neuron during primary and augmenting responses is shown in Fig. 10. Weak VL stimulation first evoked in this cell a prolonged EPSP which increased in magnitude with progressively stronger stimulation until cell discharge was secured. Build-up of additional EPSP's and IPSP's was observed to the point of supramaximal VL stimulation. Then single shock VL stimulation elicited a short-latency EPSP with two superimposed discharges and a long-latency IPSP (Fig. 10, B, 1).

The initial surface-positive component of the primary response was associated with the EPSP, whereas both the negative and late, prolonged surface-positivity were temporally related to the IPSP. Low-frequency (7/sec) VL stimulation resulted in loss of the IPSP, enhancement of EPSP's (and cell discharge) and attenuation of long-latency surface-positivity. It is seen from this that the transition from one to another type of evoked cortical potential is reflected in part in reorganization of excitatory and inhibitory synaptic bombardment. The consequence of this is primarily seen in alterations in components of evoked potential which are temporally related to PSP's exhibiting the maximal change.

IPSP's have been recorded in different patterns of synaptic activities in both corticospinal neurons and interneurons in motor cortex during virtually all phases of primary and augmenting responses to VL stimulation (Purpura, 1964). IPSP's have also been observed in interneurons during recruiting responses and generally as late components of synaptic activities in Betz cells.

Although different EPSP–IPSP patterns have been observed in different populations of cortical neurons during evoked thalamocortical activities, emphasis is to be placed on the remarkable stability of PSP patterns in a neuron or group of neurons during the development of a particular variety of evoked activity. Such stable patterns have been recorded in which inhibitory as well as excitatory synaptic activities have predominated. This finding is illustrated in recordings from cells impaled seriatim in the course of single penetrations of cortex.

In the experiment of Fig. 11 intracellular recordings were obtained from four neurons with vertical separations of about 50–100 μ. Relatively constant augmenting responses were elicited throughout the period in which intracellular recordings were made. It can be seen that only the superficially located element in this series exhibited short-latency EPSP's and repetitive discharges during augmentation. The others exhibited complex IPSP patterns which were remarkably similar in overt characteristics. This was especially prominent in responses to the third stimulus of the repetitive train (Fig. 11, B-D).

It is reasonable to conclude that, as in the case of thalamic neurons, the synchronously developing IPSP's in this population of cortical neurons contribute significant positivity to extracellularly recorded responses.

What is apparently of major importance in determining the magnitude, polarity and time course of cortical surface or focal potentials is the sum of EPSP's and IPSP's

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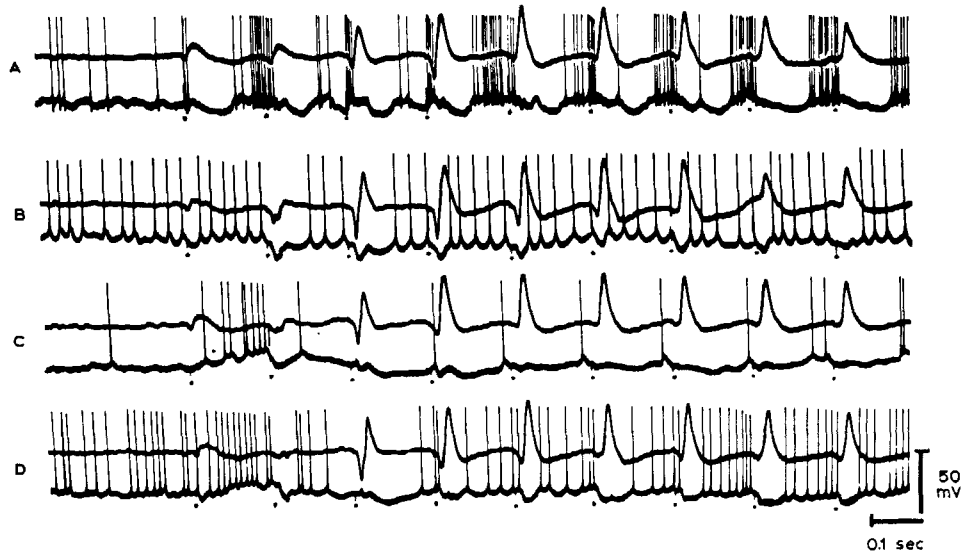

Fig. 11. PSP-patterns evoked during augmenting responses in four neurons impaled during a single penetration of cortex. Approximately 10–20 min elapsed between recording each series of responses. (A) cell located at a depth of 0.8 mm exhibits high-frequency repetitive discharges during surface-positivity, and late IPSP's during surface-negativity. Discharges re-appear prior to stimulation. (B) cell at a depth of 0.85 mm. Short-latency complex IPSP's observed throughout period of stimulation. (C) cell at 0.95 mm shows IPSP's during augmentation. (D) cell at 1.00 mm exhibits IPSP's during all phases of cortical surface response. Note similar IPSP-patterns observed in (B–C) during third response of each series. From Purpura *et al.* (1964).

generated in the immediate vicinity of the recording electrode and the contribution of electronic potentials arising from distant synaptic activities. Extracellular summations of EPSP's and IPSP's initiated at axosomatic and axodendritic sites are thus shown from these and other studies to be the major elements in the production of evoked potentials with characteristics similar to those of spontaneous brain waves (Purpura, 1959).

COMMENT

The foregoing survey of a recent series of studies of intracellularly recorded activities of thalamic and cortical neurons has provided new evidence for the important role of inhibition in a wide variety of complex electrocortical events. Two aspects of these studies are particularly relevant to the analysis of neurophysiological mechanisms underlying thalamically evoked EEG-synchronization: *(1)* the finding of prominent inhibition, of the postsynaptic variety, of intrathalamic organizations as a feature of the subcortical stage of thalamic neuronal synchronization; and *(2)* the identification of inhibitory postsynaptic potentials as components of all types of evoked thalamocortical responses. It follows from these findings that the development of EEG-synchronization involves a dramatic reorganization in the patterns of synaptic drives in thalamic as well as cortical neurons. Particularly noteworthy is the fact that the

synchronization of thalamic neuronal discharge results from activation of inhibitory elements which tend to restrict the effects of excitatory synaptic drives to relatively short periods between prolonged IPSP's.

The patterns of evoked EEG-synchronization initiated by low-frequency stimulation of the thalamic reticular system have long been considered similar to those observed in the drowsy or lightly sleeping animal. Although it has not been possible to define the behavioral effects of midline thalamic stimulation in the present studies of intracellular activities, it would be suprising indeed if the analogous EEG-synchronization associated with light sleep in unrestrained animals involved thalamic and cortical synaptic mechanisms fundamentally different from those described here. This also applies to the findings noted above in respect of the intracellular studies of reticulocortical activation. If the present findings may be extended to analyses of sleep with EEG-synchronization, then the finding of widespread inhibition of thalamic neurons as the major factor in eliminating spontaneous on-going activity and synchronizing neuronal discharge may be considered the essential element in the chain of neural events underlying the transition from wakefulness to sleep. And the fact that inhibitory processes are also demonstrable as components of the neural events involved in reticulocortical arousal serves to underscore the historical significance of Sechenov's discovery of central inhibition.

SUMMARY

The studies summarized here were carried out in 'encéphale isolé' cats. Intracellular recordings were obtained from over 200 thalamic neurons and 134 neurons in motor cortex of which 25 were identified as Betz cells by antidromic pyramidal tract stimulation. Analysis of evoked membrane potential changes in cortical neurons was facilitated by injection of hyperpolarizing currents through recording electrodes. Additional details of this work and its relationship to investigations of others are to be found in the reports of Purpura and Cohen, 1962; Purpura and Shofer, 1963, 1964 and Purpura *et al.*, 1964.

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Slow Surface Negative Potentials of the Cortex and Cortical Inhibition

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A stimulus of great intensity applied to the surface of the cortex elicits a negative potential of unusually long (hundreds of msec) duration (Chang, 1951; Goldring and O'Leary, 1960), the nature, origin and physiological value of which remain obscure. This electric reaction becomes particularly interesting in the light of the problem of cortical inhibition.

METHODS

Subjects and operation. Cats were used throughout under deep nembutal narcosis (80–130 mg/kg). The animals were fixed in a stereotaxic apparatus which was earthed. The cerebral cortex was exposed and its temperature controlled; by the end of the operation it was usually 29–32°. For recording from the exposed cortex, silver ball Ag-AgCl electrodes of 0.5 mm diameter were used. The stimulating triple electrode consisted of silver wires of 100 μ in diameter, cemented together into a triangle; the sides of the triangle were 50 μ.

Stimulation. Two stimulating electrodes were connected with one pole of the stimulator and their potential was monitored by two potentiometers; the third electrode was connected to the other pole (*cf.* Landau, 1956). The stimulation employed consisted of square wave pulses from the stimulator with a radiofrequency output, with output resistance of 400 Ω and with an output for sweep synchronization.

The first electrode ('active') was placed near the stimulating electrode, the second on the cortex at a distance of 11 mm or more. Cortical potentials were amplified with a DC amplifier, Model UIPP–2, having a maximal amplification coefficient of 50 000, with a symmetrical input whose resistance was 1 MΩ.

Recording was done either with the help of a cathode-ray oscillograph type ENO–1 with a driving sweep at a slow or fast speed, or on a loop oscillograph type N–102. Recordings of slow potentials from different layers were obtained through metallic electrodes insulated with glass, and discharges of single neurons through glass pipettes. Potentials in these experiments were amplified with an RC amplifier UBP 01–1 (Biofizpribor) with a cathode follower at the input.

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RESULTS

Under deep nembutal narcosis a stimulus (0.05 msec) of near-liminal intensity applied to the cortical surface elicited near the stimulating electrode a negative potential of 20–30 msec duration, a dendritic potential (DP) whose amplitude increased with an intensification of the stimulation (Fig. 1; 1–3). The negative potential weakened and its latency increased on shifting the recording electrode; it was monitored over a distance of up to 6 mm (Chang, 1951; Roitbak, 1955; Ochs and Booker, 1961). The following mechanism of the initiation of DP is most probable: impulses from the stimulated fibres of the first layer reach axodendritic synapses thus giving thea ppearance of postsynaptic potentials of apical dendrites (Eccles, 1951; Roitbak, 1953;

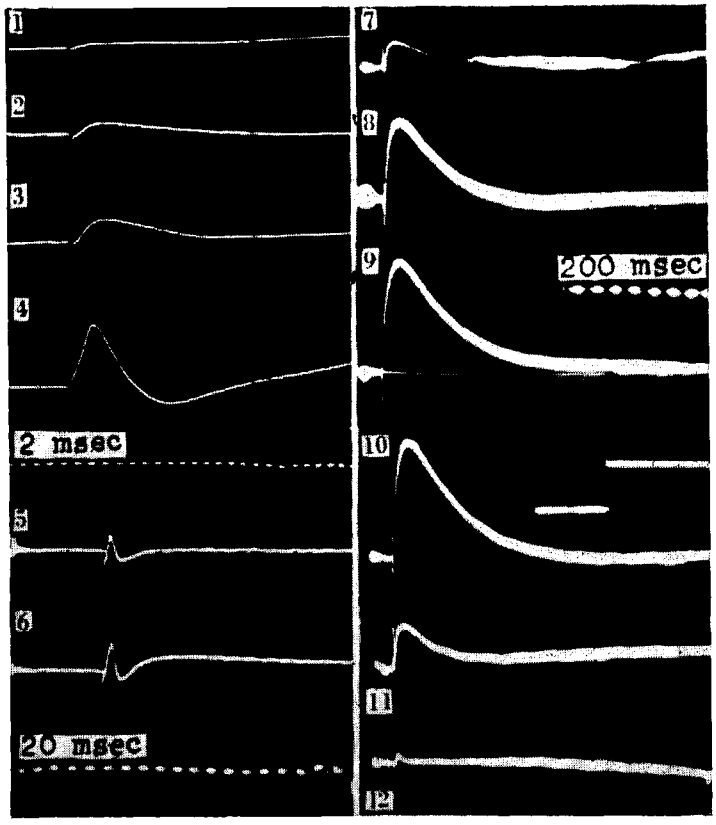

Fig. 1. Characteristics of slow negative potentials. Deep nembutal narcosis, electrodes on gyrus suprasylvius, distance between the stimulating and the 'active' recording electrodes, 0.75 mm. (1–4), thresholds of elicitation of dendritic potential (DP) and slow negative potential (SNP). (1) intensity of stimulus 1 V (0.05 msec), threshold of eliciting DP; (2) 3 V; (3) 4 V; (4) 10 V, threshold for eliciting SNP. The rest of the records are taken from the experiments on another preparation. (5–6) appearance of SNP as the result of summation of the action of subliminal stimuli. (5) intensity of stimulation 7.5 V (0.15 msec), DP appears; (6) effect of two stimuli with an interval of 5 msec: SNP appears too. (7–9) SNP amplitude increases with intensification of stimulation. (7) 5 V (0.5 msec); (8) 10 V; (9) 20 V. Voltage calibration 1 mV. (10–12) locality of SNP. (10) leading off electrode is0.75 mm apart from the stimulated point; (11) 2 mm apart; (12) 3 mm apart. Negative is up in all records.

Purpura and Grundfest, 1956). A further intensification of the stimulation evokes a positive potential after the DP followed by a second negative oscillation of a longer duration (Fig. 1: 4).

Characteristics of the slow negative potential

The slow negative potential (SNP) appeared when the intensity of the stimulation was approximately ten times more than the threshold for eliciting the DP; with further intensification of the stimulation the SNP increased within certain limits, reaching the amplitude of 2–3 mV; the SNP duration increased too (Fig. 1: 7–9) and sometimes

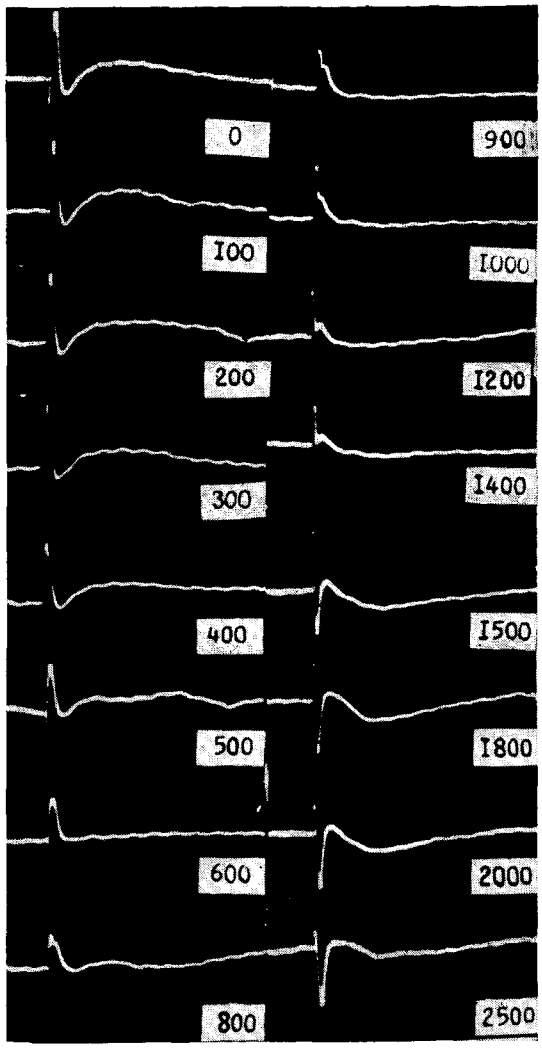

Fig. 2. Reversal pattern of SNP. Deep nembutal narcosis. Recording needle electrode and stimulating electrode are on lateral gyrus, 2 mm apart from each other. Intensity of stimulation 40 V (0.05 msec). The needle electrode is inserted into the cortex. Under each oscillogram the depth of penetration is marked in μ.

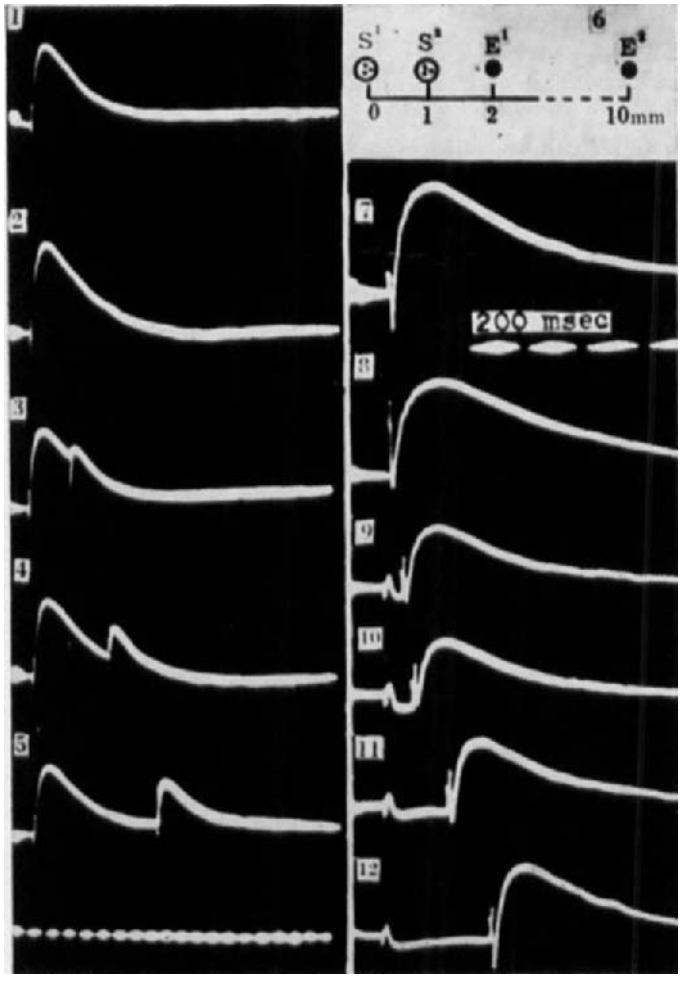

Fig. 3. On the left, effect of paired stimuli delivered to one cortical point. Distance between the stimulating and 'active' recording electrodes 0.75 mm; intensity of stimuli 10 V (0.5 msec). (1) One stimulus; (2) two stimuli, interval of 5 msec; (3) 500 msec; (4) 900 msec; (5) 1500 msec. Time 200 msec. On the right, modification of the stimulation effect in connection with appearance of SNP under the stimulating electrode evoked by stimulation of another cortical point. Another preparation. (6) Scheme of placement of two stimulating (S^1 and S^2) and recording (E) electrodes. (7) Response in E^1 to stimulus S^2 (30 V; 0.2 msec). (8) SNP in S^2 to stimulus S^1 (30 V; 0.2 msec); in E^1 no slow negative potential is recorded. (9) Response in E^1 to stimulus S^2 applied after stimulus S^1 with an interval of 50 msec, (10) 100 msec; (11) 200 msec; (12) 350 msec.

lasted up to 3–4 sec (Fig. 1: 9), but usually it was of 300–500 msec duration (Fig. 5). The SNP appeared with a greater (about 15 msec) latency; it attained its maximum during 50–90 msec and fell exponentially. The SNP may occur as a result of summation of the action of 2 stimuli, each subliminal for its appearance; it was clearly manifested when the interval was 5 msec (Fig. 1: 5 and 6). So the SNP appears as a result of a considerable intensification of the stimulation or of repetitive stimulations.

The SNP decreased sharply upon extension of the distance between the points of

the stimulation and the recording, and was not usually recorded when this distance exceeded 3 mm (Fig. 1: 10–12). So the SNP is a more local reaction than the DP: the SNP may be recorded from the circumference of 33 mm^2 of the cortical surface, whereas DP is recorded from the circumference of 120 mm^2.

When the electrode is inserted into the cortex, the SNP gradually weakens, and at the depth of 600 μ may completely disappear; then with deeper penetration, a positive potential may be recorded; at a depth of about 2500 μ it disappears (Fig. 2). Thus the SNP is generated by the elements of upper layers. Configuration of the electric field points to the conclusion that, with appearance of a SNP, there takes place a depolarization of the upper portions of the elements along the whole thickness of the cortex, *i.e.* of apical dendrites of pyramidal neurons. The above example shows that the SNP may 'reverse' the sign at a higher cortical level than the DP.

Effect of paired stimuli

The SNP to the second stimulus was reduced even when the intervals were above 2000 msec; the shorter the interval, the greater the degree of reduction (Fig. 3: 3–5); with intervals of 20 msec and less, the response to two stimuli exceeded that to a single stimulus in magnitude and duration. This summation phenomenon was pronounced at the interval of 5 msec (Fig. 3: 2). Similar results were obtained when the stimuli were delivered to two cortical points, with the recording in between them (see the scheme in Fig. 5).

The experiments whose recordings are illustrated on the right in Fig. 3 were performed as follows (see the scheme in Fig. 3). On one line two stimulating (S^1, S^2) and one 'active' recording electrodes were placed so that S^2 would be in the area where stimulation through S^1 elicits a SNP; and the recording electrode (E^1) at such a distance that stimulation through S^1 did not elicit a SNP, but through S^2 did. Stimulation at S^2 was delivered after a certain interval following that in S^1. As shown, the SNP recorded in E^1 weakened thereupon, and the degree of reduction was proportional to the amplitude of the SNP in S^2 (Fig. 3: 7–12). By a similar arrangement of the experiment, when a stimulus of small intensity was delivered through S^2 which evoked in E^1 only a DP, it was shown that no possible interval between the stimulus in S^1 (which elicited a SNP in S^2) and that in S^2 could bring forth any change in DP. Thus, when a SNP occurred at a given cortical point, the stimulus delivered to this point evoked a weakened SNP in neighbouring points, but fully maintained its effectiveness in eliciting DP.

Steady negative deflection

Repetitive stimulation of the cortical surface evokes a steady negative deflection (Bishop and Clare, 1953; Beritov and Roitbak, 1955; Goldring *et al.*, 1961). Steady deflection is not a result of DP summation. Fig. 4 (7) shows that at 50 per sec frequency the DP to single stimuli weakens rapidly; steady deflection appears when DP's reach the vanishing point, 100 msec after the beginning of stimulation. Steady deflection is

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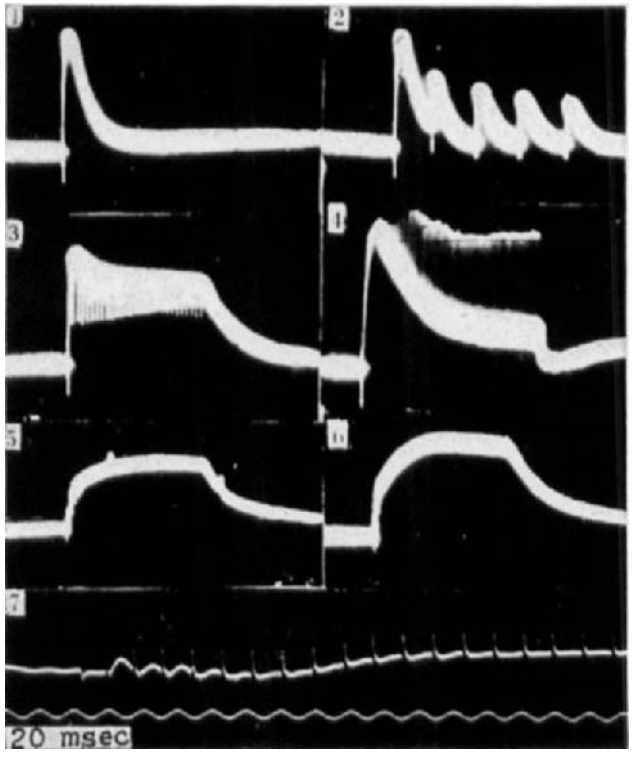
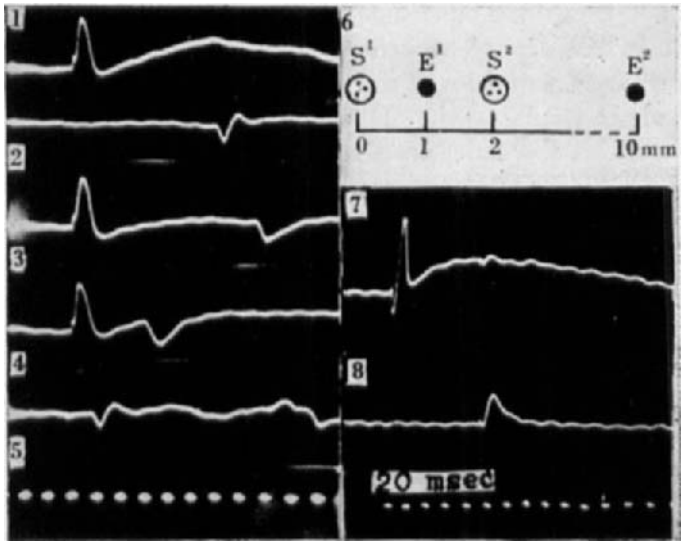


Fig. 4. Steady negative deflection. Electrodes on suprasylvian gyrus. Distance between stimulating and 'active' recording electrodes 0.75 mm. (1) One stimulus (10 V, 0.5 msec); (2) stimulation frequency of about 0.5 per sec; (3) of about 10 per sec; (4) 160 per sec. Recordings 5 and 6 are obtained with another preparation. (5) Stimulation frequency of 10 per sec (20 V, 0.05 msec); (6) 20 per sec. Duration of shot 10 sec; calibration as in Fig. 1. Recording 7 is obtained on another preparation. Beginning of stimulation, frequency 50 per sec.



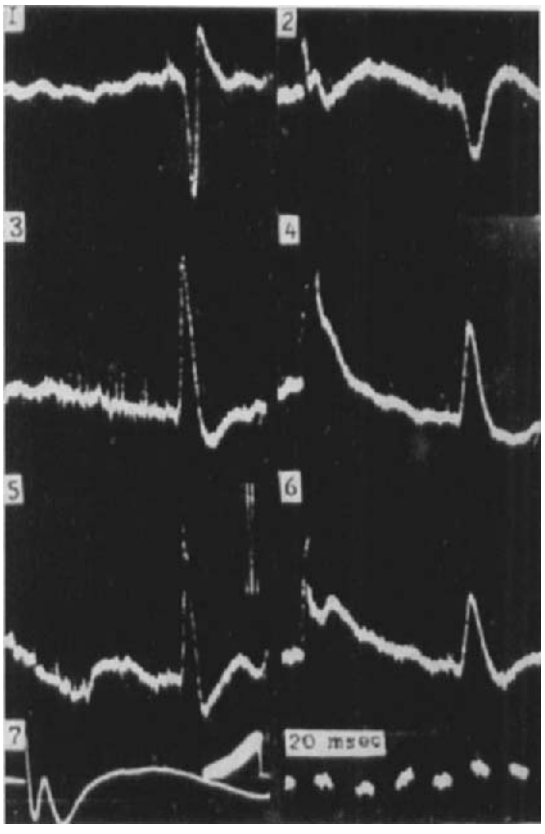


Fig. 6. Modification of primary response during SNP. Cat is relieved from nembutal narcosis. Leading-off glass microelectrode and stimulating electrode are on the posterior sigmoidal gyrus in area 1 of somatosensory region, the distance between them 0.5 mm. (1) Microelectrode is near to the cortical surface (150 μ), response to electrical stimulation applied to the skin of the contralateral fore paw; (2) effect of the same stimulation 100 msec after the stimulus applied to the cortical surface (0.2 msec, 30 V); (3–6) microelectrode is plunged in to a depth of 1600 μ; repetition of the same combination of stimuli; (7) macroelectrode instead of microelectrode; recording from the cortical surface through an amplifier with a large time constant; response to a cortical stimulation is demonstrated (0.2 msec, 30 V). Voltage calibration is 0.25 mV on 5, and 0.5 mV on 7.

the result of SNP summation — the conclusion also drawn by Goldring and O'Leary. Steady deflection as well as SNP is recorded at smaller distances than DP.

Steady deflection increased during 0.5–1.5 sec; the faster the stimulation rate, the

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Fig. 5. Modification of evoked potentials during slow negative potential. On the left, modification of primary responses. Electrodes on the posterior sigmoidal gyrus; the recording one is 1,2 mm apart from the stimulating; the second recording electrode is on the middle part of the suprasylvian gyrus. (1) Response to a stimulus applied to the cortical surface (40 V; 0.05 msec); (2) primary response to electrical stimulus delivered to the skin of the contralateral fore limb; (3) cortex + skin, interval between stimuli 150 msec; (4) 60 msec; (5) primary response to cutaneous stimulation. On the right, modification of DP. (6) Scheme of electrode placement. (7) Effect of the same stimulation 100 msec after the stimulation through electrode S^2 (40 V; 0.05 msec); (8) Response in E^1 to stimulation delivered through electrode S^1 (20 V; 0.05 msec).

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quicker it reached its maximum; with the frequency of 10–30 per sec it held out at the attained level during many seconds of stimulation (Figs. 4: 5 and 6). At frequency above 50 per sec steady deflection, after reaching its maximum, began to fall, relatively quickly at the beginning (Fig. 4: 4); after cessation of the stimulation it disappeared during 2–3 sec. Repeated stimulations with an interval of several seconds produced a markedly weakened effect.

Changes in evoked potentials against the background of SNP

Against the background of the SNP there takes place a marked depression in DP. It is observed both when the DP is evoked by means of the second stimulus delivered through those electrodes through which the SNP is elicited, as well as when stimulation is delivered to another cortical point (Fig. 5: 6–8). Thus, during the SNP the ability of apical dendrites to generate electrical potentials in response to arriving impulses is sharply inhibited.

During the SNP a regular change of primary responses is observed. When a cutaneous stimulation followed a cortical one with such an interval that the primary response appeared against the background of the SNP, the negative phase of the primary response disappeared and the positive phase had a greater amplitude and duration (Fig. 5: 3 and 4). At other times, at the peak of SNP there sometimes occurred a marked weakening not only of the negative but also of the positive phase. This is illustrated in Fig. 6. In these experiments the microelectrode was placed on the cortical surface; when it was inserted into the middle layers, a negative potential was recorded in response to cutaneous stimulation; this potential was followed by a positive phase of small amplitude (Fig. 6: 3 and 5); when the same stimulus was delivered after the cortical stimulation which elicited the SNP (Fig. 6: 7), the amplitude of the negative potential decreased, its duration increased and the positive phase disappeared (Fig. 6: 4 and 6). In other experiments, especially with recording from deep layers, the changes manifested themselves only in an increase in the duration of the negative potential and in the disappearance of the positive one. Thus changes in the primary response in connection with SNP which are recorded on the cortical surface reflect the changes that take place in its depth, in the region of generation of the initial phase of the primary response (Roitbak, 1955). If cortical stimulation were of such intensity as to elicit a DP without a subsequent SNP, it exerted no effect on the primary response.

The action of some pharmacological agents

Local strychnine poisoning somewhat augments DP and markedly increases the additional negative potential (a hump on the descending part of the DP), the latter being provoked by the discharge of the cells of surface layers (Roitbak, 1955); thereupon the positive potential after the DP disappears, and remarkably, the SNP disappears too (Fig. 7: 1–4).

Application of morphine leads to a weakening of the SNP even when DP is not yet considerably changed; a prolonged application of morphine gives an effect similar to that of strychnine (Fig. 7: 5 and 6). Under the action of γ-aminobutyric acid (GABA)

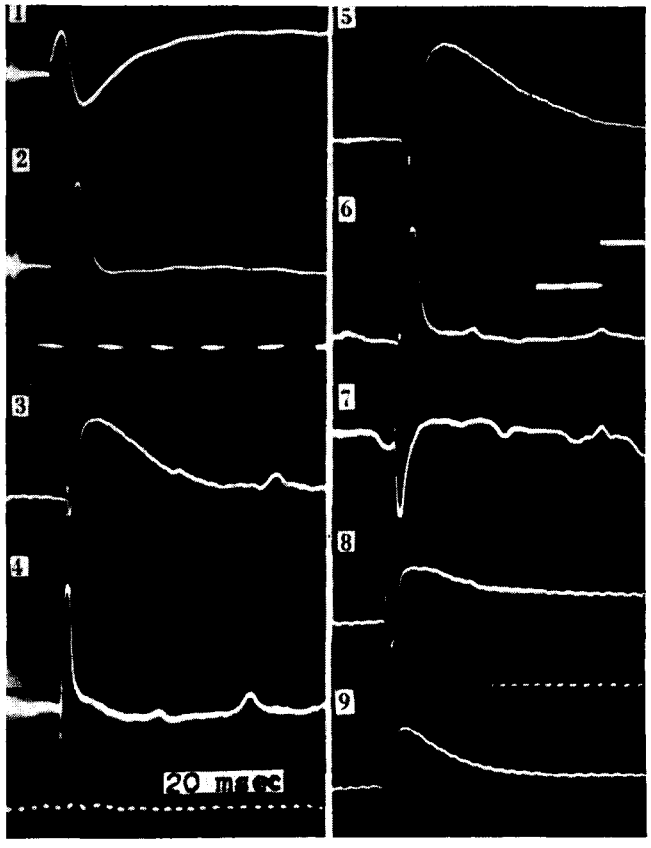

Fig. 7. Effect of pharmacological agents on slow negative potential. Electrodes on suprasylvian gyrus, the 'active' recording one being 0.5 mm apart from the stimulating electrode. The responses to the stimulus (30 V, 0.05 msec) before and after local poisoning under the recording electrode are given. (1–4) Effect of strychnine. (1) Before poisoning; (2) after strychninization (0.1%) during 1 min; (3) another preparation, before poisoning; (4) after strychninization during 2 min. (5–6) Effect of morphine; (5) the same preparation, electrodes on another area of gyrus, before poisoning; (6) after 5 min morphine poisoning (1%). (7–9) Effect of GABA; (7) the same preparation, 2 min after recording 6 had been obtained, poisoning of the same point with 1% GABA was performed; response to the same stimulation; (8) another preparation, before poisoning; (9) after poisoning with 1% GABA.

the sign of the DP 'gets inverted'*. In contradistinction to this, the sign of SNP never inverts. Those concentrations of GABA which provoke 'inversion' of the DP do no usually noticeably influenced the SNP (see also Goldring and O'Leary, 1960); greater concentrations of GABA reduce the duration and still greater concentrations

* According to Grundfest and Purpura, DP reflects the predominance of depolarizing over hyperpolarizing synapses, the action of which remains veiled. Depolarizing synapses are selectively blocked by GABA; and in response to the same stimulus a positive potential appears (Purpura, 1959). However, a number of facts indicate that the positive potential, which under certain experimental conditions replaces the DP, is the consequence of excitation of the elements of deep layers (Jasper, 1960; Goldring and O'Leary, 1960; Bindman *et al.*, 1962; Roitbak, 1963).

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reduce the amplitude of the SNP. GABA 'inverts' the sign of the initial negative complex enhanced under the influence of strychnine and morphine (Fig. 7: 7).

SNP and discharges of neurons

In these experiments, so far not numerous, the micropipette was plunged into the cortex; by its side on the surface was placed an electrode for SNP recording; a stimulating electrode was placed 1–2 mm away. Sometimes in response to stimulation provoking a SNP there occurred a discharge of neurons at the tip of the microelectrode. This discharge occurred either during the DP or during the positive phase following the DP, but never during the SNP, and disappeared when the SNP developed. 'Spontaneous' discharges, if any, ceased during the SNP. With a stimulation frequency of about 10 per sec, 'spontaneous' discharges disappeared completely; at this time a steady negative deflection was recorded from the cortical surface. The discharges were restored 1 sec or more after the cessation of stimulation. Fig. 6 shows that from the depth of 1600 μ 'spontaneous' discharges were recorded (3 and 5); during the SNP evoked by stimulation of the cortical surface they were not recorded (4 and 6).

DISCUSSION

Origin of SNP

Analysis of SNP recordings reveals their similarity to electrotonic potentials of dorsal roots as concerns their shape, order of duration, the effect of paired and tetanic stimulation, stability to nembutal etc. (see recordings of dorsal root potentials in the works of Barron and Matthews, 1938; Beritov and Roitbak, 1947; Bonnet and Bremer, 1952; Wall, 1962; Eccles *et al.*, 1963; and others). The cause of catelectrotonus of the posterior root fibres is not yet clear; the latest of the suggested hypotheses proposes that their depolarization is evoked by a synaptic action exerted on them by special internuncial neurons (Eccles *et al.*, 1962, 1963).

The data cited support the view that in the cortical region where the SNP appears, fibres of the first layer do not change excitability and conduction. Consequently the SNP cannot be the result of depolarization of the first layer fibres. SNP's occur due to depolarization of apical dendrites. Most established is the opinion that negative potentials of the cortical surface reflect postsynaptic electrogenesis of apical dendrites, *i.e.* the total of elementary dendritic potentials (Purpura, 1959). But, as has been shown, the SNP is not the result of DP summation.

Recently important new data have been obtained about the cortex. It has been proved that branchings of the axons of stellate cells form contacts with glial cells, termed 'gliapses' by Galambos (1961). Pyramidal neurons are thickly covered with glia and synapses; glia (oligodendrocytes) come into intimate contact with apical dendrites (Scheibel and Scheibel, 1958). On the other hand, it was found that in response to a single electrical stimulus a glial cell develops an electrical potential of several seconds' duration (Hild and Tasaki, 1962). In response to a burst of impulses neurons generate a slow potential the duration of which is always within 100 msec.

It is tempting to believe that the SNP (and δ-waves of the EEG) are conditioned by the activity of glia. It may be assumed that the SNP (*i.e.* prolonged depolarization of apical dendrites) occurs during glia activation as a consequence of a release of some chemical substances into the gliadendritic cleft*.

Thus, proceeding from the hypothesis suggested, the SNP is not a synaptic potential; this opinion is substantiated also by the SNP stability to GABA which is an effective blocker of axodendritic synapses.

The mechanism postulated is evidently set in motion during an intense excitation of the complex of cortical neurons either by application of a strong stimulus, or by repetitive stimulations. This mechanism may start functioning under natural conditions of cortical stimulation: in the visual area a slow negative potential appears in response to a flash (after the primary response) (Livanov, 1962; Pearlman, 1963).

SNP and inhibition

In the spinal cord slow negative potentials of dorsal roots are accompanied by the process of inhibition and are assumed to be its cause (Beritov, 1946; Bakuradze *et al.*, 1947; Roitbak, 1950; Eccles *et al.*, 1962, 1963). The inhibition involves either the synaptic apparatus (Beritov and Roitbak, 1955) or presympathetic endings of afferent fibres (Howland *et al.*, 1955; Eccles *et al.*, 1963). Under barbiturate narcosis this inhibition is deeper and longer than in unanaesthetized preparations (Howland *et al.*, 1955).

Earlier an assumption was made that steady depolarization of apical dendrites exerts on the bodies of pyramidal neurons an action similar to that of a DC cathode applied to the cortical surface (Roitbak, 1955). Under the cathode's action there takes place a depression of both 'spontaneous' (Burns, 1958) and evoked (Bindman *et al.*, 1962) discharges of cortical neurons. The primary responses change in the same way as against the SNP background (Figs. 5 and 6).

During the SNP the negative phase of the primary response in the depth of the cortex is inhibited; this phase reflects local excitation (excitatory postsynaptic potentials) of the elements of the 4th and 3rd layers which appears under the action of afferent impulsation. During SNP 'spontaneous' discharges of cortical neurons are depressed. Our preliminary data fully corroborate the results of Li and Chou (1962). However, a study of a number of other works, in which electrical stimulation of the cortical surface was delivered during the recording of cortical neuron discharges, revealed that under those conditions of stimulation which result in depression of the discharges, for a period of up to several hundreds of msec the SNP occurred (Jung

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* We know of similar concepts on the origin of the steady negative shift during slow spreading depression (Ochs, 1962; Bures *et al.*, 1963), of 'superslow' rhythmic potential oscillations (Aladjalova, 1962) and background 'spontaneous' rhythmics (Sokolov, 1962). Sokolov came to the conclusion that it is impossible to account for posterior root electrotonic potentials by postsynaptic potentials. On the basis of the aforesaid participation of glia in their generation is quite obvious, the more so as in the gelatinous substance a great number of oligodendrocytes have been found and the connections of neurons with these glial cells are especially well pronounced.

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et al., 1957; Krnjevic and Phillis, 1963; Phillips, 1956). It may be accompanied by an increase in neuron membrane polarization; strychninization removes this inhibiting effect (Sawa *et al.*, 1963). This may be explained according to the dendritic hypothesis, namely that apical dendrites appear to be the apparatus of inhibition: electric currents which appear during dendrite depolarization exert a blocking action on the synaptic transmission (Beritov, 1961). The glia–dendrite mechanism suggested might be the basis of cortical inhibition with a safeguarding function. It is noteworthy that the SNP is inhibited by strychnine and morphine (the latter removes a general inhibition in the spinal cord) (Valdman and Arushanian, 1963; Kashakashvili, 1963).

On the basis of microelectrode studies in the retina Svaetichin *et al.* (1961) came to the conclusions that in the retina the glial cells perform a metabolic control of neuron excitability, that the process of inhibition is of glial nature and that neuropharmacological agents exert their first effect on the glia.

Finally it may be assumed that in the origin of spinal cord general inhibition ('presynaptic inhibition', according to Eccles's terminology) activation of glia participates as well, which provides depolarization of dendritic processes of the dorsal horn neurons and of presynaptic parts of afferent fibres.

SUPPLEMENT

Basic facts and theoretic suggestions of the present report are published in the *Pavlov Journal of Higher Nervous Activity* (Roitbak, 1963b). Some similar data and also some similar theoretical considerations, in particular on the analogy of SNP and dorsal root electrotonic potentials, and on the analogy of the depression of cortical neuron discharges associated with the SNP and the presynaptic inhibition in the spinal cord, are found by Stohr *et al.*, 1963.

SUMMARY

A stimulus of great intensity applied to the surface of the cortex elicits a negative dendritic potential (DP) lasting 20–30 msec and followed by a slow negative potential (SNP) of unusually long duration (Chang, 1951; Goldring and O'Leary, 1960), the origin and significance of which is obscure.

Our experiments were carried out on cats under nembutal anaesthesia, with Ag–AgCl electrodes and a DC amplifier. The results obtained show that the SNP arises at a greater intensity of stimulus than required for the DP. The SNP can be recorded at a distance of up to 3 mm from the stimulating electrodes, its latency being about 15 msec. SNP increases for 50–80 msec, reaching 2 mV or more, and lasts 300–3000 msec. It also arises in response to subthreshold stimuli following one another at a short (5–20 msec) interval. At the stimulation frequency of 10–500 per sec, a long-lasting negative shift is recorded. With paired stimuli, the SNP elicited by the second stimulus is reduced throughout the duration of the SNP evoked by the first stimulus. Thus, by its duration, and its relation to paired and tetanic stimulation, the SNP is similar to electrotonic dorsal root potentials of the spinal cord. On the other hand,

if one seeks an analogy among the components of EEG, the SNP can be compared with δ-waves. It is extremely sensitive to some pharmacological agents: it is weakened or abolished by local application of strychnine and morphine to the cortex; but it is comparatively resistant to γ-aminobutyric acid. Against the background of the SNP, the DP evoked by stimulation of other points of the cortex is depressed and the primary responses are altered: their negative phase is depressed while the positive one is augmented and prolonged. A similar change of primary responses occurs during natural sleep, upon extinction of cortical reflexes and under the action of the cathode. According to our data the same conditions of stimulation that evoke a SNP suppress the discharges of cortical neurons. Patterns of depth-reversal of the SNP indicate that there takes place a depolarization of apical dendrites of pyramidal neurons.

It is assumed that the SNP reflects depolarization of apical dendrites resulting from the activation of glia around them. Upon intensive excitation of a group of cortical neurons, excitation *via* neuroglial connections is transmitted to oligodendrocytes. This causes their long-lasting depolarization, up to a few seconds (Hild and Tasaki, 1962), release of chemical substances in the glianeuronal space and, consequently, depolarization of apical dendrites. Thus, the SNP is not the result of a direct post-synaptic process. Depolarization of apical dendrites and presynaptic parts of nerve fibers, entails inhibition of the corresponding pyramidal neurons. The supposed mechanism of mass inactivation of pyramidal neurons may be the basis for certain kinds of cortical inhibition.

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Basic (Alpha) EEG Rhythm as Electrographic Manifestation of Preventive Inhibition of Brain Structures

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When studying the regulation of a number of homeostatic functions one may notice a rather pronounced regularity: weak stimuli and small doses of biologically active agents elicit shifts contrary in their character to those provoked by stimuli of medium intensity. Fig. 1 shows average changes of acute leukocytic reactions in 72 rabbits in response to administration of 5% sodium nucleonate. One can see that small doses (4–8 mg/kg) evoke a statistically authentic decrease in the leukocyte count in blood; medium doses increase the leukocyte count, while administration of superstrong doses results in leukopenia. Investigations carried out in Udelnov's laboratory proved this to be so for a number of cardiovascular reactions (Udelnov, 1961; Kulaev, 1958; Lagutina, 1958; Kulaev and Lagutina, 1960; Rodionov, 1957; Kulagina, 1963; etc.). The same regularity markedly manifests itself in the control of the functional state of the central nervous system itself under the influence of neurotropic agents. It was shown that small doses of a typical stimulator, caffeine, increase inhibition of brain structures,

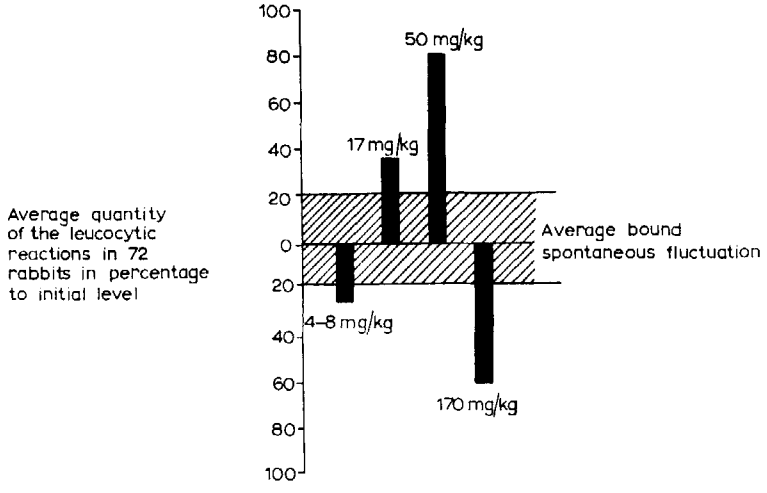

Fig. 1. Mean value of acute leukocytic reactions in 72 rabbits upon administration of different doses of sodium nucleonate. The striped band shows average limits of spontaneous oscillations in leukocyte count in blood.

that is, they prolong the effectiveness of the animal hypnosis in rabbits caused by fixation (Fig. 2).

Fadeyeva (1948) showed that small doses of strychnine decrease the average value of the conditioned alimentary salivary reflexes, whereas large doses first enhance the conditioned reflexes, then sharply suppress the conditioned reflex activity of the experimental animals (Fig. 3).

The regularity mentioned above seems to be biologicallyuniversal. Similar phenomena may be observed even in protozoa, as well as in an isolated nerve conductor, as

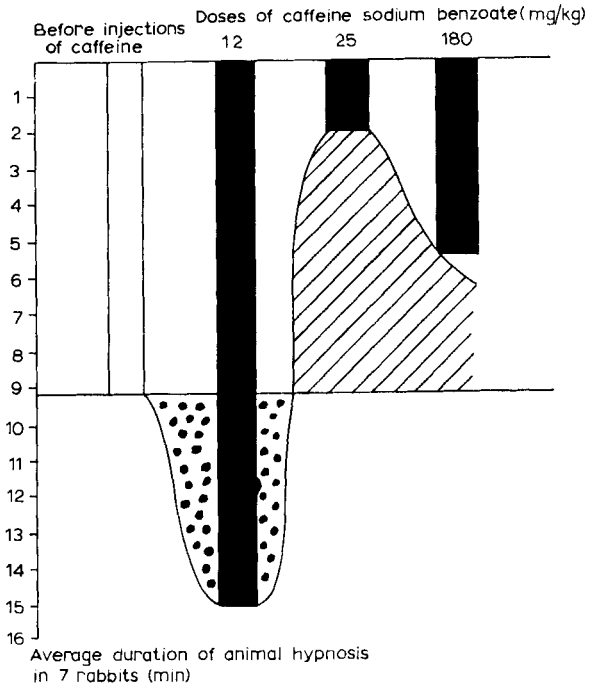

Fig. 2. Changes in the average duration of hypnosis in rabbits upon administration of different doses of caffeine sodium benzoate.

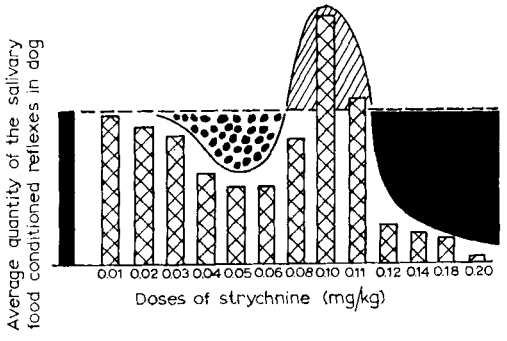

Fig. 3. Average changes of salivary alimentary conditioned reflexes in dogs upon administration of different doses of strychnine. (After Fadeyeva, 1948.)

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was shown by N. E. Vedensky's disciples A. A. Ukhtomsky, L. L. Vasilyev, N. V. Golikov and others. The shifts of homeostatic function, under the action of stimuli of moderate intensity and small doses of chemical substances, were called by us 'preventive inhibition' to lay the emphasis on the active nature of the resistance to occurring changes. It should be stressed that in our understanding preventive inhibition does not mean a particular physiological mechanism, but a regulatory principle, the determined-by-evolution program of involvement of apparatuses of homeostatic stabilization. By its functional architectonics preventive inhibition resembles, to a great extent, the scheme of a regulator with switching off of the disturbing action. At different stages of phylogenesis, in regulation of different functions of one and the same organism this scheme is manifested by physiological mechanisms infinitely different in their internal structure, and it would be rather naive to look for the signs of anodal subnormality in each of them. Intracellular biochemical circuits of feedback, certain types of interneuronal organization and interaction of specialized macrostructures, these are the possible variants of constructive realization of preventive inhibition.

In each of these variants the mechanisms of preventive inhibition still play their main physiological role, *i.e.* the function of keeping the organism from reacting to weak biologically insignificant stimuli, effecting the 'economic principle' (Pavlov) in the activity of the central nervous structures, and stabilizing the central and peripheral homeostasis.

Here a question arises: which electrophysiological phenomenon is most equivalent to activation of preventive mechanisms in the higher parts of the brain upon adequate, natural for-the-latter stimulation?

The results cited below were obtained on studying conditioned defensive reflexes in humans. In 8 young intact subjects recordings of EEG, skin-galvanic reflex and EEG with discrete frequency analysis were performed. After the background was recorded, each subject was told that at the end of the count down, from 20 to 0, he would receive a strong electric shock. Even a merely visual study of EEG registered the following pattern (Fig. 4). The beginning of the conditioned stimulus (the count) is accompanied by accelerated heart beat (revealed in the reduced duration of blocks composed of 5 successive strokes), by skin-galvanic reaction and a pronounced

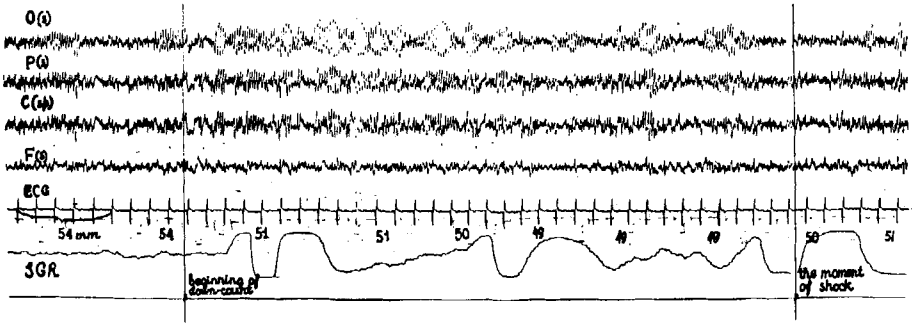

Fig. 4. Changes in EEG, heart rate and skin-galvanic reflex (SGR) during conditioned stimulation (count) in subject V.B.

augmentation of the α-rhythm. The sooner the moment of electric shock, the greater is the defensive excitation, the faster is the heart beat and the stronger is the skin-galvanic reflex. As to the EEG, here the former exultation of the α-rhythm is replaced by a distinct desynchronization.

The results obtained with the help of the analyzer present much more convincing data. Fig. 5 shows changes in the heart rate (top) and α-rhythm in percent as compared with the average background taken as 100% (bottom graph). One may see that acceleration of heart contractions, reaching its maximum during the count, is accompanied by a tendency towards depression of the α-rhythm. But still more important is the observation that in the period between the instruction and the beginning of the count, when the frequency of heart contractions is already rather considerable, the α-rhythm not only is not depressed, but even markedly augmented as compared with the background. An analogous pattern (Fig. 6) was obtained in another subject.

Graphs in the upper part of Fig. 7 show the average rate of heart contractions

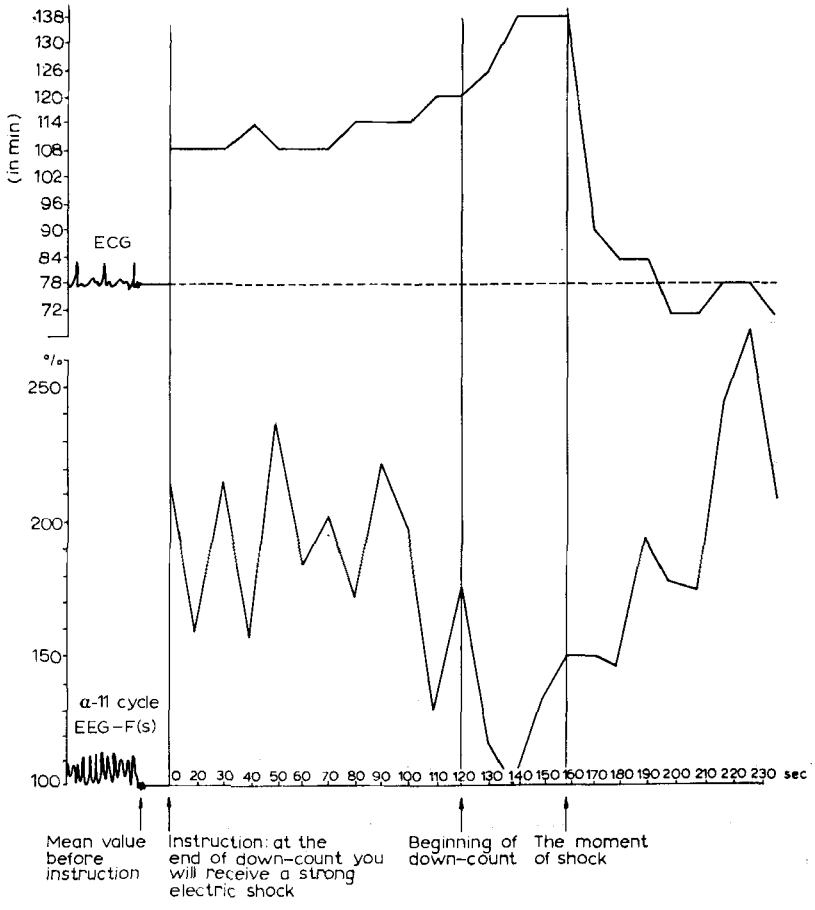

Fig. 5. Changes in heart rate (upper graph) and α-rhythm (lower graph) during the experiment on subject M.X.

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before the instruction, in the period between the instruction and the beginning of the count, and during the count which was to be followed by an electric shock. As may be seen, the nearer the pain reinforcement the faster the heart beat. The analysis of the EEG frequency spectrum shows that the period between the instruction and the beginning of the count (broken line) is characterized by augmentation of the α-rhythm, while the conditioned stimulus (the count) preceding the pain reinforcement provokes an increase of fast oscillations (continuous line on the bottom graph, Fig. 7). Analogous results, obtained in another subject, are illustrated in Fig. 8.

Hibel *et al.* (1954) in acute experiments on cats detected a parallelism between oscillations of the sympathic tonus (judged from the changes in blood pressure and in the eye pupil) and EEG activation. It is well known that vegetative shifts of synaptic nature are often accompanied by the α-rhythm depression. But how may one account, on the basis of peripheral vegetative reactions, for a pronounced augmentation, orderliness and exultation of the α-rhythm against the background of increased

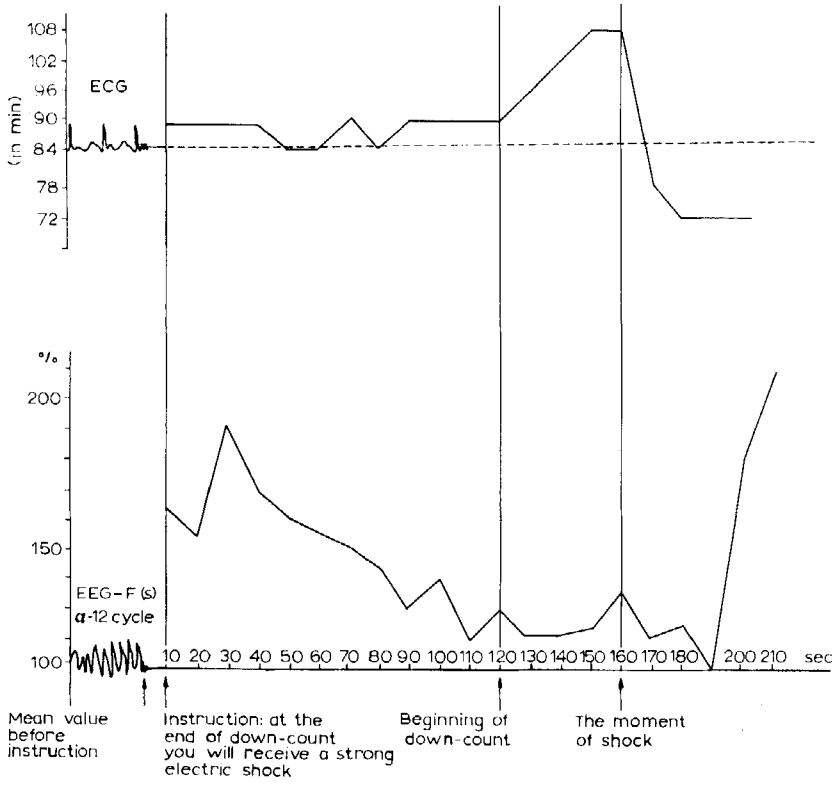

Fig. 6. Changes in heart rate (upper graph) and α-rhythm (lower graph) during the experiment on subject V.B.

excitation? Is it not much more common to record a distinct α-rhythm in the state of relative rest? Here it is pertinent to recall the results obtained by Rothballer (1956), Latash (1961), Kassil and Latash (1960) about biphasic changes of EEG upon administration of small doses of adrenalin. They showed that while an increased concentration of adrenalin in the blood results in typical desynchronization, small doses of this agent provoke the initial stage of synchronization, EEG deactivation. It is noteworthy that with administration of adrenalin the phase of synchronization coincides with the depression of hormone secretion of the adrenal cortex, and the phase of desynchronization with increased secretion (Graschenkov *et al.*, 1960). Thus we come to the conclusion that in the electrical activity of the brain to an increased, as compared to the initial background, excitation, regardless of whether it is natural impulsation or excitation caused by administration of exogenous adrenalin, frequently the first response is augmentation of the α-rhythm. But if the α-rhythm is augmented when it is fair to admit activation of the mechanisms stabilizing the intra-

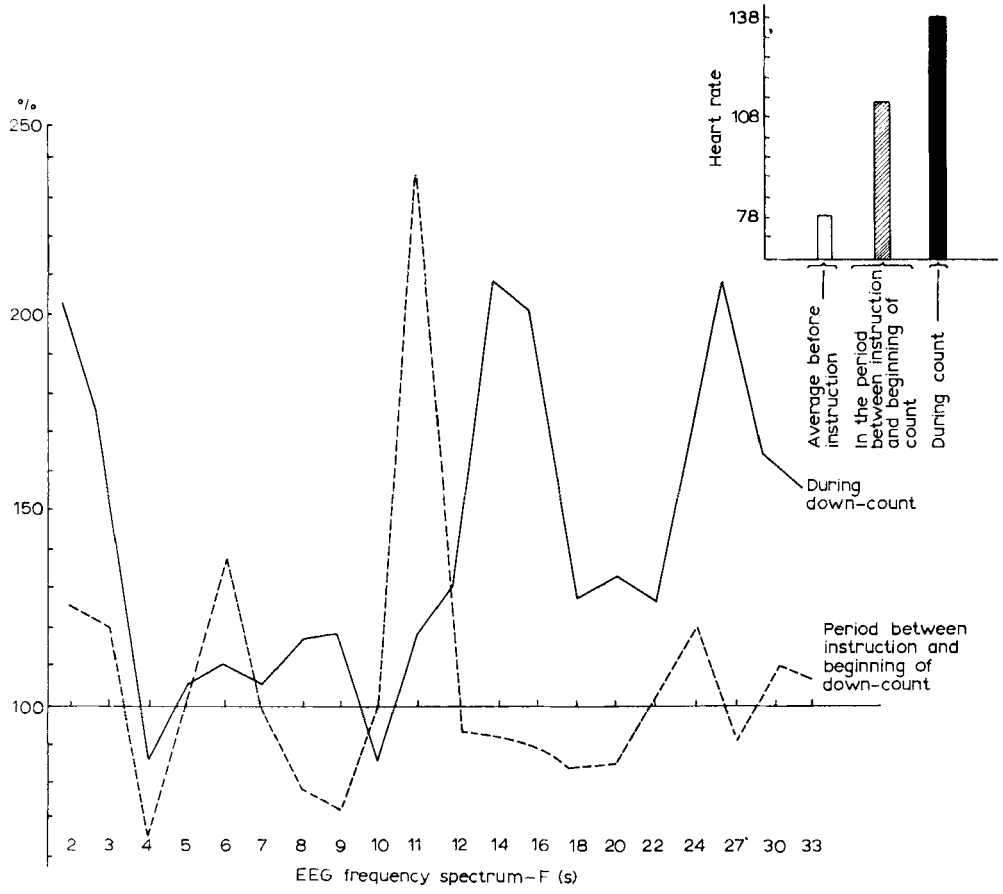

Fig. 7. Changes in heart rate (top) and EEG frequency spectrum (bottom) during 40 sec of the period between the instruction and the beginning of count (striped column, broken line) and during the count (black column, continuous line) in subject M.X.

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cerebral homeostasis, then is it not possible to consider the α-rhythm generally as an electrographic manifestation of the activity of those apparatuses which constantly perform the barrier function for the arriving-into-brain impulsation? Really, if one assumes the α-rhythm as an external manifestation of a constantly pulsating barrier, it will become clear why it is so well pronounced in the visual area of the cortex which is the last link in the chain of information arriving into the brain. Under usual circumstances the activity of the mechanisms of preventive stabilization as a rule is overcome by the inflow of excitation. Only in darkness and in relative rest do they come to the first plane. However, long inactivity of the mechanisms of preventive stabilization

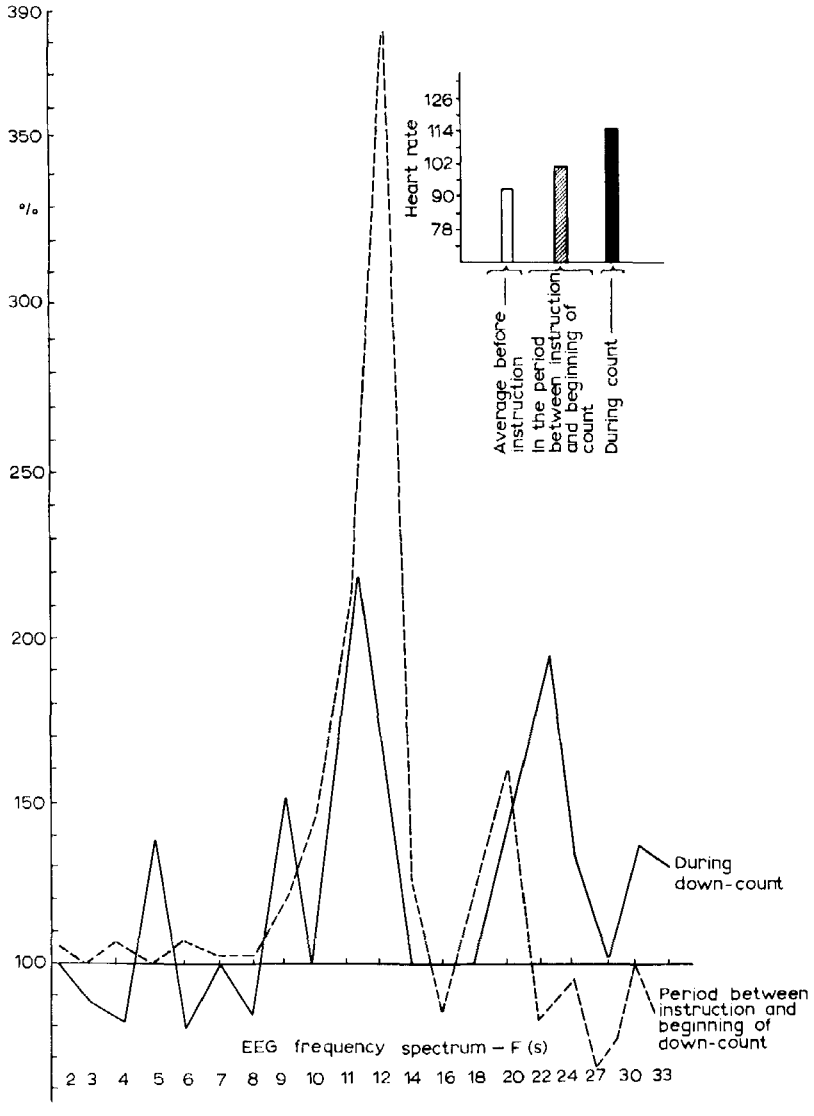

Fig. 8. The same as in Fig. 7. Subject V.B.

in blind animals and in animals kept in darkness leads to their functional atrophy, and, naturally, to disappearance of the α-rhythm (Adrian and Matthews, 1934; Novikova, 1960, 1963; Novikova and Belyaev, 1963; etc.). From this viewpoint the well-known phenomenon of driving of the rhythm of flashes may be considered as a peculiar 'brain flickering', similar to preventive blinking movements in humans and animals exposed to a continuous flickering of a strong light. The acceptance of α-rhythm as an electrographic correlate of the preventive inhibition agrees well with the results of Ivanitsky, who observed a regularization of the α-rhythm in patients suffering from light reactive psychosis, where other indications pointed to an enhancement of inhibitory processes (personal communication).

The acceptance of the inhibitory nature of the emergence of the α-rhythm does not contradict the concept of the 'biological clock', just as the hypothesis of the preventive function of central inhibition is in no way an obstacle in the profound study of its co-ordinative role. Finally, we are ready to share the concept on closed nerve cycles to be the structural basis for emergence of rhythmic electrical activity (Rusinov, 1954, 1960; Dubikaitis and Dubikaitis, 1963; etc.), with one reservation. We consider the mechanism of inhibitory autostabilization to be one of the most important links in these cycles. Shifts of the functional state of this particular link caused by modifications of external impulsation, largely determine various changes in the α-rhythm.

SUMMARY

Critical analysis of numerous literature data as well as our own experimental results induce us to consider the α-rhythm as an electrographic manifestation of preventive inhibition in cerebral structures called to provide their defense from excitatory impulses continuously arriving into the brain.

Systematic investigations of effector manifestations of preventive inhibition (Simonov, 1962) have revealed its following characteristics.

(1) The preventive inhibition, following the 'economic principle' (Pavlov) in the activity of the central nervous system, defends nervous structures from reactions to weak or biologically insignificant stimuli. This is the feature that distinguishes it from supramarginal inhibition appearing in response to superstrong stimulations.

(2) The preventive inhibition is especially pronounced under the action of weak stimuli or as the first phase of reaction to stimulation of medium strength.

(3) The preventive inhibition, as distinct from the supramarginal one, may be removed by sufficiently strong stimulation.

(4) In order to manifest itself the preventive inhibition arising in response to coming excitation requires a definite intensity of this excitation.

(5) The mechanisms of preventive inhibition possess the capacity to augment their activity under the influence of continuous excitation (adaptation) or repetitive stimulations (habituation).

(6) After cessation of stimulation generating the preventive inhibition the latter persists owing to its inertia.

Acceptance of the decisive role of mechanisms of preventive inhibition in the or-

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ganization of brain rhythms elucidates numerous and sometimes obscure bioelectrical phenomena, such as: the augmentation of the α-rhythm amplitude under the action of moderate stimuli or in the first phase of stronger stimulations; the most marked expression of the α-rhythm in the visual cortical area which receives the greatest number of external stimulations; the augmentation of the α-rhythm in the resting state and its disappearance after the subject has remained continuously in darkness (Novikova, 1960), as the result of gradually developing functional atrophy of the mechanisms of preventive inhibition; recruiting of the flickering rhythm as an expression of activation of mechanisms of preventive inhibition timed to the moment of stimulation ('brain flickering'); the blockage of the α-rhythm in patients with emotional vegetative instability (Latash, 1961) and, on the contrary, its regularization in certain groups of patients with reactive psychosis; the most pronounced 'spontaneous' rhythms in anterior brain parts, etc.

Accepting the concept of closed nervous circles to be the structural basis of rhythmic electrical activity, we consider the mechanism of inhibitory autostabilization the most essential link of these circles.

The rhythms arise not as the result of repetitive return of excitation to the initial point but owing to the fact that at one moment the mechanisms of preventive inhibition dominate over the continuously inflowing impulsation and at another they are overcome by it.

The augmentation of excitation firstly activates the mechanism of autostabilization, causing an exultation of the α-rhythm, and then overcomes it thus accounting for more frequent low-amplitude oscillations. On the other hand, the intensification of inhibitory mechanisms or short-lasting limitation of coming excitation leads to a slowing down of the rhythm and augmentation of the amplitude.

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The Possible Histological Basis of Inhibition

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When considering the possible morphological substrates of inhibition — in order to bring some system into the complex issue — we may ask certain very definite questions such as: is the inhibitory effect (1) a manifestation of specific neurons, or (2) of specific kinds of nerve endings, or (3) only due to localization of the synaptic nerve endings on specific sites of the postsynaptic neuron?

(1) Specific inhibitory neurons

The existence of specific neurons with the capacity to inhibit all other neurons with which they establish synaptic contact — even those of their own kind — is a logical necessity arising from the fundamental organization of the nervous tissue on one hand, and from certain tasks that have to be performed on the other. Some implications, in nervous coordination, of the well-known anatomical fact that muscles are rarely arranged in fixed antagonistic groups, but there are higher degrees of freedom of movement, requiring changeable groupings of muscles working together or against each other, were first systematically treated by Paul Weiss (1941). We have considered the consequences of this on the inhibitory mechanisms in an analysis of oculomotor reflexes (Szentágothai, 1952). The general assumption that otherwise excitatory pathways may have inhibitory collaterals has been refuted and the existence of a separate inhibitory pathway postulated — with at least one additional neuron incorporated — at a time when there was no direct physiological evidence of the specific inhibitory premotor neuron systems. Later, after the fundamental discovery of the specific inhibitory neurons in the pathway of reciprocal inhibition (Eccles *et al.*, 1954) we (Szentágothai, 1958) reconsidered the matter in the light of this new information, and elaborated a simple scheme of a premotor mechanism suitable for moving four muscles that act on a ball-joint in all possible combinations. I want to challenge those working in cybernetics and familiar with Booleian algebra to devise simpler circuits that would do the same job. The diagram (*l.c.* Fig. 6) postulated one thing in addition to the knowledge concerning inhibitory internuncial neurons already available, *i.e.* the capacity of these neurons to inhibit each other. This assumption is verified to some extent by physiological evidence on disinhibition of motoneurons by inhibition of inhibitory internuncials by Renshaw neurons (Wilson and Burgess, 1962).

It is remarkable that all specific inhibitory neurons hitherto identified, are short ones which, in general, bridge distances of a few millimeters. This offers some clues for the investigation of these neurons by histological means: by cutting all important extraneous connexions of certain parts of the gray matter, a goal achieved for example by isolating slabs of nervous tissue from the remaining part of the CNS, but leaving intact their blood supply, all nervous elements that originate outside can be brought to degeneration. In such isolated slabs, nerve cells and their synaptic connexions can survive indefinitely and after about two months of survival time all nervous elements encountered intact, particularly synapses, may safely be considered as originating from neurons that have survived in the slab. This procedure is therefore suited to separate from a large number of synapses normally present those that belong to short neurons or short connexions, *e.g.* initial collaterals of long neurons. If it were true that specific inhibitory neurons are short ones, one could hope for the preservation of the inhibitory synapses in relatively larger proportion than that of the excitatory ones that are derived mainly from distant connexions and therefore may be expected to degenerate in such circumstances.

We have been exploiting this mode of approach for several years in different regions of the CNS with the principal aim of identifying histologically inhibitory synapses that have been recognized or postulated on the basis of physiological experiments —mostly with microelectrodes. In spite of many efforts and some quite interesting results we failed until now to bring the inhibitory synapses to direct observation in motoneurons. We were able to identify in an isolated ventral horn preparation (Szentágothai, 1958) the excitatory synaptic knobs of motor axon collaterals on a group of small neurons located in the region where the ventral root fibres gather before leaving the ventral horn. It is highly probable that these small neurons are the Renshaw cells, which have not been identified previously by histological means. Instead of the expected inhibitory synapses of Renshaw neurons on the motoneurons only an extremely delicate plexus of preterminal fibres could be made visible around the motoneurons. Our explanation at that time was that the inhibitory contacts on motoneurons might be too small to be seen with the aid of the light microscope. This was supported by the observation of a similar plexus of nearly submicroscopical fibres in the oculomotor nucleus that could be traced back by degeneration to the nucleus of Darkshewitsch, which had already been shown to have an extremely powerful inhibitory action on the oculomotor nucleus (Szentágothai and Scháb, 1956; Scheibel *et al.*, 1961). A similar mode of presynaptic arborization was found in the column of Clarke (Szentágothai, 1961), and we have good reason for supposing this to be the histological counterpart of the short internuncial neurons surmised by Curtis *et al.* (1958) exercising inhibition on Clarke neurons. Our conclusion that the inhibitory synapses might be submicroscopic in size has recently been questioned by Whittaker and Gray (1962) on the ground that electron microscopy reveals many more synaptic terminals than ever imagined from light microscopy. This being primarily due to the lack of neurofilaments in a significant part of the synaptic terminals, it was suggested that our failure to detect the inhibitory endings might be the consequence of inhibitory synapses being devoid of neurofilaments and therefore

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not stained in our silver preparations. It is thus clear that only electron microscope analysis of these models will help us to identify the inhibitory synapses in motoneurons.

Fortunately two types of neurons have quite recently been identified as having inhibitory functions: the basket cells of the hippocampus (Andersen *et al.*, 1963a) and the basket cells of the cerebellum (Andersen *et al.*, 1963b). Both neurons are in fact relatively short and their synapses being known for a long time, we are now relieved of the unrewarding task of histologically identifying inhibitory synapses of motoneurons and may immediately proceed to a more close investigation of these two known inhibitory synapses.

(2) The finer structural properties of the inhibitory synapses

Both known inhibitory synapses have already been investigated with the aid of the electron microscope, and have been identified as belonging to Gray's 2nd type (Hamlyn, 1963; Szentágothai, 1963a). This would appear to give some support to the working hypothesis of Eccles (1964) according to which inhibitory synapses might be those of the 2nd type and excitatory ones those of the 1st type. As all spine synapses are of type 1, and the numerous spine synapses of the Purkinje cell dendrites with parallel fibres being well known to be excitatory in nature, this assumption could be supported also from the other side. Conversely, however, all synapses contacting the main dendrites of Purkinje neurons — from which at least those belonging to climbing fibres are certainly excitatory — appear to be of the type 2 (Figs. 1 and 2). The situation, therefore, is not clear, and the majority of axo-somatic synapses, especially in higher centres, being of the 2nd type it may perhaps be simply a general rule that synapses with cell bodies and proximal parts of dendrites are more likely to be of type 2 and those with more distal parts of dendrites and especially spines of type 1. This would also be in accordance with the occasional observation of the same terminal knob having a 2nd type contact with a cell body on one side and a 1st type with a dendrite on the other.

But the character of the postsynaptic membrane and the width of the synaptic cleft are not the only differences which might distinguish several kinds of synaptic contacts. Differences in the main synaptic organelles: the vesicles, their density, and occurrence of neurofilaments might be of equal importance. As generally known there are two main types of synaptic vesicles: the ordinary ones of about 400 Å diameter and the larger vesicles of 1500–2500 Å diameter with dark central body — the so-called dense core vesicles or osmiophilic vesicles, in some places and with some reason also called neurosecretory vesicles. The latter are most common in nervous structures rich in catecholamines, such as hypothalamus and vegetative nerve elements, so that they might indicate some adrenergic transmission mechanism. The smaller type of dense core vesicle may occur in any type of synapse, but generally not in larger concentration and almost always scattered among the ordinary ones, often at the far side from the synaptic contact region. In the known or suspected inhibitory synapses no peculiarities of the vesicles were encountered. The number and density of vesicles is often in inverse

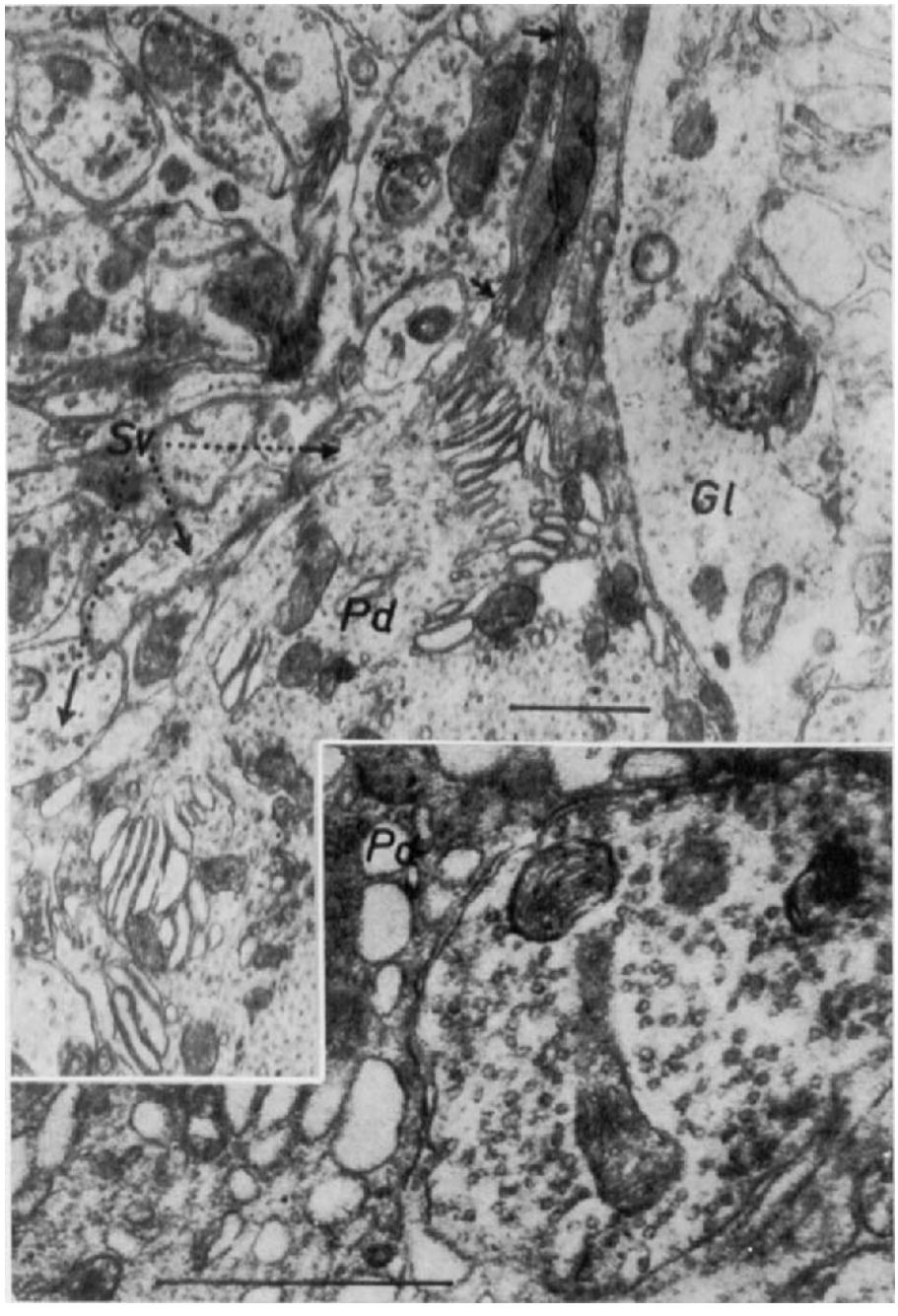

Fig. 1. Synaptic terminals of recurrent Purkinje axon collaterals on the main dendrites of Purkinje neurons. Upper micrograph shows a primary Purkinje dendrite (Pd) in transverse section from which a secondary dendrite is branching off in the upward direction. A synaptic ending attached (between the arrows) to the origin of this dendrite is a 2nd type contact after Gray. Similar endings containing synaptic vesicles (Sv) are attached to the primary dendrite. To the right the Purkinje cell dendrite is completely enveloped by glial tissue (Gl). The lower picture shows a Gray 2 type contact on a primary Purkinje dendrite (Pd) in larger magnification. Cat cerebellar cortex. Scale 1 μ.

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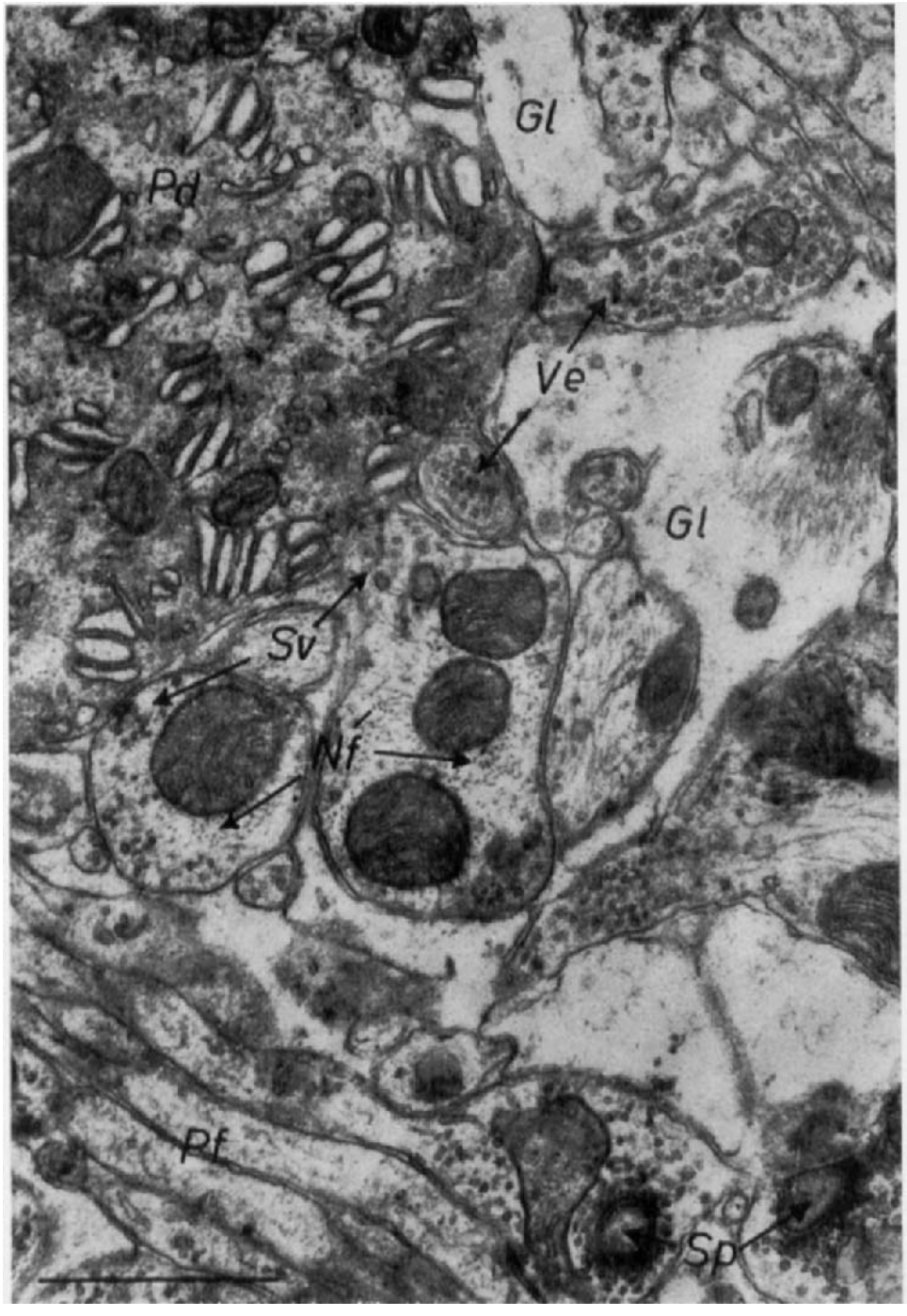
Gl
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relation to the content of neurofilaments. Strangely the terminals of the basket synapse in the cerebellum are unusually rich in neurofilaments, vesicles occurring only in the immediate neighbourhood of the contact region. This appears to be in sharp contradiction to the observation of Whittaker and Gray (1962) hinting at the possible non-filamentous nature of inhibitory synapses. An alternating arrangement of neurofilamentous and vesicular synaptic contacts has been described on the cerebellar Golgi neurons (Szentágothai, 1965), and both types occur on the main dendrites of the Purkinje cells (Fig. 2). Through the use of degeneration, the filamentous ending, in both types, has been identified (Hámori and Szentágothai, 1965b) as coming from climbing fibres, whereas the vesicular ending might be the termination of recurrent Purkinje axon collaterals. The climbing fibre being excitatory (Eccles *et al.*, 1964) there is obviously no relation between amount of neurofilaments and excitatory or inhibitory character of terminals.

(3) Localization of the presynaptic terminal on the postsynaptic neuron

According to the hypothesis put forward by Gesell (1940) the best strategic localization of inhibitory synapses would be on the axon hillock. This hypothesis has the merits of being based on a clear biophysical concept and could explain inhibition irrespective of whether the mechanism of transmission were an electric or a chemical one. Some direct physiological evidence of the inhibitory nature of a specific synaptic terminal of Mauthner's neurons coiled around the axon hillock (Retzlaff, 1957) has given some support to this hypothesis, and now the inhibitory nature of the Purkinje cell basket synapse — as will be shown later in this paragraph — would give additional support. However, even a cursory survey of the comparative histology of synapses strongly discourages any generalization of this kind. Many synapses having a whole system of coils around the axon hillock (the synapses in sympathetic ganglia of frogs) or baskets arranged around the axon hillock (in the ciliary ganglia of birds and reptiles, Szentágothai, 1964; Hámori and Dyachkova, 1964) are obviously excitatory in nature, being the only ones of these neurons. But there is perhaps no real contradiction in a biophysical sense, as the original hypothesis of Gesell implies that there are excited foci on the dendrites, *i.e.* relatively far from the axon hillock. The Purkinje basket synapse would beautifully fit into the hypothesis, due to the extremely large number of excitatory synapses on the dendrites, especially with the parallel fibres, the almost complete sealing of the surface of its main dendrites and the upper part of its body by glial elements and the location of the synaptic terminals of the basket axon branches

Fig. 2. Transverse section of main Purkinje dendrite (Pd) in the region indicated in Fig. 3. Terminal axon branches containing numerous neurofilaments (Nf) can be recognized as climbing fibres as they disappear in the chronically isolated folium. At places of close attachment to the Purkinje dendrite they contain few synaptic vesicles (Sv). More vesicular axonal profiles (Ve) (see also Fig. 1) can be identified as local axon collaterals as they survive in chronically isolated folia. Most parts of the dendritic surface of Purkinje cells are covered by glia (Gl). At the bottom of the figure several parallel fibres are cut longitudinally (Pf), and dendritic spines (Sp) — here probably from basket dendrites — are seen to be invaginated into thickened parts of the parallel fibres. The spine synapses are always of the 1st type. Adult cat. Scale 1 μ. (From Hámori and Szentágothai, 1956b.)

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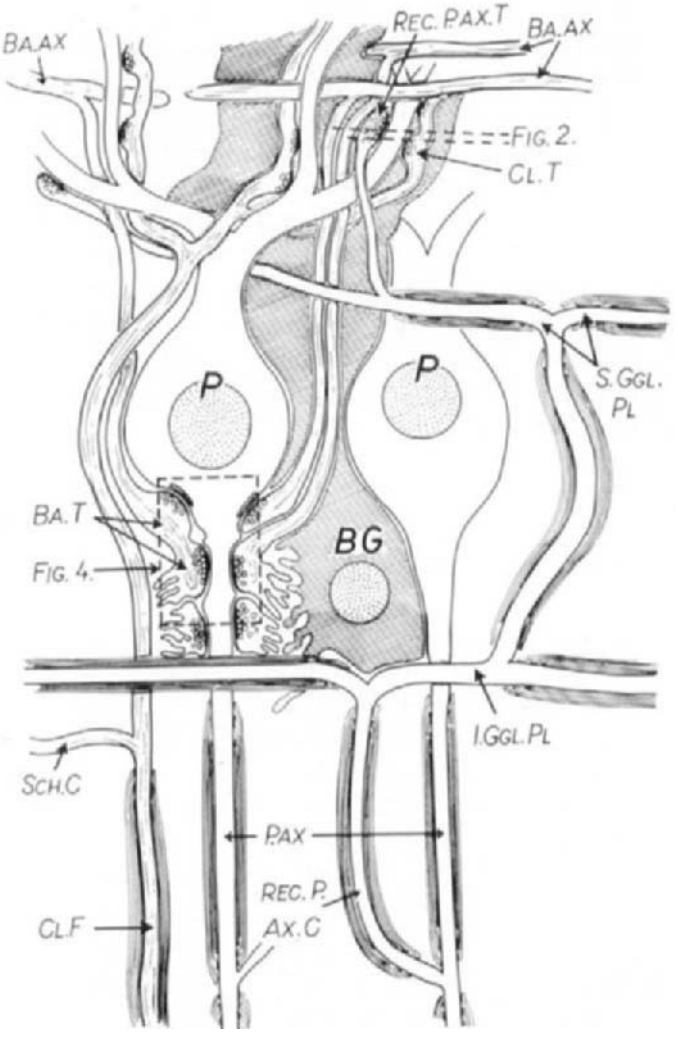

Fig. 3. Diagram illustrating synaptic relations on the somatic and proximal process regions of Purkinje neurons (P). The descending branches of basket axons (Ba.ax) have synaptic attachments (Ba. T) to the bottom region of the cell body and to the origin of the basal process of the Purkinje cell. This basal process which continues into the axon (P.ax) has no true axonal structure in its initial part. The recurrent Purkinje axon collaterals (Rec.P.ax.C.) are myelinated and reach their final contacts on the Purkinje dendrites (Rec.P.ax.T.) after having participated in an infraganglionic (I.Ggl.Pl) and a supraganglionic (S.Ggl.Pl) plexus. These axons are poor in neurofilaments and — after having given off their Scheibel collaterals (Sch.C) to Golgi cells — ascend along the main dendrites of the Purkinje neuron where they establish repeated contacts 'de passage' (Cl.F./T.). Most parts of the surface of the Purkinje cell bodies and the main dendrites are enveloped by Bergmann glia (BG) cells (hatched). The sites of the electronmicrographs of Figs. 2 and 4 are indicated by dashed lines.

around the origin of its axon. The synaptic terminals, as mentioned, are very rich in neurofilaments and have contacts of Gray's 2nd type. According to observations with the light microscope the ends of the basket axon branches follow the Purkinje axon

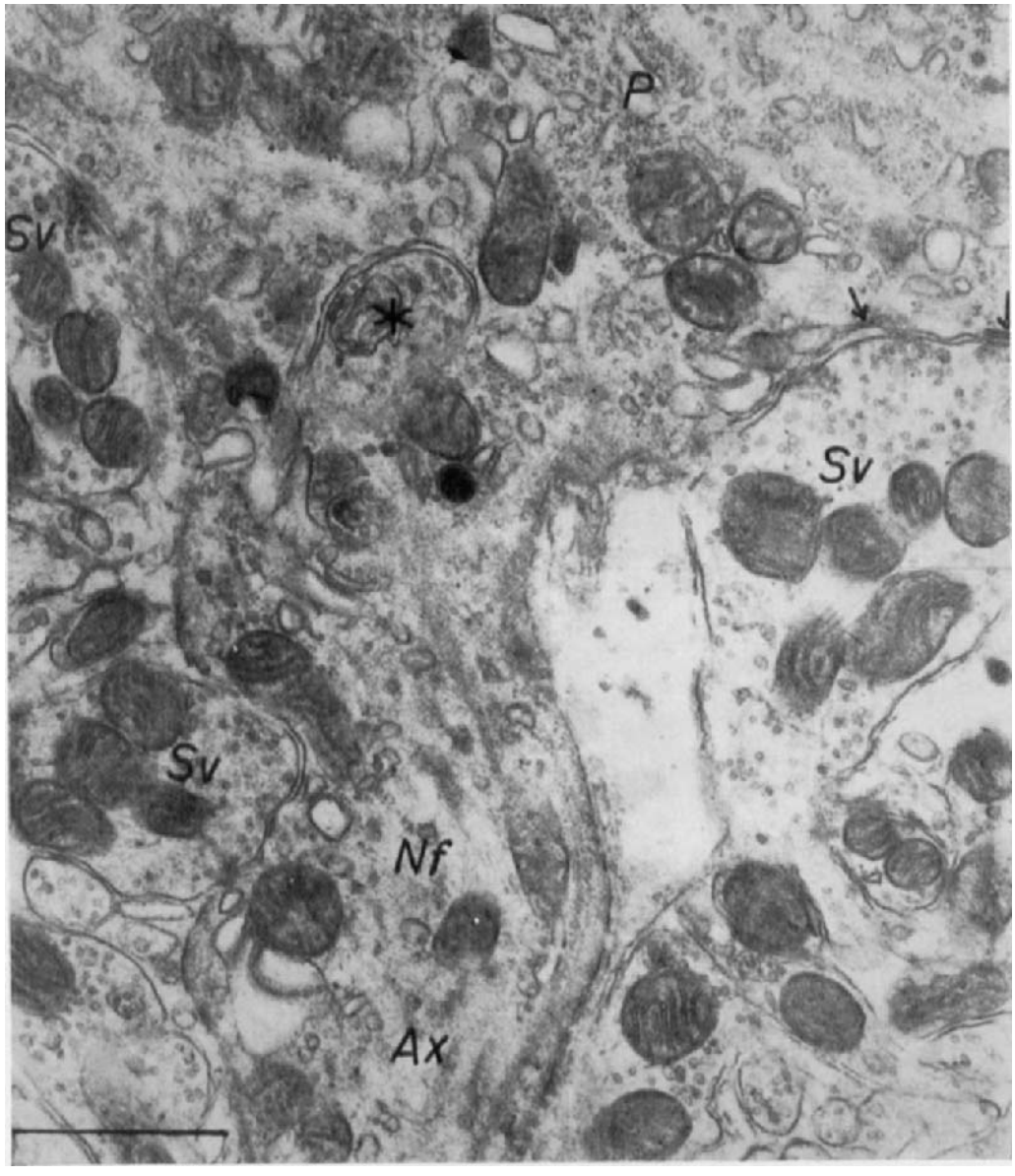

Fig. 4. Bottom and basal process of Purkinje cell (as indicated in Fig. 3). Basket axon terminals containing synaptic vesicles (Sv) are synaptically attached to the bottom region of the Purkinje cell body (P) and basal process which finally continues into the axon (Ax). Although containing neurofilaments (Nf) the initial part of the basal process is not truly axonal in structure, but resembles more an elongated part of the cell body. The asterisk indicates tangentially cut synaptic terminal that is impressed into the lower pole of the cell body. Arrows indicate flattened subsynaptic sacs, characteristic for postsynaptic sites of Purkinje cell bodies. Adult cat. Scale 1 μ. (From Hámori and Szentágothai, 1965a.)

for a considerable distance into the granular layer surrounding it as a dense conus. This conus is of complex structure and it is very difficult to understand the intricate

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relations of basket axon terminals, glial elements and Purkinje axons. Figs. 3 and 4 give some preliminary information about this inhibitory synaptic arrangement, taken from a paper in preparation (Hámori and Szentágothai, 1965b). In spite of its complexity and specificity there are no features of this synapse that could not be detected with some variation in some other synapse of excitatory nature.

The synapse of basket cells in the hippocampus (Hamlyn, 1963) is not located on the axon hillock, but distributed on the whole surface of the soma. This would suggest that the location of the synapse on the axon hillock, even in neurons with large and complex dendritic trees and numerous and many kinds of different excitatory axo-dendritic synapses, is by no means a condition of an inhibitory function. One might therefore consider the possibility that axo-somatic synapses are inhibitory in general. But this again would not be consistent with the fact that most axo-somatic synapses, for example of motoneurons (of the 'boutons-terminaux' type) are of excitatory nature (Szentágothai, 1958, 1961). We might, therefore, propose the preliminary working hypothesis that inhibition of the large neurons of higher integrative centres — such as the pyramidal neurons of the cerebral cortex and the Purkinje neurons of the cerebellum, as well as perhaps neurons in the tectum — having on their dendritic trees an immense number of excitatory axo-dendritic synapses, is brought about by axo-somatic synapses. In addition to the two known examples of basket synapses in the hippocampus and the cerebellar cortex some of our recent histological observations on the axo-somatic synapses of pyramidal cells in the cerebral cortex might be used in favour of this hypothesis. Some years ago I had the opportunity to report here in Moscow (Szentágothai, 1962a) our investigations made on isolated slabs of cortical tissue. Meanwhile we have continued these investigations with the aid of the electron microscope and have made the strange observation that, while the great majority of the axo-dendritic synapses had disappeared two months after the isolation, the axo-somatic synapses of pyramid neurons were well preserved and thus must mainly have originated from the short local neurons of Golgi type II stellate cells. These synapses are of Gray's 2nd type. It will be recalled that this was first assumed by Cajal (1911) who maintained that the pericellular baskets around pyramidal cells are the endings of local neurons with short axons. There are, of course, also axo-dendritic synapses established by local connexions. As seen in the Golgi picture of isolated slabs the initial axon collaterals of pyramidal or second layer star pyramidal cells can be traced to the dendrites of other neighbouring pyramidal cells, where they establish the usual types of axo-dendritic synapses either with the dendrites themselves or with the spines. These synapses correspond to the relatively few Gray 1st type axo-dendritic synapses found intact in isolated cortical slabs. This of course does not prove in itself that the axo-somatic synapses established by neurons with short axons on the pyramidal cells are inhibitory, but the analogy to the basket synapses in the hippocampus and the cerebellar cortex is nevertheless a very close one (Szentágothai, 1965).

Quite another aspect of the problem of inhibition, brought about by synaptic terminals of specific localization, is that of presynaptic inhibition (Frank and Fuortes, 1957; Eccles, 1961). Synapse-like contacts between axon terminals, occasionally encountered in the spinal cord (Gray, 1962, 1963) have been suggested as the possible

morphological basis of presynaptic inhibition. According to our recent observations, axo-axonic contacts with specialized regions of attachment and accumulation of synaptic vesicles appear to be particularly frequent in the substantia gelatinosa. They are frequent too in the lateral geniculate body (Szentágothai, 1962b; Colonnier and Guillery, 1964) so that if they were really the histological substrate of presynaptic inhibition, a very powerful presynaptic inhibition should be expected at these places. Axo-axonic contacts are often only part of a rather complex synaptic arrangement, which led us (Szentágothai, 1962b) to the concept of the 'complex synapse', *i.e.* a characteristic structural arrangement of the ends of several different axons and the terminal branches of one or more dendrites in a specific tissue unit.

One of these complex or glomerular types of synapse has long been known: the cerebellar glomeruli. They are a characteristic arrangement of a centrally situated mossy terminal and surrounding dendrite endings of granule neurons as well as those of descending Golgi neuron dendrites. The terminal arborizations of Golgi axons participate in the cerebellar glomeruli too, and their possible physiological significance as a feedback inhibitory mechanism of the main cerebellar input system *via* mossy fibres, granule neurons and parallel fibres has been discussed on various occasions (Szentágothai and Rajkovits, 1959; Szentágothai, 1962b, 1963a, b). The Golgi endings have been identified under the electron microscope first tentatively on circumstantial evidence (Szentágothai, 1962b), and more recently on the basis of degeneration techniques (Hámori, 1964). Similar glomeruli are found in the lateral geniculate body (Szentágothai, 1962b, 1963a, b) as the main point of articulation of the optic pathway. The endings of optic fibres occupy the centre of these glomeruli, their periphery being built up mainly of dendritic terminals and of axon endings of non-optic nature. A multitude of additional non-optic endings — deriving mainly from cortical recurrent and local Golgi II neuron axons — covers the surface of the dendrites outside the glomeruli. Glomeruli of reverse build are encountered in the pulvinar of the thalamus (Majorossy *et al.*, 1965). Here the centre of the glomerulus is occupied by a club-shaped dendrite branch surrounded by numerous axon endings of various kinds and origins. Characteristic axo-axonic contacts of specialized structure are very frequent in these glomeruli too. Most of the glomerular synapses are surrounded by and separated from their environment by a clear glial capsule, which underscores their character as independent tissue units. Although it is highly probable that inhibition must, in some way or another, play an important role in these glomerular synapses, there is no direct physiological evidence of this kind available at present. However, the known circuit of neurons of the cerebellum (mossy fibre → glomerulus → granule neuron → parallel fibre → Golgi neuron → glomerulus) forming a loop closed in the glomerulus as the key-point (already appreciated by Cajal in 1911), is highly suggestive of an inhibitory feedback mechanism. Similar neuronal circuits with key-points in the other glomerular synapses point in the same direction. Unfortunately, apart from the axo-axonic contacts mentioned, there is no clear histological difference between the several axon terminals in the same glomerulus that could be used as criterion of different (excitatory and inhibitory) functional capacities. It might be that histochemical analysis of these endings will show decisive differences. Promising results in this direction

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have been reported in the cerebellar glomeruli (Mead and Van der Loos, 1964).

SUMMARY

Histological evidence, on the whole, supports the notion that inhibition requires specific inhibitory neurons. There are many histological reasons to assume that most of the specific inhibitory neurons either have relatively short axons or are even of Golgi's type II.

There is as yet no useful structural criterion on the basis of which inhibitory synapses could be distinguished from excitatory ones. The size as well as the character of synaptic vesicles, the width of the synaptic cleft, thickenings of the postsynaptic membrane, specific subsynaptic formations, the more neurofilamentous or predominantly vesicular character of the axon terminal do not appear to be in any correlation with excitatory or inhibitory function of the synapse.

There is no clear evidence for the importance of the localization of the synaptic contact. In spite of some well established examples of inhibitory synapses arranged around or at least close to the origin of the axon, this cannot simply be generalized, either in the direction that all synapses of this kind are inhibitory, or even less by assuming that all inhibitory synapses are localized around the origin of the axon. Synaptic contacts that surround the origin of the axon may be excitatory (as in ciliary ganglion synapses of birds and reptiles) and inhibitory synapses are known to be situated on all parts of cell bodies and even on dendrites. Still one might venture to say that in neurons having many different kinds of synapse inhibition is more likely to be exercised on the cell body.

In the particular case of presynaptic inhibition the localization of a synaptic knob on top of another would be an example of the crucial importance of the location of the inhibitory contact. Axo-axonic contacts with asymmetrically specialized regions of attachment undoubtedly exist and are even frequent especially in glomerular synapses. Unfortunately, however, they are most abundant at sites where presynaptic inhibition has not yet been found, and they are rare — if not lacking completely — in regions where presynaptic inhibition undoubtedly occurs, as for example in spinal motor nuclei.

Taken as a whole, the body of histological information does not seem to indicate inhibitory function to be primarily a question of some specific geometry of the synaptic contacts. It looks much more as if it were fundamentally some specificity — very probably of chemical nature — of the neurons and their synaptic contacts that is decisive for their excitatory or inhibitory effect on other neurons. Some years ago (1961) we called attention to experimental embryological data (Székely and Szentágothai, 1962) showing that inhibition may occur in groups of disarranged nerve cells having completely haphazard connexions lacking any order in the location and arrangements of synaptic contacts. Analysis of nervous tissue cultures with more sophisticated physiological methods points in the same direction (Crain and Peterson, 1964). This shows that the capacity to inhibit other neurons is more likely to be rooted in the metabolic constitution of certain neurons, than in the geometry of their ar-

rangements. Obviously there must be very definite geometric order in inhibitory connexions, however, not merely to have inhibition at all but to have it at the correct place in order to secure purposeful function of the neuronal network. It would therefore be erroneous to interpret any specific localization of an inhibitory synapse as being in direct correlation with the basic mechanism of inhibition. Specific excitatory and inhibitory mediators released from the respective presynaptic terminals, and perhaps specific sensitivity of certain loci of the postsynaptic neuron surface to one or the other of these mediators, is the most likely assumption that can be reconciled most easily with all histological facts known about inhibition.

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The Frequency Response of Horizontal Pursuit Movements of the Human Eye and the Influence of Alcohol

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After the earlier work of Dodge (1907), Ohm (1955), Von Romberg and Ohm (1949) Stroud (1950), and Von Romberg *et al.* (1951), who had made some orientative observations about the behaviour of human eye-balls when following a sinusoidally moving target, I was able to publish in 1958, on the occasion of the 24th conference of the German Physiological Society, some detailed results about the frequency response of the pursuit movements of the human eye. In the meantime other scientists such as Sünderhauf (1960), Trincker *et al.* (1961), Mackensen *et al.* (1961), Fender and Nye (1961), Stark *et al.* (1962), and Young and Stark (1963), have repeated such experiments, especially from the biocybernetic aspect.

It has long been known that, when a moving target is followed by the eye, 2 forms of movement are produced by the contraction of the 6 exterior eye muscles: (1) a continuous smooth movement, and (2) saccadic quick movements, the so-called 'flicks'. We know further that most eye movements, by which we trace out for example the stationary figure of a circle, are principally saccadic, and that a smooth movement only occurs in two cases: (a) when — as in these experiments — the head of the subject remains still and the eyes try to follow the continuously moving target, and (b) when the head performs a movement and the eyes, aiming at a fixation of resting objects in the outer world, try to compensate the head movement. The saccadic movements are characterized by a high angular velocity up to 400 degrees/sec, which is constant for a given amplitude of movement and is characteristic for a certain person; it cannot be changed arbitrarily. In a pure form saccades are observed when the fixation point is changed, that is when we transfer our gaze from one subject to another. During the saccadic movements no conscious optical perception takes place. The flicks occur so quickly that no correction during their movement is possible. Only within 160–170 (130–210) msec after the end of a saccadic movement can a visually controlled change of the position of the eye-ball be elicited. The saccadic mechanism corresponds to an 'Abtast System' (sampled data system) with a period of about 0.2 sec. The pursuit movements of the eye when following a moving target are composed — corresponding to the character of the leading movement — in a complicated manner of both types of eye movement, the smooth and the saccadic. A corrective function of the saccadic movements plays an important role (see below). There is a fundamental difference between predictable and non-predictable movements of the target. Predictable

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movements are those for which an extraordinarily effective 'prediction-mechanism' in higher levels of our brain extracts and stores so much information from the former rate of movement that it can predict the future movement with a certain degree of accuracy. The sinusoidal movement is an especially easily predictable input signal. Westheimer (1954) and later Mackensen *et al.* (1961) have shown that, when the leading sinusoidal target becomes invisible, the eyes perform the pursuit movement in sinus form quite undisturbed for a duration of about 1 sec and pick up the target, when it reappears, at the right place and at the right time. From own experiments and that of Sünderhauf (1960), we may conclude that a sinusoidal movement, when it suddenly appears after a latency period of 250 to 300 msec, is accomplished rapidly and accurately by the eyes, that is with corresponding frequency and phase lag.

Non-predictable input signals were applied for testing the pursuit movements of the eyes by Westheimer (1954) and Stark *et al.* (1962). The behaviour under these conditions is different than when a predictable movement is offered. Both kinds of signal should be employed when this complex apparatus is tested.

Meanwhile we were able to enlarge our own experiments and confirm the observations on the frequency response of horizontal pursuit movements of the eyes. We used a larger number of persons and have investigated the influence of alcohol on the accuracy of this mechanism. The results of these observations are reported below.

METHODS

For the recording of the eye movements we used a photoelectric method which was originally proposed by Matthes *et al.* (1941). The advantage of high sensitivity and frequency fidelity is combined with the preservation of physiological conditions. No artificial bodies such as mirrors, lenses or others were brought into contact with the eye-ball.

Onto one eye of the subject we projected a horizontal beam of infrared irradiation (4 times 1 mm) in the manner of Fig. 1. The beam impinged half on the sclera and half

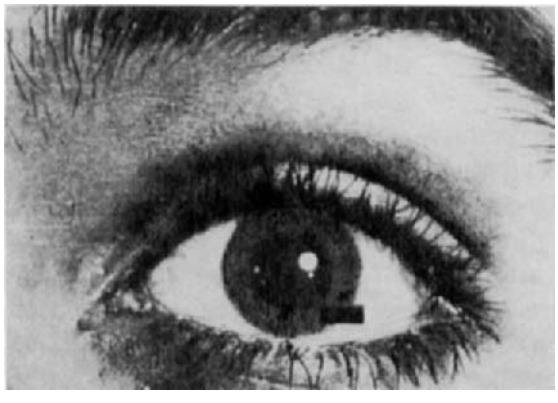

Fig. 1. Method of recording of horizontal eye movements. Projection of a horizontal rectangular beam of infrared irradiation on the sclera and iris border.

on the iris. The intensity of diffusely reflected irradiation is determined by the proportion of the more strongly-reflecting scleral part to the iris part. The reflection becomes greater when the eyeball is so moved that the scleral part gains in size. An infrared-sensitive photocell placed closely before the eye (Fig. 2) receives the reflected irra-

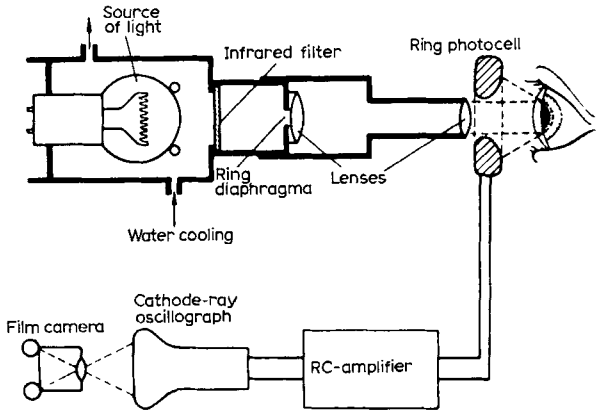

Fig. 2. Schematic view of the whole apparatus for recording. For explanation see text.

diation. The photo-stream passed to an RC-amplifier and the changes were represented on a cathode-ray oscillograph and photographed on a moving film. Thus the horizontal components of the eye movements may be recorded continuously. An absolute gauging of the amplitudes is not possible, because the recording conditions are different from person to person. With the same person, amplitudes are comparable and may be gauged by recording eye movements between 2 fixed marks.

For the experimental series we used—because of its compelling optokinetic effect, from which no person could easily withdraw—a sinusoidally moving black and white chequerboard pattern, which was projected onto a white wall in a distance of 345 cm. The excursions of the sinus movements were 53 cm; this corresponded to a visual angle of 8.8 degrees. The single squares were seen across an angle of 1.2 degrees. Frequencies between 0.3 and 4 c/s were offered. For 1 c/s the maximal angular velocity of the swinging chequerboard when going through zero position was 27.6 degrees/sec, for 2 c/s 55.3 degrees/sec and so on. We recorded the movements of the left eye which was covered by the photocell, and these we used as a measure of the monocular fixation by the right eye. We had already shown that for fixation with both eyes virtually the same results are obtained.

In order to compare the eye movements with the target's position, we fixed an optical interrupter on the diapositive which was moved in front of the projector. Thus we obtained short time marks for the extreme left position of the chequerboard pattern on a second cathode-ray beam. A disturbing superimposition on the record curves due to involuntary head movements (10 c/s' Drischel and Lange, 1956) was prevented by fixing the head with a chin-support and a frame.

The evaluation of the recorded curves was made as follows. To measure the am-

plitudes of the sinusoidal eye movements we registered with equal amplification a gliding pattern of increasing frequency (see below). The amplitudes at low frequencies (0.3 to 0.5 c/s) were put at $A_0 = 1$, and the other amplitudes were related to this value; for 8 to 10 frequencies we determined the mean relative amplitudes. The phase angle was measured at 8 to 10 fixed frequencies, and because of the variability of the angles we calculated for each frequency the mean phase angle from 15–25 single determinations. In the last case we used a varied degree of amplification in order to allow exact measurements even for small amplitudes.

RESULTS

Qualitative observations

As Fig. 3 shows, the amplitudes of the eye movements decreased with increasing frequencies of the sinus-giver and at about 3.5 to 4.0 c/s they became so low that they

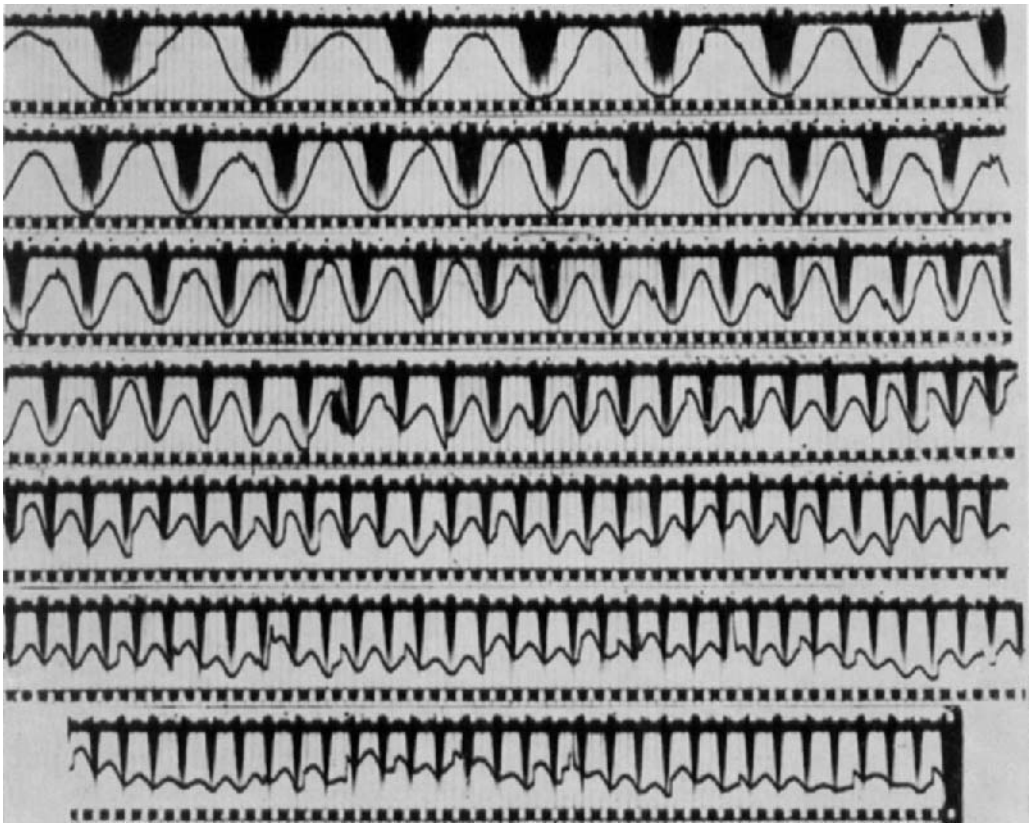

Fig. 3. Record of the pursuit movements of the eye at increasing frequency of the sinusoidally moving target. The black marks indicate extreme left positions of the chequerboard pattern. Deviations of the recorded line (eye movements) below indicate movements of the eye to the right. Time marks 1/10 sec. Equal degree of amplification.

could not be discriminated exactly. The sinusoidal responses of the eye movements then decayed and gave way to an irregular sequence of larger or smaller saccadic movements. Regular to and fro saccades were sometimes observed. Within the frequencies of 0.3 to 3.5 c/s the eyeballs followed the leading sinusoidal movement with astonishing precision, but there were differences between the several persons used as subjects as to smoothness and accuracy. Typical for a given individual is the frequency with which little saccadic movements of 20 to 50 min of arc were scattered about the smooth basic curve. The number of saccades usually diminished when the subject tried to look freely and not intently at the moving chequerboard pattern; they increased in number when he made an effort to follow the target more exactly.

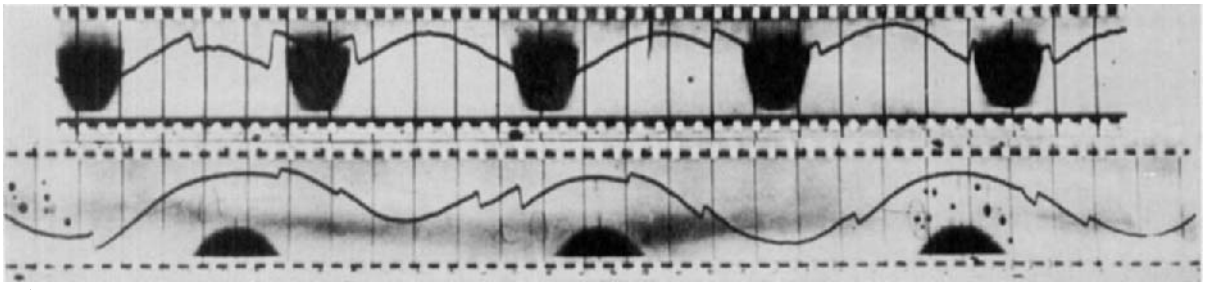

Fig. 4. Sinusoidal pursuit movements of the eye (higher speed of recording). Note the independence of smooth, gliding movements from saccadic eye movements.

In all our records the relative independence of both forms of eye movement from one another was most striking. Often — as in Fig. 4 — after a saccade the smooth sinusoidal movement continued undisturbed, with a shift only in the level of the record line. The smooth movement between two saccades seems as though stamped out. Saccadic movements occurred in the direction of the smooth movement as well as in the opposite direction. The frequency of saccades in general and the preferred direction showed individual differences.

Quantitative results

(1) The Bode diagram

In Fig. 5 the arithmetic means of the results for 3 subjects are plotted in the so-called Bode diagram. On the common abscissa the frequencies of the chequerboard pattern are marked in c/s. From the upper part, the amplitude–frequency relation, we see that with increasing frequency the amplitudes of the eye movements decreased in an S-form manner and reached zero for frequencies between 3.5 and 4 c/s.

The lower part of the diagram contains the phase–frequency relation, the phase being represented by multiples of π, that is a half period of the leading sinusoidal movement. The phase lag (negative phase angle) became greater, the more the offered frequency was increased. The position of the phase–frequency curve in the diagram and its shift to the right or to the left mark individual differences between subjects as to the accuracy of pursuit eye movements.

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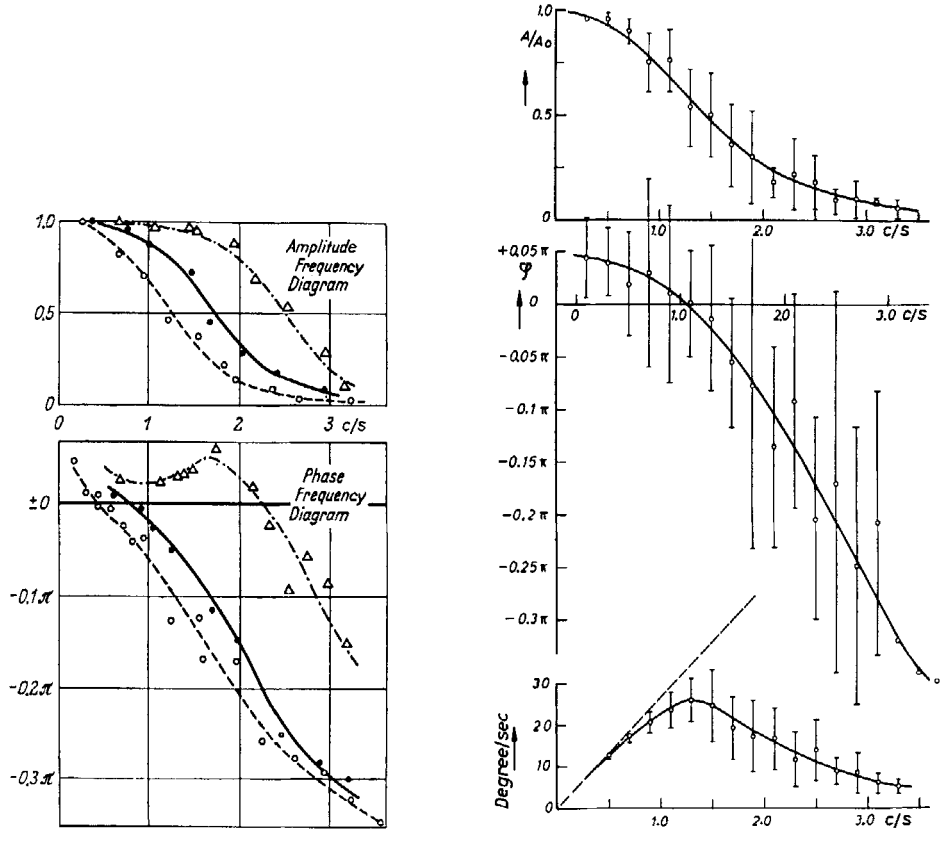

Fig. 5. Bode diagram for the sinusoidal pursuit movements of the human eye. Three subjects. Upper part, amplitude–frequency relation; below, phase–frequency relation. Amplitudes related to amplitude A_0 at very low frequencies ($A_0 = 1$). Phase angles represented by multiples of π (= half sinus period).

Fig. 6. Bode-diagram for the sinusoidal pursuit movements of the human eye. Arithmetic means of 27 subjects with standard deviations of the mean. Upper part, amplitude–frequency relation; middle, phase–frequency relation; below, behaviour of the maximal angular velocity of the eyeball when going through zero position (degrees/sec) in relation to frequency; dotted line, maximal angular velocity of the target.

Von Romberg *et al.* (1951) first drew attention to the surprising fact that in the region of lower frequencies, usually below 0.8 or 1.0 c/s, positive phase angles were observed for nearly all subjects. This means that the eye precedes the leading movement if this is slow enough. The eye is in phase at a mean frequency of 0.93 c/s (S.D. = $\pm$ 0.25 c/s). The means of all the results for 27 subjects together with the standard deviations of the mean are plotted in Fig. 6; both upper parts form the Bode diagram. Typical individual differences concern the reduction in amplitude, which may set in earlier or later, and various steepnesses of the phase–frequency curve as well as its shift to the right or to the left. We therefore put the question: are there consistent relations between the speed with which the amplitudes are reduced with increasing frequency on the one hand, and the steepness of the phase–frequency curve on the

other hand? We determined for each person: (1) the frequency at which the amplitudes were reduced to half the value of the initial amplitude at low frequencies — on the average this was 1.64 ± 0.21 c/s; and (2) the difference in the phase angle for a step of 1.5 c/s on the frequency axis, usually determined between 1.0 and 2.5 c/s. In a correlation diagram we found that the slower the amplitudes are reduced with increasing frequency (shift to the right in the amplitude–frequency diagram), the steeper, on the average, is the slope of the phase–frequency curve, and the earlier the phase lag. The converse also holds true.

(2) Amplitude–frequency relation

In order to clarify the significance of the amplitude reduction, we determined for a series of frequencies: (a) the angular velocity of the chequerboard pattern as it went through the zero point ($v_{R\,max}$; and (b) the maximal angular velocity of the eyeball as it went through zero ($v_{B\,max}$) (Fig. 7). The velocity of the pattern is a linear function

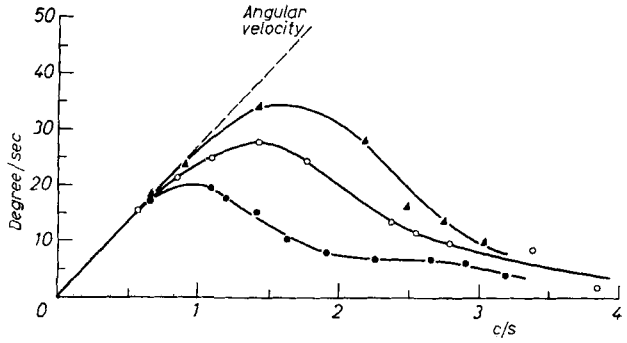

Fig. 7. Maximal angular velocity of the eyeball when going through zero position (degrees/sec in relation to frequency). Three subjects. Dotted line, maximal angular velocity of the sinusoidally moving target.

of the frequency; it results in a straight line with a slope of 27.6 degrees/sec. For the maximal angular velocity of the eyeball the following formula is valid

$$v_{B\,max} = \frac{360^\circ \cdot r' \cdot f}{a},$$

where r' is the amplitude A of the bulbus movement related to the full amplitude $A_0 = 1$ at low frequencies, f the offered frequency, and a the distance of the pattern from the eye in cm. The following principal behaviour (Fig. 6) was observed. The maximal velocity of the eyeball when going through zero for lower frequencies follows the velocity of the leading pattern (dashed line) very well, but then it remains behind this and reaches a maximum — for each subject at different heights — and eventually falls for higher frequencies to reach a steady state which lies at a different level for each subject, reaching zero for some.

Usually there was a high positive correlation between the value of the maximal

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velocity of the eyeball and the frequency at which this maximum was reached: the higher the former, the greater the latter. The arithmetic mean of the peak of maximal velocity of the eyeball lay at 26.9 degrees/sec (S.D. = $\pm$ 5.2 degrees/sec. The frequency of the peak was at 1.32 $\pm$ 0.15 c/s.

The maximal velocity of the eyeball is a characteristic individual peculiarity for each person, and determines the frequency up to which the eye may follow the leading movement with accuracy as to amplitudes. The frequency at which the amplitudes are reduced to half the value of A_0 shows a regular increase with increase in the maximal velocity of the eyeball.

(3) Phase–frequency relation and saccadic movements

In order to investigate this relation we also determined the absolute values of the phase lag and gain in msec. The positive phase angle at low frequencies was found to correspond to a value of about 100 msec; the values differed considerably from subject to subject. On the other hand, the negative phase angle for higher frequencies tended asymptotically to reach a final value usually at 50 msec (30–70). This value seems also to have an individual significance.

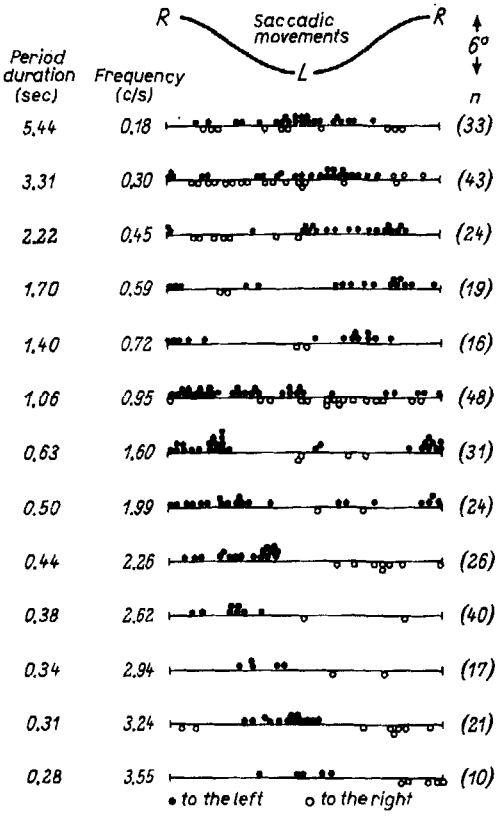

Fig. 8. Distribution of saccadic movements to the left (●) and to the right (○) over one sinus period of the target in relation to frequency. Right column, number of sinus periods evaluated.

We have attempted a detailed analysis of the saccadic movements that are superimposed on the smooth movements. We put the question: are the saccades distributed equally and arbitrarily over one period of the smooth sinusoidal movement, or are there points of predilection for the occurrence of the saccades? For this purpose we determined the distance of all the observed saccades from the beginning of the sinus period of the leading movement, at a certain frequency. We calculated them in multiples of 2π (a full sinus period). Fig. 8 shows the result for one subject, which is more or less typical for all subjects. The distributions of the saccades to the left (filled circles) and to the right (empty circles) are not equal, but show certain regions of greater frequency, which means that there are moments at which there is a greater tendency for the originating of saccadic movements. This tendency is correlated regularly with the pattern frequency: when the latter is increased (from above to below) the maxima of saccadic tendency shift to the right, so that (filled circles) they enter the beginning of the sinus period at about 0.9 c/s. This means that at low frequencies saccades to the left only occur exceptionally, when the pattern (and the eyeball) moves from left to right; here the saccades are in the opposite direction to that of the smooth movement. At higher frequencies (below) saccades to the left fall into the movement from right to left; they are equally directed. This behaviour supports the view of a corrective function of the saccadic movements. They diminish the phase lag or gain of the smooth movement.

The frequency of turnover at which saccades in opposite direction change into saccades of similar direction shows individual peculiarities. A maximum occurrence of saccadic movements was found for most subjects between 1 and 2 (mean 1.4) c/s of the leading sinusoidal pattern movement.

Influence of alcohol on the frequency response of horizontal pursuit eye movements

In 2 series of experiments we tested the influence of ethanol on the accuracy of pursuit eye movements. The same 10 subjects each received in the first series 100 ml of 38% alcohol per os, and in the second series 200 ml. At 30, 60, 90, 120 and 180 min after the drink, complete determinations of the frequency response of the eye movements were accomplished. At the same times blood was taken from the cubital vein for alcohol level determinations according to Widmark. The recordings for a full determination of the frequency response occupied about 10 to 15 min. The initial values were determined before the drink.

A typical example of the results of these series is shown in the Bode diagram of Fig. 9 (200 ml.) Top, amplitude–frequency relation; middle, phase–frequency relation; bottom, behaviour of the maximal angular velocity of the eyeball. Under the influence of alcohol the following changes were observed: (1) The amplitudes were reduced earlier than before, that is at lower frequencies; the amplitude–frequency curve is shifted to the left; (2) The phase–frequency curves under alcohol were shifted to the right and are less steep than before; the phase lag became less, an apparently improving effect, which can only be understood in connection with the reduction in amplitude and the significant diminution in the maximal velocity of the eyeball;

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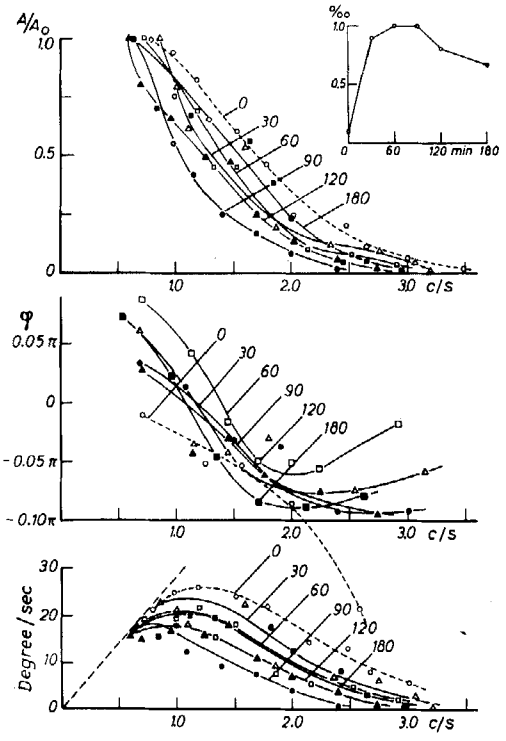

Fig. 9. Bode diagram for the influence of 200 ml alcohol per os on the horizontal pursuit movements of the eye (one subject). Top, amplitude–frequency relation; middle, phase–frequency relation; below, maximal angular velocity of the eyeball. Inset (top right), course of blood alcohol concentration, in parts per thousand, during the observation (180 min). The numbers on the curves mark the time after imbibition of alcohol in min.

(3) The latter, the decrease in maximal eyeball velocity, is characteristic of the influence of alcohol; this seems to be the important factor for the reduction in the accuracy of the fixation and pursuit mechanism of the eyes by this pharmacon.

The degree of the change in all parts of the diagram corresponds very well with the blood alcohol level; the maximal changes are reached 60 to 90 min after the dose of 200 ml alcohol. It can be shown that the dynamics of this extremely sensitive neural apparatus is affected by blood alcohol levels as low as 0.2 to 0.3 parts per thousand, as may be proved by the Bode diagram.

Fig. 10 shows the statistical results of our alcohol experiments. The means for all subjects are here related, in contrast with the representation of the Bode-diagram used hitherto, to the initial values (dotted lines at zero). The deviations from these initial values were determined. Deviations in the upward direction mean greater reduction in amplitudes and diminution in the steepness of the phase–frequency curve or shift to the right. The opposite applies to deviations below. The reduction in amplitudes is not so pronounced in the series of 100 ml doses of ethanol. The phase–frequency curves on the contrary show a greater shift to the right and diminution

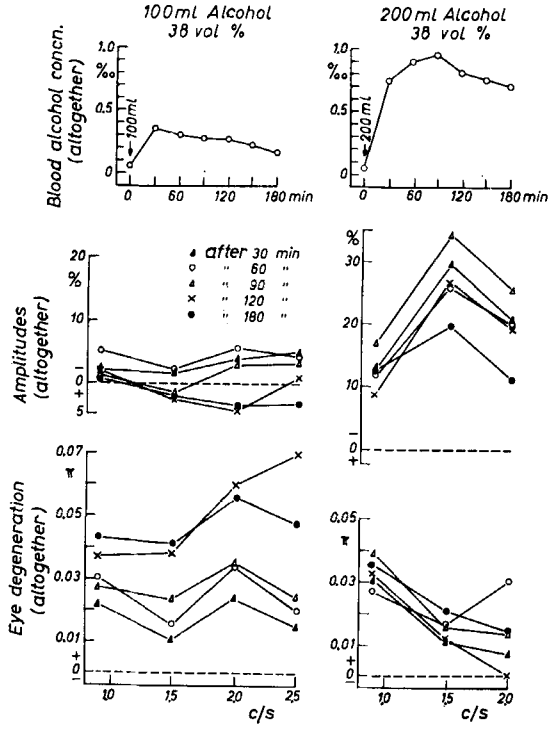

Fig. 10. Arithmetic means of the alcohol experiments (100-ml and 200-ml series), related to the initial values before alcohol intake (dotted lines at zero). Middle, amplitude–frequency relations; below, phase–frequency relations; top, blood alcohol concentration curves.

in the steepness for 100 ml alcohol. In the top of the diagram the means of the blood alcohol curves are represented.

DISCUSSION

Our findings are in good agreement with results that have been reported in the literature in the meantime (Sünderhauf (1960); Trincker *et al.* (1961), Mackensen *et al.* (1961), Fender and Nye (1961), Stark *et al.* (1962), Young and Stark (1963)). Trincker *et al.* (1961) found that for very low frequencies (0.1 c/s and less) the amplitudes of the pursuit movements are lower than for 0.15 to 0.6 c/s (mean 0.3 c/s), where a maximum in the form of a plateau is reached. This is understandable, since for extremely slow movements the object ceases to be effective as an optokinetic stimulus. The results of the different investigators are not entirely comparable with one another, because different optokinetic patterns have been used, such as a light spot, a vertical light strip and a black and white vertical strip pattern. Furthermore the excursions of the sinusoidally-moving pattern had different amplitudes, from 1.1 up to 60 degrees. Sünderhauf (1960) found that in the Bode diagram for excursions increasing from 5 to 20 degrees the amplitude–frequency curve is shifted to the left, that is to lower frequencies, and that the phase lag becomes greater. On the whole,

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however, all results on the dynamics of pursuit eye movements are in good accord.

That smooth and saccadic movements of the eyes are controlled by different central nervous mechanisms is well known, although we do not yet know all the functional and topographical details. Obviously the saccadic mechanism is the older phylo- and ontogenetically. In childhood saccadic movements are observed from the 13th day onwards, whereas smooth pursuit movements are seen only at the age of 3 months. The extremely different kinetics of the 2 types of movement prompt the question, are the same elements of the eye muscles, the motor units, able to perform both forms of contraction? There is some evidence for morphological and functional differentiation in the fibres of the external eye muscles.

Stark *et al.* (1962) reported results on the character of the eye movements when non-predictably moving input signals were followed. These authors used the superposition of 4 to 9 sinus movements of incommensurable frequencies. The time pattern was produced by an electronic computer and offered to the eye as an analogue signal. The pattern was random: it could not be predicted by the brain mechanisms. The responses of the eye consisted in continuous smooth movements dominating for slow parts of the leading movement, and saccadic movements that occurred at faster rates of the input signal. The Bode diagram showed, in contrast with the response to simple sinusoidal inputs, a greater phase lag than in our experiments, and a positive phase angle for lower frequencies was missed altogether. The amplitudes of the eye movements showed a quicker reduction than in our observations. In the experiments of the American authors the prediction mechanism of the brain was functionally eliminated by the non-predictable input signal. In our laboratory we shall now test the pursuit mechanism of the eyes to stochastic input signals that are produced by a special chance generator.

Under the influence of alcohol the complex integrative mechanism of pursuit eye movements is affected even at blood alcohol levels so low that we do not yet see any measurable alterations in most other physiological systems of the organism; they are referred to as psychological deficits only. The accuracy of the pursuit movement is limited first by the significant decrease in the maximal speed of movement with which the eyes are able to follow at various frequencies. This leads to a decisive reduction in amplitudes, and the sinusoidally moving target cannot be followed with the same accuracy as before. Obviously the intricate prediction mechanism of the brain is also damaged by alcohol and is partly abolished. The cybernetic analysis of the dynamics of pursuit eye movements is suitable to separate in a purely functional manner the regulating efficiencies of higher and lower integrative levels of the central nervous system. This can be done on the one hand by studying the response of the eye to predictable and non-predictable input signals of different kinds, secondly by influencing the system dynamics by pharmacological means as we have done in our experiments with alcohol. We hope that in this way we may enlarge our knowledge on the dynamic properties of the investigated mechanism, and that this better knowledge will lead to some practical consequences.

SUMMARY

(1) By photoelectric means we undertook a continuous recording of the horizontal components of pursuit eye movements. The optokinetic reaction of the eyes was elicited by offering to human subjects a sinusoidally and horizontally moving black and white chequerboard pattern with 8.8 degrees total excursion. The results were represented as the frequency response of the system in the manner of the Bode diagram.

(2) The amplitude of the eye movements was reduced with increasing frequency of the pattern in an S-form and fell to zero at about 3.5 to 4 c/s.

(3) The phase lag of the eyes became greater when the pattern frequency increased. For lower frequencies, less than 0.8 to 1.0 c/s, slightly different for every person, the system showed positive phase angles; the eye preceded the pattern movement. This is due to an extraordinarily effective 'prediction mechanism' for the easily predictable sinusoidal movement.

(4) The maximal angular velocity of the eye when going through zero position grew with increasing frequency up to about 1.3 c/s and reached a peak of 26.9 degrees/sec on the average; for higher frequencies it fell to lower values or to zero. Individual peculiarities, which limit the faculty to pursue rapidly moving objects with the eyes, were observed.

(5) Saccadic movements, superimposed on the smooth sinusoidal movements of the eyes, showed a regular, non-arbitrary distribution over the sinus period, which depended on the frequency of the pattern. This suggests a corrective function of the saccadic movements.

(6) Under the influence of alcohol we observed the following changes in the Bode diagram: (a) a reduction in the pursuit amplitudes of the eyes occurred at lower frequencies than before; (b) the phase lag became less; (c) the maximal angular velocities of the eyes were significantly diminished; and (d) the peak of the velocity curve was lower and shifted to lower frequencies.

(7) The extremely sensitive fixation and pursuit mechanism of the human eye was altered by blood alcohol levels of 0.2 to 0.3 parts per thousand. It is concluded that alcohol injures above all the prediction mechanism which must be ascribed to higher integrative levels of the central nervous system.

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Concept of the Conditioned Reflex in the Light of Instrumental Reactions

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The task we set ourselves was to investigate the manner in which both instrumental or operating and classical conditioning could be explained by the same theoretical position. We think P. K. Anokhin's concept of conditioned reflex may offer the way to solve this question.

METHOD

We 'educated' dogs to run across a long path to obtain meat in two different places at opposite sides of the trajectory. The departure point was a cage situated along the path asymmetrically, at unequal distances from the opposite sites of feeding. In the first stage of the experiment we used two stimuli: the time and the individuals who opened the door's cage (time of day and individuals were maintained constant) (Diagram 1).

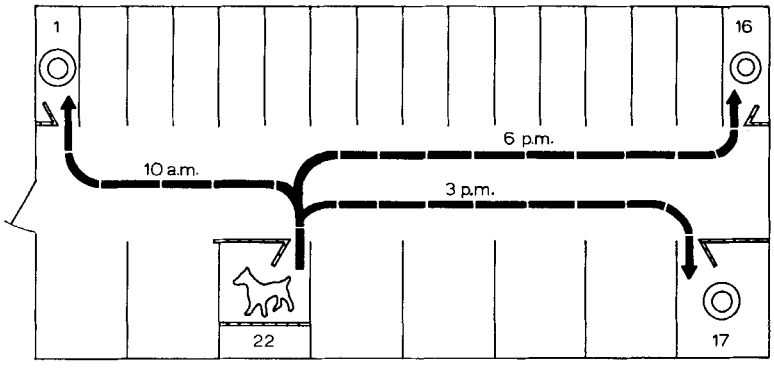

Diagram 1.

In the second stage we placed a gong at each of the two feeding sites, so that the animals had to distinguish the direction of the two different acoustic stimuli; a lever was put inside the cage so that the dogs could open the door by themselves, and a black curtain blocked the visual stimuli to the cage from the outside. Inside the cage

there were other stimuli: the opening of the door, the noises made by the process of unlocking the door, two lights and a buzzer (Diagram 2).

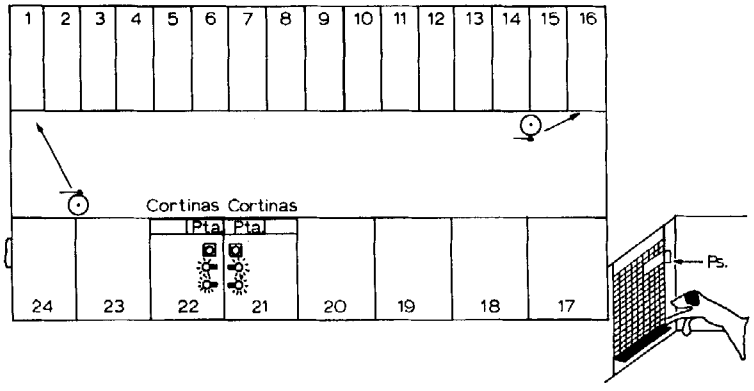

Diagram 2.

RESULTS

We established conditioned reflex responses to combinations of different stimuli (individual No. 1 at 10 o'clock, individual No. 2 at 3 o'clock and individual No. 3 at 6 o'clock). In these circumstances the dog went accordingly to different places (1, 17 and 16) (Diagram 3).

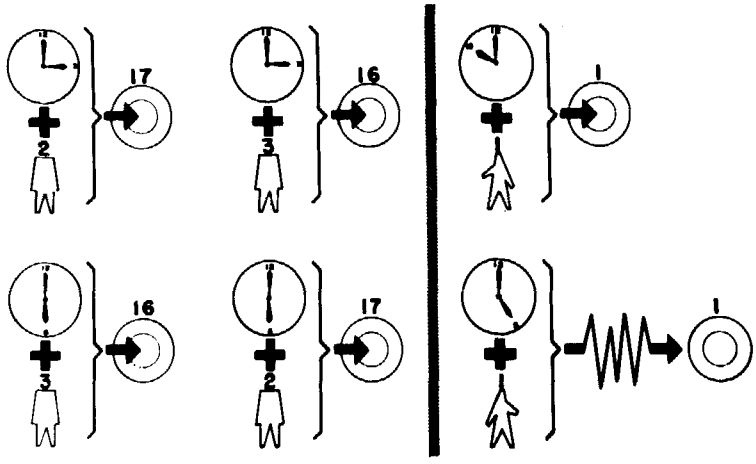

Diagram 3.

When a change was introduced into a combination through the appearance of the laboratory assistants (which we call 'individuals') at different hours (that is, individual No. 2 at 6 o'clock and individual No. 3 at 3 o'clock) the animal went to the place indicated by the individual and not to that related to the time of the latter's appearance (Diagram 4).

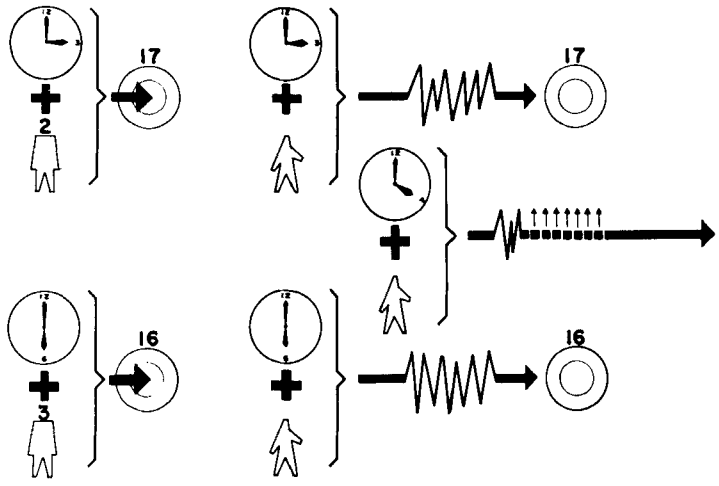

Diagram 4.

Nevertheless the time factor was operative in other experiments. The same dog went to the time-associated place when we used neutral individuals at the previously learned feeding time.

In the second stage we applied the combination of buzzer, light, lock's sound, and opening of the door, and the individuals indicated a unidirectional place of feeding.

In these experiments the door was opened mechanically by a device in the control room and not by the individuals as previously. As soon as the dogs began to show spontaneous conditioned reflex activity we noticed that they did not wait for the individuals to lead them to the feeding places. They jumped out at the noise made by the opening lock and ignored the individuals. Contrary to previous behavior, the dog's actions were not reinforced by the appearance of the individuals.

However, the lock's sound and the opening door eventually had no behavioral effects, that is the dogs did not go to the feeding places when we applied these stimuli. When they were stimulated by the buzzer they immediately pressed the lever in order to proceed towards the feeding places. This occurred only with the dogs that had previously learned to press a lever to open the door.

It took about 40 trials to confirm the inhibition of the individuals' stimuli on 5 dogs, 74 for the lock's sound (that is on the dogs that had learned how to open a door), and 2 months of continuous learning to establish the inhibition of opening the door. Certain other elements of the general circumstances were inhibited in some degree. The inhibition was produced spontaneously by the natural effect of the events.

We later changed this unidirectional setting for a bidirectional one (that is, the system of two gongs at opposite ends of the path), and we obtained definite specific responses to both, observing 100% correct responses after we inhibited the buzzer by extinction. The responses remained stable until we changed the conditions so that a so-called 'alimentary extra stimulus'–that is, the clatter of a dish–not connected with the previous experiments, was applied together with the gong but clattering from

the opposite direction. This stimulus had the initial effect of drawing the dogs in the wrong direction. The non-alimentary extra stimuli accidentally had the known effect of external inhibition and non-specific responses.

In an attempt to explain the mechanism of the above observations our hypothesis was that instrumental behavior was related not only to Pavlov's general laws but also to Anokhin's concept of the afferent synthesis and the feedback of the response upon the previous patterns of conditioned reflex activity, that is, the theory of relations between the 'return afferentation' and the 'acceptor of action'.

We consider it to be a correct view that familiar psychological conditions such as 'trial and error', 'law of effect', 'insight', 'transference', 'readiness' and others appear naturally, especially during the experimental study of the effects of blocking responses on the subsequent conditioned reactions.

On various occasions we placed obstacles in the dog's path towards the site of food, each time employing the gong stimulus. We found that the dog's responses diminished in direct relation to the difficulty of the obstacles, that is until all responses were extinguished.

On other occasions we modified the preceding experiments by blocking the cage door while the previously conditioned bell was rung. Our results confirmed those of the preceding experiments; that is, the dog's responses to the bell were completely extinguished. In contrast with the last experiments, where it was possible for the dogs to overcome the obstacles, in this experiment they had no control over the obstacle. Clearly this new extinction was not achieved by the classical non-reinforcement method. These experiments illustrate the well-known 'law of effect'.

We discovered that the dogs responded correctly from many points of the pathway. We later systematized these experiences by moving the cage to different locations along the path. The dogs gave the correct response provided that the stimuli (in this experiment, the gong) were constant. Also, the correct response was related to the location of the animal and his sensory equipment to perceive the direction of the stimuli. This experiment illustrates the theory of 'transference' of learning.

The fact that two dogs could take advantage of their previous learning (in another cage) to open the door of the experimental cage exemplifies 'transference'. But here they had to solve another problem. They were able to open the door at any time; so we had many irrelevant responses which we call 'non-specific'. Finally, as the food-seeking reflex developed, they stayed in, without opening the door. Only later did they respond opportunely to the buzzer stimulus.

In the brief span between the buzzer's sound and the mechanical opening of the door the dogs inside the cage ran to the door, trying to get out, but they were in a similar condition to that described before, that is, the door was blocked. In order to get out it was necessary for them to relate the buzzer to the lever, or, in psychological terms, 'to obtain insight' into the situation.

The dogs that had previously learned to open a door succeeded respectively in 11 and 21 trials. A dog that had a training period half that of the others, succeeded in 33 trials, whereas those dogs that had received no training were never able to open the door. Even after 6 months of trials they could not open it.

CONCLUSIONS

(1) When we try to understand instrumental behavior, it is necessary to analyze the total situation. It was clear in every stage of our experiments that the animals' responses showed a direct relation to the situation as a whole. It was also clear that the animals' responses revealed a direct relation between the feeding goal and the stimuli which defined it, which precisely indicated the feeding place.

(2) However, the concept of situation should not be based upon a predetermined cause of behavior, but should be considered as a process conditioned by the nervous reflex activity of the animals and their previous history. Some dogs succeeded and some failed in the same situation: some pressed the lever to get at the food, and some did not.

(3) The classical puzzle-box, the mazes, the Skinner box and other similar methods present situational problems which resemble those we saw when we blocked the animals' responses. We suggest that the instrumental seeking situation could be explained as determined mostly by a naturally conditioned reflex, which develops as a special case of the relation between the return afferentation and the acceptor of action.

(4) An instrumental seeking situation develops according to Pavlov's laws of the generalization of conditioned reflexes and differentiation (first and second stage of our experiment) determined by the conditions of the entire setting and its definite stimuli, in every moment of the reciprocal relations between this setting and the animal.

(5) It seems to us that extinction and differential inhibition were results of the return afferentation effect on the acceptor of action. The afferent synthesis could also explain the diversity of responses in an instrumental setting.

(6) The 'transference', 'insight', and solution of problems in our experimental setting (that is, already established conditioned responses) remind us of Pavlov's principle of generalization and systematization of relationships. Following the orienting reaction after blocking, the dogs' successful responses finally harmonized previous learned relations (past afferentations) with the present relations, as if a complex process were developing through the return afferentation. It seems to us that two previously learned 'patterns of action' were integrated into a new one determined by the present situation.

On the basis of our experiments we believe it is only possible to solve the neurophysiological and psychological controversies in relation to instrumental experimentation according to a general conditioned reflex theory.

SUMMARY

(1) We reproduced the well-known psychological experiments of 'trial and error', 'insight', 'transfer' and the 'law of effect' during the course of the formation and development of instrumental alimentary reflexes in dogs, for the purpose of examining these experiments in the light of unitary criteria.

(2) Many of the situations examined could be explained through the laws of classi-

cal conditioning and the new criteria derived from them, when the experiments under study were examined globally and in historic perspective. Nevertheless, an examination of an individual instrumental experience offered obstacles to a classical pavlovian interpretation, in view of the diversity of acting stimulants, the multiplicity of external factors and internal concurrents in the animal's response and the feedback mechanisms involved.

(3) The new concepts of the conditioned reflex about the existence of a stimulative afferent synthesis and of the intervention of the return afferentation (P. K. Anokhin) in the complex relationships of the animal with its surroundings, were found useful for a more comprehensive appreciation of the phenomena of all active conditioned reflexes, both in their historical aspect as well as of a concrete instrumental experience.

Relations between Behaviour in Dogs and Electrical Activities in Various Parts of the Brain

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For a long time relations between electrical activities in various parts of the brain and various aspects of behaviour have been studied in animals. These mainly concern investigations of relations between electrical activities and: (1) states of 'consciousness' or states of 'alertness'; (2) behaviour of animals during 'conditioning' or during 'learning'; (3) behaviour of animals during various forms of stimulation, either directly of brain structures, or indirectly *via* sense organs.

Particularly since the second world war these three categories have been subject to intense investigation. Many symposia have been organized and the number of publications is too large to summarize. During the Marseille Colloquium on 'Conditionnement et Réactivité en Électroencéphalographie' in 1957 and the Moscow Colloquium on 'Electroencephalography of Higher Nervous Activity' in 1958 a large amount of data have been presented on relations between electrical activities of the brain and 'conditioning' or 'learning' processes. Since then, studies in this direction have increased considerably in most parts of the world, particularly of course, in the Soviet Union as far as conditioning is concerned. The literature on these investigations has also become so extensive that no attempt at reviewing will be made. Moreover, the investigations to be reported here have gone into a somewhat different direction.

Since 1956 we have investigated the relations of behaviour and electrical activities in the brain of animals that are not subjected to intentional conditioning or learning procedures and that are not subjected to intentional stimulation. Or in other words, the investigation concerned the relations between electrical activities in the brain of animals that are behaving freely, with as little restraint as possible and without special training procedures. We have tried, therefore, to record electrical activities while the animal is behaving as 'normally' as possible, and during 'instinctive' behaviour.

The investigations have become feasible because of the development of radio telemetering the electrical phenomena of the brain. As the apparatus necessary for this is still relatively large, notwithstanding intensive miniaturization, it has been necessary to use a comparatively large experimental animal and thus dogs have been chosen. To facilitate the stereotactic introduction of the electrodes into the brain only dogs of a certain breed were used. Because they are pleasant animals which get along well with different people we have chosen German boxers.

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METHODS

Two kinds of electrodes are used:

(1) Comparatively large stainless steel electrodes in the occipital, temporal and frontal bone of the skull.

(2) Strands of stainless steel wires. The wires have a diameter of 100 μ. They are insulated, except for 1 mm at the tip. The strands consist of 7 or 12 wires. The interelectrode distances are $2\frac{1}{2}$ mm or 1 mm. Gold or platinum wires are sometimes used. The ends of the wires are soldered to a connector plug. This plug is fixed with stainless steel screws and with dental cement to the skull of the animal. In some animals a total of 16 electrodes is placed; in others 50.

The method for fixing the plug to the skull has been developed by Van der Vliet (Thesis, in preparation; and Van der Vliet and Mechelse, 1962). In this way it is possible to record the electrical activities of various brain areas over periods up to $3\frac{1}{2}$ years in the same dog.

The method for the introduction of the electrodes is essentially the same as the method used by Sem Jacobsen *et al.* (1955), Dodge *et al.* (1954) and by others for implanting electrodes in the brains of patients.

The strands of stainless steel wire are introduced stereotactically. For obtaining the parameters of the brain structures the 'Atlas of the Dog's Brain' by Lim *et al.* (1960) has been used. Recently the Atlas by Adrianov and Meringe (1959) has been at our disposal.

These atlasses show that considerable interindividual variability of the parameters exists. This interindividual variability makes a precise placement of the electrodes in the desired structures difficult. As the period of investigation is so long, it is important to have as much information as possible on the electrode positions during the life of the animal. For these reasons the following controls are carried out.

(1) Before the actual operation X-ray pictures are taken of the dog's skull. First lateral X-ray pictures are taken while the dog's head is fixed, but is not placed yet in the stereotactic apparatus. The X-ray is centered in the extension of a line connecting left and right meati acustici externi. In this way the X-ray is centered on the point used as stereotactic zero reference. Then the dog's head is fixed in the stereotactic apparatus and a series of X-ray pictures is taken in the directions of the three stereotactic coordinates. In this way an evaluation is obtained of mutual relations of bony landmarks. On the basis of these data the values of the parameters obtained from the atlasses may be corrected. Recently the corrections have been improved by making airencephalograms.

(2) During the operation the place of the needle, through which the electrodes are introduced, is checked physiologically and anatomically. To this end an electrode is introduced through the needle, protruding 1 mm beyond its tip. The point of this electrode is small, approximately 20–40 μ. By means of this electrode the electrical activity (Albe-Fessard *et al.*, 1962) may be recorded and also the tissue impedance may be measured (Robinson, 1962). The needle with the electrode is lowered millimeter by millimeter and thus an impression is obtained of the electrical activities

encountered and a graph is made of the tissue impedances. The impedance measurements give an indication of the nature of the material through which the point passes. Spinal fluid has a relatively low impedance, gray matter has a higher impedance and the impedance of white matter is highest. The recording of the electrical activities allows a distinction between cell and fibre structures and, moreover, allows the recognition of structures in which responses to stimuli may be evoked.

When the needle has arrived at its place, the electrode is removed and in its place a strand of wires is introduced. Then, the needle is removed while the strand of wires is left in place.

(3) Some weeks after the implantation of the electrodes the X-ray photography described in (1) is repeated. This gives the possibility to check whether the electrodes have been placed at the calculated positions.

The electrical activities are recorded with a 16-channel electroencephalograph (Offner Dynograph, R-type). By means of a tape-recorder (EEG Magnetograph, Bekkering, 1956) 8 EEG channels are recorded as well as 1 speech channel. The speech channel is used to report verbally on the behaviour of the dog. The electrical activities are recorded also by means of a 2-channel radio telemetering system. Last year an 8-channel telemetering apparatus was used (Kamp, 1963a, b). (A detailed description of the apparatus is available on request.) The use of the method has influenced the investigation considerably and, therefore, a general description will be given here.

The system is entirely transistorized. It consists of 8 EEG amplifiers, 1 modulator and 1 transmitter. The apparatus weighs 200 g and is carried by the dog on its back (Fig. 1). It does not hamper the dog's movements and the animal does not appear to take notice of it. The action radius of the transmitter is approximately 50 m. The cable (17 wires) connecting the telemetering instrument with the connector plug on the dog's head is constructed so as to produce no movement artifacts. This is obtained mainly by ensuring that no electrostatic charge can be built up on the insulation of the wires. To this end, the insulated wires are embedded in material with low resistance (Kamp *et al.*, 1965). In this way the EEG of 8 areas may be recorded without artifacts

Fig. 1. The dog (German boxer) carries the 8 channel telemetering apparatus on his back. He is sniffing and biting at a meshwire container in which fish is placed. During this time the activities of 8 brain areas are recorded.

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while the animal is moving about freely, walks, runs, plays etc. in the building or outside.

Analyses of the electrical activities may be made by means of the following: (1) Continuous frequency analysis (Bekkering *et al.*, 1957); (2) Topographical analysis (Petsche and Marko, 1955); (3) Average response computers (according to Kozjevnikov (1958) and according to Buller and Styles (1959) with the modifications described by Cooper and Warren (1961)); (4) Auto- and cross-relation methods (Storm van Leeuwen *et al.*, 1963, 1965).

RESULTS

In the description of the dog's behaviour some terms are used (always in quotes) as 'interest', 'staring vacantly', 'tenseness', 'walking with a goal', 'walking aimlessly' etc. These expressions are used in relation to the dog's posture and behaviour. Evidently, this entails a subjective evaluation of these parameters by the observer. However, as stated in the section on methods, the observer reports verbally on the dog's behaviour. This report is recorded on the same tape as the 8 EEG channels. Moreover the speech is also recorded with one of the EEG pens. Although this gives a distorted graph, the moment of speech is indicated exactly. By means of the tape recorder it is possible to fill in later the words spoken during the verbal report. As the observer is prevented from looking at the trace during the recording an objective evaluation of relations between the observer's report and the electrical activities is obtained.

The following relations have been observed:

(1) If the animal is quiet, is sitting or lying down and closes its eyes, rhythmical activity at approximately 10–14 c/s occurs in the occipital areas (Fig. 2). This activity disappears if the dog opens its eyes or if it falls asleep. Topographical analyses

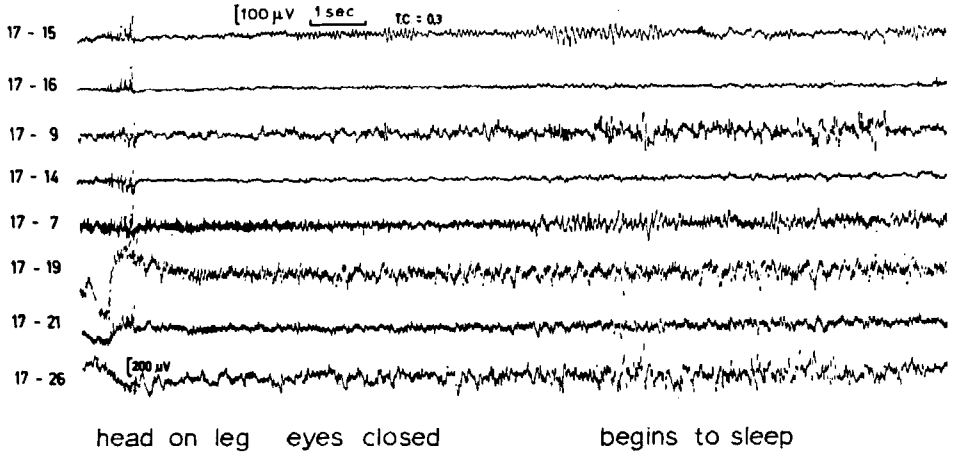

Fig. 2. Occurrence of 12–14 c/s activity in occipital area (upper lead) when the dog shuts his eyes, and decrease of frequency and disappearance of this α-rhythm when the animal falls asleep and slow waves begin to appear in leads from various subcortical structures, lower traces. (Electrode 17 placed on frontal bone, is used as common reference.)

(which are to be published elsewhere) elucidate that the rhythm 'sweeps' over the cortex similar to α-rhythms in man. Because of their properties these rhythms are so similar to the α-rhythms in man that we regard them as identical and propose to call them α-rhythms.

(2) If the dog has its eyes open and appears to look with interest at something, isolated waves occur in the occipital cortex (lateral gyrus). These waves are generally positive in relation to surrounding structures; they have durations of approximately 1/7–1/10 sec and they have amplitudes of about the same size as the α-rhythms (usually around 100 μV). Moreover they have a similar topographical distribution. These isolated waves appear to be connected with eye movements. They have the same properties as the λ-waves in man (Gastaut, 1951; Evans, 1953; Green, 1957), and, therefore, we propose to call them λ-waves of dog. Studies with radio telemetering of the EEG have elucidated that these λ-waves are enhanced considerably if the animal is moving in bright light (Fig. 3). They occur more frequently if the dog is walking carefully in high grass 'finding his way' than if it is 'walking aimlessly' on the pavement. If the dog is standing still and is 'staring vacantly', few or no λ-waves are seen, but if it looks with 'interest' as for instance when another dog is brought into the 'arena' then many λ-waves occur even while the dog is standing still. If the dog is shown different objects, the nature of the object appears to be of little importance for the provocation of λ-waves. A female dog, a male dog, the hand of the investigator, a

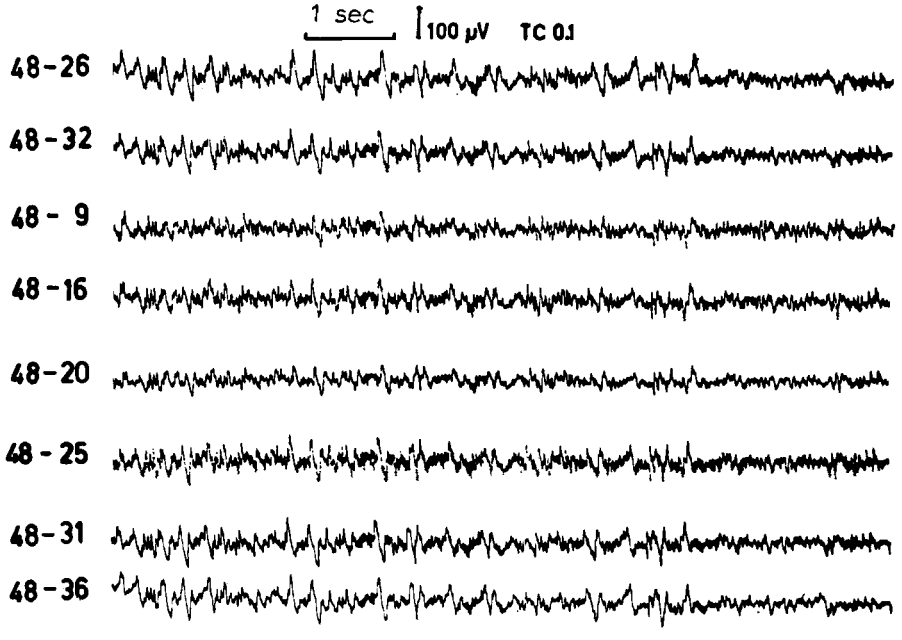

Fig. 3. Activity recorded from 8 electrodes on occipital cortex. Note occurrence of λ-waves with amplitudes up to 100 μV while the dog is walking outside in bright sunlight. (Positivity upwards. Electrode 48 placed on frontal bone is used as common reference.) In this figure and in the following the paper speed is 3 cm per sec, the amplification 100 μV per cm.

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piece of cheese, or a piece of paper may provoke λ-waves as long as the animal looks at the objects with 'interest'. This 'interest' does not appear from the dog's eye movements alone, but also from its general posture of attention: ears forward, head forward, standing on toes, etc.

(3) If the dog starts walking or is walking 'purposefully', activity at 5–6 c/s occurs in certain hippocampal structures. This activity occurs whenever the dog starts walking or starts activities connected with walking such as running and jumping. If the dog is walking 'aimlessly' the activity decreases or disappears. The activity is always encountered when the following sequence of events takes place. The dog is playing with a ball. After a while it becomes somewhat tired and lies down with the ball still in its mouth, chewing it. Then, it stops chewing, the ball drops out of its mouth and rolls away. The dog then looks at the ball rolling away, rises, walks to the ball,

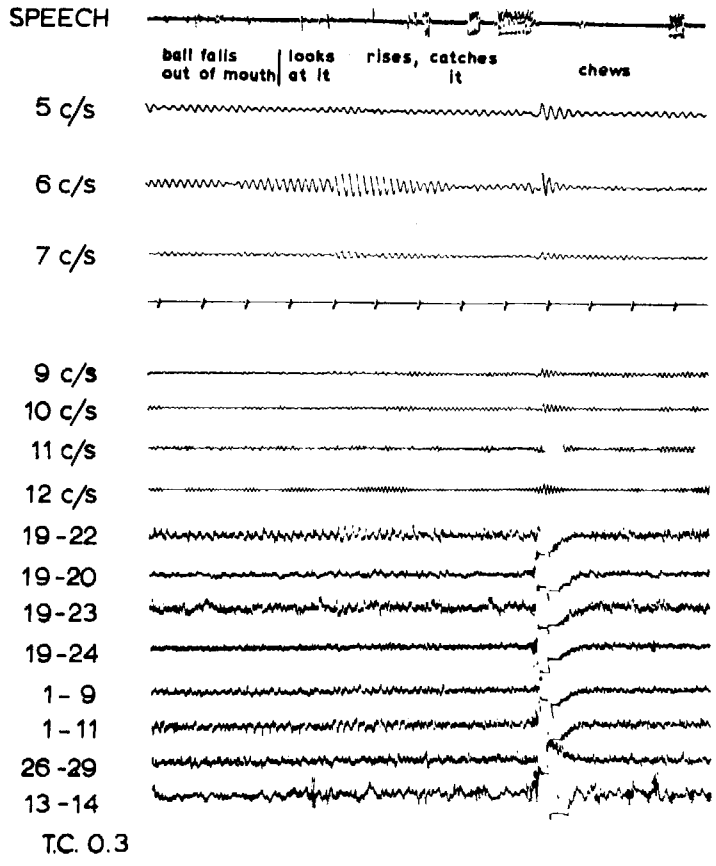

Fig. 4. After the ball has fallen out of its mouth the dog looks at it, rises, walks to the ball and retrieves it. Afterwards it lies down and chews the ball. Note occurrence of 6 c/s activity in hippocampus (lead 19-22), when the dog looks at the ball, rises and catches it. This activity disappears when the dog chews the ball (note transmitter artifact). In this period there are few λ-waves in the occipital cortex (lead 13–14). There is some 5–6 c/s activity in other hippocampal areas (leads 1–9 and 1–11). Upper trace, recording of speech. Next 7 traces are direct write-outs of frequency analysis of leads 19–22.

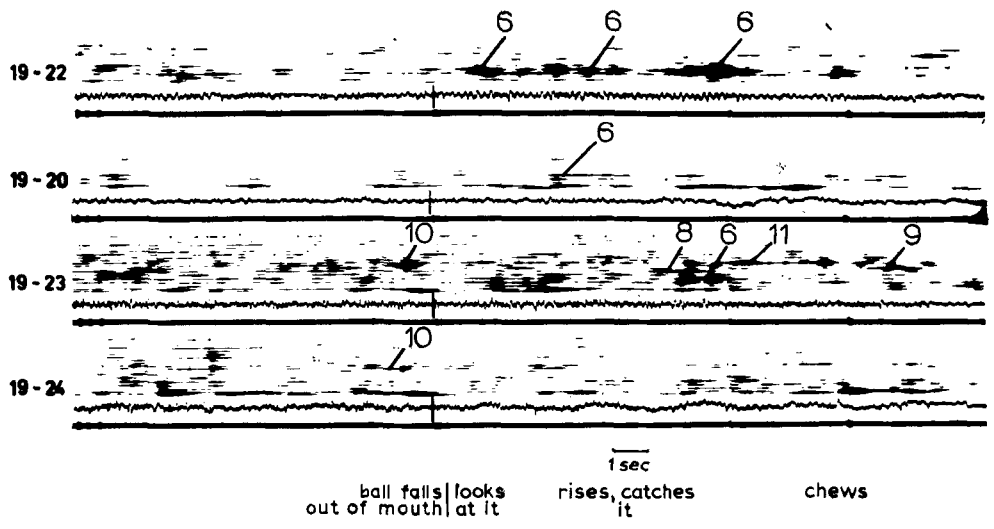

Fig. 5. Continuous frequency analysis of 4 channels during similar sequence of events as described in Fig. 4. Note occurrence of 6 c/s activity when the dog rises and walks to the ball, and disappearance of this activity when he starts chewing the ball.

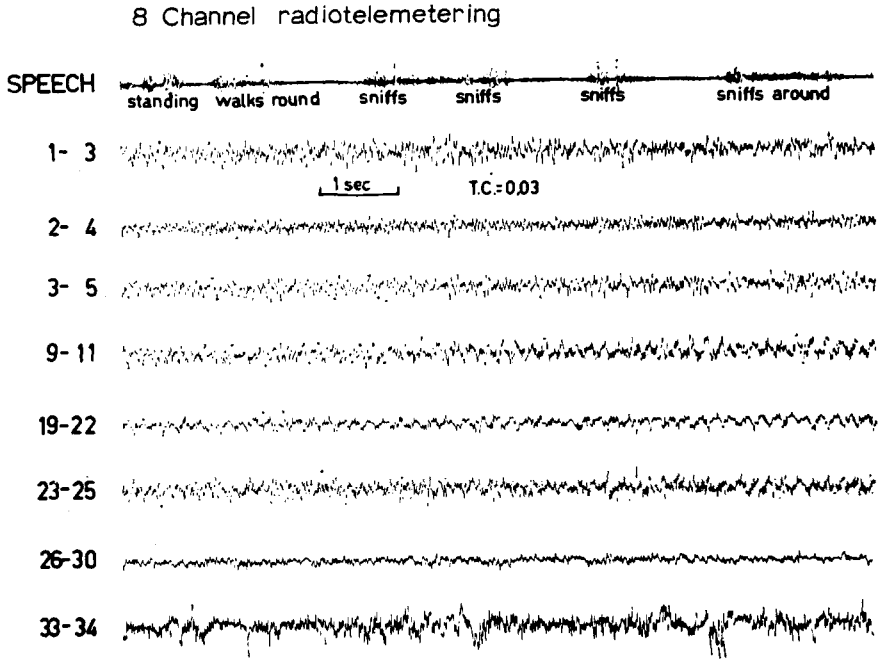

Fig. 6. Occurrence of continuous 5–6 c/s activity in certain hippocampal structures (lead 19–22) when the dog is 'tense' and 'nervous'. The dog is in the photographic darkroom, is walking and is sniffing intensely at the objects. After the dog has started walking 5–6 c/s activity occurs in another hippocampal structure (lead 9–11). In amygdaloid structures (leads 1–3, 2–4 and 3–5) fast activity is present, but no change while sniffing is taking place. In occipital leads (33–34) some muscle potentials are recorded, but few or no λ-waves occur.

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catches it and starts chewing again. At the moment the dog rises and walks to the ball, the activity at 5–6 c/s occurs (Figs. 4 and 5). It disappears when the dog has picked up the ball and starts chewing.

(4) In another hippocampal area activity at 6 c/s occurs whenever the dog appears to be generally 'tense' or 'nervous'; for instance when it is in new surroundings, or when it looks with intense 'interest' at something. In this latter event many λ-waves occur simultaneously in the occipital areas. This 6 c/s activity is pronounced if the animal looks 'intently' at a ball in the hand of the investigator. The activity also occurs if the dog is placed in a 'new' situation, for instance in a photographic darkroom. The animal walks round, sniffing vigorously at the objects it encounters, appears to be 'nervous' and wants to get out (Figs. 6 and 7). The activity is even more pronounced if the light is switched off and the dog becomes more 'nervous', whimpers and scratches at the door. In these latter circumstances, no λ-waves are present.

The 5–6 c/s activity, related with 'purposeful' walking and the 6 c/s activity related with 'tenseness' may occur at the same time for instance when the dog is walking to a 'goal' and is 'tense'; during short periods they may even appear to occur phase-locked. The two activities, however, may also occur independently, either one being present, while the other is not.

(5) A peculiar activity occurs in certain parts of the amygdaloid nucleus if the dog is sniffing at something (Figs. 7–9). The form of this activity is not easily described. The most striking feature of the activity is a considerable increase in amplitude.

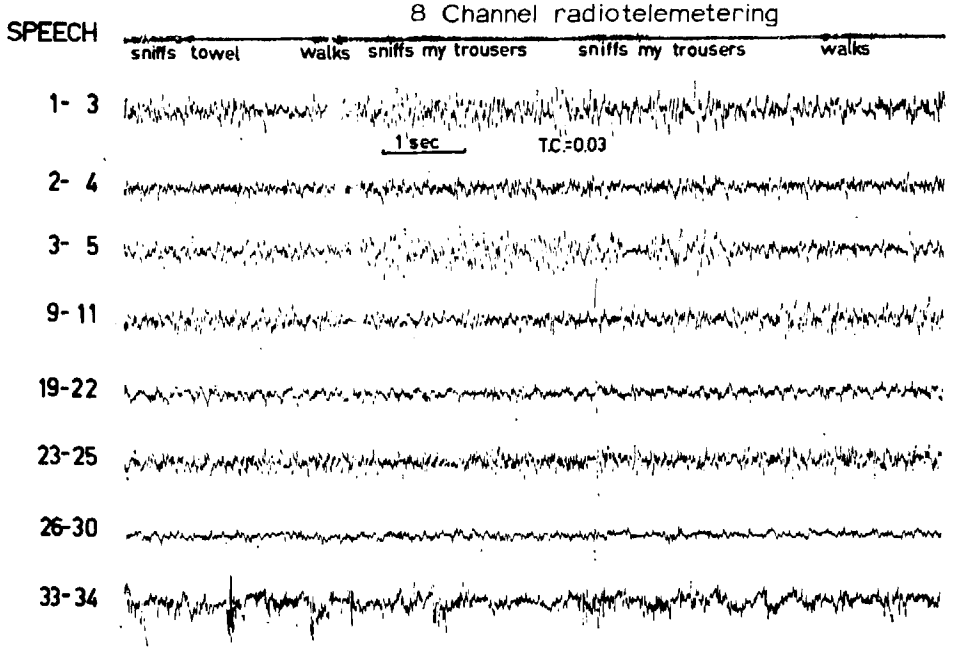

Fig. 7. (Continuation of Fig. 6.) Occurrence of 'sniffing' activity in amygdaloid structures (leads 1–3, 2–4 and 3–5), when the dog is in the darkroom and sniffs at the investigator's trousers. Muscle action potentials in occipital leads (33–34), but no λ-waves. Disappearance of 5–6 c/s activity associated with 'tenseness' (lead 19–22) when dog is sniffing at trousers.

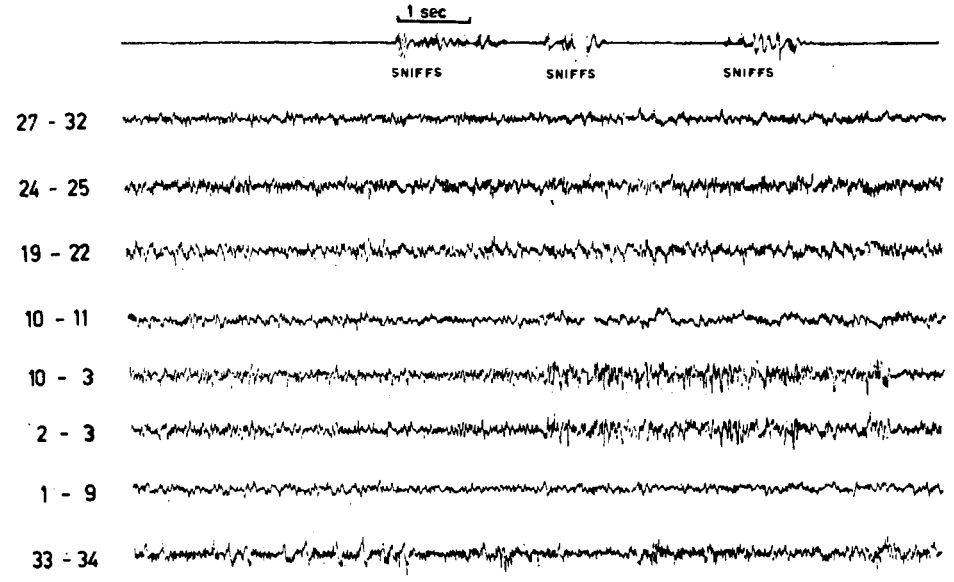

Fig. 8. Occurrence of irregular activity in amygdaloid structures (leads 10–3 and 2–3) when the animal is sniffing while no λ-waves are present (leads 33–34) (2nd and 3rd 'sniff'). If λ-waves are present (1st 'sniff') the amygdaloid activity does not take place.

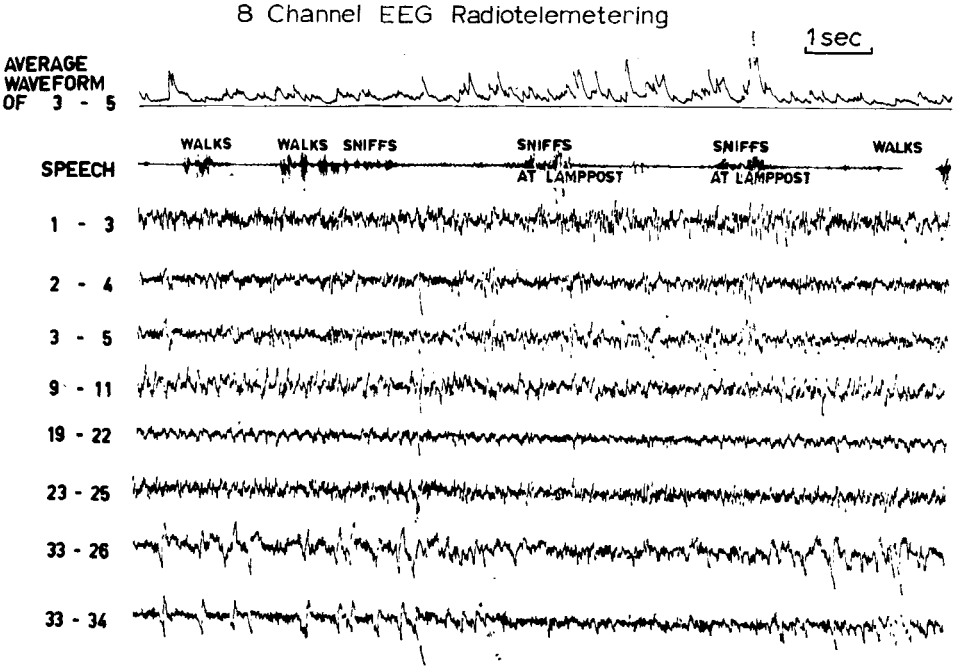

Fig. 9. Occurrence of irregular activity in amygdaloid areas (leads 1–3, 2–4, and 3–5), when the animal sniffs while no λ-waves are present ('sniffs at lamppost'). The upper trace is the integral of the rectified activity of leads 3–5. An upward deflection indicates an increase in activity. When the dog walks 5–6 c/s activity is present in hippocampal areas (lead 9–11 and 19–22) and λ-waves occur in occipital areas (leads 33–26 and 33–34). These activities disappear when dog is sniffing at lamppost.

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Continuous frequency analysis reveals that this activity consists of many frequencies occurring simultaneously. The activity has the properties of noise, and therefore frequency analysis is not a suitable form of analysis for this phenomenon. The activity may be presented well, however, with a method which records the integral of the rectified waves (Fig. 9).

The evaluation of this activity has produced difficulties, because the activity does not always occur when the animal is sniffing. If the dog sniffs at food, meat or cheese etc. in the hand of the investigator, the activity arises. While the dog is eating the activity disappears. If the dog is sniffing at other smelly substances in the hand of the investigator, for instance tobacco, a piece of cotton, or wool drenched with alcohol or with acetone, the activity also takes place. The activity, however, does not appear if the dog smells at the piece of cotton wool with acetone thrown on the ground but arises immediately if the investigator picks up the piece of cotton wool and presents it by hand to the dog. This experiment has been carried out many times with the same outcome. If the dog walks outside on the pavement and encounters a piece of meat, cheese or fish, usually the activity occurs. If the walks in high grass and encounters a piece of meat etc. the activity usually does not ensue. If the dog is taken into the darkroom (Fig. 6) and sniffs vigorously at the ground, curtains etc. the activity does not present itself. If, however, in these circumstances it encounters the investigator and sniffs at his trousers, the activity immediately arises (Fig. 7).

After experiments were carried out in which the dog was presented with various smells and in which effects of different conditions have been studied, the following was observed. If the dog is presented with a smell and sniffs, the activity may occur if at the same time some other activities are not present. For instance, if the dog is sniffing while λ-waves are present in the occipital cortex, no activity related with sniffing occurs in the amygdaloid structures (Figs. 8 and 9). Nor does the activity take place if the dog sniffs while marked 6 c/s activity related with general 'tenseness' is present (Fig. 6). Similarly the amygdaloid activity is not seen simultaneously with the occurrence of 5–6 c/s activity related with 'purposeful' walking. If the dog is walking in the grass, usually many λ-waves occur; apparently the dog is looking with 'interest' and if he sniffs simultaneously no amygdaloid activity takes place. Simillary if the dog is in the darkroom and much 6 c/s activity related with general 'tenseness' takes place no amygdaloid activity occurs. If the animal, however, sniffs at the investigator's trousers (Fig. 7) the 6 c/s hippocampal activity related with 'tenseness' disappears and the amygdaloid activity related with sniffing makes its appearance. Apparently the occurrence of the other activities excludes the appearance of the amygdaloid activity if the dog sniffs.

Although eye movements have not been recorded, an attempt has been made to investigate whether the principle of exclusiveness applies also for the other activities encountered.

The λ-waves, as stated above, do not occur if the amygdaloid activity related with sniffing is present; the λ-waves may be seen when 5–6 c/s hippocampal activity related with walking is present and they may be seen simultaneously with 6 c/s hippocampal activity related with 'tenseness', but not if the animal is very 'tense' and 'nervous' in

the darkroom (Fig. 6). Evidently α-rhythms do not occur if the other activities are present, because during the presence of these activities the animal has his eyes open. Attempts to produce sniffing while the animal's eyes were shut, have failed.

The activities are not entirely exclusive. Occasionally, over short periods of time, some of the activities may occur simultaneously.

The phenomena described have been observed mainly in one dog. This dog, however, has been investigated for $2\frac{1}{2}$ years. In this period more than 100 EEG's have been recorded. The activities seen in the first records were similar to those in the last. In the first records, moreover, the topography, form and reactivity of the activities are the same as those in the last records. Most of the activities have also been observed in other dogs, but further investigations are necessary and are being carried out.

DISCUSSION

The results described have two points of importance. The first is the finding of activities related with behaviour as (i) the occipital λ-waves with eye movements made with 'interest'; (ii) the 5–6 c/s hippocampal activity related with 'purposeful' walking; (iii) the 6 c/s hippocampal activity related with 'tenseness'; and (iv) the irregular amygdaloid activity related with sniffing.

The second point is the mutual exclusiveness of some of these activities.

If these findings are compared with similar results obtained by others, the following observations should be mentioned.

The occurrence of waves at approximately 10 c/s in dogs has been observed by various other authors. This activity has been described for instance by Werner *et al.* (1962) and has been related with states of sleepiness or beginning of sleep. As far as the present authors know, no attempt has been made previously to identify this rhythmic activity with the α-rhythms in man.

The occurrence of λ-waves has not previously been described in dog. However, they have been the subject of extensive study in cat, particularly by Rhodes *et al.* (1962) who have demonstrated that these waves are related to eye movements. It seems to the present authors that the observations made by Rhodes *et al.* in the cat are confirmed by the observations in dogs. Moreover, these observations seem to indicate that the λ-waves may be used as an indication of the degree of 'interest' with which the animal is looking. The more λ-waves, the more 'intense' the interest.

The occurrence of 5–6 c/s activity in the hippocampus related with 'purposeful' walking has been described in cat and in monkey by Adey (1961) and Adey *et al.* (1961) in training experiments. The phenomenon described by him is similar to the phenomenon observed by us in the dog.

The occurrence of 6 c/s activity with general 'tenseness' appears to be similar to the activity termed θ-activity by Green and Arduini (1954), observed in the hippocampus of rabbits and described extensively by Green *et al.* (1960 and 1961). These authors have related the activity with general 'alertness' or with 'arousal'. In their experiments the animals were not able to move freely. For this reason a precise comparison between the results obtained in their experiments, in Adey's and in ours

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is not easily made. The hippocampus is a large structure. In many parts, perhaps in all parts, activities at approximately 6 c/s may occur. These activities particularly take place in states of increased 'alertness' or increased 'attention'. The 6 c/s activities arising in the various parts of the hippocampus are not precisely related, and are not mutually interdependent all the time, although they may appear to be related some of the time. It seems to the present authors that in general the notion of 'arousal', 'attention' or 'alertness' is not a rigid phenomenon, adjustable only in intensity, but rather should be regarded as a pliable phenomenon which in man and in animal may have certain directions. The animal may become 'alert' and have an 'intention' to walk to a certain 'goal' without 'anxiety' and 6 c/s activity may occur in a particular part of the hippocampus. Or, the animal may be more generally 'tense' or 'anxious', and 6 c/s activity may occur in another hippocampal area. Evidently the two behavioural aspects may take place simultaneously, and it is not too far-fetched to suppose that in such circumstances the different hippocampal areas have simultaneous 6 c/s activities. In the 'directing of attention' an almost infinite number of tendencies seems possible. It seems unlikely that such 'directing of attention' would be regulated by a smaller or larger number of discrete centres, but more likely by a changing population of units, the largest density deciding the main 'direction'.

The occurrence of the amygdaloid activity related with sniffing 'with interest' has not been described previously to the knowledge of the authors. As stated above, this activity generally does not occur simultaneously with λ-waves, the one activity appearing to prevent the occurrence of the other. It seems to the present authors that this alternative occurrence may be regarded as the electrical counterpart of the 'directing of attention' to visual or olfactory messages. If this be so all the activities described — the λ-waves, the 5–6 c/s hippocampal activities and the amygdaloid activity — although different in frequency, form and topography may be regarded as the electrical signals accompanying 'directing of attention'. Apparently the dog, like man, preferably directs attention to one modality at a time though it appears not to be impossible to give attention simultaneously to more modalities for a short while. Based on these considerations the occurrence of an activity related to 'listening' may be predicted. As yet such activity has not been encountered.

The phenomena may be of importance in conditioning experiments. Perhaps the 'directing of attention' as indicated by the electrical phenomena is an aspect of the 'orientation reflex' described by Pavlov. In the classical conditioning experiments the outward circumstances are kept rigorously constant and in these, therefore, 'directing of attention' may not play a major part. In all investigations where control over the outward circumstances cannot be exerted so rigorously, this 'directing of attention' may influence considerably responses to stimuli and may contribute to the variability of the responses.

SUMMARY

An investigation has been carried out in dogs with electrodes implanted in various brain structures over periods up to 3½ years. A description of the recording methods

is given. Radio telemetering of 8 EEG channels has played an important part in obtaining electrical data during 'free' behaviour of the dogs. The following relations have been observed: *(1)* At eye closure rhythmic activity occurs in the occipital cortex which is identified with α-rhythms in man. *(2)* In agreement with observations by Rhodes *et al.* (1962) in cat, λ-waves in occipital areas are encountered which are related to eye movements made with 'interest'. *(3)* In agreement with Green *et al.* (1960) and Adey *et al.* (1961), rhythmic activities in hippocampal areas are observed related with 'tenseness' and with 'purposeful walking'. The mutual relations of these hippocampal activities are discussed. *(4)* In amygdaloid areas activities are encountered associated with sniffing 'with interest'.

The mutual relations of the activities encountered are discussed. Some of the activities occur alternatively: the occurrence of one activity appears to exclude the occurrence of another. The hypothesis is proposed that these phenomena may be regarded as the electrical counterpart of 'directing of attention'.

ACKNOWLEDGEMENT

We should like to express our sincere gratitude to Dr. O. L. Adrianov and Dr. T. A. Meringe for presenting us with the 'Atlas of the Dog's Brain'.

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Reflex and Brain Stem Inhibition of Sham Rage Behaviour

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The classic experiments of Cannon and Bard (Cannon and Britton, 1925; Bard, 1928) on release of rage behavior following decortication or thalamic transection of the brain have initiated abundant, though controversial, literature on descending inhibitory influences exerted by neocortical or rhinencephalic structures upon the hypothalamic mechanisms regulating emotional activity. Little attention has been paid to the possible existence of ascending excitatory and inhibitory influences acting upon diencephalic rage behavior, in spite of the fact that recent years have seen a rise of interest in ascending mechanisms regulating wakefulness and sleep (Rossi and Zanchetti, 1957; Zanchetti, 1962). The experimental outline of various reflex or brain stem mechanisms capable of either inducing or suppressing waking behavior has prompted the question whether the same or similar ascending influences are exerted upon the diencephalic rage behavior as well. During the last few years, our group in Siena has intensively investigated this problem. Excitation and inhibition of sham rage behavior have been obtained both by reflex and lower brain stem stimulation. These results are briefly presented here, with particular emphasis on inhibitory phenomena.

All the experiments here reported have been performed on acutely decorticated cats. The cat's head was fixed in the frame of a stereotactic apparatus, while the body was placed in a hammock in such a way as to permit convenient observation of the animal's rage behavior. Arterial pressure was continuouly measured from a cannulated femoral artery through a capacitance manometer, and respiration was recorded by means of a crystal capsule transducer connected to the side-arm of the tracheal cannula. Graphic evidence of the somatic discharges paralleling autonomic activity in sham rage outbursts was obtained through the electrical activity of one or more muscles of the forelimbs, adequately amplified.

Our attention was called to a report by Bonvallet *et al.* (1954) showing that natural stimulation of carotid sinus pressoceptive afferents results in EEG synchronization, an effect which has been considered to depend on reflex inhibition of activating reticular neurons. Our experiments (Bartorelli *et al.*, 1960) have indicated that the diencephalic mechanisms regulating sham rage behavior are also subjected to a restraining influence from the carotid sinus pressoceptors. Indeed, in acutely decorticated cats transient pressoceptive inactivation by carotid occlusion below a carotid sinus blind sac induced outbursts of sham rage otherwise indistinguishable from those occurring spontaneously (Fig. 1). Sham rage outbursts followed carotid occlusion even when ca-

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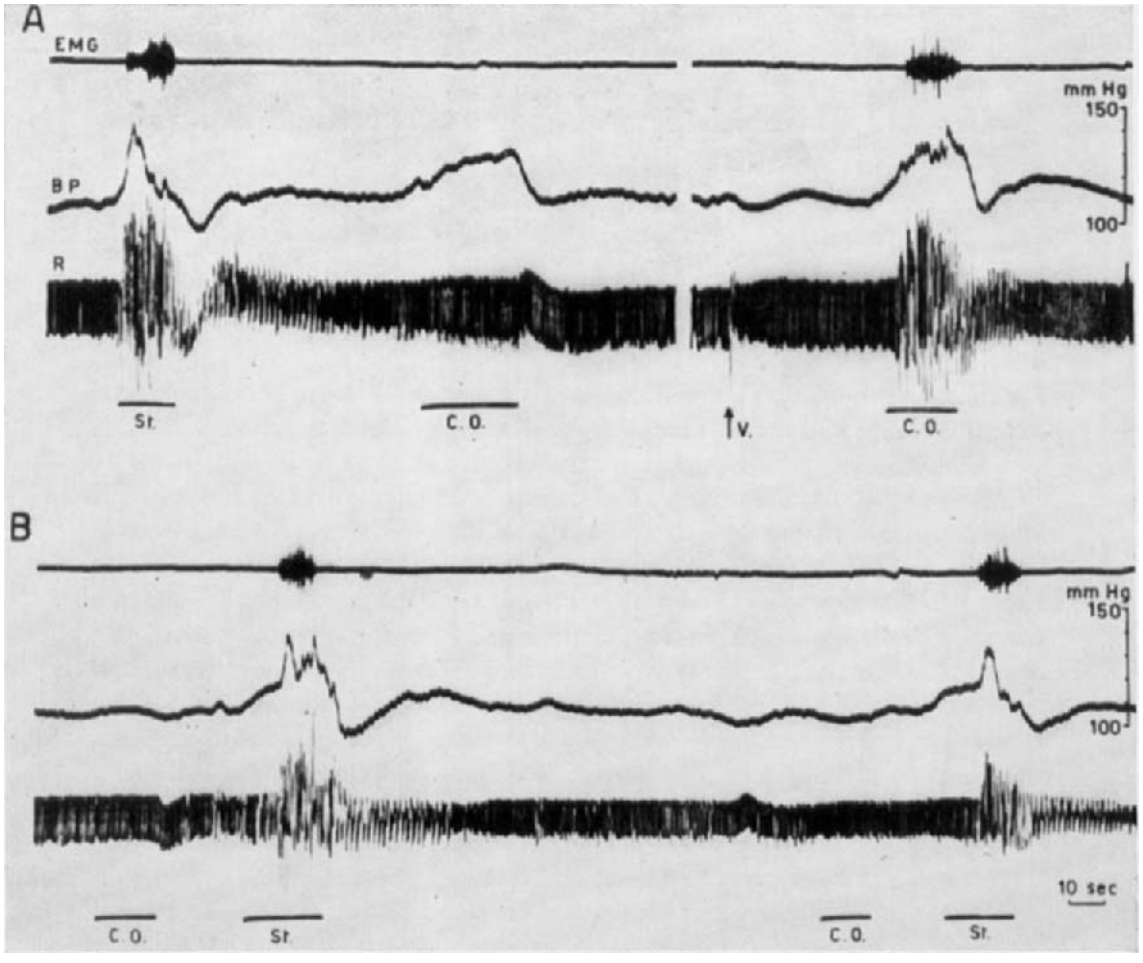

Fig. 1. Sham rage patterns induced by bilateral carotid occlusion (A) and disappearance of the effect after bilateral carotid sinus denervation (B). Decorticated cat. C.O. = carotid occlusion; St. = noxious stimulation; V. = bilateral cervical vagotomy. Note that after section of the carotid sinus nerves, noxious stimuli are still effective in eliciting sham rage, while carotid occlusion yields no results. EMG = electromyogram of the left triceps brachii; BP = femoral arterial pressure; R = respiration. (From Bartorelli *et al.*, 1960, by courtesy of Arch. ital. Biol.)

rotid body chemoceptors had been inactivated by embolization with a lycopodium suspension, an observation showing that this effect of carotid occlusion results from transient suppression of pressoceptive discharges, independently of chemoceptive co-excitation. This conclusion was further substantiated by the observation that pressoceptive stimulation by increased intrasinusal pressure succeeded in blocking spontaneously occurring outbursts of rage (Fig. 2).

In other experiments (Bizzi *et al.*, 1961), natural stimulation of carotid body chemoceptors was found to excite the sham rage behavior of the decorticated cat, an effect which can be compared to the arousing action displayed by these afferents in the 'encéphale isolé' animal according to Hugelin *et al.* (1959).

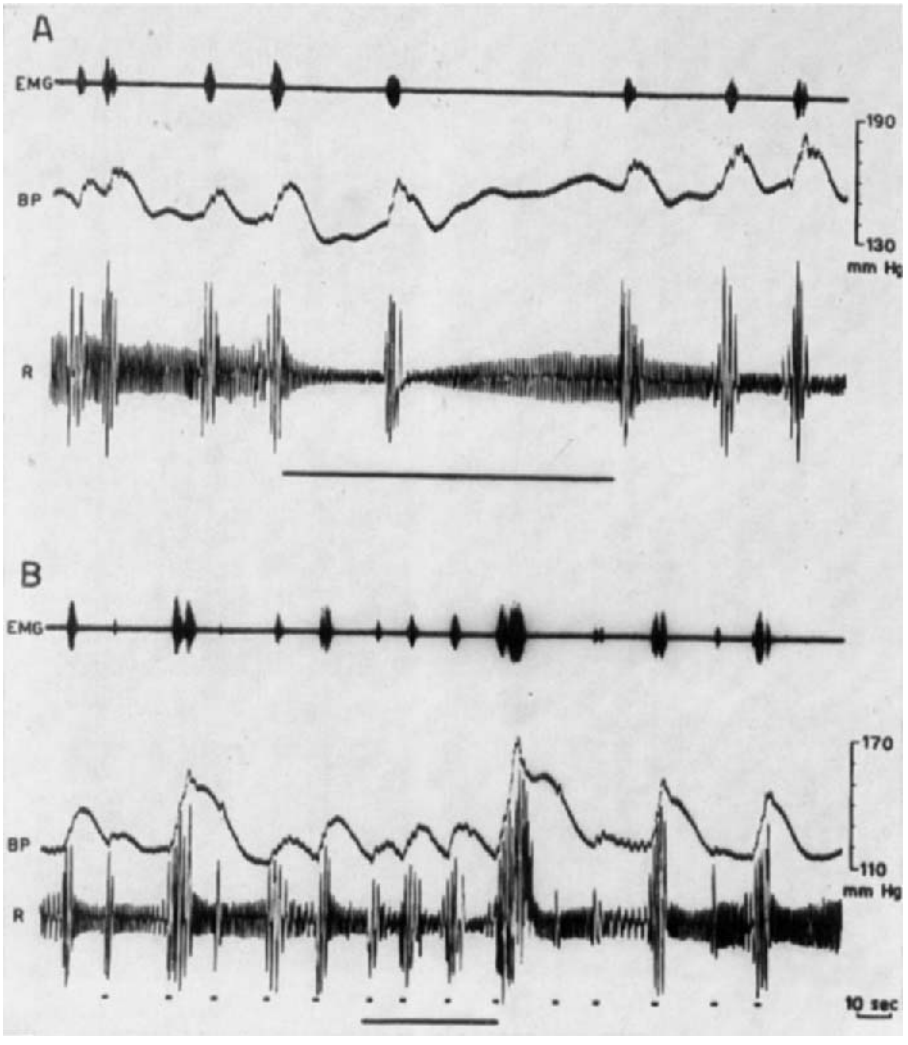

Fig. 2. Effects of increased intrasinus pressure on 'spontaneous' and evoked outbursts of sham rage. Decorticated vagotomized cat. Left carotid sinus denervated; right carotid sinus blind sac; chemoreceptors inactivated. (A) Partial inhibition of spontaneously occurring outbursts of sham rage during increase in pressure inside the right sinus from 0 to 200 mm Hg (continuous line). (B) No definite effect of comparable pressoceptive stimulation (continuous line) on outbursts of sham rage driven by rhythmic scratching of the neck (dots). Other abbreviations as in Fig. 1. (From Bartorelli *et al.*, 1960, by courtesy of Arch. ital. Biol.)

Recent studies (Baccelli *et al.*, 1963, 1964) have shown that both the inhibition and the excitation of sham rage behavior induced by natural stimulation of pressoceptors and chemoceptors in the carotid sinus region can be duplicated by electrical stimulation of two different groups of fibers in the aortic nerve. Low voltage stimuli, exciting low threshold pressoceptive afferents, are accompanied by clear-cut and prolonged inhibition of recurring rage outbursts, while slightly stronger stimuli, involving intermediate threshold chemoceptive afferents, yield a definite potentiation of rage activity (Fig. 3).

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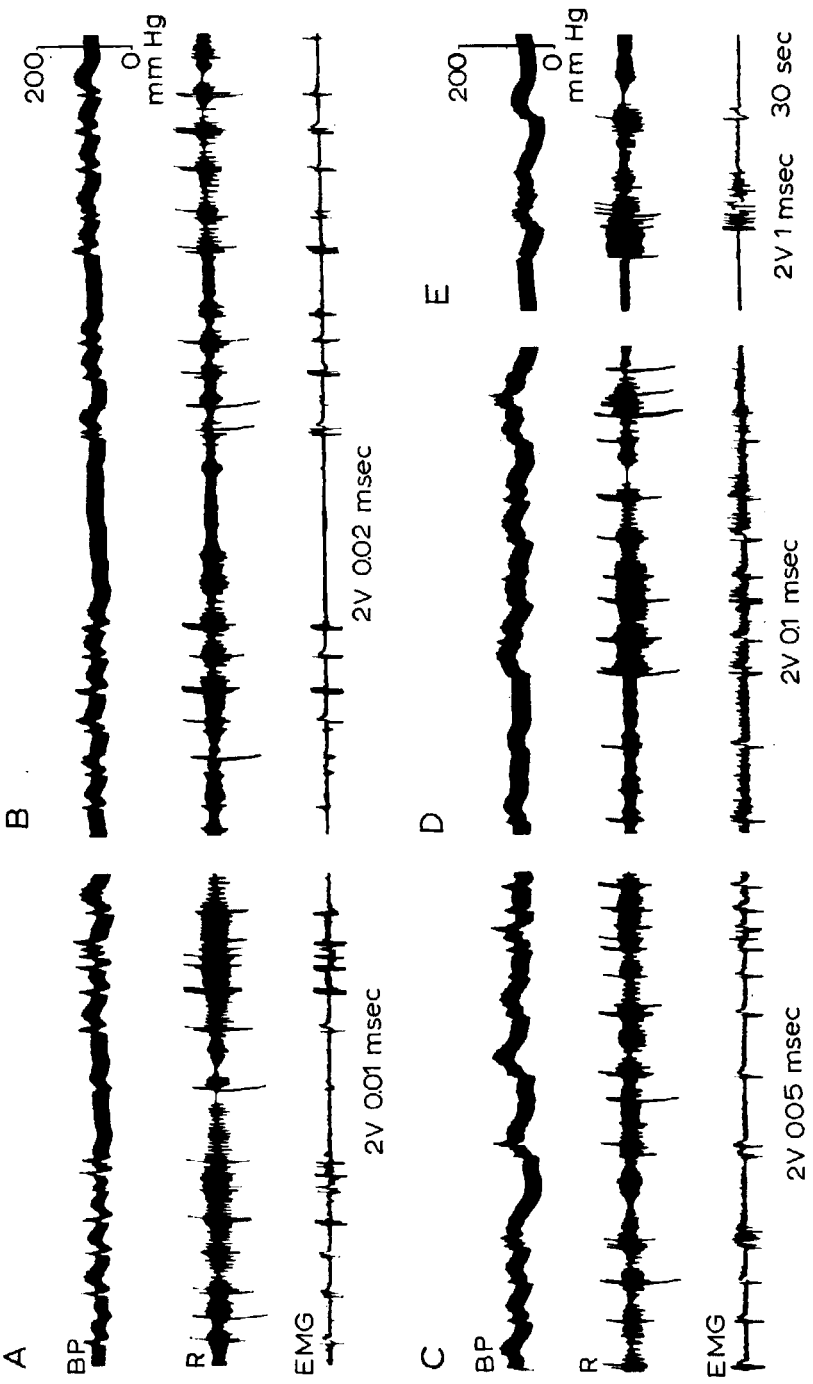

Fig. 3. Inhibition and excitation of spontaneous rage behavior upon stimulation of the left aortic nerve with impulses of increasing strength. Decorticated cat. 100/sec stimuli, the strength of which is indicated above the signals. (A) and (B) inhibition of rage outbursts (particularly evident with the stimulus strength used in (B). (C) transitional effect. (D) and (E) excitation of rage behavior. Other abbreviations as for Fig. 1. (From Baccelli *et al.*, 1965, by courtesy of *Amer. J. Physiol.)*

It is interesting in this connection that the inhibitory action of low threshold pressoceptive fibers is completely overwhelmed by excitation when the intermediate threshold chemoceptive fibers are co-stimulated.

Excitation and inhibition of sham rage behavior were also elicited in the decorticated cat when several loci in the lower brain stem, namely in the pons and medulla, were stimulated by co-axial bipolar electrodes (Bizzi *et al.*, 1963). Both excitation and inhibition of sham rage could be observed using stimuli which did not induce any gross modification of postural tone, although stronger stimuli did usually produce postural reactions often interfering with the manifestations of rage. As far as the excitatory effects are concerned, it suffices to say here that they could be induced by stimulation within the lateral and the medial portions of the medullary reticular formation. The reflex nature of these rage responses was demonstrated by their disappearance when the upper brain stem was reversibly inactivated by an intracarotid injection of barbiturate (Fig. 4).

Inhibition of sham rage could be obtained only from the medial tegmentum. Thresh-

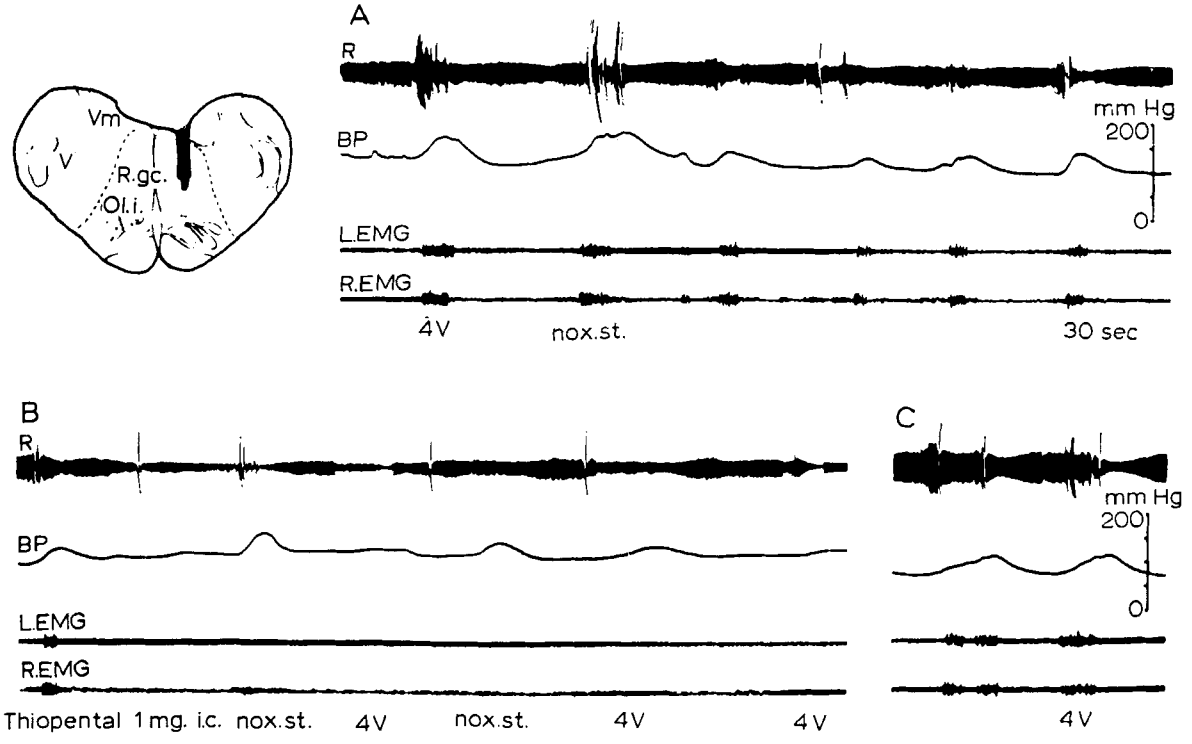

Fig. 4. Sham rage outbursts reflexly induced by stimulating the medial reticular formation (nucleus reticularis gigantocellularis). Decorticated cat. (A) Control period. 4 V indicates reticular stimulation (25/sec, 1 msec, 4 V) yielding rage activity. nox. st. = noxious stimulus of the tail. Sham rage outbursts not otherwise labeled are spontaneous. (B) Introduction of 1 mg thiopental into the central stump of the right lingual artery is marked by the continuous line. Subsequently no sham rage outburst is induced by noxious or reticular stimuli. (C) Two minutes after end of (B) the effects of thiopental are dissipated: reappearance of spontaneous and reticularly induced rage outbursts. Abbreviations as for Fig. 1. (From Bizzi *et al.*, 1963, by courtesy of Arch. ital. Biol.)

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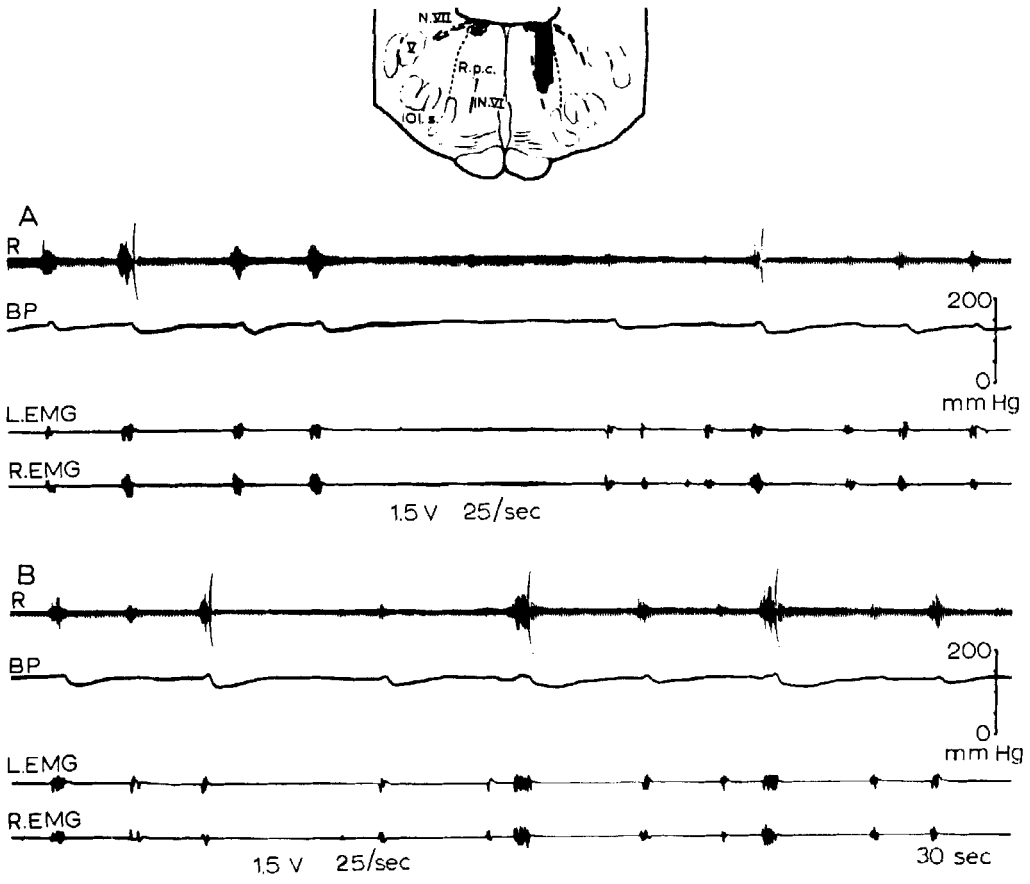

Fig. 5. Prolonged inhibition of spontaneous sham rage activity during stimulation of nucleus reticularis pontis caudalis. Decorticated cat. (A) and (B) show two successive instances of stimulation of the same reticular site. The continuous line indicates reticular stimulation, the parameters of which are written above. (From Bizzi *et al.*, 1963, by courtesy of Arch. ital. Biol.)

old stimulation of several points in the medial medullary and pontine reticular formation blocked the spontaneously recurring outbursts of rage, when present (Fig. 5), or those evoked by tactile or noxious stimuli (Fig. 6). Clawing and struggling movements of the limbs, snarling facial expression, mydriasis, hyperpnea, hypertension and all other components of rage outbursts could similarly be blocked by stimulating the brain stem. On the other hand, when timed during an interval of quietness in the preparation, the same stimulus was without any effect on postural tone, respiration and arterial pressure.

Although the cerebellum is known to exert both inhibitory and excitatory influences on sham rage behavior (Moruzzi, 1950; Zanchetti and Zoccolini, 1954), the effects of brain stem stimulation did not result from co-excitation of cerebellofugal fibers of passage, as both inhibition and excitation of sham rage could be induced by bulbar and pontine stimulation in chronically cerebellectomized animals.

The location of the brain stem sites yielding excitation (filled circles) or inhibition

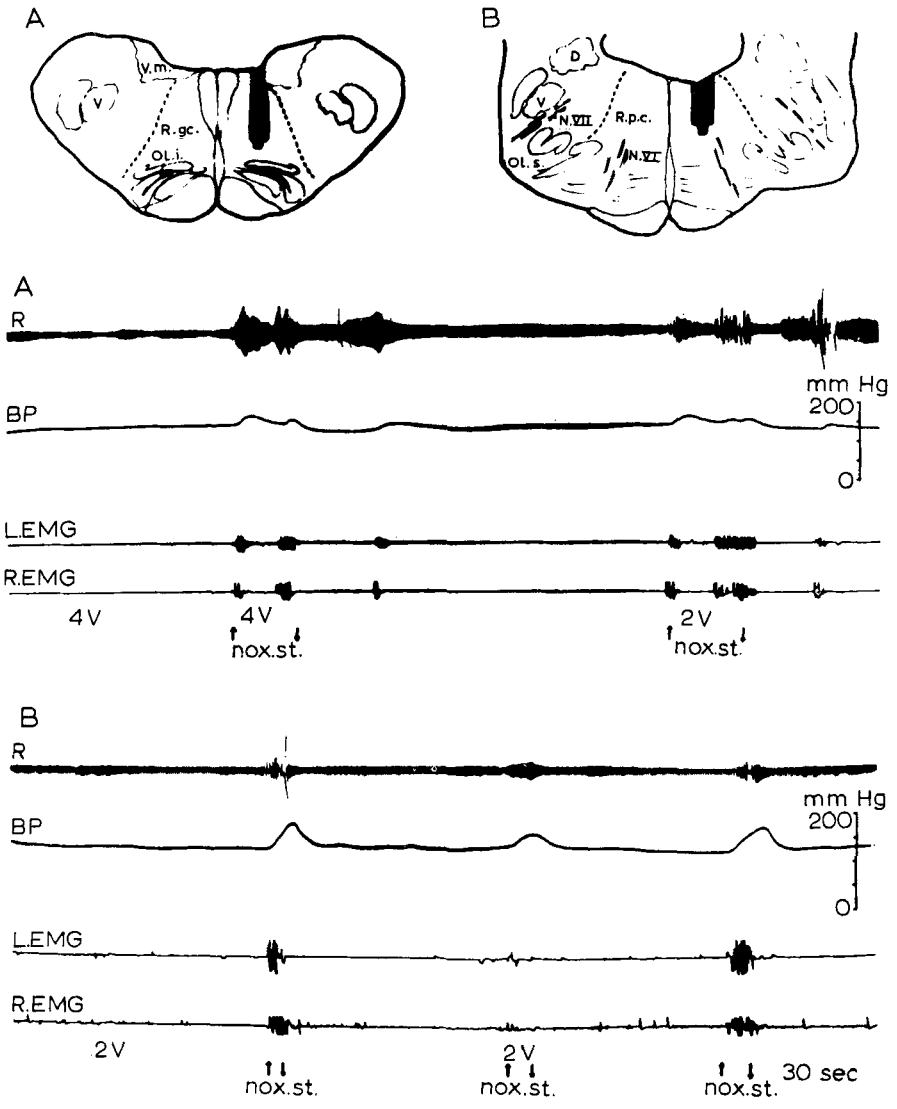

Fig. 6. Reticular inhibition of sham rage outbursts evoked by noxious stimuli. (A) and (B) refer to two different animals. Noxious stimulation of a hind limb (A) and of the pinna (B) is performed between arrows. Electrical stimulation of the nucleus reticularis gigantocellularis (A) and of the nucleus reticularis pontis caudalis (B) is carried out with 1 msec pulses, 25/sec, and with the voltage indicated. (From Bizzi *et al.*, 1963, by courtesy of Arch. ital. Biol.)

(crosses) of rage behavior is summarized in Fig. 7. Whereas excitation could be induced both from the medial and the lateral reticular formation, and from extrareticular structures as well, inhibition was only obtained by stimulating medial reticular sites. Within the medial portions of the tegmentum, excitatory and inhibitory points appeared to be intermingled without any regular order. However, a certain degree of anatomical segregation of the two influences is suggested by experiments like that of Fig. 8.

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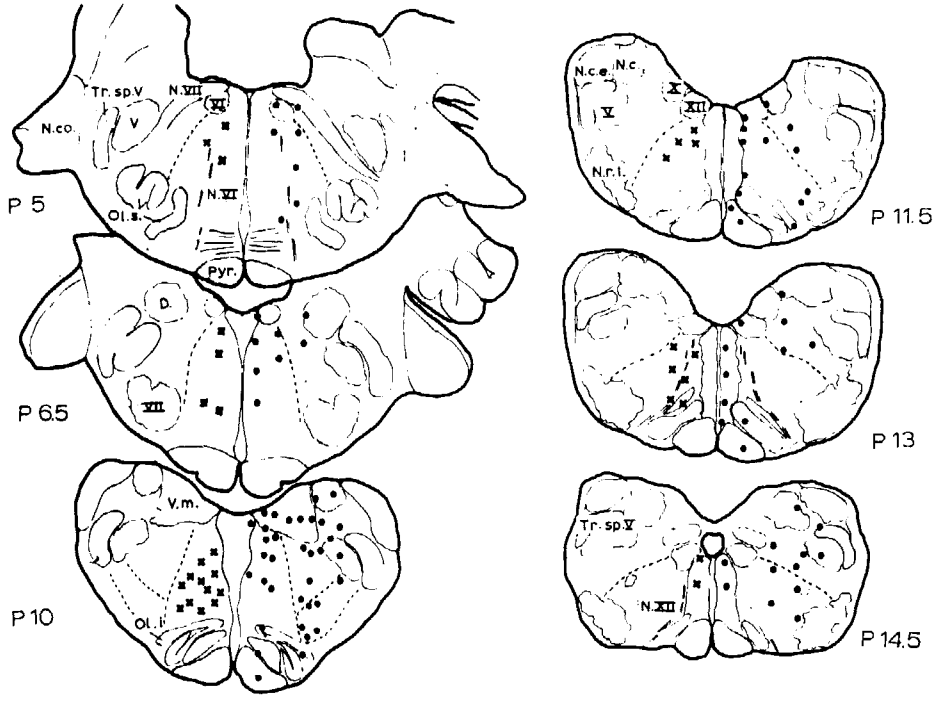

Fig. 7. Anatomical distribution of bulbopontine structures yielding excitation (filled circles) or inhibition (crosses) of sham rage. Excitatory and inhibitory points are separately represented on the right and left sides of each drawing. Numbers refer to the distance from the interaural plane of the Horsley–Clarke instrument. (From Bizzi *et al.*, 1963, by courtesy of Arch. ital. Biol.)

In one and the same animal it was often possible to change an excitatory into an inhibitory effect, or *vice versa*, by simply moving the stimulating electrode as little as 1–2 mm without leaving the cytoarchitectonic limits of a given nucleus. On the other hand, a change of effect was never obtained from any reticular site simply by modifying either the intensity or the frequency of the stimulation, except for the appearance of postural reactions.

As to the level at which sham rage is inhibited by the reflex and brain stem mechanisms we have investigated, it seems likely that we are dealing with an ascending inhibitory influence, rather than with a descending one such as that of the bulbar inhibitory center of Magoun and Rhines (1946). Indeed neither postural nor autonomic effect was ever observed when the inhibitory stimulus was given in a period of quietness in the preparation. Moreover, the possibility of simultaneously blocking all somatic and visceral components of rage activity clearly, though not crucially, points to an influence acting more directly upon the diencephalic mechanisms of rage. Finally, this conclusion is also supported by other evidence showing that the reflex afferents and the brain stem regions yielding the inhibitory effect are also possessed of other influences, which are undoubtedly ascending in type, like those on the electroencephalogram, wakefulness and sleep.

More direct comparison of the rage inhibiting action of the lower brain stem to the

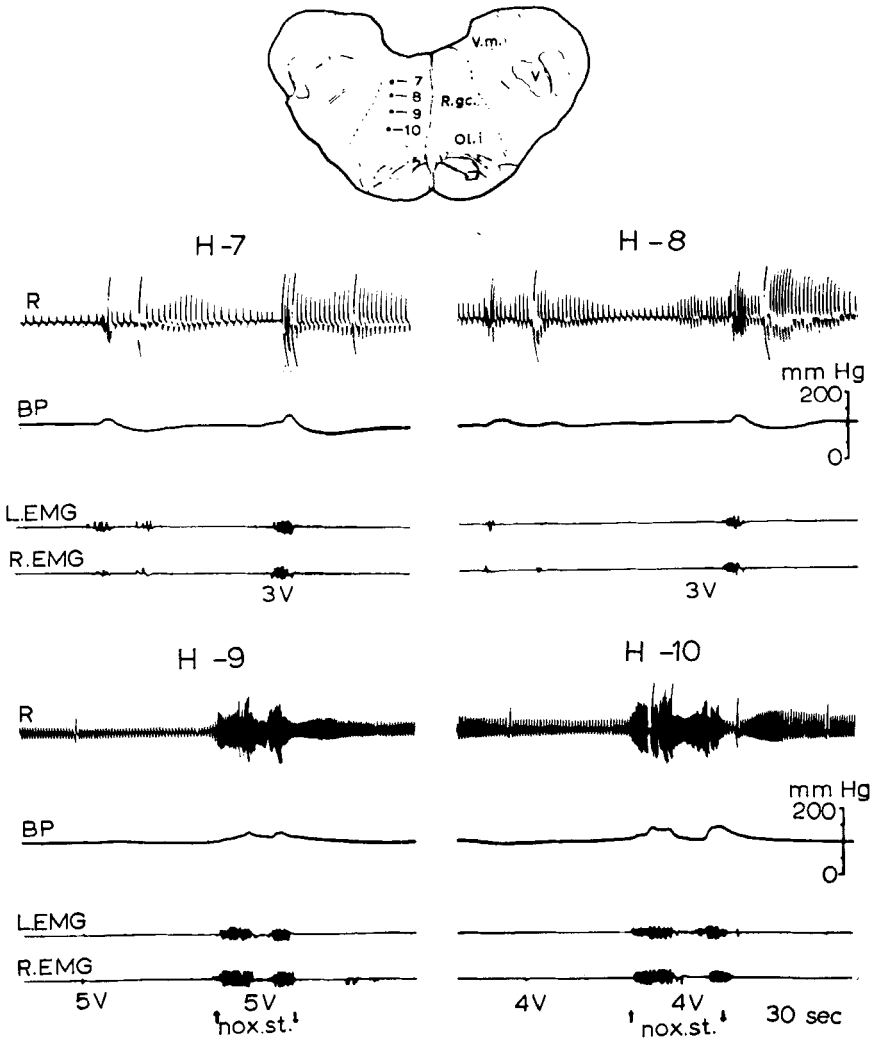

Fig. 8. Excitation and inhibition of sham rage from adjacent sites in the nucleus reticularis gigantocellularis. Numbers above each of the four graphs refer to sites marked with dots in the anatomical inset. Each electrical stimulation is effected with 25/sec, 1 msec pulses, the voltage being indicated in the figure. At H —7 and H —8 bulbar stimulation evoked sham rage outbursts; at H —9 and H —10 bulbar stimulation inhibited rage activity induced by pinching the tail. Nociceptive stimulation is marked nox. st. between arrows. All tracings are from a single animal. (From Bizzi *et al.*, 1963, by courtesy of Arch. ital. Biol.)

tonic and phasic synchronizing and hypnogenic influences which are known to be located in the same parts of the brain (Batini *et al.*, 1959; Dell *et al.*, 1961; Favale *et al.*, 1961; Magnes *et al.*, 1961) is rather unwarranted at present (Guazzi *et al.*, 1964). However, the recent finding by Bonvallet and Allen (1963) that discrete bulbar lesions are often followed by bursts of cortical activation associated with motor and visceral effects, is worthy of comment. These rhythmical discharges call to mind the recurring outbursts of sham rage that we have observed after transient or permanent inactivation

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of pressoceptive afferents (Bartorelli *et al.*, 1960), and might well result from partial elimination of the inhibitory mechanisms we have recently investigated by stimulation techniques.

The experiments of Bonvallet and Allen are also relevant to another aspect. As bulbar coagulation could release reticular activity also in mesencephalic preparations, these authors have suggested that the bulbar restraining influence is exerted directly at reticular levels. It is possible, therefore, that the pressoceptive and brain stem inhibitory influences on rage behavior are also largely mediated through a depression of the reticular activating system, a hypothesis that is supported by the demonstration that sham rage activity is dependent upon a tonic barrage of ascending reticular impulses (Malliani *et al.*, 1963). However, in our opinion the promptness by which bulbopontine stimulation can often block an active rage outburst makes it likely that inhibitory effects may also be exerted at higher levels, upon the diencephalic pacemaker of rage activity.

SUMMARY

The ascending mechanisms regulating autonomic and somatic hypothalamic activity and sham rage behavior have been investigated in the acutely decorticated cat, and compared with those involved in regulation of wakefulness and sleep. Excitation and inhibition of sham rage behavior have been obtained both by reflex and lower brain stem stimulation. Inhibitory effects are reported and discussed in detail.

The diencephalic mechanisms regulating rage behavior have been found to be subjected to a restraining influence from the carotid sinus pressoceptors. Indeed transient pressoceptive inactivation by carotid occlusion induces outbursts of sham rage even when the carotid body chemoceptors have previously been inactivated; on the other hand, pressoceptive stimulation by increased intrasinusal pressure blocks spontaneous rage outbursts. Low voltage stimuli of the aortic nerve, exciting only low threshold pressoceptive afferents, can also induce prolonged inhibition of rage activity.

Inhibition of sham rage could also be obtained by electrically stimulating the medial portions of the bulbopontine reticular formation. Effective stimuli, when tested during an interval of quietness in the decorticated preparation, were without any action on postural tone, respiration or arterial pressure.

Although the brain stem sites yielding sham rage inhibition were intermingled with others whence rage behavior was excited, the two influences appear to be anatomically segregated, as a change of effect could never be obtained by modifying the electrical parameters of stimulation, but only by moving the stimulating electrode, even as little as 1–2 mm.

ACKNOWLEDGEMENT

This research has been sponsored jointly by the Aeronautical Systems Division, AFSC and Air Force Office of Aerospace Research, OAR, through its European Office, Office of Aerospace Research, United States Air Force, under Contract No. AF 61 (052)–253, and by Consiglio Nazionale delle Ricerche.

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Average Evoked Responses to Somatic Stimulation in the Cat: Changes in Relation to State of Vigilance

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INTRODUCTION

In the unanesthetized and unrestrained animal, somatic stimuli give rise to responses, not only in structures that are directly fed through primary pathways, but also in structures that are connected to secondary or 'associative' pathways. This fact has been demonstrated by several experiments (Albe-Fessard *et al.*, 1960, 1961a, b; Hirsch *et al.*, 1962; Pompeiano and Swett, 1962; Okuma and Fujimori, 1963; Albe-Fessard and Fessard, 1963.)

These early findings suggested that the amplitudes of the evoked associative components vary differently from those of the primary components as the animal's state changes along the wakefulness–sleep dimension. In the present paper, we have tried to quantify some of the earlier findings through the use of appropriate recording and averaging techniques; furthermore, we have tried to compare the behavior of simultaneous events in different cortical and thalamic structures.

PROCEDURES

The experiments were carried out on 4 implanted cats during 26 recording sessions. The multiple-electrode placements enabled us to record simultaneously both spontaneous and evoked activity of several cortical and subcortical structures during different stages of wakefulness.

Deep recordings were made with concentric bipolar electrodes; at any time during experimentation, the inner electrode was movable vertically over a range of 2 mm. (See technique in Albe-Fessard *et al.*, 1961a.) Cortical recordings were monopolar, with the reference leads in the sinus; the electrodes (silver) were implanted into the bone, and rested upon the dura mater.

The cats were placed in a soundproof room which remained moderately illuminated throughout the experiment. The experimenters were able to observe the animals without being seen themselves.

We have distinguished 4 more-or-less typical stages of behavior along the wake-

fulness–sleep dimension. The animal may be awake, drowsy, or in 1 of 2 stages of sleep. In the sleeping cat, the electrocortical activity may be predominantly characterized by low frequency waves ('sleep–slow phase') or by higher frequency waves ('sleep–fast phase') (Dement, 1958; Hubel, 1960; Jouvet, 1962). We have classified the state of an animal by observing simultaneously the cat's behavior, the continuously recorded electrocorticogram, and, finally, by a measure of integrated electrocortical activity. We used a device which enabled us to distinguish three categories of activity: low-voltage fast activity, high-voltage slow activity, and the type of fluctuating activity which is associated with drowsiness. These various indicators made it fairly easy to identify drowsiness and the sleep–slow phase. The distinction between the states labeled awake and sleep–fast phase was less certain on the basis of this measure of electrical activity; but, in these instances, observations of the cat's overt behavior, such as small myoclonic twitches, yielded the information necessary to make a clear distinction. In some experiments, recordings of the neck EMG enabled us to verify the presence of so-called paradoxical sleep.

During the states defined above, we sampled both spontaneous and evoked activity. The evoked potentials were observed in response to bipolar stimulation of the superficial radial nerve; stimulating electrodes had been implanted according to a method previously described. In general, the voltage at which the nerve was stimulated was twice the value needed to yield a clearly detectable evoked response in the primary cortical receiving area. In all instances, this voltage was less than the amount needed to produce a flexion reflex.

We have recorded from the following structures: the primary thalamic relay nucleus (VPL), the cortical area Somatic 1 (S1), and different associative structures both in the thalamus (centre median, VL) and in the cortex (gyrus marginalis anterior, sigmoid anterior).

Both spontaneous and evoked activity were recorded on magnetic tape and later analyzed. During the experiments, 'on-line' averaging of records from one location was done for monitoring purposes. Averaged responses were computed for each state and neural structure for intervals during which both behavior and electrical activity remained substantially unchanged. In general, the averages were computed from 50 sample responses; exceptionally, only 25 responses were used.

In later sessions, the cats were anesthetized: at first, with chloralose (80 mg/kg); then, after a day during which alertness had apparently recovered, with Nembutal (35 mg/kg). This procedure was designed to permit us to compare spontaneous and evoked activity in the unanesthetized sleeping cat with comparable activity in the anesthetized cat. Control observations of electrical activity were carried out after recovery from each anesthesia.

At the end of this experimental series, the cats were perfused under anesthesia with 10% formol; the location of the cortical electrodes was visually verified; the location of the deep electrodes was checked in Nissl stain sections.

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RESULTS

Our observations fall into two categories: changes in electrical activity from structures that belong to (1) the primary system or (2) the associative complex.

(1) Evoked activities in primary structures

Here, we obtained most data from the primary cortical area that lies forward of S 1 at the lateral extremity of the cruciform gyrus (Fig. 1). Recordings from the primary thalamic relay nucleus (VPL) were taken in 1 cat only.

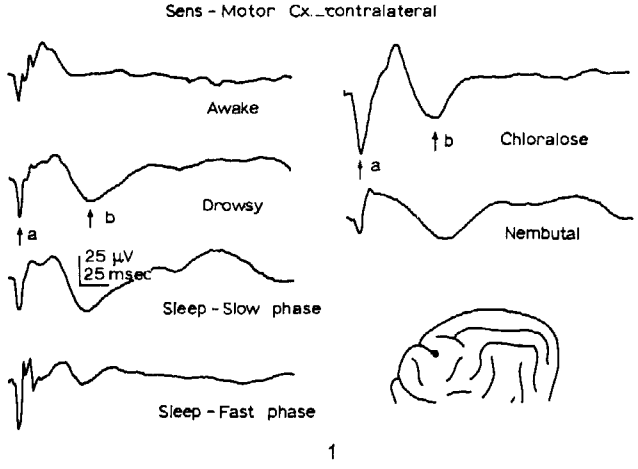

Fig. 1. In this and the subsequent figures, the animals carried implanted electrodes but were otherwise unrestrained. Downward deflections indicate positivity with respect to the reference lead. The traces recorded at the level of the sensorimotor cortex (see the location of the active electrode on the insert map) represent the averages of 50 responses to stimulation of the contralateral radial nerve for different stages of sleep and wakefulness. Comparable records were also taken under chloralose and Nembutal anesthesia. In Figs. 1–6, the shock stimulus was delivered at the beginning of each trace. For identification of a and b see text.

Cortical responses. These consisted of two major components, one of short latency (labeled a on Fig. 1) and one that has longer latency and duration (labeled b). The short-latency component varied in a manner that was characteristic of the state of the animal. In Fig. 1, it changed relatively little as the cat became drowsy or entered the state labeled sleep–slow phase; however, it increased noticeably during the sleep–fast phase (Figs. 1, 2 (right column) and 8). This increase during 'paradoxical sleep' was a particularly stable phenomenon; the mean value of the amplitude of the short-latency component increased to about 1.5 or even 2 times the value found in the awake cat.

The long-latency component varied in a different manner: its behavior resembled the changes reported below as characteristic of associative responses. The amplitude of this long-latency component was small in the awake cat and during the state labeled sleep–fast phase; it was large during drowsiness and sleep–slow phase.

Chloralose anesthesia resulted in a small, further increase of the amplitude and a

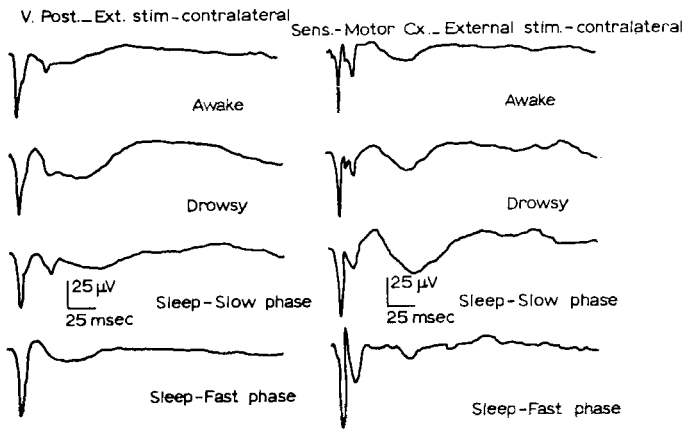

Fig. 2. Changes with state in averaged responses computed from simultaneous records taken along the primary pathway: electrodes in thalamic relay nucleus (V. Post.: ventralis posterior) and cortex (sensorimotor CX). Electrical stimulation of the skin of the contralateral foreleg.

broadening and smoothing of the waveform of the early-latency component (Fig. 1). During chloralose anesthesia, we also observed a slow component similar to that which appeared during the sleep–slow phase. Barbiturate anesthesia reduced the early component and delayed and stretched the later one.

The thalamic response exhibited much less striking variation in amplitude; this fact has been shown previously. The response was greatest when the animal was awake and during the sleep–fast phase and decreased when the animal was drowsy or in the sleep–slow phase (Fig. 2).

(2) Evoked activities in associative structures

These structures are divided into two groups: those that seem without any component that passes through the lemniscal pathway (cortex marginalis anterior, centre median, and nucleus parafascicularis); and those in which both primary and associative re-

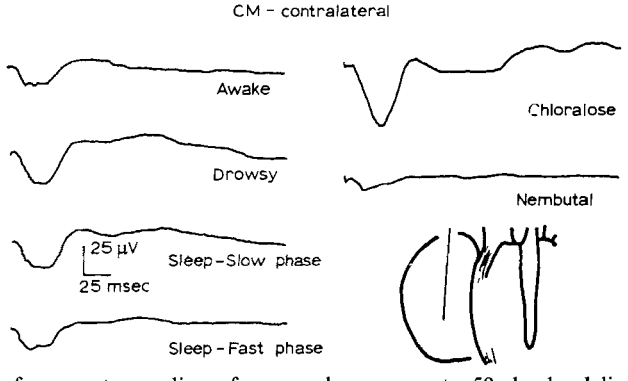

Fig. 3. Records from centre median of averaged responses to 50 shocks delivered to the contralateral radial nerve. All records are from the same cat taken under conditions comparable to those of Fig. 1.

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sponses are observable (anterior sigmoid gyrus). For clarity of exposition we shall here report first the simpler changes observed in the thalamus before proceeding to the cortical observations.

Thalamic responses. The following observations were made in a region which we shall call, for brevity's sake, the centre median (CM) in the cat. This region is equivalent to the centre median and nucleus parafascicularis in the monkey. In the awake animal, the response is small; its amplitude increases during drowsiness and the sleep–slow phase, but is again reduced during the sleep–fast phase (Fig. 3). The response grows noticeably under chloralose and is very much reduced under Nembutal. The sharp localization of the CM response is shown in Fig. 4. In the same animal, the electrode was lowered during several successive experimental sessions: when we compared responses obtained during the sleep–slow phase (*i.e.* when the responses are biggest), the deepest of our CM locations showed the largest response. The same result was also obtained under chloralose anesthesia.

Cortical responses. These responses were observed at the area marginalis anterior which, under chloralose, yields the largest converging responses among associative

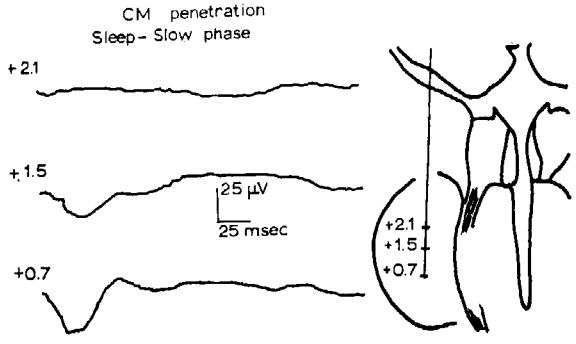

Fig. 4. Averaged responses to stimulation of the radial nerve obtained during 'sleep–slow phase' for different depths of penetration of the posterior part of the CM. (See insert map.)

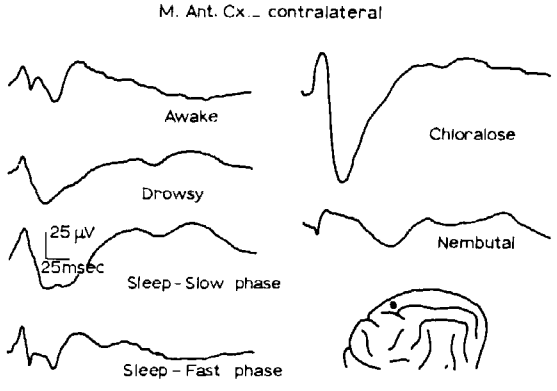

Fig. 5. Records from cortex marginalis anterior of averaged responses to 50 shocks delivered to the contralateral radial nerve. Conditions are comparable to those described in the legend of Fig. 1. (All records are from the same cat.)

structures (Amassian, 1954; Albe-Fessard and Rougeul, 1958; Thompson *et al.*, 1963). At this level, there was observed in the awake cat a doubly-notched positive response just after an initial, small, negative deflection which represents the activity of the neighboring primary area (Fig. 5). This W-wave in the awake cat was replaced by a larger, relatively short-latency wave during drowsiness and the sleep–slow phase; the W-response reappeared during sleep–fast phase. During chloralose anesthesia, the observed response resembled that found in the drowsy cat except for a further increase in amplitude. During Nembutal anesthesia, the deflections were smaller and the latencies much longer; the whole pattern bore little resemblance to the ones discussed above. It is impossible to say whether the late evoked component is the same one observed during sleep, but delayed, or whether it represents an entirely new component. The changes in evoked potentials that were observed at the area marginalis anterior during different stages of vigilance and under the influence of anesthetics were similar to those seen at the level of the centre median. This similarity points towards the existence of a pathway between the centre median and the cortex marginalis anterior. However, the greater complexity of the cortical waveform, especially while the cat is awake or in the paradoxical phase of sleep, leads one to assume that afferent somatic pathways other than those that go through the centre median may reach the cortex marginalis anterior.

Winters (1964) has studied changes in potentials that are evoked by clicks at the area marginalis anterior; his findings are similar to those we have just described.

(3) Structures which exhibit both primary and associative responses

(a) Electrodes placed at locations in the anterior sigmoid gyrus yielded, under chloralose, a double response when the contralateral radial nerve was stimulated. This double response often broadened into a single one. In the unanesthetized animal, the two response components were present: the first component behaved like the short-

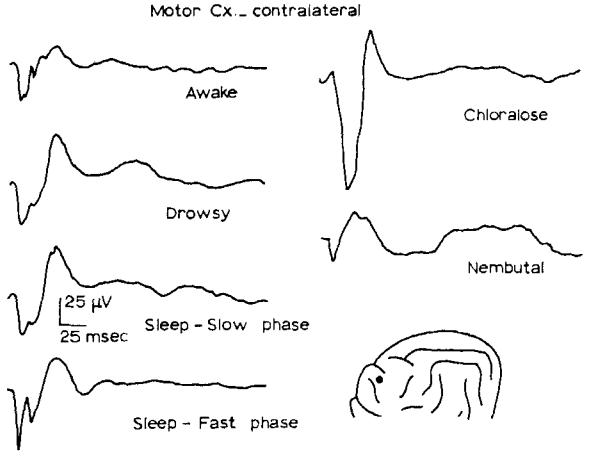

Fig. 6. Averaged responses from the motor cortex to 50 shocks delivered to the contralateral radial nerve. The records were taken under conditions comparable to those of Fig. 1.

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latency component of Fig. 1 (at the extremity of the cruciform gyrus); the second component increased during the sleep–slow phase and decreased during the sleep–fast phase. It is this latter component that seemed to increase during chloralose anesthesia and to disappear under Nembutal (Fig. 6).

In the same cat, under chloralose anesthesia, we were able to demonstrate the different natures of the two components by experiments in which the two radial nerves were stimulated in combination. Fig. 7 shows that the response to ipsilateral stimul-

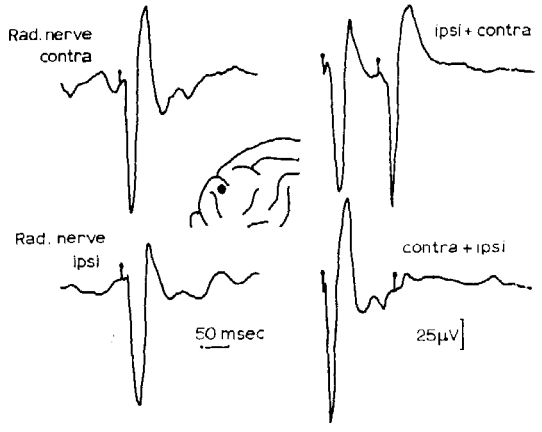

Fig. 7. Records from the same cat as in Fig. 6, taken under chloralose. This figure makes evident the presence of two components — the primary and the associative — in the responses evoked at the level of the motor cortex (see insert map) by stimulation of the radial contralateral nerve.

ation was completely abolished by a prior contralateral stimulus. In contrast, the response to a contralateral stimulus exhibited only a minor change in wave shape when preceded by an ipsilateral stimulus. (For further discussion see Albe-Fessard and Fessard, 1963).

(b) At the thalamic level, we studied evoked responses in the VL. Our findings show little invariance and do not lend themselves to clear interpretation without further experiments.

DISCUSSION

The variations in amplitude we have observed confirm and extend previous reports

(1) Our observations demonstrate that the slow waves which increase under chloralose are also present in unanesthetized preparations and have greatest amplitude when the animal is in a state of sleep characterized by slow activity; these results agree with other findings obtained by different techniques. These results, however, need further discussion, since some workers (Bickford *et al.*, 1964; Davis *et al.*, 1964) have been inclined to interpret some response components, especially in the case of acoustic stimulation in man, as EMG's due to reflex activity.

Several arguments seem to contradict such an interpretation for the present experiments. Firstly, the various associative responses have been previously obtained from

curarized preparations, *i.e.* in the absence of all muscular activity. Secondly, the intensity of stimulation was less than that which gives rise to a detectable muscular reflex. Finally, such an interpretation (which depends heavily on the ambiguities of monopolar recording) does not apply to recordings from deep thalamic structures with concentric, bipolar electrodes.

Guilbaud and Yamaguchi (1964) have undertaken a more detailed critical examination of this problem, involving the use of a variety of reference leads (frontal sinus, neck muscles, and masseter muscles) and a check on whether stimulation of the radial nerve can give rise to myographic responses from different head muscles. Their experiments have demonstrated that the cortical associative responses depend upon the activity recorded at the focal electrode and not upon activity that comes from the location of the reference electrode. Moreover, with our intensities of stimulation, no response can normally be seen in the neck or masseter muscles. In exceptional instances when such a response is found (Fig. 8), its behavior does not parallel that of cortical responses.

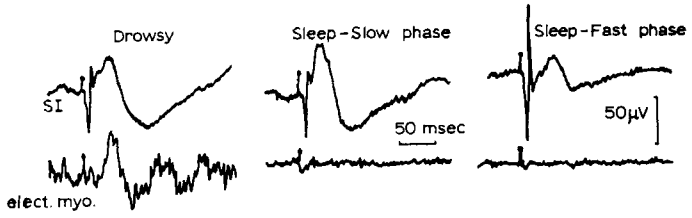

Fig. 8. Effect of stimulation of the superficial radial nerve upon the activity of the sensorimotor cortex and the EMG (bipolar electrodes in the neck muscles) for several stages of sleep. Each trace is an average of 25 responses.

(2) The increase in amplitude of the primary cortical component (somatomotor cortex) during the sleep–fast phase confirms the previous findings by Pompeiano and Swett (1962) and Allison (1965). A similar finding has been described for the cortical responses to both visual (Evarts, 1962; Cordeau *et al.*, 1965; Rossi *et al.*, 1965; Palestini *et al.*, 1964) and auditory stimuli (Teas and Kiang, 1964). It seemed worthwhile knowing whether amplitude increases in averaged responses correspond to a general increase in the amplitude of the individual responses or whether they result from a different distribution of amplitudes, *i.e.* from differences in variability. A recent reexamination of this problem by Guilbaud and Yamaguchi (1964) shows that the amplitudes of the first primary component vary over a more restricted range during sleep–fast phase, and that the maximum amplitude found does not exceed the maximum amplitude found in the sleep–slow phase (Fig. 9).

Finally, the primary component in the VPL is largest in the awake cat, and this agrees with results obtained in this same structure by Favale *et al.* (1963) and Okuma and Fujimori (1963) and with results by Mancia *et al.* (1959) in the lateral geniculate body.

(3) The different effects of chloralose and Nembutal on primary and associative evoked responses have been previously used to differentiate between them. Our work

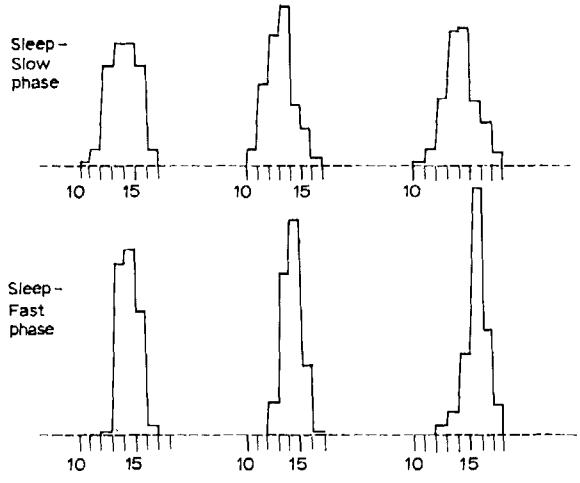

Fig. 9. Amplitude distribution of the first surface-positive component of 80 evoked responses: recordings from sensorimotor cortex, stimulation of contralateral radial nerve. Three samples of such amplitude distributions were obtained for each phase of sleep and classified into 25 levels. The amplitude histograms demonstrate that the distributions are narrower during sleep–fast phase and that the largest amplitudes observed are comparable in both phases of sleep. (Courtesy Guilbaud and Yamaguchi, 1964.)

confirms these previous results. Responses from associative structures are notably enhanced during chloralose anesthesia, while their amplitudes are reduced under Nembutal (see also Brazier, 1963). The same holds true for primary responses, but the effects are smaller.

In general, we have found that responses under chloralose resemble responses to the same stimuli in the sleep–slow phase; however, their wave forms are simpler and smoother (Fig. 1). This finding suggests that during anesthesia some of the components have dropped out. Consequently, if the electrical activity of an unrestrained animal during normal sleep is clearly more complex than that observed under Nembutal, it is also more highly patterned than that observed under chloralose. In particular, certain slow components which fail to appear under chloralose are detectable in sleep at the levels of the motor and marginal cortex.

Finally, at various cortical locations, Nembutal anesthesia brings out a long-latency, slow wave that seems to correspond to the secondary discharge described by Forbes and Morison (1939).

SUMMARY

(1) Electrodes were implanted into primary or associative structures of 4 cats. At the level of the thalamus, the structures were the center median, the VPL, and the VL; at the level of the cortex, the area of the anterior sigmoid gyrus, the lateral extremity of the cruciform gyrus, and the area marginalis anterior.

(2) Spontaneous activity and that evoked by stimulation of the radial contralateral nerve were recorded on to magnetic tape from cats which were awake, drowsy, and

asleep (either in sleep–slow phase or in sleep–fast phase). Averages of 50 responses were computed for each condition; data were also collected from these same animals under chloralose or Nembutal anesthesia.

(3) The average amplitude of the first positive deflection evoked in primary cortical areas is small while the cat is awake; it increases slightly as the animal falls asleep and is in the sleep–slow phase; it increases significantly during the sleep–fast phase. The amplitudes of corresponding thalamic responses exhibit less change. In addition, slow response components appear at the level of the primary cortex during the sleep–slow phase as well as during chloralose anesthesia.

(4) A small positive deflection appears in the CM of the awake cat; it grows during the sleep–slow phase and decreases again during paradoxical sleep; a partly similar but more complex phenomenon takes place at the area marginalis anterior.

(5) Amplitude fluctuations in relation to the sleep–wakefulness dimension are more complex at the anterior sigmoid gyrus and in the VL nucleus. Further studies are needed before conclusions can be reached.

(6) In the discussion, our findings are compared with those previously reported; in particular, the effects of chloralose anesthesia are compared with the changes in evoked potentials which occur during the sleep–slow phase.

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GENERAL PRINCIPLES OF SELF-REGULATION IN CORTICO-SUBCORTICAL CORRELATIONS

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Conditioned Reflex and the Problem of Thalamo-Cortical Interrelations

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Analysis of cortico-subcortical interaction in the integrative activity of the brain is one of the pressing problems of neurophysiology. New data have recently been obtained in the study of this problem by neurophysiologists and neuromorphologists.

In the present paper we shall touch upon two aspects that arise from our morpho-physiological studies on the interrelation of thalamus and brain neocortex.

(I) The experiments on destruction of cortico-subcortical, and in particular thalamo-cortical, connections showed that the manifestation and the volume of analytico-synthetic activity might depend on structural and functional peculiarities of unconditioned reflexes (alimentary or defence with all its local or global variations), which form the basis for conditioned reaction. Our experiments showed that deep disconnection of dogs analyzer cortical ends (optic and acoustic from cutaneous and motor), if it involved the cortex and the white matter at the level of anterior sylvian gyrus, resulted in destruction of the fibres of the central auditory pathway (Fig. 1) and in subsequent nearly complete damage of the medial geniculate body. Bilateral lesion of the central auditory pathway in animals entails complete inability to differentiate acoustic stimuli for a long time. This is manifested under conditions of unrestrained behaviour (general food-obtaining reaction) in the impossibility of obtaining an inhibition of the differentiative running behaviour, whereas every acoustic stimulus evokes conditioned running (Fig. 2). This peculiar ability for a generalized reaction to acoustic stimuli after bilateral lesion of the central auditory pathway was also observed by Mering (1963). In our experiments, however, the process of differentiation of acoustic stimuli was possible (though weakened) under the conditions of the classical secretion method in those dogs that did not differentiate them under the method of conditioned running.

Certain differences in the character of conditioned reflex activity were found in animals in which disconnection of analyzers was accompanied by interruption of the connections running from the ventral complex of the thalamic nuclei to the brain cortex and/or by profound changes in the cellular structure of the somatosensory cortex. Conditioned reflexes then recorded in the method of unrestrained behaviour were not considerably damaged, or sometimes a disturbance of the process of internal inhibition was observed (Fig. 3).

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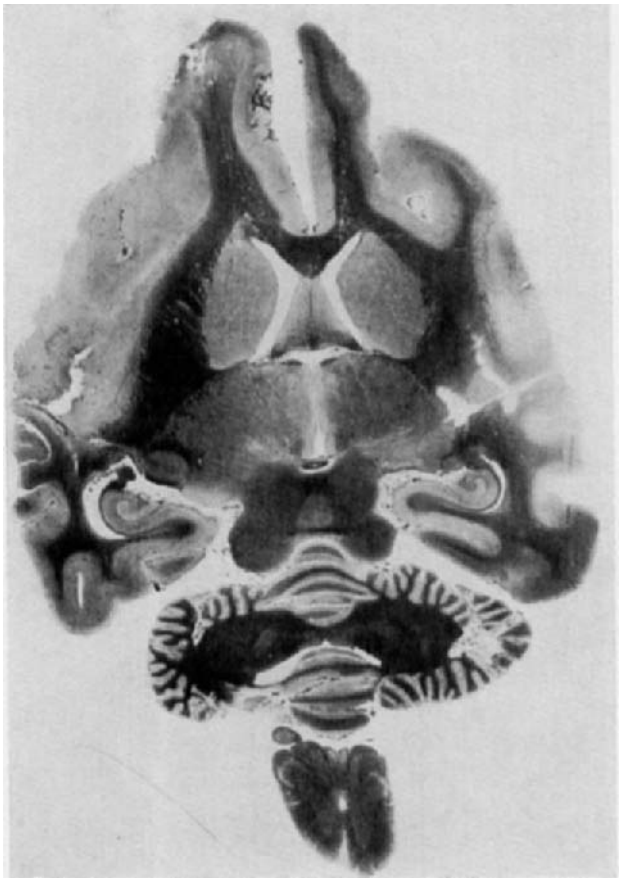

Fig. 1. Bilateral incision of the fibres of the central auditory pathway upon disconnection of cortical areas in dog 1.

Destruction of cortical and thalamo-cortical connections mostly led to a weakening of the excitation process, to the fall in the magnitude of positive local motor defence conditioned reflexes and to their instability. Under these conditions, in one of the dogs, preservation of differentiations acquired with time not an absolute, but a relative character (Fig. 4). Another dog displayed a complete and stable disappearance of the local defence conditioned reflex (lifting of extremity) with preservation of the respiratory component of the conditioned response.

These observations suggest that one effector reaction is insufficient to permit a full judgement of the character and volume of the conditioned reflex activity. Disturbances of the conditioned reflex activity after destruction of the cortical projection zones of the conditioned and unconditioned representation, or of their connections with corresponding formations of the diencephalon, may manifest themselves in various ways, depending on the methods applied. Such differences are conditioned by the localization peculiarities of the paths and morphophysiological mechanisms of this or that response activity, in particular by its predominantly cortical or subcortical nature.

A general food-obtaining reaction is a complex global conditioned reflex and, as evidenced by a number of experiments, may be secured with participation of different cortical or subcortical structures.

The local motor defence reflex (lifting of the extremity to electrocutaneous stimulation) is evidently manifested within a narrower anatomical arc and may be considerably damaged upon destruction of its separate links.

The defence reflex in the form of movement of the extremity, though occurring with participation of epicritical (cutaneous) and nociceptive stimuli, has a small receptive field and a local nature of response, and for its distinct manifestation requires preservation of the sensorimotor cortex and its connections with the thalamus.

Great attention is being paid to the detailed study of the connections between the thalamus and the neocortex, which are essential in the realization of different aspects

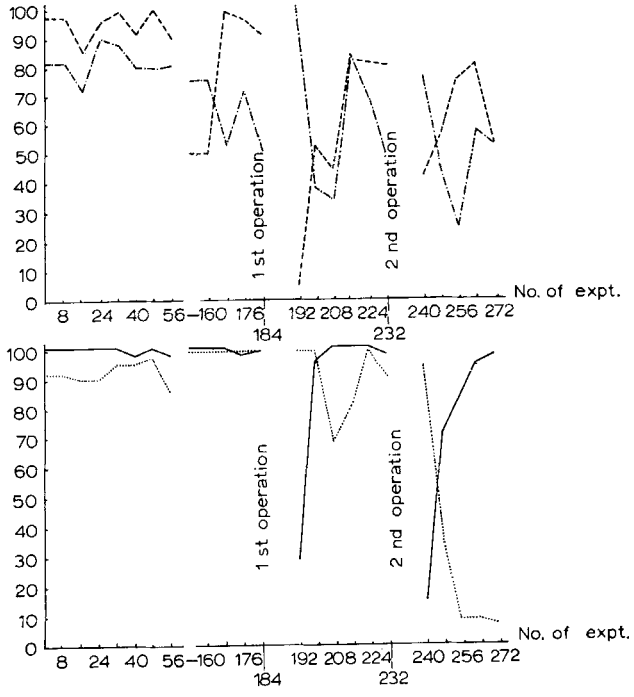

Fig. 2. Dynamics of conditioned reflexes to acoustic stimuli in dog I before and after operation in the right (first operation) and left (second operation) hemisphere. Ordinate, mean value (in %) of correct reactions (the data of 8 experiments). Abscissa, the number of experiments. ——, positive conditioned motor alimentary reflex to the sound of bell ringing 1; . . . . . , differentiation of sound (ringing) in the motor-alimentary method; - - - - -, positive conditioned secretive alimentary reflex to ringing 1; - · - · - · - · - , differentiation of sound (ringing 2) in the secretive alimentary method.

of the animal's alimentary activity. Numerous electrophysiological experiments (Bailey and Bremer, 1938; Patton and Amassian, 1952; Chernigovsky, 1956, 1959) as well as the data obtained in our laboratory (Kovalenko, 1962), have disclosed the important role of the orbital and anterior sylvian cortex in the manifestation of con-

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ditioned and unconditioned salivary reflexes in dogs. Removal of these areas or destruction of their connections with the thalamus brings about a sharp and prolonged reduction in the quantity of conditioned salivation, and modification of the dynamics of unconditioned secretion, as a result of the elimination of cortical influence. Such operations also involve destruction of co-ordination of unconditioned chewing, and the act of eating is impeded. With the help of the method of retrograde degeneration, Kovalenko found that the area of orbital and anterior sylvian gyrus of the cortex receives fibres from the cells of the medial portion of the arcuate ventral nucleus, and partly from the medial and external parts of the ventral nucleus, *i.e.* from those thalamic structures where, according to the literature data, gustatory and some other sense conductors from the intra- and peri-oral part of the muzzle terminate. Efferent connections of these cortical areas with the hypothalamus (Lyubimov, 1958) point to their importance in the efferent components of the alimentary reflex.

(II) After ablation of different portions of the anterior cerebral cortex and application of the method of retrograde degeneration of thalamic cellular structures, we observed

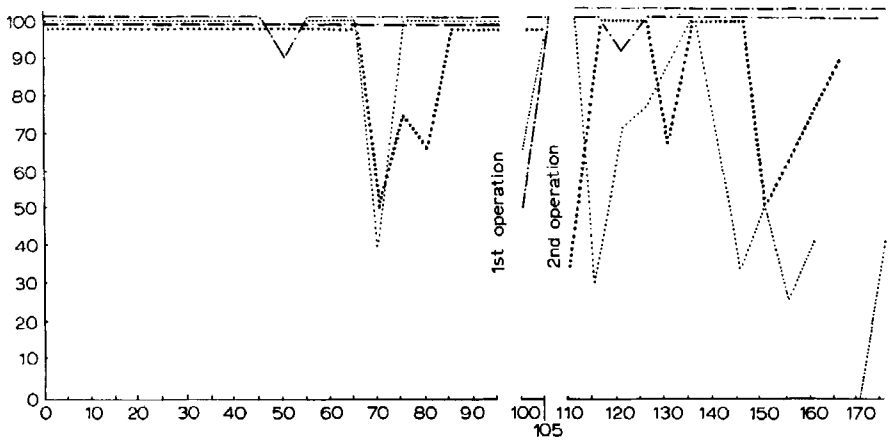

Fig. 3. Dynamics of motor alimentary conditioned reflexes in dog 2 before and after operation (mean of 5 experiments). Conditioned positive reflexes: - · - · - · -, ringing 1; – · – · , light +. Differentiations: · · · ·, ringing 2; · · · ·, light.

the disappearance and distinct rarification of cells not only in 'specific' but also in 'non-specific' thalamic nuclei. Some electrophysiological data (Dell *et al.*, 1952; Gastaut *et al.*, 1957; Jasper, 1958; Karamyan and Belekhova, 1963; Rusinov, 1963) show that the thalamic reticular system evidently has a certain topographic organization connected with its cortical projections, especially to frontal, motor, parietal and anterior limbic areas.

The results of our morphophysiological investigations, first published in 1958, to a certain extent substantiate these physiological findings. The study carried out on 12 hemispheres have shown (Fig. 5) that the lateral section of the interior portion and part of the anterior portion of the dorsomedial thalamic nucleus (MD) are connected

with frontal cortical fields; while their exterior and exterior-posterior portions are connected predominantly with field $Prc_2(6)$* of the motor (precoronar) cortical area. A considerable number of cells of the anterior medial nucleus (anm), especially its lower portion, send axones to parietal cortical areas (just as cells of the 'specific' anterior ventral nucleus (anv) do). Axones of the majority of cells of the anterior (vna) and of some of the cells of the medial (vnm) portions of the thalamic ventral nucleus, attributed by a number of authors to the 'non-specific' thalamic system,

Fig. 4. Dynamics of motor defensive conditioned reflexes in dog 3 before and after operation. Mean of 5 experiments: ———, positive reflex to ringing 1; - - - - -, acoustic differentiation (ringing 2).

terminate within the precoronar cortex, most probably in the $Prc_1(4)$ field in the main, as well as partly in the $Prc_2(6)$ field. Some of the vnm cells are also connected with the orbital gyrus cortex (Kovalenko, 1962). The majority of the cells of the relay parts of the ventral nucleus, *i.e.* arcuate (vnar) and exterior (vne), send their axones also to the precoronar and partially to the postcoronar cortical areas. Intralaminar nuclei, *i.e.* the central lateral nucleus (cnl) and the paracentral nucleus (pcn), have direct, though not so considerable, connections with pre- and postcoronar cortical areas, *i.e.* with the cortical ends of the motor and cutaneous analyzers. Some cells of the anterior-exterior, and to a lesser extent of anterior-interior parts, of the reticular nucleus (n.ret) send axones to the cells of the frontal and precoronar cortical areas. It may be assumed that different parts of the reticular thalamic nucleus are diffusely connected with those structures of the neocortex to which the main adjacent relay thalamic nuclei are projected more locally (Figs.5, 6). Changes in non-specific nuclei are always accompanied by changes in specific thalamic nuclei. The majority of thalamic diffuse nuclei, whose relatively local projection to the cerebral cortex has been shown in our experiments, are close in their neuronal structure to the main thalamic relay nuclei. Both nuclei consist of I type Golgi cells whose axones, as a rule, have no collaterals; of typical II type Golgi cells and, in a much lesser degree, of the so-called 'reticular' neurons (Leontovich, 1959). The latter, following from the work of Zhukova and Leontovich (1960), are characteristic of the reticular formation

* Nomenclature of thalamic nuclei and neocortical fields is given according to the 'Atlas Mozga Sobaki' (Adrianov and Mering, 1959).

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structures. Only the parafascicular nucleus, centrum medianum nucleus of Luysi and the reticular nucleus (Leontovich, 1959) have a reticular character in their structure. According to our results, the first two of these nuclei have no direct connections with the neocortex, and the third has more diffuse connections, than other thalamic nuclei.

The data on the similarity between the neuronal structure and the efferents of non-specific thalamic nuclei may be complemented with those pointing to the similarity of their afferent connections. It has been shown that the fibres of the spinal thalamic and trigeminal tracts (Magoun and McKinley, 1942; Getz, 1952; Nauta and Kuypers, 1958; Scheibel and Scheibel, 1958), as well as fibres of some visceral pathways (Papez, 1944; Delov *et al.*, 1959; Hugelin *et al.*, 1959; Durintan, 1962) may terminate on both specific and non-specific cells of thalamic nuclei.

All this, along with the facts on genetic proximity of different structures of the

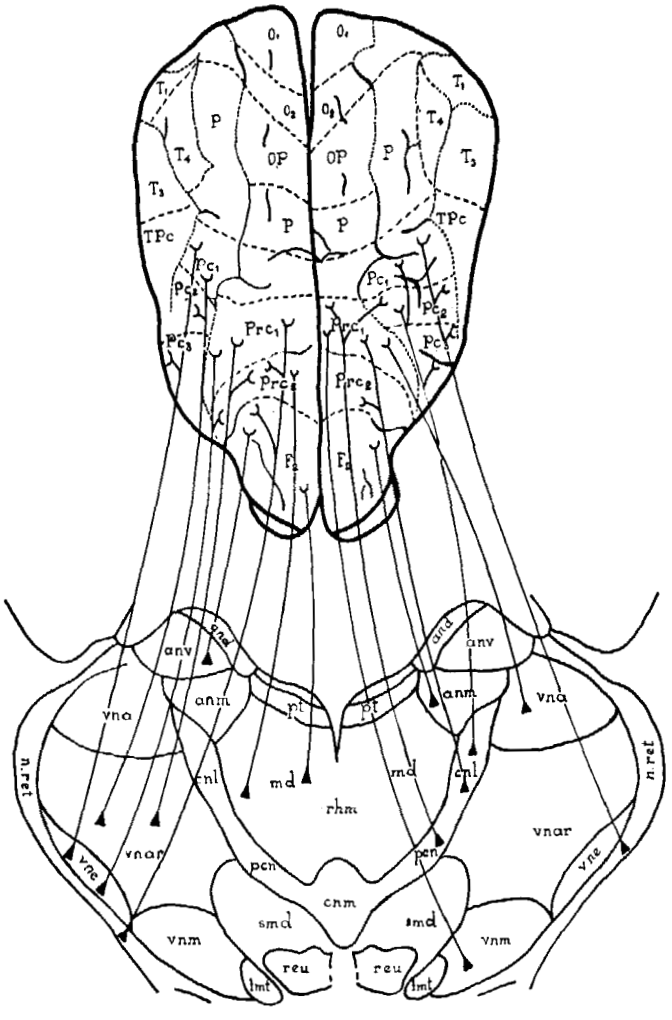

Fig. 5. Scheme of connections between thalamus and anterior parts of cerebral cortex in dog.

dorsal thalamus in the process of evolution (Kurepina, 1940, 1959; Rose and Woolsey, 1949; Kuhlenbeck, 1954; Zurabashvili, 1959), prevents the possibility of uniting the numerous and multiform thalamic nuclei into one diffuse non-specific thalamic system.

Our principal scheme (Fig. 6) of the efferent connections of thalamic nuclei, supplementary to the scheme in Fig. 5, points to a great complexity of topographical relations. For instance, some 'non-specific' nuclei may be connected with the neocortex, as well as with allocortex and the striatum.

Such kind of complex connections of non-specific thalamic nuclei with the neocortex might in time elucidate the effects of the two-way influences (inhibitory and excitatory) recorded by neurophysiologists and exerted by these nuclei on the cerebral cortex (Krupp and Monnier, 1963) as well as the effects of modification of the cortical response upon a slight shift of the site of the application of a polarization current to non-specific thalamic nuclei (Rusinov, 1963).

Analysis of thalamo-cortical connections shows that there exists a probability of rather close interrelations between 'specific' and 'non-specific' thalamic nuclei, particularly within: (a) the complex of thalamic anterior nuclei; and (b) the complex of ventral thalamic nucleus with its specific (arcuate and exterior) and non-specific (anterior and medial) parts which give the main projections to motor and general sensory cortex. From the point of view of their connections the central lateral and

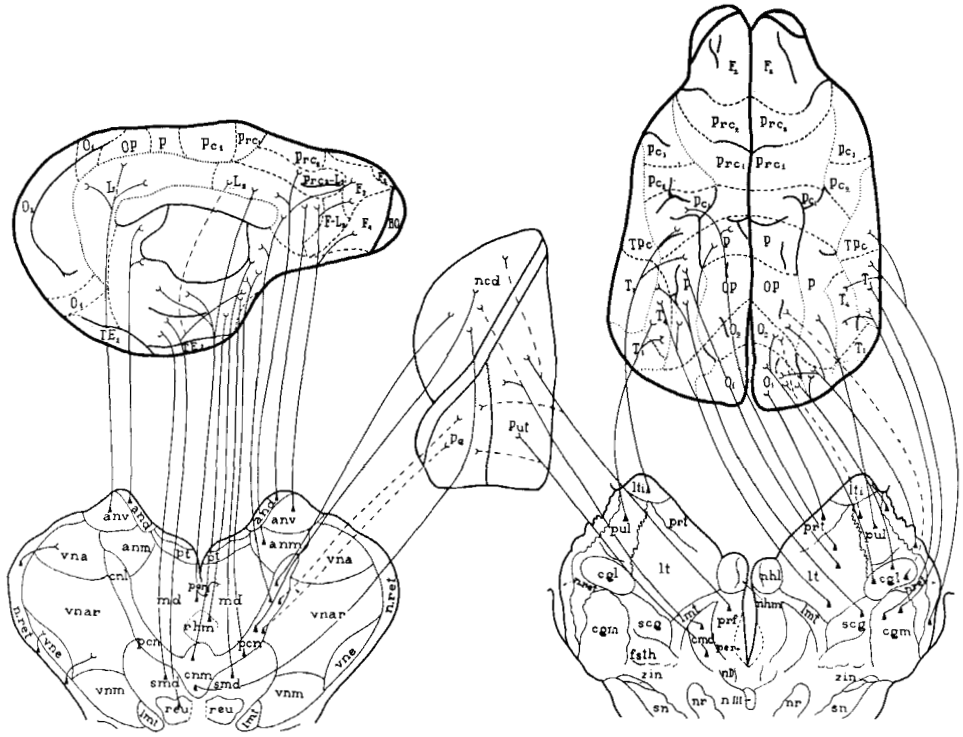

Fig. 6. Principal scheme of connections of thalamic nuclei with posterior and inferior parts of cerebral cortex and with strio-pallidum (according to the literary data).

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paracentral nuclei come very close to this complex. Taking into account literature data on the relation of non-specific thalamic nuclei to the analysis of nociceptive and visceral stimuli, and quite possibly, to the adaptive trophic function of the sympathic nervous system (Karamyan, 1959, 1962; Tonkich, 1960) it is logical to conclude that distribution of somatic and older vital influences within the same cortical structures may very well take place at the thalamic level. A non-specific influence should be considered here, not only in the plane of biological specificity of the action of the reticular formation (Anokhin, 1962), but also in the plane or more of less distinct topographic (analyzer) specialization.

In conclusion we should like to touch upon some, to our mind, most essential premises of thalamo-cortical interrelations in mammals.

Firstly, there is a close interdependence in the activities of the diencephalon and cerebral cortex manifested in topographic differentiation of thalamo-cortical connections of both specific and to some extent non-specific thalamic projections.

Secondly, there is a different structural and functional specificity of different thalamic formations (as compared with those cortical areas to which they are projected). Some of the thalamic formations, for example, the lateral and medial geniculate body, arcuate part of the ventral nucleus, and possibly the anterior nuclei of the thalamus, are the formations that provide only the mechanism of relay of stimulations into corresponding cortical areas. Their functions are closely adjusted to the functions of the corresponding cortical areas. Other formations, even though possessing distinct cortical projections (the main part of the dorsomedial nucleus, lateral group of nuclei and some 'non-specific' nuclei of the thalamus) evidently have a more pronounced functional specificity as compared with the cortical structures to which they are projected. Specificity of cortical and thalamic levels is determined by the peculiarities of their neuronal structure, by the complexity of their efferent and associative relations, etc.

Thirdly, along with direct influences of the diencephalon on the cortex, there is the possibility of the existence of indirect ones. This concerns certain non-specific thalamic nuclei (for example, the parafascicular complex and some other diffuse nuclei, which exert their influence through other thalamic formations or through strio-pallidum), as well as the epithalamus, hypothalamus and subthalamus which also consist predominantly of reticular neurons (Leontovich, 1959).

And finally, an important condition for thalamo-cortical and cortico-subcortical correlations is the existence of numerous centrifugal influences studied in the laboratories of Bremer (1955), of Narikashvili (1961, 1962) and by others.

Both morphological and physiological studies have recently revealed a great diversity in these corticofugal influences from the point of view of their topography and their functional significance (specific response activity, activation of the orienting reflex, inhibitory and excitatory cortical influences on different subcortical areas).

It appears to us that we should extend the principle not only of specific but also of non-specific (tonic) influences on corticofugal systems, thus including the possibility of cyclic, coupled movements of both specific and non-specific influences between

different levels of the brain stem and subcortical structures, between the cerebral cortex and these subcortical structures which must be of great and, according to our results, versatile importance for different forms of integrative activity.

SUMMARY

After disconnection of the cortical analyzer zones and lesion of the central auditory pathway in dogs differentiation of acoustic conditioned stimuli is impeded. The disturbance manifests itself in different degrees depending upon the method of conditioning. One and the same animal is able to differentiate acoustic conditioned stimuli when alimentary salivatory reflexes are formed yet cannot differentiate them under conditions of unrestrained behaviour. With the method of motor defensive reflexes (after Protopopov) the differentiation may be incomplete.

Removal of the orbital and anterior sylvian cortex (Kovalenko, 1962a, b), as well as an interruption of the connections between these structures and the ventral thalamic nucleus, leads to a decrease in conditioned salivation from the ipsilateral salivatory gland to the positive stimuli and to a change in the dynamics of unconditioned secretion, as a result of the elimination of cortical (conditioned) influence.

Interruption of connection between the ventral thalamic nucleus and sensorimotor cortex changes the positive motor defensive conditioned reflexes, but at the same time has no marked effect on the dynamics of the positive conditioned reflexes in unrestrained animals.

Thus the effector reaction does not allow full judgement about the character and volume of the analytico-synthetical activity. The conditioned reflex activity depends on the structural and functional organization of the unconditioned reaction (or of the complex of conditioned and unconditioned reactions), on which the conditioning is based. The dependence becomes particularly apparent after the destruction of the subcortico-cortical projections which provide for the interaction of different levels of unconditioned and conditioned reactions, or after primary damage of the cortical regions bearing a great importance for the unconditioned reactions. These differences of conditioning depend on the localization of the pathways and morphophysiological mechanisms of various unconditioned reactions, as well as on biological peculiarities of alimentary and defensive unconditioned reflexes.

The author's morphological data on the similarity of the influences exerted by 'specific' and 'non-specific' thalamic nuclei on the cerebral cortex, and analysis of the literature on the evolution and neuronal structure of different thalamic nuclei, do not permit agreement with the hypothesis on the 'diffuse non-specific thalamic system'. We assume the existence of different structurally and functionally differentiated systems of thalamic structures, each of them including both some 'specific' nuclei and a greater or lesser portion of the neurons of 'non-specific' nuclei. The 'non-specific' nuclei cannot be regarded as higher correlative and integrative centres.

The complexity of functional organization of an analyzer depends not only upon its afferent but also upon its efferent systems running to different levels of the brain stem. One has to assume that when a conditioned reflex is performed, various brain

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structures come into the state of a long cyclic excitation involving subcortico-cortical, cortico-cortical and cortico-subcortical systems.

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Nodular Mechanism of Functional Systems as a Self-regulating Apparatus

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This symposium is dedicated to the memory of a man who by his researches into the reflexes of the brain and discovery of the process of central inhibition had written one of the most brilliant pages in the great history of culture of the human race.

I. M. Sechenov was the first scientist who co-ordinated in the study of the work of the brain fine analytical researches, such as the discovery of inhibition of spino-cerebral reflexes, with the study of brain reflexes, which includes complex forms of man's psychic activity. It is this aspect of the unity of analysis and synthesis in the study of the brain that I would like to discuss in my humble report, dedicated to the memory of our great countryman.

All experiments in neurophysiology aim ultimately at comprehending the law of the entire organism's behaviour, the work of the entire brain. This is true also as far as the microelectrode researches of single neurons of the central nervous system are concerned. Indeed, even the most valuable results of analytical research, aerlier or later, but unavoidably, must raise a question as to what place they occupy in the functioning of the entire brain and what is the degree of their participation in the adaptation activity as far as the external world is concerned.

The conditioned reflex, therefore, as regulatory of the entire organism, has become a favourite object of research of purely neurophysiological laboratories that never raise the question to the study of higher nervous activity, as such. The reflex proved to be that connecting link, which establishes transition from a fine neurophysiological experiment to whole behavioural acts of an animal, *i.e.*, implements that final aim of neurophysiological research, which was formulated above.

If we take the latest and most outstanding works of neurophysiological character, performed by professional neurophysiologists (Fessard, Jasper, Gastaut, Morell, Young, Buser, Hernández Peón and others), then the most interesting part of these researches consists in an attempt to reveal fine neurophysiological mechanisms of the very formation of the conditioned reflex. Moreover, some of the above-mentioned neurophysiologists, making use of contemporary achievements of neurophysiology, in 1958 proposed theories of inhibition of conditioned reflexes (Fessard, Gastaut).

The gap between the finest neurophysiological researches and appraisal of the entire behaviour is so great that it worries neurophysiologists. It has become obvious that a necessity has arisen to work out such a concept, that, on one hand, would represent an expression of a principle of integral behaviour, and, on the other hand, would

create facilitated conditions for analysis of details and for finding microprocesses in nervous activity. In other words, it becomes more and more expedient to formulate, speaking figuratively, a 'big address' for fine analytical researches, an exact place for the given concrete analytical research in gross physiological architecture. It is hardly necessary to emphasize that with this approach in use even the finest research gains in the definition of its place and role in maintaining the brain's integral activity.

Proceeding from this urgent necessity of uniting the deductive and inductive approaches to appraisal of material, some years ago we formulated the principle of 'functional system', in which we find the manifestation of general physiological architecture for any act of behaviour or adaptability; at the same time it makes it possible to outline in a neurophysiological sense concrete schemes of research work on a fine analysis of specific nodular mechanisms and architecture.

By 'functional system' we understand such a dynamic organization of processes and mechanisms, which, responding to queries at a given moment, ensures the organism some sort of adaptable effect and simultaneously determines the currents of an adverse effect, *i.e.* resultant afferentation and central nervous system, informing the latter of sufficiency or insufficiency of the obtained adaptable effect. In other words, any functional system, inborn or dynamically formed in the given situation, possesses traits of self-regulation with the main mechanisms characteristic of it.

For a more complete acquaintance of our functional system, as a unit of physiological integration, we refer the reader to our former publications specially dedicated to this question (Anokhin, 1935, 1937, 1947, 1949, 1958, 1961, 1963a, 1963b).

The general architecture of the conditioned reflex, as an elementary act of behaviour, serves as a basis for neurophysiological analysis of functional system. We regard the conditioned reflex as a partially closed formation, in which, as in any other functional system, the final effect or the result of an action, immediately after receiving it, is reflected in a volley of afferent impulses, directed in reverse sense in regard to impulses, which formed an action. These afferent impulses inform the central nervous system as to the degree of sufficiency or insufficiency of the obtained adaptable results, *i.e.*, in the sense of their behaviour they are resultant, inasmuch as they reflect all parameters of the results obtained.

Below we present a principled scheme, which most fully reflects the physiological architecture of the conditioned reflex and at the same time gives an idea about a number of such main mechanisms which are specific only for the functional system, as an integral formation, and not characteristic of separate, anatomically isolated processes and mechanisms (Fig. 1).

Here it is important to point out that the presence of such a general physiological architecture of the act of behaviour, or as it is called Magoun 'model', creates a definite strategy in filling up that gap which at present separates the behavioural act from those most detailed neurophysiological researches and which became possible after successes were attained by contemporary electronics and stereotaxic technology.

Having as a prospect a clearly designated physiological architecture of conditioned reflex, we can correct each fine neurophysiological research in correspondence with the tasks of understanding the whole.

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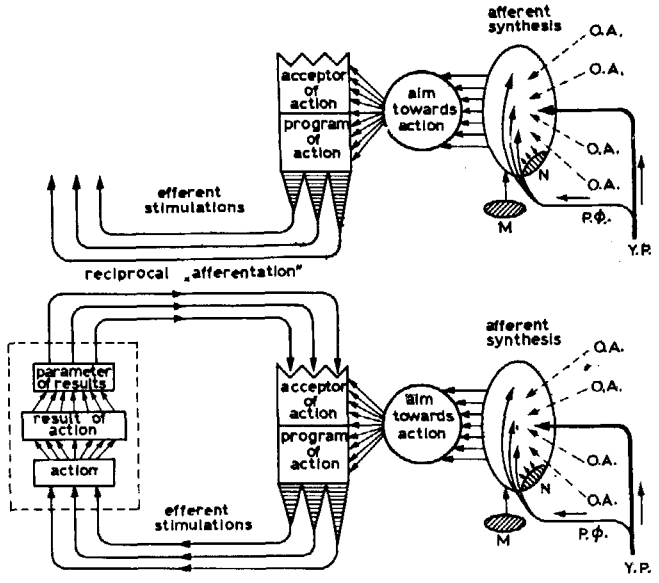

Fig. 1. Principal scheme of physiological architecture of the behavioural act, created in response to the conditioned stimulus. All stages are designed in the figure. I, Stage of formation of efferent excitations. II, Stage of closing a working cycle with the aid of reverse afferentation. In the figure it is clearly seen that the action acceptor, as a model of future results, is formed at the moment when formation of an action starts. M, motivation complex; N, memory as substrate of last experience. It can be observed in the scheme, if the behaviour act is developed on the basis of 'inclination' (hunger, thirst, etc.), then the stage of afferent synthesis is preserved, and the collations of the results with the action acceptor may be consistent and multitudinous (stage after stage reverse afferentations).

In this sense it is necessary to understand neurophysiological (Fessard and Gastaut, 1958, Magoun, 1963) and neurocybernetic views on the physiological architecture of conditioned reflex proposed by us.

The physiological architecture presented above has a number of main mechanisms which may serve as a source for making analytical researches. Some mechanisms or phases of development of physiological architecture of conditioned reflex are so fundamental and so characteristic of any integral behavioural act, that they must be taken and examined separately. However, in this paper I cannot present all the material obtained by us for the characteristics of all properties of the functional system as a whole. I shall confine myself to enumerating and characterizing the role of these phases and main mechanisms with a view later to concentrating the whole attention on experimental materials, characterizing the work on one of these main mechanisms, namely of the one that precedes and defines the trend of the next stages of the whole behavioural act.

The first stage of any behavioural act consists in the synthesis of all the afferent information, that reach the nervous system from external and internal spheres and in the aggregate compose a necessary stage for forming 'a decision', as an impetus to further formation of effector apparatuses of the behavioural act. We must proceed from an obvious premise that in the sphere of decision it would not be possible to

formulate even one aim of behaviour or 'decision to act', if before this, synthesis had not been made of the whole information available at this moment from an animal's external and internal world.

During the growth of cybernetic researches and their contact with biology and physiology, the 'problem of decision' became one of the central problems of a new approach to deciphering mechanisms of the behaviour of animals and especially of man's behaviour. The programme of efferent processes, forming an action with simultaneous formation also of the apparatus of the action acceptor, is an objective physiological expression of 'decision' or 'aim'.

Experimental researches conducted with the aid of various methods, from the classical method of conditioned reflexes up to microelectrode researches inclusive, show that there exist at least four forms of initial information, that enter as the organic first stage components and that never fail to precede the formulation of the aim to action.

The initial stimulus, serving as a concrete impetus to start the formation of the behavioural act, is the first component of the afferent synthesis. A conditioned stimulus may be such an initial stimulus, but it may also be any other stimulus from the external world or the internal medium of the organism, which appears to some degree unexpectedly and enters into the already available broad system of afferent excitors, formed previous to the action of the initial stimulus.

Environmental stimulus serves as a second component of the afferent synthesis. It is not so clearly defined as an initial stimulus, and it includes all those factors that in total represent the general situation, in which the given initial stimulus has begun to function. For work with conditioned reflexes in accordance with the classical method this second category information may be represented by numerous stimuli, which are evoked from the environment of experiment and from all that preceded the formation of an experiment (the room's appearance, table, experimenter, configuration of the passage through which the animal is conducted into the experimental room, etc.). Each of these stimuli was conditionally acquired during the animal's past experience and, composing an organic part of general environmental afferentation, serves as an unavoidable background for its subsequent synthesis with the initial stimulus.

That summary information, which reflects the organism's condition at the given moment in the form of specific excitations ascending in the cortex, serves as the third component of the afferent synthesis, organically entering the process of elaboration of information. Such a state may be dictated by the action of humoral factors in the organism itself and even by the whole complex of environmental stimuli, but having formed such a state it may also subject to itself in the process of afferent synthesis the environment and initial stimuli. The character and the trend of the behavioural act may to a considerable degree be determined by this initial condition. To some extent this initial condition may be compared with what Pavlov called 'basic trends' (Pavlov, 1916) and what some American research workers call 'motivation' (Miller, 1960; Pribram, 1964; etc.).

The fourth component, which is also used at the afferent synthesis stage is the ani-

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mal's or man's past experience, put away in the 'memory's storehouse' and brought out in the process of afferent synthesis for the formation of a decision to act. The usual elaborated conditioned reflex is also developed on the basis of former hardened structural relations and, consequently, an elementary conditioned reflex is already formed in some degree on the basis of the memory's data. The use of memory is far more extensive and diversified when the behavioural act is built into the natural environment in the form of free behaviour and on the basis of the whole complex of a large quantity of external and internal information.

As can be seen from the enumeration of four components of the initial stage of development of behavioural acts, it is a truly all-embracing one. It is precisely at this stage, as in no other stage of development of the behavioural act, that Pavlov's prevision of the decisive (the 'so-called creative') role of the afferent function of the cortex of the great cerebral hemispheres applies. In a way, the most essential thing at this stage is that all the enumerated fragments above comprise a real organic unity, flowing into a single channel, resulting in the formation of the aim of behaviour.

The characteristic feature of the afferent synthesis would be incomplete if we omitted that exclusive role which the orientation-research reaction plays here.

Whenever the process of afferent synthesis is held up or proves to be incomplete, the orientation-research reaction joins in and contributes to the development of additional rising excitations, thus determining an active selection of new information and the process of its cortical synthesis.

As an example of organic unity of the above-mentioned components of afferent synthesis we may cite the results of Shumilina's systematic experiments (1949), conducted in our laboratory, for defining the role of the frontal parts of the cortex in forming a conditioned secretory motor reaction in an environment of active and relatively free choice of one of the sides of reinforcement. These experiments showed that stimuli are organically united in the form of a conditioned stimulus with that specific environment of selection, in which the experiment was conducted.

In place of an initial stimulus here we have a conditioned stimulus, for instance a bell; in place of environmental stimuli we have here both the general stimuli from the entire environment of the experiment, as well as the specific environmental stimulus in the form of alternative selection of one of the sides of a table in correspondence with the conditioned signal.

Removal of frontal parts from a dog that had a well established selection of a corresponding feeding place, demonstrated that here we have an organic interaction of the above-enumerated components of afferent synthesis. Immediately after the removal of frontal parts the animal loses its accustomed integration between the initial and environmental stimuli, and begins to run ceaselessly from one side to the other, performing pendulum-like movements. The environmental stimuli, which were a part of the afferent synthesis as specific stimuli, acquired a role of initial stimuli, and therefore the animal, on being placed on a table, makes movements in order to chose this or the other feeding pan (Fig. 2).

This experiment convinced us that in the stage of afferent synthesis the central nervous system forms a genuine unity of environmental and initial stimuli. Only on

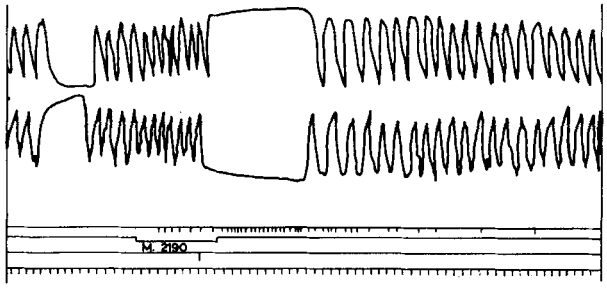

Fig. 2. The dog's behaviour on a two-sided table after the removal of frontal parts of the brain cortex. There can be seen an alternating 'choice' of sides of the table in the intervals between the conditioned stimulus. During the time the conditioned stimulus acts there can be clearly seen more frequent runs and prevalence of the signalled side. What is characteristic is the retardation of the reaction of selection immediately after eating food and greater frequency in intervals. The conditioned secretion has not suffered at all.

this basis does it become clear, though seemingly paradoxical, that being in an experimental environment, which has such a decisive influence on the quality of a conditioned reflex reaction, nevertheless a normal animal in the intervals between the conditioned reflexes sits quietly and does not give the conditioned reflex secretion. The latter flows only at the moment of action of the initial stimulus.

The results given here regarding the role of the frontal parts of the cortex, as implemented in afferent synthesis, are given only to emphasize the significance of that process which is played during the first stage of the formation of the behavioural act. As regards the concrete composition of all afferent information and its neurophysiological analysis, to this the later part of my paper will be dedicated. But now I shall dwell briefly on the form of enumeration on other main mechanisms of the physiological architecture of the conditioned reflex, shown in Fig. 1.

Immediately after the end of afferent synthesis and the emergence of an aim to action, almost simultaneously there are formed two closely-connected neurophysiological mechanisms. One of these mechanisms contains in itself afferent parameters of future results of action, while the other mechanism contains all details of the programme of distribution of efferent excitations, implementing the work of peripheral apparatuses, forming an action and consequently results of that action.

As can be seen from the principal architecture of the behavioural act, the apparatus, anticipating the efferent properties of future results, is a sequence of processes of afferent synthesis that most fully reflects the aim to action itself. To some degree it is a direct reflection of organic synthesis of what has been acquired by the given animal in its 'recent' and 'old' memory. This apparatus, in conformity with its physiological significance, was named by us an acceptor of action. From this name one can see that it takes upon itself real afferent signals from real results of action and compares them with the afferent parameters of required results contained in it.

It follows that, in conformity with our concept, the action acceptor primarily represents dynamic formation, in its composition anticipating combination of afferent

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parameters of precisely those results that were required by the extraordinary situation which arose at the given moment.

Consequently, there are as many action acceptors as there are new situations, as there are afferent syntheses, and as there are aims in the changing stream of an animal's and man's behavioural acts. And just as the results of numerous actions cannot be the same, so the afferent parameters of these results cannot be the same.

From this point of view one must not speak, as is sometimes done, about the 'localization' of the acceptor of action. It cannot represent something forever localized, like 'phrenological bumps', inasmuch as the evaluation of the results of action will be implemented in one example, for instance in visual and tactile afferentation, and in another in auditory and spatial afferentation, etc. One thing is undoubted: integration of all these parameters of results of action, as Shumilina's experiments show, occurs mainly in the frontal parts of the cortex.

As may be seen from the principal architecture, the results obtained, influencing with their parameters the animal's various receptive surfaces, form a stream of afferent impulses, which in the system of the whole behavioural act flows in reverse direction in relation to excitations, that formed an action.

By comparing parameters of reverse afferentation with an action acceptor the behavioural act closes; should they coincide an animal or man is anabled to go over to the next stage of behaviour.

From all that has been said here it is clear that afferent synthesis, formation of an aim to action, formation of an action acceptor and of a programme of action, attaining of results and diverse information about these results compose absolutely necessary main mechanisms of any behavioural act. They may vary in volume and composition, but not one reflex or behavioural act can be of adaptative significance, if it does not contain all these links of physiological architecture.

As can be seen from the general architecture of the act of behaviour, the reflex act or 'reflex arc' in its old Descartes concept is, of course, included in the functional architecture and can be excluded at any moment, if, with the view of carrying out research, we wish to confine ourselves to the linear distribition of excitation, beginning from the initial stimulus and ending only with reflex action.

However we must remember that each real action of an animal, which is an expression of efferent excitations only, unavoidably ends by obtaining results, and by information about these results going into the central nervous system. Consequently, setting up of a 'three-part arc' may now be performed only temporarily and only for research purposes. Recognition of its universal educational significance would now contradict that enormous scientific material and new generalizations, that have appeared during the last ten years in many spheres of science (see, for example, Anokhin, 1935; Wiener, 1958; Magoun, 1963; Drischel, 1960; Klaus, 1961; and many others).

Filling in the neurophysiological detail of each of the main mechanisms of functional architecture of a behavioural act is an extremely big piece of work. It would suffice for many laboratories and perhaps even for entire generations of researchers. The important thing is that each concrete research be defined exactly in conformity with its

place in this big architecture, so that it received, so to say, its 'address', which must determine the tactics of research, *i.e.*, orientation in collecting material and explanation of its physiological meaning.

Let us return to the first stage, to the afferent synthesis, and try to understand the composition of the information, which in its entirety determines this stage. According to the work of Magoun and Moruzzi, the ascending information, reaching the cortex of the great hemispheres, consists of two extensive streams of information, which have an entirely different informative significance. The first channel, which ensures the advance of information in the cortex of the great hemispheres in accordance with the lemniscus system to this cortex, was named a specific channel of afferentation, inasmuch as owing to this afferentation the organism obtains information about the fine sensorial modality, ascending excitation. The channel that has been named 'non-specific' begins from collateral branches of the lemniscus system and through subcortical nuclear formations enters the brain cortex. Thus, the first general characteristic of the stage of afferent synthesis in forming a behavioural act consists in expedient utilization of both these channels of afferent signalling and in the study of their interrelation at the level of the cortex and 'the nearest subcortical structures'.

The more detailed study of ways and characteristics of arising afferentations shows, however, that practically all the apparatus of subcortical nuclei thus or otherwise takes part in transmitting information to the cortex of the great hemispheres. Suffice it to recollect the second discharge of Forbes (the urethane second discharge), which was studied in our laboratory by Lu-Juan-Hui, in order to appreciate the multiple character the afferent excitations acquire on reaching the cortex of great cortical hemispheres. The researches of Purpura and other scientists have shown that practically all subcortical nuclei take part in this or other stages in the transfer of peripheral information to the cortex of the great hemispheres.

It follows that even a plain single stimulus, applied to some receptor's surface, unavoidably penetrates into the brain cortex in the form of thousands of rising excitations, directed to its diverse synaptic organizations. In substance this penetration gives us a genuine idea of the volume of afferent synthesis which takes place mainly at the level of the cortex of the great hemispheres.

Our own researches on the so-called non-specific rising activity have demonstrated that the amount of information having a general character is much greater than only one homogeneous non-specific activity, as is assumed by the theory of non-specific rising activity.

It was found that each activating influence, ascending in the cortex of the great hemispheres and having an overall influence on its synaptic organizations, has a specific quality, for which it was necessary to introduce the concept of biological modality in contrast with 'sensorial modality'. This biological modality of the generalized activation of the cortex may be of protective, pain, orientating, sexual, or alimentary character, etc. And each of these excitations, reaching the cortex of the great hemispheres, excites here precisely those extensive synaptic organizations and intraregional connections, that were already formed earlier, first on the basis of inborn connections with subcortical structures, and later on the basis of acquired connections.

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It is not difficult to imagine what different interrelations meet the rising excitations, originating in specific subcortical formations, having a definite biological quality. This is the arena of an afferent synthesis, where the interrelation of the most varied and multitudinous excitations of formation of decision and aim is in complete correspondence with the volume and quality of the afferentations received at the given moment.

In recent years we conducted a study of these rising afferentations in two ways. On the one hand, we compared the quality of rising afferentations, originating in various centres of diverse inborn reflexes, especially in food seeking and protection. This is, so to say, the projection of biological subcortical excitations on the cortex. On the other hand we also analysed the very station of destination, *i.e.*, the interrelation of excitations, coming to cortical neurons and distributed on these neurons in conformity with their functional meaning.

In the first direction special attention is merited by the systematic experiments of our co-worker Sudakov who studied alimentary activation, arising from the hypothalamus, to frontal parts of the cortex of the great hemispheres.

He demonstrated that in a hungry cat under urethane narcosis the frontal parts of the brain cortex are in the state of desynchronization, while other parts of the cortex of the great hemispheres remain in the characteristic sleep state of slow electrical activity (Sudakov, 1962). The artificial 'satiation' of an animal under narcosis led to a decline in activation and emergence of slow electrical activity. In the same manner the coagulation of lateral nuclei of the hypothalamus always produced emergence of slow electrical activity into the brain's frontal part. Comparison of both these experiments enabled us to draw the conclusion that activation is indeed of a specific alimentary character, and the initial point of activation, so to say, its 'pacemaker' is in the lateral nucleus of the hypothalamus, which corresponds to the alimentary centre, discovered by Annand, Brobek and Andersen.

If an animal, which is under urethane narcosis and displaying hunger activation, is subjected to tetanic sciatic stimulation in the brain's frontal parts by electric current then the whole cortex of the great hemispheres proves to be in a state of general desynchronization. In this we may see the generalization of the ascending excitation throughout the whole cortex of the great hemispheres. But then we must recognize that at this moment a hungry cat has in the frontal parts of its brain cortex the simultaneous presence of two activating excitations: one alimentary organized before the stimulus, arising from the hypothalamus, and the other protective, originating on the basis of gentle sciatic stimulution (Fig. 3).

The question is into what relations these two excitations enter in synaptic organization of the frontal parts of the cortex of the great hemispheres. Are we witnessing the incompatability of these two excitations varied in biological modality, or can they coexist as in convergence on the same cortical neurons?

The search for an answer to this question developed in two ways. On the one hand, for the dissociation of two rising activations, alimentary and pain, we applied aminazine (chlorpromazine), as a substance of which we had already had experience and that showed selective blocking action on the pain ascending activation. On the other hand, we undertook microelectrode researches on solitary cortical cells of frontal

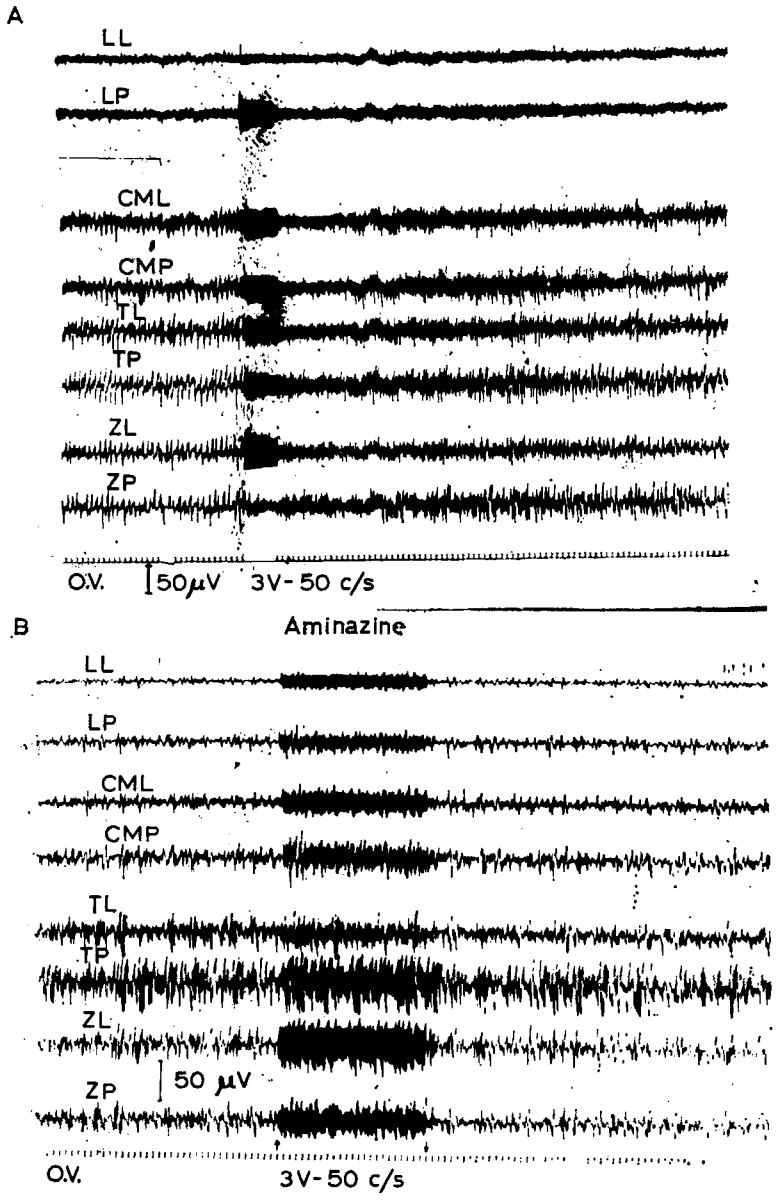

Fig. 3. Comparison of the chemical nature of pain and alimentary activation. A, activated state of all parts of the brain after a brief application of the sciatic pain stimulation of 3 V and 50 c/s. B, the same experience with pain stimulation after a preliminary aminazine injection. One can notice the elimination of the activated state in all parts of the cortex with the exception of frontal parts.

parts of the cortex of the great hemispheres both against the background of separately produced rising activations of varied biological modality, and against the background of their simultaneous influences on cortical neurons.

Experience has shown that there are at least two biologically different rising

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activations, which may enter into the most varied interactions in the cells of the cortex of the great hemispheres.

Thus, for instance, aminazine fully blocks that subcortical substrate, which ensures in the cortex the generalized pain activations, but at the same time leaves untouched the activation of frontal parts of the brain's cortex, depending on the rising excitations of the alimentary centre of the hypothalamus. Through this the important fact is stressed that rising activations of diverse biological modalities are adressed to various synaptic organizations of the cortex cells of the great hemispheres (Fig. 3).

Perhaps the most interesting consequence of the experiments described above is the observation that synaptic organizations of one and the same cortical cell may be an arena of struggle of two rising excitations having different biological modalities. This phenomenon was named by us 'multibiological convergence' in correspondence with the established terminology for convergence of various sensorial excitations in one and the same nerve cell (Fessard, 1961; Young 1963) (Fig. 4).

Fig. 4. Schematic depiction of two categories of convergence in cortical cells. I, multisensorial convergence; II, multibiological convergence. Marks: a, zone of convergence; c, pain subcortical centre; d, alimentary subcortical centre.

We were led to this experience by new variants of the experiments described above. Thus, for instance, Panfilov and Loseva (1964) conducted experiments on the prolongation of the starvation time. In all the experiments described above cats were hungry for 2 days before the experiment started. But in experiments conducted by Panfilov and Loseva they hungered for 4 to 6 days. As the researches showed, the general picture of electric activity differed considerably from that picture, described above for a 2-day starvation (Fig. 5, A) (Panfilov and Loseva, 1964).

First it was found that activation did not confine itself to the frontal parts, but embraced the whole cortex of the great hemispheres and consequently became generalized (Fig. 5). Such a picture was not unexpected. If a 2-day starvation led to a regional activation of the frontal parts of the brain cortex, then it was natural to expect that, with the increase in excitability in the sphere of the hypothalamus nuclei from a more powerful stimulation by famine blood, there should also have appeared a more extensive generalization of excitations in the brain cortex. Two possibilities of this transfer from regional activation to a generalized one were feasible.

(1) In the first place there could be widening of a stream of rising activations in connection with the increase in excitability of the alimentary centre of the hypothalamus, *i.e.*, generalization of excitability of a 'vertical type'.

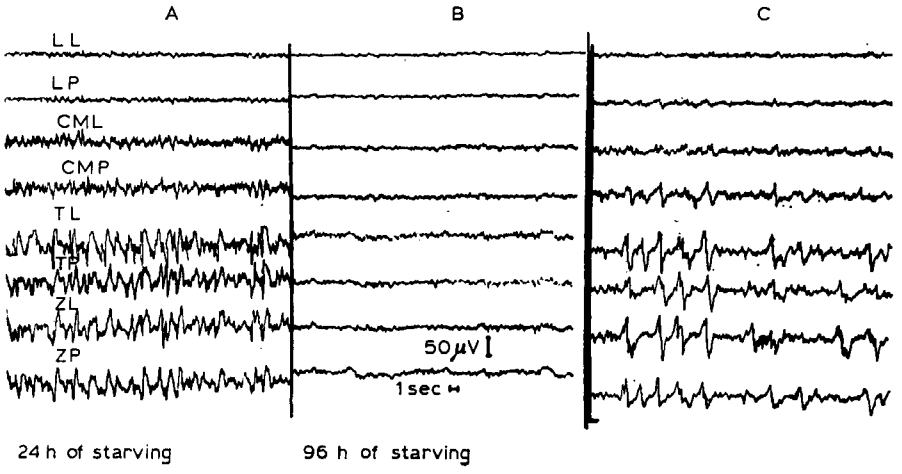

Fig. 5. An example of long hungering and discovery of the chemical nature of the activation evoked by it. A, 24-h starvation. Activation is observed in frontal parts and slow activity in all remaining zones of the cortex. B, 4-day hungering. Activation is spread over the whole cortex of the great hemispheres. C, the same animal after the aminazine injection. One can observe the restoration of the slow electrical activity.

(2) It could be presumed that the excitability irradiation throughout the brain cortex from the frontal parts of the cortex activated by the hypothalamus for the first time could also occur on the basis of the classical theory of the spread of excitations in the cortex, *i.e.*, of a 'horizontal type' (Fig. 6).

In both these possibilities the biological modality of generalized excitations, despite the various mechanisms of their spreading, should have been similarly preserved, *i.e.* alimentary. To check this assertion we made a special control experiment.

In view of the diverse chemical properties of alimentary and protective excitations described above, it might be expected that the injection of aminazine into an animal that had gone without food for 4 days, would not be able to eliminate the preformed generalized activation.

However, the experimental result was different. Injection of aminazine gave totally unexpected results: as with pain generalization, which sets in after the stimulation of the sciatic nerve, aminazine put an end to the whole activation in the occipital, temporal and parietal regions, but left untouched the frontal parts of the brain cortex.

We were thus confronted with a new aspect of interrelation in the cortex of the great hemispheres among the rising activations of diverse biological modality.

It is clear that the rising alimentary activation from the hypothalamus has only a regional importance in the form in which it manifests itself after a 2-day hunger. And as the hunger period grows, there occurs in the region of the hypothalamus a rise in the initial alimentary excitation, which leads to irradiation of excitation into the adrenergic substrate of the reticular formation, possessing the ability to produce rising activation, already generalized throughout the cortex of the great hemispheres.

Results of experiments definitely influence us to such a concept of phenomena witnessed, especially since this concept coincided to a considerable extent with the

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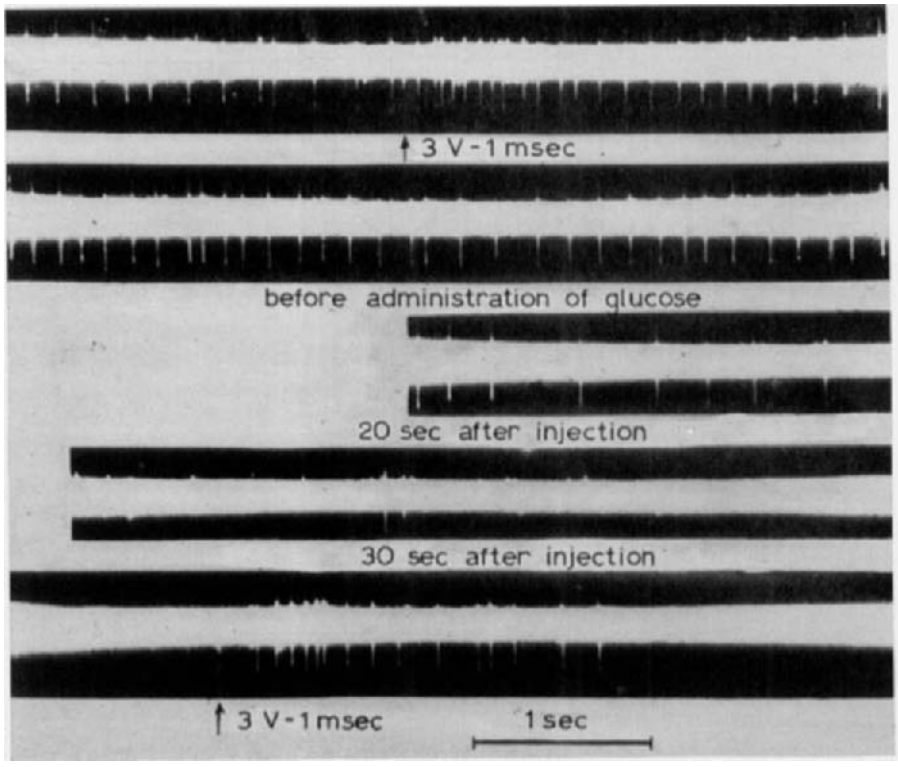

Fig. 6. Microelectrode research of individual neurons in the frontal parts of the cortex. The figure shows a neuron, activated by the ascending alimentary excitation of the hypothalamus. The sciatic nerve stimulation increases the discharge frequency, which points to multibiological convergence. The subsequent injection of glucose leads to gradual elimination of background discharges. Additional sciatic stimulation performed for control purposes results in the restoration of discharges, which points to a full preservation of the cell in respect to other non-alimentary ascending excitations.

biological meaning of the phenomenon. Most probably the long-famished animal is in a more intense general condition and its food-acquiring reaction is more active, requiring the intervention of the sympatho-adrenal system (Kennon, 1928).

Returning to the initial statement of a question regarding afferent synthesis, we can thus observe that one of the undoubted mechanisms of this synthesis represents the interaction of rising activations of varied biological modality in synaptic organizations of the brain cortex. This conclusion was inescapable and made us start experiments requiring the use of the microelectrode technique.

We were of the opinion that a study of the regime of the activity of one and the same cortical neuron, influenced by various biological modalities rising from the hypothalamic activations, could yield more exact data on the interaction of these activations through the synapses of one and the same neuron (Fadeev's experiments). We knew, of course, that these interactions take place in the synaptic organizations of one and the same cortical neuron. But it is precisely these interactions that compose the neurophysiological basis for analysis and synthesis of rising afferent influences —

synthesis without which neither 'the decision' nor 'the aim to action' can be formed, nor can the formation of subsequent stages of the behavioural act begin. Therefore the study of real interactions of biologically characterized rising excitations at the level of the cortical neuron is a totally unavoidable stage on the road of learning of afferent synthesis in its entirety.

Systematic microelectrode researches were conducted in our laboratory by Fadeev in conditions of special formation of rising activations with a clearly defined biological modality.

First it was proved that it is possible to apportion at least 5 types of cortical cells in conformity with their relation to rising excitations of varied biological modality.

In obtaining from a hungry animal a cortical cell with spontaneous discharges, we had to be sure that these spontaneous discharges are exactly a result of concrete alimentary rising influences in the sphere of the alimentary centre of the hypothalamus. This was ascertained through the gradual elimination of phonal discharges after an intravenous injection of glucose (Fig. 7). Having convinced ourselves that we had an 'alimentary' cell activated by rising influences from an alimentary centre in the hypothalamus, we inflicted a pain stimulation on the sciatic nerve and watched the reaction of the same nervous cortical element.

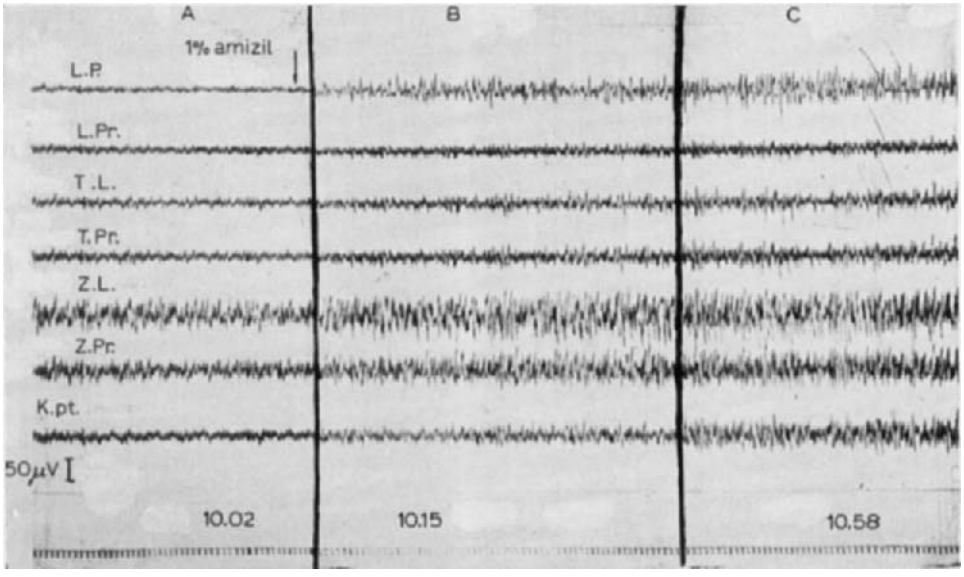

Fig. 7. Experience of application of amizil to the frontal parts of the cortex of the great hemispheres in conditions of their alimentary activation. A, state of electrical activity before the application of amizil. B, 13 min after the application of amizil (the upper part). C, 56 min after the application of amizil. One can see the slowing down of electrical activity at the point of application of amizil.

The most interesting observation made from these experiments was the convergence of the pain rising excitation precisely to those cortical neurons which up to this time were aroused deliberately by the alimentary rising excitation from the region of the hypothalamus. In other words we observed the convergence to one and the same

cortical cell of excitations of varied biological modality, which, as we mentioned above, we called 'multi-biological convergence'.

Such a convergence takes place in two ways. In one the pain rising excitation inhibits the spontaneous alimentary discharges, and in the other it increases the frequency of these discharges. In either type of action we must presume the meeting of two heterogeneous influences on one and the same nerve cell.

The interactions of this sort of excitation at the cortical level show that in the process of afferent synthesis various complexes involve not only individual neurons, but also individual synaptic organizations of one and the same neuron.

It is true that according to Bullok (1957), who demonstrated an extraordinary chemical and functional heterogeneity of the membrane of one and the same nerve cell, we can no longer be surprised that various chemical processes may take place in it simultaneously.

Consequently we have now on hand new possibilities for explaining analytical and synthetic processes of the brain cortex.

Here too the problem becomes prominent of the convergence of heterogeneous rising excitations in one and the same cortical cell. And, indeed, how is the cell 'taken apart' as to rising excitations varied in nature? How can these excitations, being chemically heterogeneous, in their entirety determine the exit to a definite axon specific for the given neuron excitation? What is the mechanism of this work of the neuron in bringing numerous excitations 'to one denominator', and what informative value has each of them in this peculiar synthesis?

As can be seen from these questions, the neuron represents a fully developed cell of synthesis, and, receiving (through dendrites and the neuron's body) heterogeneous excitations, generates on an axon an excitation, which in some still incomprehensible manner combines in itself in latent form all the properties of excitations converged upon it.

Today it is clear that in order to understand the afferent synthesis of the whole brain it is necessary also to know the particulars of those mechanisms on the basis of which heterogeneous and numerous chemical processes of the membrane of the nerve cell are transformed into impulses on the axon, well charged with information.

Obviously this represents a new and fascinating aspect in comprehending those synthetic processes which define the natural passage to the next stages of development of the behavioural act, to forming 'the aims of behaviour', the apparatus for envisaging results and formulating a concrete programme of action.

In this exceptionally ramified way we may be helped only by a systematic approach to a problem, but just now, to our regret, we must satisfy ourselves with only initial approaches to this problem.

As a first attempt to undertake the chemical characterization of processes that take place at the level of synaptic organizations of brain cortical cells, we utilized the method of application to the cortex of diverse inhibitors of synaptic activity in conditions of deliberately required convergences on cortical cells of excitations that had biological and sensorial modalities known beforehand. The experiences of many authors in application of diverse substances to the cortex with a view to making

analyses permit us to hope for success in our own experiments (see Richrich, 1960).

The general course of the argument preceding the start of these experiments was as follows. If the rising excitations of varied origin converge in the sense of biological and sensorial modality to one and the same cell of the brain cortex, then it is natural to expect that the synaptic formations themselves on the cortical cell used to conduct these excitations may be of different functional importance, they may be depolarized, hyperpolarized and in general have a wide range of changes in the chemical nature of their subsynaptic membranes. This was indicated both by our own results, as well as the data obtained by other authors (Ata-Muradova, 1963; Bullok, 1957, 1958; and others). If this be so, then by applying various specially selected substances directly to points of the cortex, we may create some dissociation of synaptic formations located in the given region. Some of them may be blocked; others, on the contrary, may even be alleviated in the conduct of excitations, and, consequently, we may observe changes in electric potential.

On the basis of these logical prerequisites we conducted experiments with application of cholinolytics to diverse spots of frontal parts of the brain cortex of animals that were in a state of hunger and, consequently, had the activation described above. If we proceed from the point that this initial activation is a result of rising activating influences on the cortical cell, then it would be interesting to discover of what neurochemical nature are the given synapses on the cortical cell.

Experience has shown that aminazine and atropine radically change the electrical activity of the point of application. Thus, for instance, a clearly desynchronized electroencephalogram turns into a slow electroencephalogram of the usual quiet type (Fig. 8).

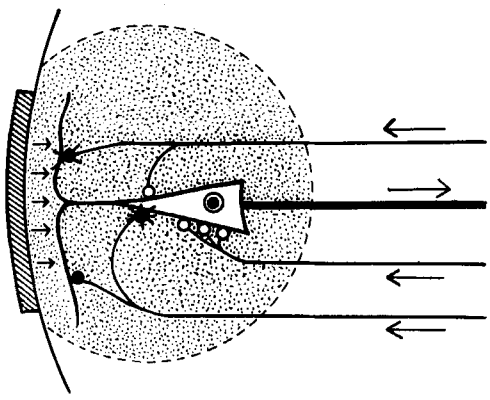

Fig. 8. Schematic depiction of the action of applied substances to the point of cortex. It shows the possible action of applied substances to the synaptic formation of varied localization and varied chemical nature.

If we are to organize this experiment in such a way as to record the produced potential from a zone of application, then we may hope to obtain an interesting result. As was demonstrated in our laboratory's former researches, in conditions of

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hunger activation of the frontal parts of the brain cortex, the potential evoked loses its usual form.

Only a positive part is clearly revealed, while the negative impulse is absent. If we are to proceed from ideas expressed in the figure, then we conclude that the digestive ascending activation 'subtracts', *i.e.*, utilizes exactly those synaptic organizations that are absent from the produced potential, obtained against the background of this activation.

If these suppositions are correct, then the animal's satiation in applying cholinolytics at the point of leading off the evoked potential should have acted in one direction. Both these factors would have to restore the usual form of the evoked potential, positive, negative and 'secondary'. And, indeed, application at this point of amizil and atropine leads to the evoked potential acquiring its usual appearance with positive and negative components (Turenko) (Fig. 9).

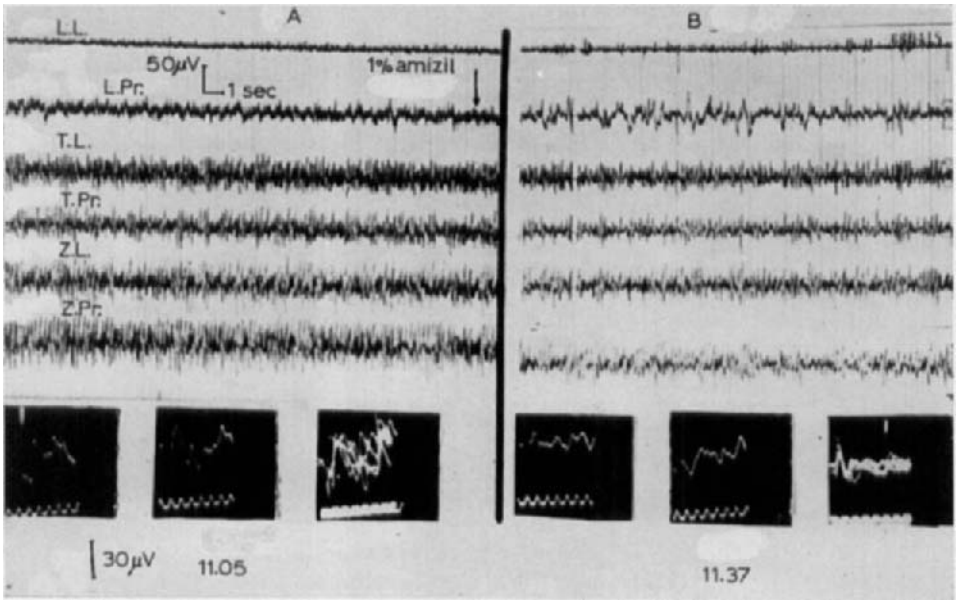

Fig. 9. Comparison of slow electrical activity with the evoked potential at the point of amizil application. We can see a distinct expression of the evoked potential 30 min after the application of amizil.

It can also be said that an animal's satiation alongside the return of the exact and slow electrical activity in the frontal parts also restores the fully valued evoked potential (Sudakov, 1962) (Fig. 10).

We obtained one more illustration, perhaps the most demonstrative, of the fact that each rising activation, having a specific biological modality, is addressed to its own specific synapses on the cortical cells.

After the application of cholinolytics converts the alimentary activation of the cortex into slow electric activity, the stimulation of the sciatic nerve against this background very rapidly transforms this slow activity into desynchronization, but

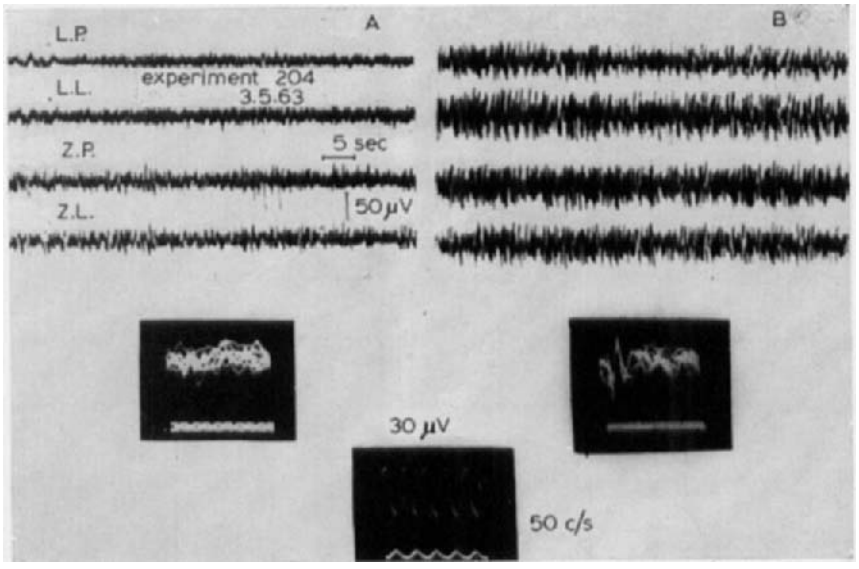

Fig. 10. Experiment demonstrating changes in the evoked potential in the frontal parts of the brain cortex during the process of the animal's transformation from the state of hunger to artificial satiation. A, state of 1-day hunger; activated condition can be seen of electrical activity in frontal parts. The evoked potential is expressed indistinctly. B, electric activity of frontal parts after the intravenous injection of glucose. Electroencephalogram shows the slowing down of electrical activity, which is accompanied by the distinctly expressed evoked potential.

this time of pain character. Thus, synapses responsible for the production of rising activation of different biological modalities proved not to be blocked (Gurenko) Fig. 11).

Considering all the observations cited above, we can say that they give us every ground for assuming that the initial alimentary activation of the brain cortex depends on excitation of cholinolytic synapses on the cells of the brain cortex, and that these synapses serve as a final station for perception of rising excitations from lateral nuclei of the hypothalamus. On the contrary, we may presume that a negative component of the potential produced is formed by synapses, being of different nature, inasmuch as it frees itself from the application of cholinolytics.

Both these results are in good agreement with our laboratory's former experiments, performed by Shumilina, 1949; Gavlichek, Makarov, Sudakov, 1962; and others. In these experiments the influence of aminazine quite distinctly revealed the difference between the activity, depending on the functions of the cholinolytic substrate and activity, connected with the activation of the adrenergic substrate. The experiments made above strengthen the opinion that activities and synaptic organizations, unyielding to the action of aminazine, may be of cholinergic character.

Experiments cited in my paper represent only a beginning of systematic researches of these mechanisms at the level of cortical synaptic organizations. And already the first results, as you have seen, permit us to presume that it is precisely at the level of the brain cortex that we have an extraordinary coincidence of diverse excitations both in a quantitative as well as in a qualitative chemical respect.

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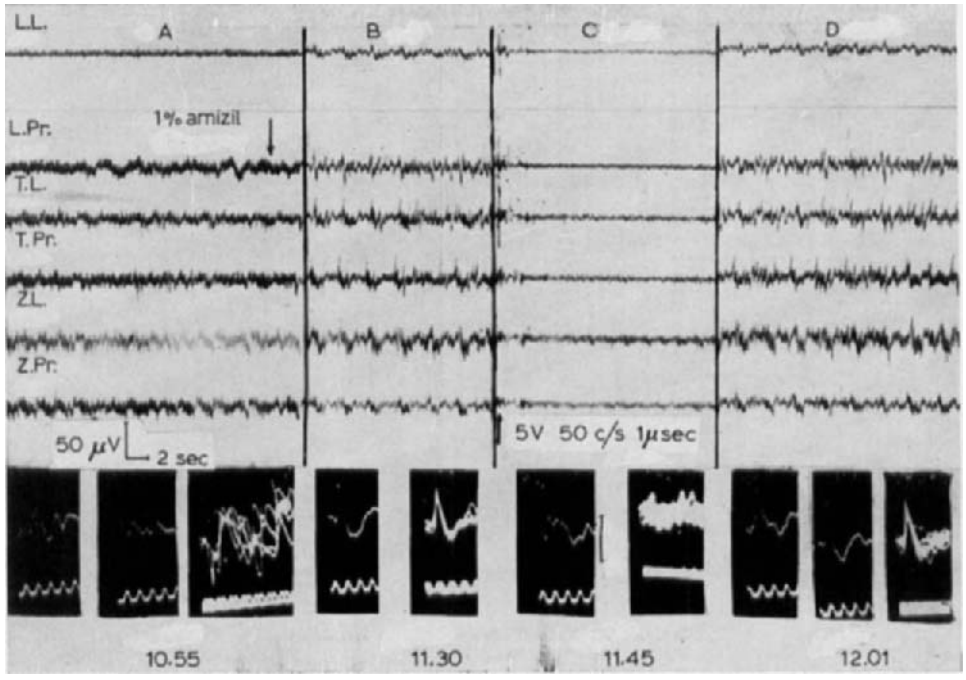

Fig. 11. Comparison of electroencephalogram and evoked potential in conditions of application of amizil and pain stimulation. A, the initial background during the 1-day hungering. The activated state in the frontal parts of the cortex is seen. B, 35 min after application of amizil in the frontal part, on the left side (upper section). Slowing down of activity in the frontal part and appearance of a distinct evoked potential can be seen. C, sciatic nerve pain stimulation. Activation can be seen in all parts of the brain cortex. The evoked potential is shown indistinctly. D, 16 min after inflicting the pain stimulation. The initial background of slow electrical activity is restored, and a distinct evoked potential has appeared. Comparison of electroencephalogram and evoked potential shows that amizil does not block the synaptic organizations, but performs pain activation in the brain cortex.

Figuratively speaking we have at the level of cortical cells an enormous quantity of excitations varied in quality, which may be synthesized on the body of one and the same cortical cell. At the same time we must not forget that a tremendous quantity of excitations, which rises to the cortex of the large hemispheres from the most varied subcortical formations and exterior receptor apparatus, determines both the direction and the main exit of effective corticofugal excitations, *i.e.*, excitations forming the behavioural act.

Only a thorough and fine analysis of all excitations ascending to the cortex of the great hemispheres and appraisal of quantitative correlations of these excitations can give us objectively tangible concepts of those multiform and numerous processes which accompany the stage of afferent synthesis. And it can hardly be doubted that only then, when all these boundless types of interrelations create a final model of the given situation, may the nervous processes swoop down in a free stream on the final stages of formation of the behavioural act.

SUMMARY

The investigation of the individual mechanisms maintaining all parts of the arborized functional system in a state of integration, logically led us to the necessity of characterizing most completely the first phase of the system activity — the afferent synthesis, which precedes the formation of the goal to act and detailed programming of all successive steps of the given action. This task should be performed through revealing the concrete neurophysiological mechanism of cortico-subcortical interaction.

With accumulation of our knowledge in the field of physiology of different brain parts and their interaction under different conditions of functioning, it becomes more and more difficult to regard the cortex and the subcortical structures as something separate, isolated. The question of the function of those structures nowadays is gradually substituted by the following question: which specific parameter is carried by the cortex or subcortex into organically integrated brain work?

As an illustration we may point out the decisive role played by the apparatus of the ascending subcortical activation in associative activity of the cortex (Magoun, 1963). The corticofugal influences on the subcortical apparatus without which the whole brain work would lose its adaptive essence is an example to the contrary.

Consequently, the problem of cortico-subcortical interactions has radically changed both in its neurophysiological aspect and in the evaluation of the integrated behavioural acts of self-regulatory character, especially of the conditioned reflex activity. It implies a new approach to interpretation of already existing data as well as to tactics of obtaining new facts which reveal integrative and self-regulatory character of brain work as a whole.

The most general neurophysiological mechanisms of the adaptive behavioural acts should include in accordance with well elaborated architectonics of these acts, the following stages.

(a) Recognition and identification of the 'exterior' situation, *i.e.* afferent synthesis, which is formed on the basis of ascending activating influences connected with the orienting reflex.

(b) Corticofugal mobilization of subcortical apparatuses in accordance with the situation.

(c) Selective excitation of the cortical neurons or synaptic organizations in accordance with the biological quality of the ascending activations.

(d) The formation of the goal to act and simultaneous formation of the afferent apparatus for the acceptor of the results of the action to be performed in future.

(e) The formation of efferent mechanisms of manifestation of the behavioural act and the achievement of the corresponding results.

(f) The reverse afferentation from these results and their comparison in the acceptor of action with previously formulated purpose.

Special experiments were performed to investigate the fine neurophysiological characteristics of these key processes. These experiments allowed us to reveal the following mechanisms in the cortico-subcortical relationships.

The specific character of the ascending activations to the cortical cells, and the

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convergence of the same cortical cells of sensory as well as specific ascending activations from subcortical apparatuses of different biological quality (multisensory and multibiological convergence).

The combination and interaction of subcortical apparatuses of different biological and chemical specifity in the process of the organism's adaptation to the peculiarities of its internal and external media.

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A Modern Interpretation of the Mechanism of I. M. Sechenov's Psychical Reflex Medium Member

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FUNCTIONAL CHARACTERISTICS OF MEDIUM MEMBER GIVEN BY I. M. SECHENOV

In his immortal work 'Brain Reflexes' I. M. Sechenov considers three types of reflexes: pure, passionate and psychical. Each type of reflex consists of three members: initial member presented by sensory excitation of the receptor or the nerve from the receptor; middle or medium member presented by more or less complex activity of the nervous centres; and final member represented by muscle motions.

According to Sechenov, pure reflex is an involuntary reflected motion which inevitably follows the excitation of the sensory nerve or receptor. This includes the defensive leg motions of the decapitated frog, sucking of new-born beings, sneezing and coughing of the animal and man, starting at sudden sound, reflected motions of the sleeping human, walking, etc. In pure reflexes the sensory excitation is transmitted to the motor nerves *via* more or less complex central apparatus without participation of the psychical element (Sechenov, 1952, p. 28).

Sechenov considers two kinds of pure reflexes: the first kind is the nervous mechanism of the pure reflex which is available from birth of the human being (sneezing mechanism, coughing mechanism, and the like). In the second kind, motor mechanism is acquired in the course of studying the motions, and is inevitably displayed in the form of some involuntary motions when 'sensory ability is blunt and those portions of the brain whose integrity as proved by the physiological experiments is essential for sensation (and consequently for consciousness) function very slowly or do not function at all' (Sechenov, 1952, p. 47). Evidently, of the first kind Sechenov was speaking about unconditioned reflex, and in the second about chain conditioned reflex developed by humans after multiple arbitrary repetition of labour motions.

The passionate reflex according to Sechenov is such a reflected motion whose medium member is formed by 'the most elementary sensual delight'. Nowadays the passionate reflex is termed emotional reaction. Sechenov stresses the large variability of this reflex with due regard to the physiological condition of the nervous centres. Such, according to Sechenov, are the well-known 'physiognomy of a hungry man' who feels delight while eating, and the characteristic physiognomy of a sated man who feels disgust for food (Sechenov, 1952, pp. 30–31). This reflex is displayed also in a laughing child who looks at painted objects. This laughing is caused by enjoyment; taking away the object will result in naughty behaviour of the child or crying, connected with displeasure or dreary feeling (p. 106). Sechenov arrives at the

conclusion that in an emotional reflex the external sensory effect is followed by strong motor reaction (p. 27). He supposes that a special mechanism is involved which intensifies the reaction. Sechenov proved experimentally that these intensifying mechanisms are arranged in the brain; they increase the effect of pleasant or unpleasant sensations (p. 28). In the emotional reflex the process develops for all the arbitrary motions: 'beginning — excitation of the afferent nerve; continuation — central activity, pleasure; end — contraction of muscles' (p. 30).

According to Sechenov, the emotional reflex is inevitably displayed also as a pure reflex to a certain sensory excitation. Being repeated, it becomes associated with a large amount of outside feelings, ideas and conceptions, and hence may be easily reproduced by wish. But the emotional nature is displayed rather weakly and may be absent altogether (p. 107). Here Sechenov means the emotional reaction acquired selectively.

The psychical reflex according to Sechenov is such a reflex which begins with the sensory excitation, continues with a certain psychical effect and ends in muscle motion (p. 52). This psychical effect, which is considered by Sechenov as a medium member, consists mainly of the conceptions about the outside world produced since early childhood. Studying or memorizing of these conceptions goes on a par with studying the accompanying motions. Since these motions are accompanied by muscle sensations, the psychical reflex includes the beginning (sensory excitation), the continuation (association of outside world conceptions), and end of the whole action (muscle sensation) (pp. 93–95).

The learned motions produced as a reply to the sensory excitation in the result of conception association are termed voluntary motions. Sechenov considers that such motions may be developed without participation of consciousness when for this or that reason some portion of the brain 'essential for sensations (and consequently consciousness) functions rather weakly or does not function at all'. As mentioned above. Sechenov speaks here about the labour motions performed automatically on the principle of the chain conditioned reflex.

All the views about the pure, emotional and psychical reflexes presented by Sechenov are still true at present. However, Sechenov clearly understands that his ideas about psychical processes lack explanation of the nature of these processes (p. 123). But he is sure that 'memory and reproduction of psychical formations' are based on the hypothesis 'on latent state of the nervous excitation' (p. 125).

In his article written 10 years later and entitled 'Means and Ways of Psychology Study' Sechenov stated that 'psychical actions should be worked out on the basis of the study of simplest psychical phenomena observed in animals and not in human beings' (p. 176). Sechenov himself did not study the psychical phenomena of animals, and so could not compare them with the structure of the brain which was not examined thoroughly enough to be used for studying the structural foundations of the psychical activity. However, Sechenov was convinced that 'there exists some relationship between the psychical actions and the so-called nervous processes in the body which are of purely somatic nature' and that the psychical activity, analogously with the nervous activity, requires (for its display) a certain time period and anatomic

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and physiological integrity of the brain (p. 179). Therefore Sechenov comes to a true conclusion that the psychical actions of the reflected type should be a subject for physiological research (p. 193).

Sechenov considers that there are nervous processes which run without sensations, without psychical phenomena. On a par with these processes there are other processes which involve sensations and psychical actions. Hence it would be logical to deduce that the nature of the psychical reflex being phylogenetically more developed differs qualitatively from the nature of the pure reflex which is phylogenetically less developed. Surely, both reflexes are stimulated by external actions and cause similar muscle motions based on the reflected principle. But the central actions proper, with and without sensations, should go on in compliance with different specific regularities.

On the basis of the psychical reflex characteristics of the medium member given by Sechenov we may draw the conclusion that the nervous substratum of the medium member which produces the psychical processes in the form of sensations, conceptions, ideas, and feelings differs qualitatively from the substratum of the pure reflex medium member which lacks the psychical property.

NERVOUS SUBSTRATUM OF PSYCHICAL ACTIVITY

A century has passed since Sechenov presented his brilliant ideas about the psychical reflex. But as I have already mentioned, Sechenov could not say anything about the nature of the medium member specific for the psychical reflex, its nervous substratum, or its physiological and structural peculiarities.

Now when we mark this anniversary it is important to show what is actually known about the nature of the medium member of the psychical reflex. We shall not discuss the history of this problem, as our modern knowledge of the psychical reflex medium member was obtained in the last 10–20 years. We shall discuss not the psychical activity in general but the lowest forms of this activity, such as sensation, perception, image or concrete notion. These psychical phenomena are characteristic both of the human and highest vertebrates. They are well studied nowadays and, as stated by Sechenov, study of the lowest forms of the psychical activity is obligatory before studying the psychical activity of an adult man which is complicated by sociological effects.

On the basis of modern experimental data it may be stated that the main nervous substratum of the subjective experience (sensations, perceptions, images) is made up of the nervous complexes in the primary zones of the perceiving regions of the cerebral cortex where the specific thalamic tracts from the receptors terminate. These nervous complexes located mainly in the 4th layer of the primary zones consist of the stellate cells with pericellular axon net. Hence it was supposed that these stellate cells function as the sensory elements which being excited produce the sensations of light, colour, sound, taste, smell, touch, warmth and cold.

This concept is based on certain morphological, physiological and psychological phenomena (Beritashvili, 1956). Most of the stellate neurons with short axons are located in the 4th layer of the perceiving region primary zones. The number of stellate

neurons located in the secondary zones and between the primary and secondary zones is considerably smaller.

The less developed the perceiving function of this or that receptor, the less developed is the 4th layer with the stellate neurons. For instance, the optical region of the large cerebral cortex of hedgehog with weak eyesight contains many fewer stellate neurons than that of the rabbit (Shkolnik-Yarros, 1954, 1958a).

In the course of phylogenetic development of the cortex, the number of stellate neurons with short axons has considerably increased especially in the primary zones. Monkeys and humans possess many more stellate cells than cats and dogs. The 4th layer in the primary zones takes the largest amount of the monkey's and man's cortex and is divided into three sub-layers consisting mainly of the stellate neurons (Cajal, Lorente de Nó, 1938; Polyakov and Sarkisov, 1944; Polyakov, 1956).

On this basis Cajal and then Lorente de Nó, Sarkisov and Polyakov believe that the prevalence in humans of stellate cells with short axons in the 4th layer of the primary perceiving zone is an anatomical expression of the improved function of the cerebrum. Further, Sarkisov considers that these stellate cells are probably the bearers of memory and keepers of the trace processes (Sarkisov, 1959).

The stellate neurons with short axons are united into a functional system which accomplishes perception and reproduction image of the outside world by means of internuncial and associative pyramidal neurons. It may be supposed that unification of the stellate neurons of the primary zone of one perceiving region for production of a certain perception is accomplished with participation of the primary zone pyramidal neurons, whereas the reproduction of images requires participation of the secondary zone. For instance, in optical perception, this unification is accomplished mainly by means of the associative and internuncial pyramidal neurons of the primary zone of the 17th field whereas the optical images are produced with the participation of similar neurons of the 18th and 19th fields which are secondary zones.

This supposition is proved by the experiments on humans.

Penfield and Jasper (1954) observed the subjective results of the electrical stimulation of the optical cortex during operations on humans and came to the conclusion that the 17th field is the first area where the optical sensations arise, while the 18th and 19th fields organize the optical perceptions and relate to binocular vision.

There is no doubt that the primary zones serve as the final stage for the afferent impulses sent from the receptors by the specific system. It is possible that only sensations of all the effective components of the outside world arise here, but primary perception of the object is accomplished, *i.e.* the large complex of sensory stellate cells unites into one functional system which accomplishes subjective reflection of the given object. It may be assumed that numerous internuncial and associative neurons with recurrent collaterals in the layers of the 5th, 4th and 3rd primary zones serve as an important structural base for integration of the excited sensory cells into the functional system of perception.

Since the given object has been perceived in a certain environment, the perception of this environment is included in this functional system. As a result of this the perceived object is projected in the given environment.

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From this functional system the excitation impulses are transmitted through special associative pyramidal neurons into the secondary zones. Here they terminate on the associative neurons which in turn send their own pulses to the given functional system of the primary zone. These bidirectional relations are very strong and stable, and they serve as a structural base for the appearance of the image of this or that object in a certain environment. Hence any component of the object or the environment proper may be employed for reproduction of the image of the given object.

The appearance of the image of complex objects of the outside world with participation of several perceiving regions is accomplished by means of pyramidal neurons of the associative fields located between these regions. This explains why electrical excitation of human temporal regions between the auditory and optical perceiving regions results in specific optical and auditory images in the form of optical and auditory recollections (Penfield, 1958).

Experiments on humans prove that when the receptors are stimulated the primary electric potentials in the primary zone and the subjective sensations of the excitations caused by this stimulation coincide. When, due to distraction of attention or intensified mental work, like solving arithmetical problems, the primary potentials caused by definite peripheral excitations become weak in the cortex, then the subjective experience caused by the same peripheral excitations also becomes weak (Hernández-Peón and co-workers, 1959).

The observations discussed above clearly show that the stellate neurons in the 4th layer of the primary zone of the perceiving regions are probably the only nervous elements which accomplish differentiated sensations, and contribute to perception of the outside world and to reproduction of the outside world images. I term these stellate neurons sensory neurons and hence the primary zone may be also referred to as the sensory zone as was proposed by Ranson (1937) and others.

STRUCTURAL AND PHYSIOLOGICAL CHARACTERISTICS OF STELLATE NEURONS

Now let us discuss an important problem: what are the structural and physiological peculiarities of the stellate cells (according to the latest data) which enable them to accomplish their sensory functions?

According to their appearance the stellate cells with short axons may be divided into two groups. One group of neurons is characterized by the fact that in each neuron the axon is branched in the region of dendrites of the same neuron. In this group the dendritic and axonal branches are so interlaced around the body of the cell and are so thin and dense that they can hardly be separated. Such cells with a dense pericellular net are termed spider cells or just Golgi cells of the second type. These neurons are mostly found in the 4th layer of the primary zone.

The stellate neurons with pericellular net prevail in the human and monkey primary zones (Sarkisov and Polyakov, 1949; Polyakov, 1956). Lower mammals such as mice and rabbits possess fewer such neurons (Lorente de Nó, 1943; O'Leary and Bishop,

1938, 1941). Hence we consider that these neurons with pericellular nets are the sensory cells with the most developed form.

Certain of the sensory neurons are characterized by their lack of axon branches from the pericellular net which would activate the other cells. The stellate neurons with the pericellular axonal net are clearly described by Lorente de Nó (1943). The axon leaves the dendrite sphere and yields the collaterals exclusively to the side of these dendrites. The other neurons with a pericellular net possess axon branches which leave the dendrite sphere to terminate just here or at some distance on the pyramidal or stellate neurons. Mostly the axon branches of these stellate neurons terminate on the nearest stellate cells to form separate groups of these cells (Shkolnik-Yarros, 1955). According to Lorente de Nó, each glomerulus consists of several dozens of stellate cells located closely to each other (1922). These cells are evidently united by means of axodendritic and axosomatic synapses as well as dendrite–dendritic synapses (Estable, 1961). The stellate cells of one group are probably the sensory cells of one modality.

The axon branches around the stellate cell not only repeatedly cross the dendrites of the given cell but even terminate on these dendrites and on the body of the cell in the form of synapses. Owing to this structure the neurons may cause the cell excitation circuit to remain closed. This provides for multiple excitation of the stellate cell during each cell activation.

This structural peculiarity of the stellate cells results in a physiological peculiarity. According to the experimental results obtained by Jasper, Li and others, some separate perceiving neurons in the sensorimotor zone (g. sigmoideus posterior of cat) usually respond by more or less continuous rhythmical discharges of the spike potentials on the background of the continuous slow potentials. This phenomenon is observed during excitation of the thalamic relay nucleus or sensory nerve of the limb by single electric shocks.

The continuous duration of the slow potentials (about 50 msec) is the result of summation of the local potentials of the cell bodies and dendrites, and evidently depends mainly on the continuous impulsation of their own axons. Spike potentials on the background of the slow potential arise from excitation of the axon branches by the stimulating effect of the slow potential upon the initial portions of the axons. The high rhythm at the beginning when the slow potential is strong and the gradual decrease in this rhythm with weakening of the slow potentials accord with the appearance of axon spike potentials under the influence of the slow cell-dendrite potential.

After local strychnine poisoning of the sensorimotor zone the intensification and elongation of the slow potentials is accompanied by a higher rate and intensification of the axon discharges (Li, 1959). The authors of this discovery single out these cortical elements as special Type 1 which relate to the cortical function of the skin and muscle sensations. They believe that these cells are the stellate pyramidal cells and numerous Golgi II cells on which the synaptically afferent terminations make contact (Li *et al.*, 1956).

We assume that these neurons which produce continuous rhythmical discharges in response to one peripheral impulse are the stellate cells with pericellular axon net

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or their glomeruli. Therefore such discharges should be typical of all the perceiving regions and not only of the sensorimotor region. They always serve to produce sensations under the influence of impulses sent by the receptors *via* the specific thalamic tracts.

These stellate neurons, under the effect of their own axon discharges, evidently become so active that they start producing the subjective sensations of light, sound, touch, etc. (Beritov, 1961).

However our present knowledge proves that the afferent impulsation from the receptors along direct rising tracts, *i.e.* through the specific system, is not sufficient for excitation of the sensory stellate neurons. This excitation largely depends on impulsation from the side of the reticular formation, *i.e.* through the non-specific system. Electrophysiological researches show that the afferents both from the specific and nonspecific systems (Creutzfeldt and Akimoto, 1958) terminate on the stellate cells of the visual zone. The afferents from the vestibular apparatus (Jung, 1959) also converge on these cells.

It is well known that the level of excitability of the cortex sensory elements required for their excitation with subjective display depends on the afferent impulsation from the side of the reticular formation.

The second group of stellate neurons is found in all the layers of the cortex. These neurons possess a small quantity of short dendrites, and the axons move off the cell in the horizontal plane or in the vertical plane to terminate in the other cells by numerous collaterals. According to Polyakov, the axon branches of such stellate cells often entangle the bodies of most pyramidal cells. Therefore it is assumed that the excitation of each such cell should result in simultaneous excitation of a large number of pyramidal cells.

Evidently the stellate cells of the second group serve to transfer the excitation impulses from the afferent thalamic fibres to the internuncial, associative and projection pyramidal neurons.

There are reasons for believing that the sensory cells with pericellular axon nets are not directly inhibited. Their excitation is temporarily discontinued due to the inhibition of the associative or internuncial pyramidal neurons which unite them or due to the disengagement of the specific and non-specific systems which activate these cells (Beritov, 1961).

Thus, the stellate cells of a definite type — with the pericellular axon net typical of the 4th layer of the perceiving regions of the highest vertebrates — are in our opinion the sensory neurons which produce the most differentiated sensations. But by no means may these properties be considered the function of the above-mentioned outside structure. This outside structure is adapted for the best way of repeated excitation of the neuron under the influence of the short-period afferent impulses acting upon it. Hence, these spider stellate cells may exist not only in the cortex but also in other portions of the cerebrum. It is assumed that production of the differentiated subjective sensations is a phylogenetically developed property of the nervous cytoplasm. Owing to the peculiarities of the molecular and submolecular structure of the cytoplasm, or more certainly the ectoplasm, the definite nervous cells could become

the base of the psychical activity which is the highest form of adaptation. However, the outside form of the sensory neurons may be quite different. Evidently, the spider shape of the stellate neurons with the pericellular axon nets is the most developed shape of the sensory cells typical of the highest vertebrates including humans.

The fact that the sensory stellate cells are arranged in united groups and form glomeruli which lack direct axon connections between each other has great biological importance. The stellate neurons of the 4th layer presumably perceive and reflect the outside world; they should be simultaneously activated under the influence of the outside world to produce concrete images of this world. The irradiation of excitation in the system of stellate neurons would interfere with the imaging perception of the outside world and with rapid change of images caused by the variability of the outside medium.

The fact that some portions of the spider stellate cells lack axon connections with the pyramidal cells must indicate that not all the sensory cells participate in direct transmission of excitation to the pyramidal cells for production of, for example, orientative motions of the head. Their main and sole function is to perform subjective reflection of the outside world, to produce the images of this world.

CHARACTERISTICS AND ORIGIN OF OUTSIDE WORLD IMAGES

We have already discussed the problem of the nervous elements which produce sensations, and we know how these sensory elements build up the functional system which performs perception of the outside world. Now we shall consider the problem of how the images of the outside world are produced from these perceptions and what are the characteristics of these images.

In humans, the subjective elements of the reproduced image are in general identical with those of the perception. In animals, the image of a certain object may contain all the components that are the subject of perception.

We have proved experimentally that the image of food perceived in a definite place includes the quality of food, its quantity and position. For instance if a hungry dog is shown a piece of meat behind one screen and a piece of bread behind another screen, it will go first to the place where it was shown the meat. If it finds no meat the dog will go to the place where it was shown the bread. Or if the dog is shown a small piece of meat in one place and several pieces in another, it will go first to the place where it was shown many pieces of meat (Beritov, 1939).

The images of the perceived objects are produced immediately during the first perception. Evidently, the sensory stellate cells which perform the sensations of one or various modalities are united into one functional system immediately after cell excitation. These images are preserved for a long time, sometimes for several months or years after perception. They are reproduced each time during repeated action of the given event or part of this event or even during a showing of the medium where the object was situated at the moment of perception. However, during perception the sensory stellate neurons are united into one functional system in another way than in the case of establishment of temporary connections of the conditioned reflex.

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During perception, the connections between the sensory neurons appear immediately at the first action of the object, and these connections are preserved for the reproduction of images sometimes for life. In fact these images do not disappear even in multiple reproduction without new perception. Evidently the connections between the sensory neurons differ qualitatively from the temporary connections formed with the projection pyramids which determine the development of the conditioned reflexes. These connections may become strong and stable only after multiple repetition. They quickly disappear if not continuously trained.

As mentioned above, the images of the perceived objects are produced by unification of the sensory stellate cells by the associative pyramidal neurons of the secondary zones. The secondary zones differ considerably in their microscopic structure from the primary zones. The most developed layer in the primary zone is the fourth which includes many more stellate cells with pericellular axon nets than the fourth layer of the secondary zone. The secondary zones in turn are characterized by an increase in the third layer which includes a large quantity of internuncial and associative pyramidal neurons. So far as the optical perceiving region is concerned, it is known that in the course of the phylogenetic development the area of the secondary zones of the 18th and 19th fields increases more than the respective area of the primary zone (Philimonov, 1948). Phylogenetically, fields 18 and 19 are younger structures than field 17 (Polyakov, 1949). Ontogenetically, field 17 matures earlier than fields 18 and 19 (Preobrazhenskaya, 1948).

All these observations prove that the development of the secondary zones are connected with the highest integration of the cortical activity *i.e.* its psychical activity.

The experiments on dogs performed in our Institute have shown that the functional unification of the sensory neurons required for perception takes place in the primary zone, for instance in field 17 for vision. The production and preservation of images which would be able to control the behaviour of the animals after the elapse of a prolonged time since perception are evidently accomplished by obligatory participation of the associative neurons of the secondary zones, for instance by participation of fields 18 and 19 so far as vision is concerned (Sikharulidze, 1962a,b).

The same observations prove that prolonged preservation of the perceived images depends not on the features of the sensory neurons or in general all the neurons and their connections in the primary zone, but on the features of the neurons and their connections in the secondary zone. Hence the production and preservation of images cannot be considered the result of trace processes in the sensory neurons. They evidently are based mainly on specific molecular and submolecular changes in the protein within cortical associative pyramidal cells. Moreover it is assumed that building up of the neuron functional system for production of this or that image may depend on change of the synaptic connections of these neurons in the secondary zone. These connections are probably capable of rearrangement and may easily become excited immediately after first activation of the functional system of the neurons of the given image. It is generally acknowlegded that the synapses of the central nervous system are very plastic. Their shape and size in various functional states may change considerably (Plechkova, 1961).

MOTOR ACTIVITY OF REPRODUCED IMAGES

Finally we shall discuss the problem: how do the reproduced images cause the motor reaction — orientative motions of eyes and head and then orientative movement in accordance with these images.

Sechenov attached great significance to the reproduced images of the outside world in the life and activity of the organism. He considered these images as the controlling mechanisms of behaviour. Hence Sechenov supposed that the images of vitally important objects may cause the same external reaction (this or that action of adaptation in the outside medium) as the perception of these objects.

We can now give a comprehensive explanation of the origin of this phenomenon. At perception of the outside world the orientative reaction occurs in which the head and eyes are turned to the side of the perceived object to ensure better perception. The respective structural mechanism exists in the same fourth layer of the primary zone of the perceiving region whither the afferent fibres from the receptor are delivered. Thus, in the optical primary zone the structural mechanism of the orientative reaction probably includes the pyramidal cells of Meinert and the so-called stellate cells of Cajal. The axons of these cells escape from the cortex to the midbrain and terminate in the colliculi superior organ. Thence the nervous tract from the Cajal cells goes on to the eye-moving nucleus and consequently this tract serves for eye fixation; from the Meinert cells the nervous tract passes to the spinal cord and probably causes movement of the head. The Meinert and Cajal cells also serve as terminal points of the afferent fibres which terminate here directly or through the transmission neurons — the stellate cells of the second group and internuncial pyramidal neurons. Hence the Meinert and Cajal cells are excited in each perception of the outside world and initiate orientative movements of the head and eyes. It may be assumed that the cortical neuronal complex which effects the visual perception of the outside world is connected with the projection neurons of Meinert and Cajal of the given orientative reaction. Therefore, each time the image of the outside world is reproduced it may be accompanied by orientative movements of head and eyes.

The perceived outside objects are projected outside, in the same places whence they have acted. The property of outside projection of the perceived images is the congenital property of the whole of the neuron functional system which performs perception of the outside objects. This is proved by the fact that new-born animals such as foals and pigs orientate in space shortly after birth. For instance the sucking pig, having run away from its mother, runs back when she starts invocatorily grunting (Beritov, 1935).

This ability of man and animal to localize the reproduced images of the outside objects in definite places in the outside medium enables them to perform orientative purposeful adaptive motions during reproduction of images of the vitally important objects.

Thus, humans and animals, being guided by the reproduced images, may accomplish movements in the outside medium towards or away from essential objects in the same manner as during perception according to the importance of these objects. This is the

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practical confirmation of Sechenov's statement that psychical activity is the main controlling mechanism of behaviour.

SUMMARY

Sechenov in his immortal creation 'Brain Reflexes' convincingly showed that all acts of conscious and unconscious activity of a human being, no matter how complicated, are due to his interaction with the outside world and therefore are reflex in origin.

Sechenov distinguished three types of reflexes: pure, passionate and psychic. These reflexes begin with the excitation of a sensory nerve or the receptor, then comes the middle link in the form of central nervous activity, followed by the final link in the form of muscle movements of the working organs.

According to Sechenov, pure reflex arises inevitably through the central nervous apparatus without any psychic element. Passionate reflex is such a reflected movement whose middle link is 'elementary sensual delights', and the final link is the unproportionally augmented reaction. By the middle link of a psychic reflex are meant psychic effects in the form of sensations, ideas, thoughts or feelings.

Sechenov believed that psychic activity is effected through a definite part of the brain, and this activity, as well as the nervous one, requires definite time and anatomo-physiological integrity of the brain. Sechenov did not identify psychic processes with nervous ones. He only found the relationship of psychic phenomena with the so-called nervous processes, the latter being purely somatic. But since Sechenov believed that pure nervous activity may take place without any sensation it may be concluded that he considered the substrate of the middle link of psychic reflex to be qualitatively different from the substrate of pure reflex, which has no psychic feature.

Sechenov could say nothing about the essence of psychic processes. He just regarded the hypothesis of the latent state of nervous excitation as the basis of memory in general and reproduction of images in particular.

After the publication of 'Brain Reflexes' many things about the middle link and the activity of nervous centres became clear. Pavlov and his school thoroughly investigated individually acquired, or conditioned reflex activity, connected mainly with the middle link of the learned form of pure and passionate Sechenov reflexes.

The middle link of the psychic reflex in animals has been the subject of our investigations for the last thirty years. Contemporary knowledge of the structural and physiological properties of cortical neurons, as well as clinical data, permit us to assume that stellate neurons, with their pericellular axon net, which constitute the main part of neurons of the fourth layer of primary cortical zones, are sensory. Their excitation produces elementary subjective experiences such as sensations of light, colour, sound, taste, odour, touch, warmth and cold. These sensory modalities are determined not by the structure of stellate cells, but by the phylogenetically formed property of cytoplasm.

During the perception of outside objects a great many sensory neurons of one or several primary zones become active. Simultaneously many internuncial and associative pyramidal neurons of both primary and secondary zones become excited by

means of which all the excited sensory neurons form a single functional system. This system becomes fixed, apparently, due to a momentary readjustment of synapses situated on associative neurons of secondary zones. As a result, repeated perception of the same object, a part of it, or its environment produces activation of the same sensory neurons and reproduction of the same perception. The reproduced perception is an image or concrete representation.

During perception the animal turns its head and fixes its eyes on the perceived object. This is achieved through excitation of special efferent neurons in primary zone which are activated also during the reproduction of the object.

The image reproduced projects to the outside world to the place in which the object was perceived for the first time. Sechenov pointed out that these two psychic processes — perception and image reproduction — are identical in their essence. At present we find that indeed their structural and physiological bases are absolutely equal. In both processes the cortex, the relay nuclei and the receptors are involved, but in the process of image reproduction relay nuclei and receptors are excited through the corticofugal pathways after excitation of the cortex.

Both the perception of the vital object and its reproduced image are accompanied by emotional excitation. If the excitation is pleasant then the animal or the child tries to get hold of this object; if the excitation is inpleasant, they turn their backs to it. These movements oriented in respect to the image are voluntary for animals and small children.

We believe that there is every reason to make the next step in the scientific comprehension of the thinking processes in adult humans.

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A Cortico-Subcortical System Controlling Differentiation Ability

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It has recently been demonstrated (Shustin, 1959; Brutkowski, 1964) that suppression of conditioned responses during inhibitory trials in a differentiation task ('go–no go') is temporarily lost after lobectomy of frontal lobes in the dog. In monkeys, Brutkowski *et al.* (1960, 1963) have established that the deficit on a differentiation task may be related to damage of the orbital as opposed to the dorsolateral frontal cortex, and that this impairment is not due to a sensory discrimination loss but rather to a difficulty in refraining on inhibitory trials from the response reinforced on positive trials. The purpose of the present study is two-fold: *(1)* to define the critical areas within the dog's frontal cortex and associated limbic and subcortical structures necessary for inhibitory performance; and *(2)* to investigate the mechanism of the impairment of inhibition.

The subjects were 41 experimentally naive mongrel dogs, approximately 2 to 4 years of age, and between 9 and 12 kg in weight. Thirty-two of the animals were tested in a regular sound-proof conditioned-reflex room. The procedure used was that described by Konorski and Miller as type II (instrumental) conditioning (1936). Before surgery, the animals were trained to place their right foreleg on a food tray to obtain food reinforcement to an acoustic stimulus (positive conditioned stimulus, CS), and to refrain from this response to an acoustic stimulus of different intensity or frequency (inhibitory CS). No food was given in inhibitory trials. Originally, two types of training trials were randomly presented. Either the positive CS was not followed by food, or, with the CS, the experimenter placed the animal's leg on the food tray and the food was delivered. Within a few days, an active instrumental conditioned response (CR) occurred, and the inhibitory CS was then introduced in some trials. In groups with lesions of the amygdaloid complex (Am and AmBL), the positive CS was a buzzer, and the inhibitory CS was a buzzer of different intensity. Daily, 5 to 7 positive and 1 to 3 inhibitory trials were given. The animals in these groups were trained to a criterion of at least 20 correct inhibitory responses in 25 successive inhibitory trials. In the remaining groups, the CSi were 1000 c/s and 700 c/s, and an equal number (10 or 15) of positive and inhibitory trials, separated by approximately 1 min intervals, was given every day. These animals were trained to a criterion of 45 correct inhibitory responses in 50 successive inhibitory trials. An error on the positive trials was recorded

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if the animal failed to place the foreleg on the food tray during the 5 sec presentation of the positive CS. Errors of disinhibition were defined as placing the leg on the food tray during the 5 sec presentation of the inhibitory CS.

Two animals, both of whom subsequently received a combined lesion of the medial prefrontal and premotor regions (group FM), were not trained preoperatively. They were fed *ad libitum* once per day. The amount of food consumed was measured daily and body weight recorded throughout a period of 3 months preoperatively. Seven animals out of 8, who subsequently received lesions of the entire amygdaloid complex (group Am), were not trained preoperatively either. Prior to surgery, these animals were observed nearly every day for a few weeks, and the response to food was carefully noted.

Upon completing the preoperative conditioned-reflex training or measurement of body weight and food intake, the animals were subjected to bilateral lesions. All lesions were carried out in one stage, except that for animals in the amygdala groups they were made in two stages. Animals were anesthetized with nembutal, and surgery was performed under aseptic conditions. Sixteen animals received lesions within the frontal cortex: *(a)* in 4 animals the proreal and antero-orbital areas on the dorsolateral surface of the frontal lobes were ablated (group L); *(b)* 4 animals received lesions of the inferior surface of the depth of the presylvian sulcus (group PS); *(c)* 4 received lesions of the proreal, pregenual and anteroprecruciate areas on the medial surface of frontal lobes (group M); *(d)* 2 received lesions of the medial subdivision of the subproreal area with a slight encroachment on the ventral portion of the pregenual area (group SP); and *(e)* 2 received an extensive lesion of both the anterodorsal portion of the medial prefrontal cortex and the premotor cortex anterior to the medial branch of the cruciate fissure and the genu of the corpus callosum (group FM). In 8 animals, the cingulate cortex was damaged: in 4 animals an attempt was made to confine the lesions to the genual region located in front and immediately superior to the genu of the corpus callosum (group G); 4 others were subjected to lesions destroying cortical tissue of the posterior cingulate gyrus.

In 3 animals, the lesions were restricted mostly to the basolateral portions of the amygdaloid complex (group AmBL), in 8 animals, the excision was extended in depth to remove the entire complex of the amygdaloid nuclei (group Am) and 6 animals were subjected to damage of the dorsomedial nucleus of the thalamus (group DM). Fig. 1 shows the lateral and medial aspects of the dog's brain.

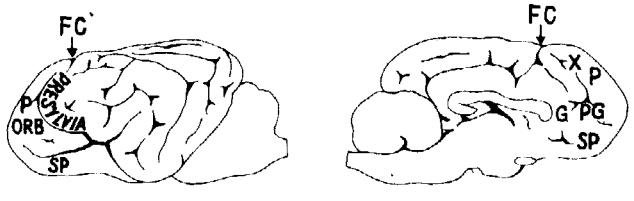

Fig. 1. Schematic diagrams of lateral (left) and medial (right) surfaces of dog's brain showing divisions of the frontal cortex according to Kreiner (1961). P = proreal area; ORB = orbital area; SP = subproreal area; X = precruciate area (premotor cortex); PG = pregenual area; G = genual area; FC = cruciate fissure.

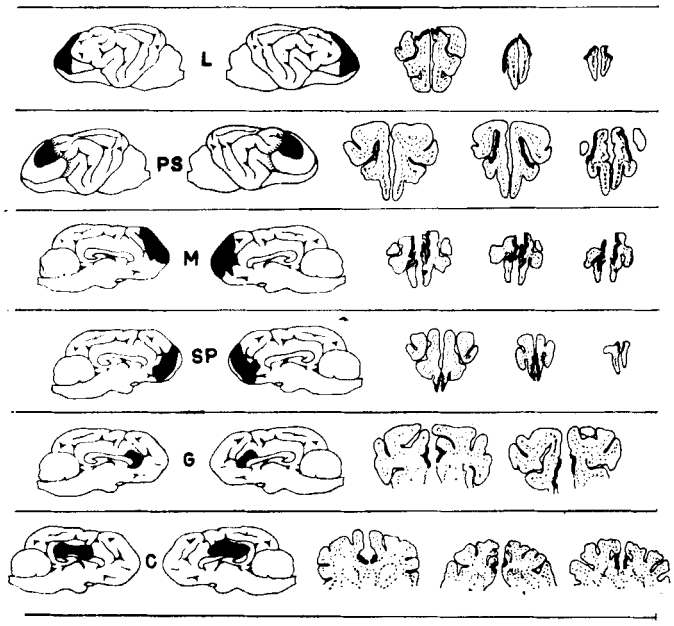

Fig. 2. Diagrams showing, by means of reconstructions and selective cross sections, the extent and depth of lesions of representative animals with resections of the frontal and cingulate cortex. Letters designate animal groups described in the text. Black indicates destruction. In PS, the composite gyrus was removed at the place indicated to show the extent of the lesion in the inferior depth of the presylvian sulcus.

The ablations within the frontal and cingulate cortex as well as those in the amygdaloid region were made by gentle subpial aspiration. For the destruction of the thalamus, the animals were placed in a stereotaxic instrument. Lesions were made by passing a 4.5 mA DC for 2 min through a stainless steel electrode 0.7 mm in diameter, insulated except for about 1.0 mm at the tip. In the case of complete dorsomedial lesions 6 electrodes, 1 mm apart, 1.2 to 2.2 mm from the midline, were placed in each hemisphere. To destroy the medial (magnocellular) portion of the dorsomedial nucleus, lesions were produced by means of 3 electrodes, 2 mm apart, 1.2 mm from the midline on each side, and 3.5 mA DC for 2 min was used. The locations of the electrodes were determined from the atlas by Lim *et al.* (1960).

After a recovery period of 1 week, all the animals were retested on differentiation under the same conditions and to the same criteria as those involved in the original learning.

Subsequent histological examination revealed that the lesions were well within the intended limits. In group Am, in addition to a complete destruction of the amygdaloid complex, the damage was found to invade the substantia innominata, the globus pallidus, the putamen and the anterior tip of the hippocampus. Figs. 2 and 3 show representative reconstructions of the placement of the lesions.

The results indicated that in the initial postoperative period, most of the animals with lesions of the thalamus or anterodorsal subdivision of the medial prefrontal cortex (group M) showed a slight retardation or an impairment (failure to respond)

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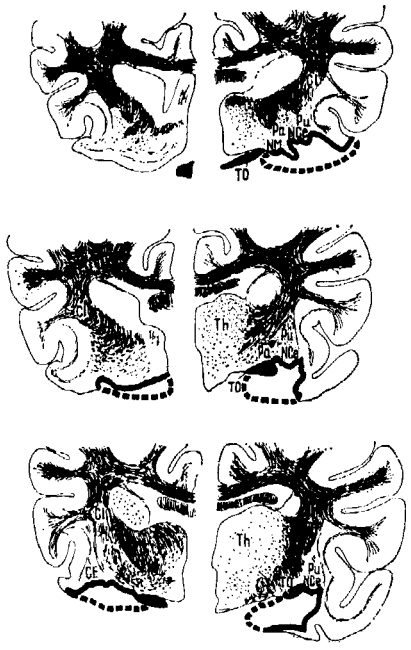

Fig. 3. Selected cross sections of the left and right hemispheres showing the extent of a partial lesion of the prepyriform-amygdaloid region, resulting in disinhibition. The solid heavy line indicates the border of the lesion; the broken heavy line, the hypothetical border of the area removed. CI = internal capsule; CE = external capsule; TO = optic tract; Pu = putamen; Pa = pallidum; Th = thalamus; NCe = central amygdaloid nucleus; NM = medial amygdaloid nucleus.

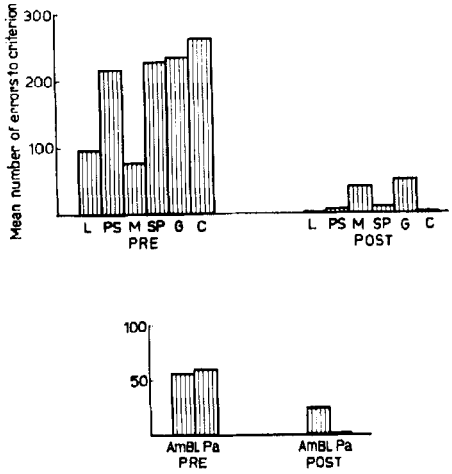

Fig. 4. Mean number of errors on inhibitory trials before (PRE) and after (POST) lesions in animals with resections of the cerebral structures described in the text. The diagram shows disinhibition errors following lesions of the medial prefrontal cortex (M), genual cortex (G), and the ventrolateral parts of the amygdaloid complex (AmBL), and failure of such impairment in the remaining operated groups.

on most of the positive trials. In a few days, however, the positive performance recovered and intertrial responses as well as errors of disinhibition then occurred in all animals in group M (Brutkowski and Dabrowska, 1963), and in 1 animal out of 6 in the group with lesions of the thalamus. Disinhibition errors were also noted after lesions of the genual area of the rostral cingulate cortex (Brutkowski and Mempel, 1961) and of the basolateral parts of the amygdaloid complex (Brutkowski *et al.*, 1960). In most of these instances, the differentiation performance returned to the preoperative level within a few experimental sessions. The animals of the remaining groups showed perfect retention of the differentiation task. Fig. 4 shows the mean number of pre- and postoperative disinhibition errors for groups L, PS, M, SP, G, C, AmBL and Pa (control lesion of the parietal cortex, involving the middle portions of the entolateral, suprasplenial and ectolateral gyri). Postoperatively 3 animals out of 8 with complete amygdalectomy stopped eating. Among them, the only animal in group Am with training in differentiation showed an irregular performance, possibly due to the absence of active eating; however, there was, in general, postoperative retention (Brutkowski *et al.*, 1962). It is interesting to note that the lesions of the thalamus were (with one exception) not followed by an impairment of inhibitory performance as might be expected on the basis of anatomical connections between frontal and cingulate parts of the cortex and the dorsomedial and anterior nuclei of the thalamus. This finding deserves emphasis in association with the earlier observation by Peters *et al.* (1956) that lesions in the medialis dorsalis in the monkey do not produce impairment of delayed response performance, another type of function which has been reported to be severely affected after frontal lobe damage. As yet, then, there is no satisfactory evidence of the participation of the dorsomedial nucleus in inhibitory processes. Although the most recent work by Syrenskii (1963) points to a slight impairment of inhibition after lesions of the medial thalamic nuclei in the dog, further systematic studies must be conducted to evaluate accurately the relation of anterior and medial nuclei of the thalamus to the performance on differentiation tasks.

The subjects of the 3 groups (M, G and AmBL) that were postoperatively affected in inhibitory trials showed a marked increase in food-directed activities in the experimental situation, characterized by an abnormal sniffing and searching behavior in the vicinity of the food cup. On the basis of such findings, it has recently been hypothesized that impairment of the inhibitory performance in animals with any of these 3 lesions is produced by an increase in drive functions (increased motivation for food, increase in fear or sexual behavior, etc.), which may be related to disruption of hypothalamic mechanisms (Brutkowski and Mempel, 1961; Brutkowski, 1964). This is consistent with the evidence indicating that basal and medial parts of the frontal cortex together with the temporal polar regions, including the amygdaloid complex, are directly tied up with the hypothalamus (Le Gros Clark and Meyer, 1950; Nauta, 1961), thereby forming a system for the regulation of autonomic and emotional functions (Fulton, 1951; Pribram and Kruger, 1954), which seemed worthwhile to warrant intensive research efforts.

Recent findings by Balińska *et al.* (1961), and Balińska and Brutkowski (1963) on

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rabbits demonstrate that, after damage to the medial hypothalamus, a disinhibition of responses to inhibitory CSi in association with hyperphagia occurs. Moreover, Balińska and Brutkowski (1963) found that rabbits with lesions of the lateral hypothalamus, who exhibit both a depression in conditioned-reflex activity and in food intake after surgery, later showed a conspicuous impairment of inhibition associated with an increased drive for food after recovery which was possibly due to application of a forced hydration technique (Balińska, 1963). Previously, Fuller *et al.* (1957) reported that hyperphagia might be produced by lesions placed in the amygdaloid complex region in the dog. It is thus possible that hyperphagia causes difficulty with suppression of CR in inhibitory trials, thereby resulting in disinhibition. This explanation does not seem applicable to the entire medial frontal cortex, including premotor cortex, however, since dogs with complete removal of the prefrontal-premotor regions on the medial surface (group FM) showed a depression in food intake and body weight (Fig. 5). Yet these results are of a preliminary nature since the lesions in these animals were much more extensive than those (group M) which were made to produce disinhibition. Studies are now being undertaken on animals with smaller lesions, tested at the same time for conditioned-reflex activity.

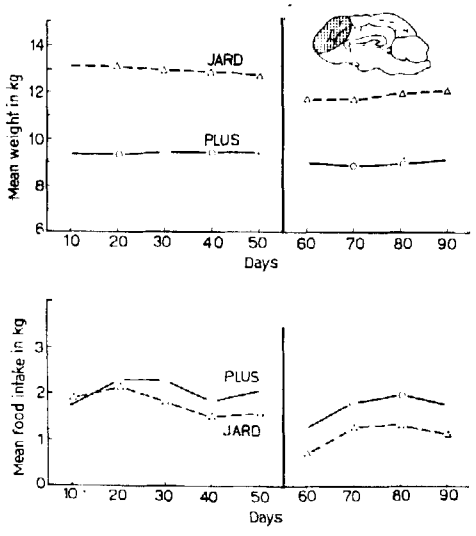

Fig. 5. Mean food intake and body weight before and after extensive bilateral lesions of the prefrontal-premotor cortex on the medial aspect of the brains of dogs Jard and Plus. The cortical lesion is designated schematically on the brain map.

Another approach to elucidate the nature of food-oriented activities in dogs with medial prefrontal lesions is that of measuring the salivary outflow during conditioning. Experiments now in progress (Wolf, Balińska and Brutkowski) tend to indicate that, postoperatively, salivation decreases in positive trials and remains at the previous level during inhibitory trials. This agrees with the earlier results of Shustin (1959), obtained on dogs with frontal lobectomies, but conflicts with the findings reported by Brutkowski (1964), who found that in some lobectomized dogs an increase in

both the positive and inhibitory CRs may be noted. The reasons for these differences are not apparent.

To sum up, the present findings indicate that the anteromedial prefrontal cortex together with the genual area of the cingulate gyrus, the basolateral amygdaloid complex and the hypothalamus constitute part of a system concerned with the type of inhibition trained in a situation in which food is presented in positive trials.

Unfortunately, the present research leaves unexplained the nature of disinhibition following damage to the medial frontal cortex. Due to the failure of an increased food intake and salivary outflow the drive inhibition hypothesis (Brutkowski, 1964) does not suffice to account for this impairment.

SUMMARY

The present investigation was undertaken to reveal the role of cortical and subcortical structures of the dog which are involved in the suppression of conditioned responses in inhibitory trials. A local conditioned reflex type II of the foreleg reinforced by food and an inhibitory reflex were trained. Daily 10–15 positive and inhibitory trials with an interval of 1 min, were used. The animals were trained to a criterion of stability of the positive and inhibitory reflexes. Upon completion of the preoperative training, each animal underwent a bilateral full or partial lesion of one of the following brain regions: the prefrontal cortex, the cingulate cortex, the dorsomedial and anterior nuclei of the thalamus and the amygdaloid complex.

Immediately after lesions of the medial frontal areas errors occurred in positive trials. Within a few days, the positive reflexes recovered, however, and errors of disinhibition then occurred. Disinhibition errors were also noted after lesions of the genual area of the cingulate cortex and the basolateral parts of the amygdaloid complex. It appeared that the impairment of inhibitory trials might be induced by an increase in food-directed activities. Complete amygdalectomy, on the other hand, was sometimes followed by a long-lasting aphagia and adipsia. Combined lesions of the medial prefrontal and premotor regions resulted in a decrease of food intake. Extirpation of other mentioned regions produced no effect either on the differentiation task or on the more general aspects of behavior.

Thus the anteromedial prefrontal cortex together with the genual area of the cingulate gyrus and the basolateral amygdaloid complex constitute a system (or part of one), concerned with the type of inhibition trained in a situation where food is presented in positive trials.

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The Role of the Amygdaloid Nucleus in Animal Behaviour

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During the last few years the amygdaloid complex has attracted great interest as a structure which is anatomically and functionally interposed between the neocortex and subcortical centres, and, as a part of the 'limbic system', exerts an integratory and regulatory influence on certain basic functions of the organism such as food intake, sexual reactions, defence against danger, and emotional expression (Papez, 1958; MacLean, 1952, 1958; and others).

The effects of amygdala stimulation as well as amygdala ablations are however multiform and often contradictory. Controversial results are probably due to the stimulation or damage of different nuclei which compose the amygdaloid complex. On the basis of anatomical and physiological investigations of other authors as well as our own experiments it is possible to distinguish within the amygdaloid complex two different parts: the phylogenetically older dorsomedial part and the basolateral one which develops progressively with higher species.

Several authors have shown that damage to the amygdaloid complex, particularly in its basolateral portion, results in hyperphagia (Green *et al.*, 1957; Morgane and Kosman, 1957, 1959; and others). These extremely interesting findings suggested that the amygdala takes part in alimentary behaviour as an inhibitory centre.

In order to discover more about the character of this hyperphagia we performed several series of experiments in which the pavlovian conditioned reflex method was used. If amygdala ablation impaired both positive and inhibitory reflexes we may suppose the effect of damage to be more general. Many different functions might be impaired, including learning and memory processes, and therefore the hyperphagia might have an agnostic character. If after operation the positive reactions were augmented, and particularly, if such augmentation had a perseverative character, hyperphagia may be due to perseverative repetition of the alimentary act and would mainly have an effector character. The disinhibition of inhibitory reactions would show weakening of the inhibitory mechanisms and would support the hypothesis that the basolateral amygdala plays an inhibitory role in the alimentary system. If that were so, stimulation of this structure should produce the opposite effect, *i.e.* inhibition of alimentary reactions.

For our studies we used cats and dogs. The first experiment was performed on 3 dogs (Brutkowski *et al.*, 1960) and concerned the effect of amygdala ablation on pre-operatively trained instrumental alimentary reactions. The results showed that

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positive conditioned responses were not changed after surgical removal of the amygdaloid complex (mostly its basolateral part). On the other hand responses to the inhibitory stimuli (both differential and conditioned inhibitory) were markedly disinhibited (Fig. 1). However this impairment did not have a perseverative character, and was limited to disinhibition of the instrumental reaction to the inhibitory stimuli.

These results seem to support the hypothesis that the basolateral part of the amygdala plays the role of an inhibitory alimentary 'centre'. The fact that the disinhibition was not complete may suggest that the inhibitory 'centre' in the amygdala is a secondary one and may act on the 'food centre' in the lateral hypothalamus either through or convergently with the 'satiation centre' in the ventromedial hypothalamus.

If the basolateral part of the amygdala is really the alimentary inhibitory centre, its stimulation should inhibit the alimentary reactions. Corresponding series of experiments were performed on six cats (Fonberg and Delgado, 1960, 1961) and then repeated on seven dogs (Fonberg, 1963a). The results show that stimulation of the

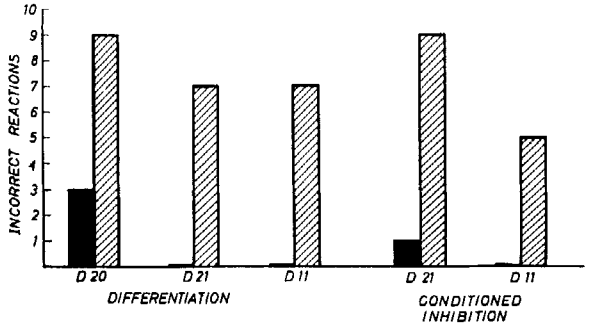

Fig. 1. The impairment of inhibitory conditioned reflexes in dogs after amygdalectomy. Each block shows the number of incorrect (disinhibited) instrumental reactions to the inhibitory stimuli in ten inhibitory trials (five experiments). Black blocks represent extent of disinhibition before operation; striped blocks, after operation.

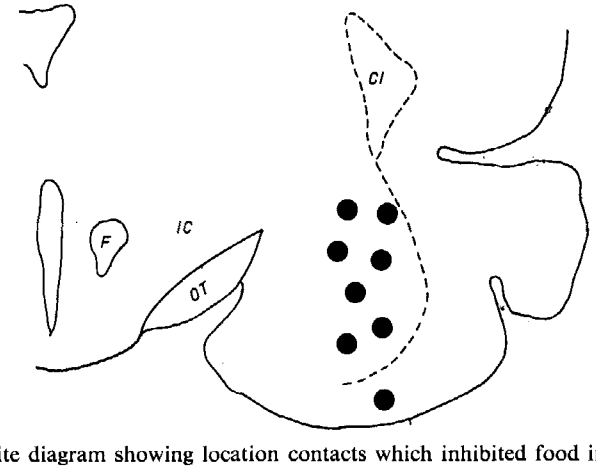

Fig. 2. Composite diagram showing location contacts which inhibited food intake and alimentary reactions at low threshold of amygdala stimulation. Coronal sections are about 12–13 mm anterior to the interaural plane.

basolateral part of the amygdala (Fig. 2) through chronically implanted electrodes causes cessation of food intake as well as performance of previously learned instrumental alimentary reactions in hungry animals. The inhibition of alimentary reactions outlasted stimulation from ten seconds to several hours or even days. The inhibition of food intake could be conditioned to indifferent stimuli and then extinguished when the brain stimulation no longer followed a conditioned stimulus. The shape of the conditioning and extinction curves and the behaviour is suggestive of normal conditioning and extinction processes (Fig. 3).

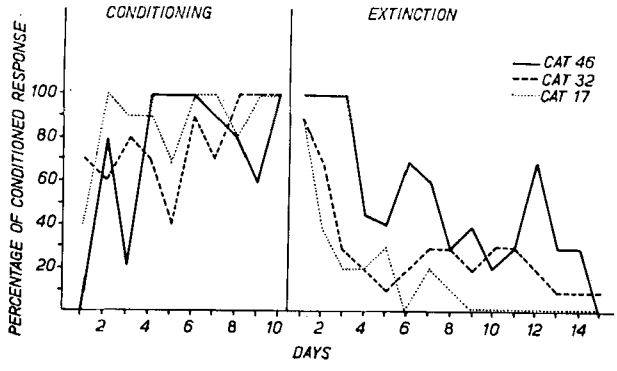

Fig. 3. *Conditioning.* The first part of the graph shows quick establishment of conditioning (inhibition of eating to the buzzer followed by amygdala stimulation). *Extinction.* The second part of the graph shows extinction of the inhibitory reaction when the buzzer was no longer followed by cerebral stimulation.

All these findings support the hypothesis that the basolateral amygdala plays an inhibitory role in the alimentary system. The stimulation did not interfere with spontaneous activity of the animals such as walking, playing, etc., and therefore it can not be considered as a kind of general inhibition or 'arrest' reaction. It was observed however, that during stimulation the animals were quieter, and apparently less fearful, and tended to lie down and purr. Thus the effect of basolateral stimulation seemed to extend to emotional behaviour.

The next series of experiments (Fonberg *et al.*, 1962) dealt with the problem of the role of the amygdala in defensive emotional mechanisms. Surgical removal of the amygdaloid complex was performed on six dogs that had been trained to perform either classical defensive responses (3 dogs) or avoidance (3 dogs). Postoperatively the animals showed marked changes in behaviour: they were tamer and less fearful. Classical conditioned defensive reactions were, after operation, completely abolished but then were gradually re-established. No changes in avoidance performance were observed (Fig. 4).

This discrepancy in the postoperative performance of the two kinds of defensive conditioned response may be due to the fact that classical defensive reactions are linked to the emotional fear state, and are therefore altered by the postoperative impairment of the emotional state. Well trained avoidance reactions, in which the connection with the fear had not been renewed for a long period of time (because the

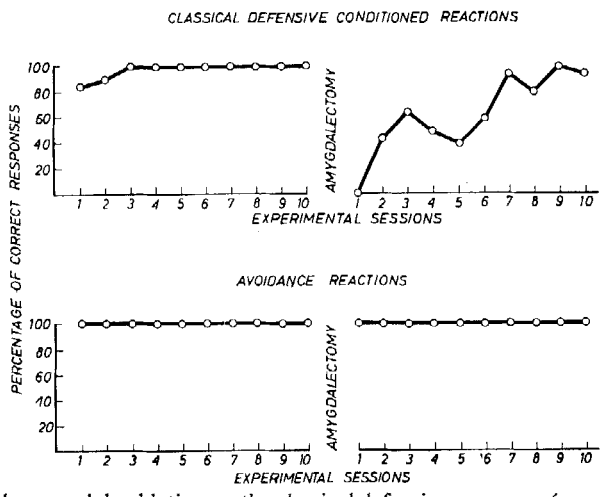

Fig. 4. Effects of the amygdala ablation on the classical defensive responses (upper part of the graph) on dogs D-8, D-9, and D-10, and on the avoidance responses (lower part of the graph) on dogs D-15, D-20 and D-21. Amygdalectomy impaired classical defensive reactions, while avoidance performance remained completely unchanged.

nociceptive reinforcement was no longer used) may be based on some other mechanism in which the emotional fear state plays little or no role.

Although our experiments seemed to support the conclusion that the amygdala plays an essential role in emotional reactions, the mechanism was not clear. We know that the effects of amygdala ablation described by various authors are contradictory. Some investigators have described a postoperative increase in fear and aggression (Bard and Mountcastle, 1948; Spiegel *et al.*, 1940; Wood, 1958) and others have reported quite opposite effects (Schreiner and Kling, 1956; Fuller *et al.*, 1957; and others). The results of the surgical ablation are always more or less global, therefore some effects may be due to the damage of other structures and quite opposite effects may be caused by irritation of surrounding areas. In order to investigate in more detail the role of different parts of the amygdala in emotional reactions we performed experiments on 15 dogs, in which several points within the basolateral and dorsomedial parts were stimulated separately.

Electrical stimulation of the dorsomedial part (Fonberg, 1963c, 1964) evoked fear (flight) and defensive reactions such as attempts to escape from the experimental situation, whining and sometimes barking. These reactions resembled those obtained from the electrical stimulation of the defensive zone in the posterior hypothalamus (Fonberg, 1963b). The effect of the hypothalamic stimulation was more dramatic however, and had a shorter latency. The effects of stimulation of the amygdala had a longer latency, fearful behaviour was not so dramatic, but fear symptoms sometimes outlasted the stimulation by several minutes. Results of investigating this aspect in dogs (Fonberg, 1963b,c) were similar to those obtained with cats by other authors. We are in agreement with Fernandez de Molina and Hunsperger (1959, 1962) who thoroughly studied the defensive system and considered the dorsomedial part of the

amygdala to be the forebrain level of the system mediating defensive behaviour, lower centres being situated in the hypothalamus and mesencephalon.

From our point of view it was interesting to see whether both the amygdala and the hypothalamic levels of the defensive system can be used with equal success as the negative reinforcement in the avoidance training. Experiments which were performed on six dogs showed that it was possible to establish instrumental reactions to the conditioned stimulus in order to avoid electrical stimulation of both amygdala and hypothalamus fear points. These results seem to indicate that fear reactions produced by cerebral stimulation of these points are not pseudo-affective motor syndromes but have real emotional and motivational characteristics.

However, avoidance training to the hypothalamus stimulation was faster and more regular than to the amygdala stimulation (Fig. 5). These findings suggest that the

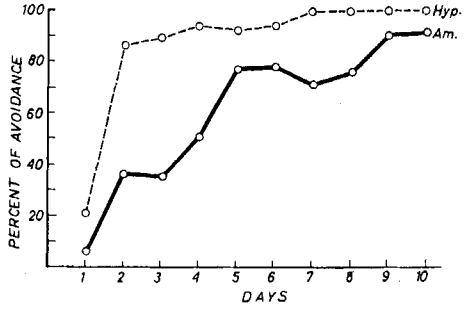

Fig. 5. Avoidance conditioning to stimulation of amygdala and hypothalamus 'fear-flight' points. The broken line shows the progress of conditioning to the hypothalamus stimulation; the solid line represents the conditioning curve to the amygdala stimulation. Each point represents the average percentage performance for three dogs in daily sessions.

amygdala has rather a secondary, regulatory role, while the hypothalamus is the main structure integrating defensive behaviour. This is in agreement with the work of Fernandez de Molina and Hunsperger (1962) who found that damage of the hypothalamic defensive centre abolished defensive reactions from the amygdala but not *vice versa*. The defensive region, as shown by the histological verification of our stimulation points, was not limited to the dorsomedial amygdaloid nuclei but extended to the piriform cortex (Fig. 6). This may explain the observation that, after amygdala ablation (mostly basolateral part, including the piriform cortex), the dogs were tamer, and the classical defensive reflexes were impaired (Fonberg *et al.*, 1962).

Stimulation of the basolateral part had an opposite effect than stimulation of the dorsomedial part on emotional reactions (Fonberg, 1962, 1963a). Basolateral stimulation inhibited various fear symptoms such as whining, screaming, attempts to escape from the experimental situation and numerous intertrial reactions, which, as we know, are good indicators of a general fear state. The inhibition of fear symptoms was observed in this group of dogs (4 subjects) in which electric shock to the skin had been used as the nociceptive reinforcement as well as in the second group (four subjects) in which fear symptoms were evoked by direct hypothalamic stimulation.

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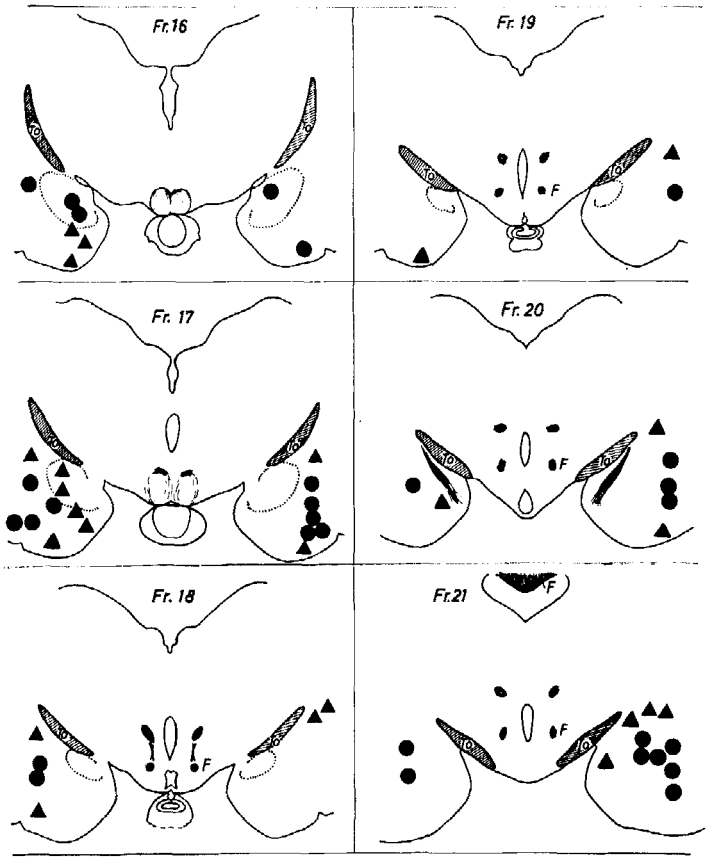

Fig. 6. Schematic diagram of the localization of stimulated points in fifteen dogs. The defensive field (triangles) is situated in the dorsomedial portion (mostly nucleus centralis) and also in the piriform cortex. The inhibitory points (circles) are found mostly in the lateral nucleus, extending to the basal nucleus.

In contrast with the great changes in fearful behaviour produced by amygdala stimulation, avoidance performance to the conditioned stimuli (Fig. 7) remained unchanged. These last findings confirm our previous results obtained on cats (Fonberg and Delgado, 1961), which showed that basolateral stimulation does not inhibit avoidance reactions.

The last series of experiments, now in progress, seem to show that basolateral amygdala stimulation reduces or even abolishes conditioned classical defensive responses. These observations may support the suggestion that classical defensive reactions are based on the fear state, while the emotional state in avoidance reactions (although it plays a role in training) is not essential for the performance of overtrained avoidance responses. Overtrained avoidance reactions may be dependent on some other mechanisms, in which the amygdala does not play an important role.

Our experiments as well as the work of Egger and Flynn (1963), who observed that

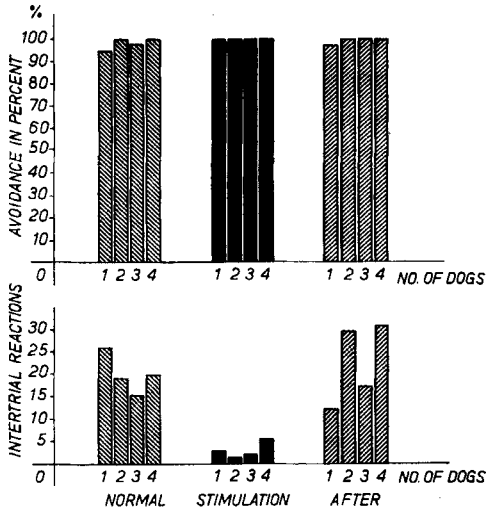

Fig. 7. Comparison of the effect of amygdala stimulation on both the intertrial reactions and avoidance performance in Group I. The mean values for each dog were obtained from five experimental sessions (50 trials before, 50 trials during and 50 trials after stimulation). The upper part of the graph represents the percentage of the avoidance performance, and the lower part the number of intertrial reactions. Although amygdala stimulation greatly reduced the number of the intertrial reactions it did not affect the avoidance performance.

basolateral stimulation also inhibited attack reactions, indicate that the inhibitory effect of basolateral amygdala stimulation is not limited to the alimentary behaviour but extends to various other behavioural patterns.

In conclusion we suggest that the amygdaloid complex does not work as a whole but is composed of at least two different portions belonging to two different systems. While the dorsomedial part consists mainly of a link in the defensive chain, the basolateral part seems to be connected with the widespread inhibitory system.

It is possible however that the inhibitory effect of basolateral stimulation is a secondary one, due to the interference with some positive emotional state, connected with reward mechanism. We know from the work of Olds (1958) and Brady (1961) that amygdala stimulation gives high rates of barpressing in self-reward experiments, and observation of our animals gave the impression that they are in a comfortable state during stimulation. If basolateral stimulation of the amygdala truly produces a highly pleasant rewarding state it may interfere both with the hunger drive and with the defensive and aggressive behaviour. In this case we may suspect that the amygdala is the upper level of a subcortical system dealing with motivation and reinforcement. While the dorsomedial part obviously belongs to an aversive system, and its stimulation serves as negative reinforcement, the basolateral part may be involved in reward mechanisms. The widespread physiological connections with different areas and particularly with subcortical structures as shown by Gloor (1955, 1957), and also interconnections between particular nuclei composing the amygdaloid complex, suggests that the amygdala exerts a motivational influence on various brain structures dealing with different functions. All these problems need further investigation.

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SUMMARY

It is possible to divide the amygdaloid complex, which plays an integratory and regulatory role in important functions of the organism such as food intake, defence and sexual behaviour, into two parts with different or even opposite physiological meanings.

The dorsomedial division is involved in defensive mechanisms. Its stimulation, producing various symptoms of fear and defence may serve as negative reinforcement (punishment) for the training of defensive conditioned reflexes both classical and avoidance. Thus we may consider the dorsomedial part as a 'defensive centre' probably co-operating with the hypothalamic and mesencephalic ones.

The basolateral part plays an inhibitory role. Its stimulation inhibits performance of the instrumental alimentary reactions as well as food intake, and its destruction produces hyperphagia and disinhibition of conditioned alimentary reactions. This would suggest that it plays the role of an 'alimentary inhibitory centre' duplicating the one in the ventromedial hypothalamus. However, stimulation of the basolateral part also inhibits fear symptoms and instrumental intertrial reactions connected with the generalized fear state. According to Egger and Flynn, basolateral stimulation also inhibits attack reactions. All these results point to a general inhibitory role of the basolateral part of the amygdaloid complex. The question arises however, whether it is the inhibitory centre *sensu stricto* which exerts true inhibition directly on various functions of the organism and in this way regulates the behaviour, or whether the inhibitory effect is indirect, due to interference of some other mechanisms. It follows from our experiments that stimulation of the dorsomedial part has properties of negative reinforcement. There is also a great deal of evidence suggesting that stimulation of the basolateral part is positively reinforcing. Therefore it is possible that the pleasant emotional state produced by basolateral stimulation may interfere with both feeding and defensive behaviour and this may account for lack of the respective reactions.

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Pseudo-Affective Reflexes of Cats produced by Extracts from the Plant *Actinidia polygama*

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A plant, *Actinidia polygama* (Matatabi in Japanese), which is found in the middle region of Japan, has been said to be specially liked by cats. It is said that cats are attracted by the odour of these burning plants and that they display a special type of behaviour — salivation, licking, playful rubbing, rolling, treading with hind legs and finally sleeping. These signs seem to be akin to the normal sexual reflexes of cats. Physiological observations of this phenomenon have been published sporadically in Japan since 1940.

Recently the effective substances of *Actinidia polygama* were extracted by Professor Sakan (Sakan *et al.*, 1959a) and identified as follows. Three substances were said to be physiologically active. One was phenylethanol (Fig. 1). The second was a lactone

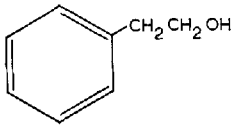

Fig. 1. β-phenylethanol (Phenethyl alcohol).

(isoiridomyrmecine) similar to nepeta-lactone (Fig. 2), an extract of a plant which occurs in Europe and America, and which is said also to be liked by cats (Pavan, 1951, 1955, 1957). Iridomyrmecine is secreted by a species of ant, *Iridomyrmex humilis* (Pavan, 1951, 1955, 1957; Cavill and Locksley, 1957) and probably has some protective function. The third substance more recently extracted from *Actinidia*

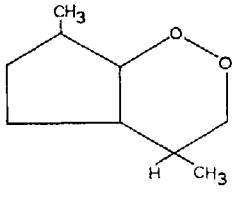

Fig. 2. Nepeta-lactone (iridomyrmecine).

polygama has been named actinidine. These three substances have now been synthesized.

The three substances were administered to cats with the object of investigating the claims that they can evoke reflex behaviour.

Phenylethanol is water-soluble and has a strong alcoholic smell. This substance was not attractive to cats when a quantity was placed near them in a small bowl, but it strongly induced salivation. When applied directly to the cat's tongue salivation

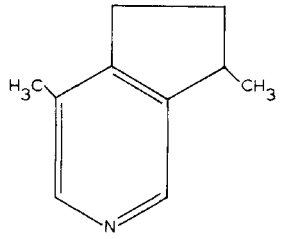

Fig. 3. Actinidine.

occurred almost instantaneously after a very short latent period. Intraperitoneal injection had no obvious effect. The salivation was therefore presumably induced by the action of phenylethanol on receptors of the tongue and buccal cavity. The reflexly evoked salivation, however, is not peculiar to this alcohol for allied alcohols with 4C and more had the same action (Table I).

TABLE I

THE ACTION OF ALCOHOLS TO PRODUCE SALIVARY REFLEXES IN CATS

Alcohols	*Salivary reflex when put in the mouth*
Methanol	(—)
Ethanol	(—)
Butanol	(—)
Propanol	(+)
Isopropanol	(+)
Phenylethanol	(+)

Nepeta-lactone is water-insoluble, but is readily soluble in ethanol. A 10% solution made by dissolving 100 mg in 0.1 ml ethanol and adding 0.9 ml water had a slight organic smell. This solution when presented to cats showed no attractive power. When 1.0–1.5 ml was poured into the mouth of a cat, it produced neither salivation nor behavioural change. When 1.0–3.0 ml was injected intraperitoneally, there was no salivary flow, and no behavioural deviation was observed.

Actinidine (Sakan *et al.*, 1959b) is apparently insoluble in water, but 0.1 ml of this oily fluid shaken with 100 ml of water produced an emulsion. The suspension had a strong and characteristic smell which remained in the room for 3 days then gradually disappeared.

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This 0.1 % emulsion of actinidine was not attractive to cats and it may even have been repellent. This lack of activity occurred with young and old cats of both sexes. When 1.0–3.0 ml was poured into the cat's mouth salivation was evoked and continued for several minutes. No behavioural change was observed, except that the animal became quieter and squatted down in a corner of the cage. When 1.0–3.0 ml of 0.1 % actinidine was injected intraperitoneally salivation did not occur. When 10–20 ml of the fluid was injected the animal vomited violently several times without salivation; after the contents in the stomach were ejected, the vomiting ceased. This might mean that the vomiting did not come from stimulation of the centre in the medulla, but it could mean that the reflex came from stimulation of the stomach, through some receptor to the actinidine localized in the systemic circulation.

CONCLUSION

Our experiments do not support the claim that *Actinidia polygama* produces behavioural changes in cats. The salivary effect of actinidine might well be due to the solvents used in extraction. These limited experiments are not conclusive, however, in disproving the folklore associated with *Actinidia polygama* in Japan and *Nepeta cataria* in Europe and America. It is possible that the effect may vary with the sexual cycle or hormonal status of the animal, or with previous conditioned states such as domestication, or the presence of human observers. However, our results do not suggest that the substances extracted from *Actinidia polygama* will produce psychophysiological effects in the cat.

SUMMARY

A plant, *Actinidia polygama*, found in the middle part of Japan is reputed to be especially liked by cats. Cats are said to be attracted by the odour of the burning plant and display a special behaviour — playful rubbing, licking, rolling, treading with hind legs — which seems to be some kind of sexual reflexes. We call them 'actinidine reflexes' after the effective substance of the plant, recently extracted by Professor Sakan and identified as $C_{10}H_{13}N$ and named actinidine.

The actinidine reflexes, called forth by the odour of the fluid, were observed in cats but not in mice, rabbits or guinea pigs. When the fluid was injected subcutaneously, intravenously or intraperitoneally, it did elicit actinidine reflexes in cats and dogs as well as in rabbits, mice and guinea pigs. The actinidine reflexes were observed when the drug was injected into the cerebrospinal fluid of cats and dogs.

An attempt has been made to determine the localization of the reflex centre in subcortical regions of animals' brains.

ACKNOWLEDGEMENT

After this work, I confirmed that the reflex above mentioned is easily aroused by chronic experiments in decorticated cats. It was published in International Sym-

posium on Cortico-Visceral Physiology and Pathology, at the German Academy of Sciences at Berlin, 1964. I am pleased to acknowledge the help of Professor F. R. Bell, Royal Veterinary College, London, in some of these experiments.

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Effects of Activating Systems on Neocortical After-Discharges

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This paper includes a brief review of our results on the influences exerted by stimulating certain subcortical structures (cerebellum, mesencephalic reticular formation, diffuse thalamic system and certain levels of the amygdaloid complex) on epileptic discharges induced by electrical stimulation or topical application of convulsive drugs on various neocortical areas. References from the literature are deliberately avoided. They are included in our previous publications quoted in the text.

(1) Stimulation of certain cerebellar lobules (folium, tuber, ansa-paramedian lobe) resulted in different effects on neocortical focal after-discharges (ADs) according as it had been applied to a curarized animal or to one liable to respond by tonigenic muscular activation to cerebellar stimulation (Kreindler and Steriade, 1960). In the intact uncurarized cat cerebellar effects were exerted depending on cerebellar stimulation parameters. Thus, the focal AD evoked by cortical faradization could be inhibited by long and strong cerebellar stimulation delivered before and during cortical stimulation; this phenomenon stood in contrast to the facilitation obtained with a short stimulation at low intensity in the same experiment for the same type of cortical AD. To ascertain the part played in such phenomena by proprioceptive reverberation, we studied the same effects in curarized animals. While the nature of the cerebellar influence on cortical AD in the uncurarized animal depends chiefly on the intensity and duration of the cerebellofugal discharge, in the curarized cat what imports is the degree of development of the cortical AD at the moment of cerebellar stimulation. Thus, while small cortical ADs were inhibited by a preceding and concomitant cerebellar stimulation, ADs exceeding a certain level both in duration and amplitude were facilitated by the same stimulation applied to the same structure (Fig. 1). Both the dynamogenesis and the inhibition produced by the cerebellum seem to occur at cortical level as we could not obtain records with such events from the thalamic relay (VPL and VL) nuclei.

(2) In the 'encéphale isolé' cat stimulation of the reticular formation and mid-line thalamic nuclei (Kreindler and Steriade, 1961), preceding by a few seconds, and/or simultaneous to the electrical stimulation eliciting a focal cortical AD, resulted in a marked enhancement of the AD, expressed by an increase in voltage, an increase in AD duration and, sometimes, by a spreading of the electrical events to cortical points

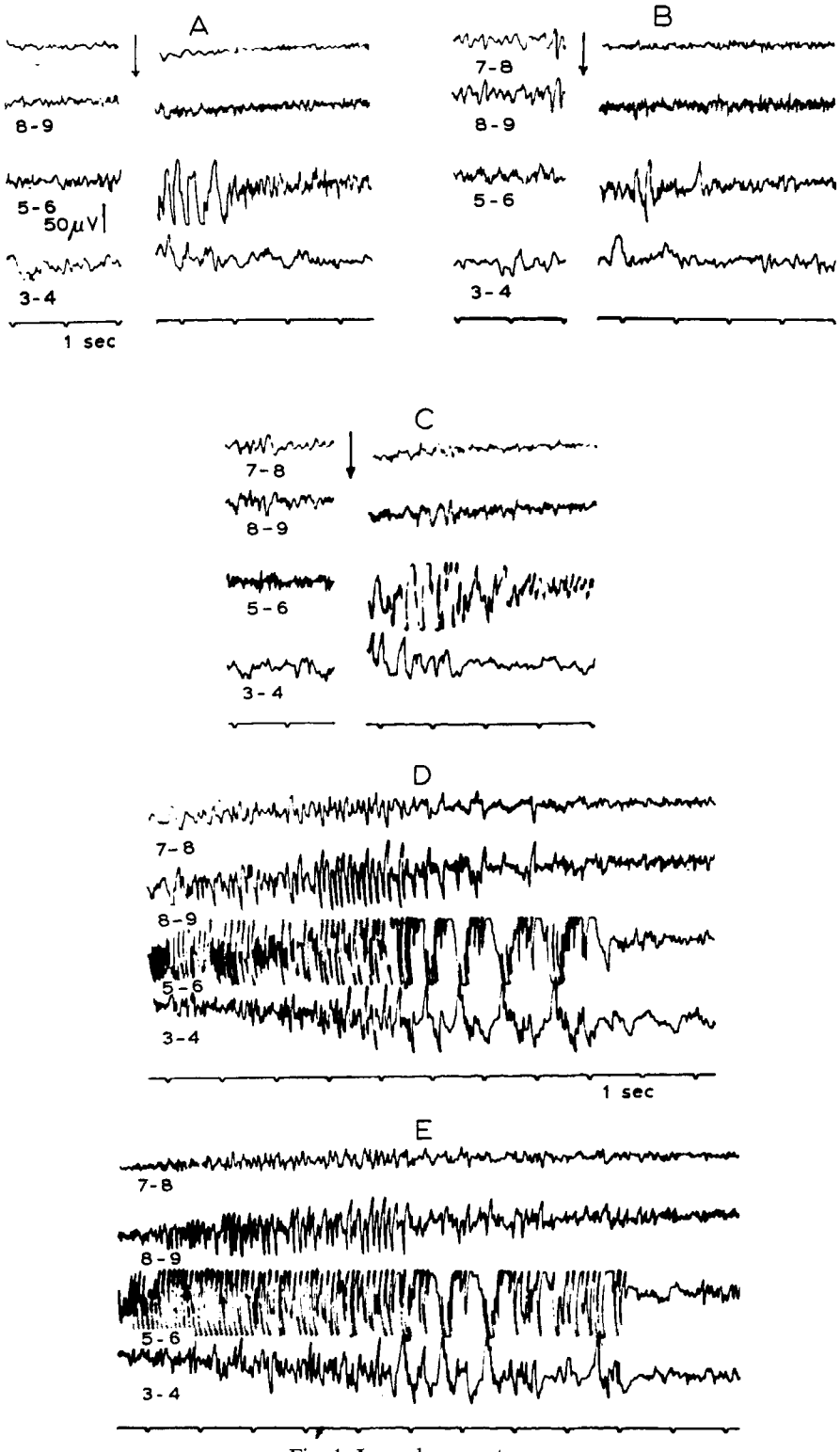

Fig. 1. Legend see next page.

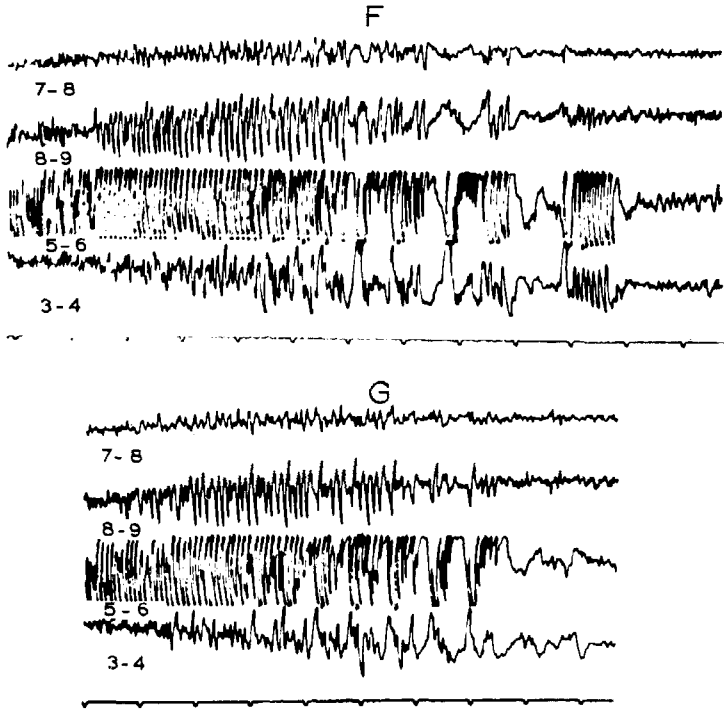

Fig. 1. Effects of cerebellar stimulation on focal cortical after-discharges in the flaxedilized cat 3–4, left sigmoid posterior gyrus. (A) liminal after-discharge (AD) induced by stimulating the left sigmoid anterior gyrus in the point symmetric to 5–6. (B) suppression of the liminal AD by a concomitant cerebellar stimulation (0.35 mA). (C) control of the test AD from (A). (D) AD elicited by a supraliminal cortical stimulation in the left sigmoid anterior gyrus. (E) and (F) facilitation of the AD by the same cerebellar stimulation as in (B). (G) control of the test AD from (D). (After Kreindler and Steriade, 1960.)

at a certain distance from the point of stimulation. This effect occurred when the cortical AD had been elicited by threshold, or slightly stronger, stimuli. A similar effect was, however, obtained in a few cases where a subliminal stimulus applied to the cerebral cortex was transformed into a supraliminal one if it had been preceded by a stimulus to the reticular formation or to the midline nuclei.

The increase in focal cortical hypersynchrony, an expression of reticular and thalamic facilitation, occurred on the background of a diffuse desynchronization (arousal reaction), characteristic of the fast repetitive stimulation of activating systems.

The facilitating effect of reticular and midline nuclei on the cerebral cortex was equally exerted, with no topographic differentiation, on after-discharges from sigmoid and marginal gyri. We saw no suppression of cortical after-discharges, as we did in experiments on cerebellocortical relationships.

(3) Reticular stimulation changed the neocortical excitability, facilitating the transition from normal to convulsive activity elicited by direct electrical stimulation (Kreindler *et al.*, 1964). The direct cortical response (DCR) to a single, either liminal or supraliminal, shock was not changed or showed an irregular alteration during

reticular stimulation. An effect of constant occurrence consisted in the disappearance of the late positive potential. Direct cortical responses evoked by rhythmic stimuli were markedly altered, and after-discharges were sometimes noticed during concomitant reticular stimulation even at cortical stimulation parameters previously unable to alter the DCR and elicit the after-discharges. In effect, the positive primary phase appearing in such conditions grew much larger and longer in duration and a second biphasic positive–negative wave developed. The latency of this second wave increased gradually from 20 to 60–80 msec. In some instances the DCR resumed its initial pattern sometimes passing through a period of disorganization of the DCR. In other instances an after-discharge appeared (Fig. 2). The same alterations also affected the

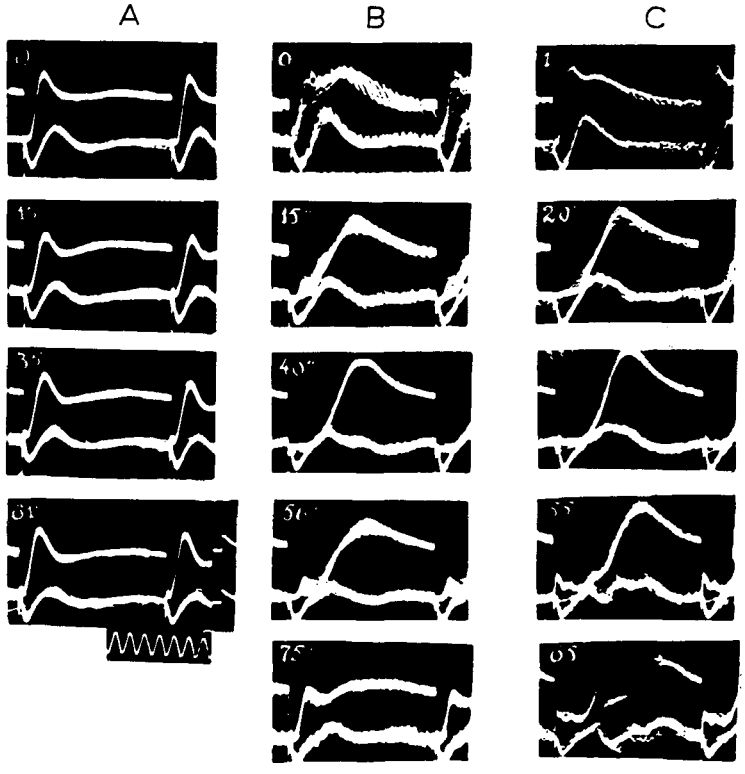

Fig. 2. Effect of mesencephalic reticular stimulation on DCR evoked by rhythmic supraliminal stimuli. (A) 10/sec cortical supraliminal rhythmic stimulation of the middle suprasylvian gyrus. Stimulation was over 60 sec in duration. No change in DCR appeared throughout the stimulation. (B) the same cortical stimulation combined with a fast reticular one. Note under the near recording electrode the increase in amplitude and a reduplication of the positive phase of DCR, and the appearance of a second positive–negative potential, gradually increasing in amplitude and duration in the negative phase. DCR displays the initial configuration at the 76th sec of stimulation. (C) the same phenomenon as in (B) with a stronger reticular stimulation. The cortical response recorded through the far electrode is polyphasic. Top tracing, record taken at a 3 mm distance from the cortical stimulated locus. Bottom tracing, at a 5 mm distance. Timing numbers in the left corner show the second at which records were taken during continuous cortical stimulation. Each record represents at least 15 superimpositions. Calibration, 10 msec, 200 μV. (After Kreindler *et al.*, 1964.)

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transcallosal potential. After several reticular stimulations, the cortical excitability showed constant changes.

Fast repetitive stimulation of the reticular formation, simultaneous to cortical rhythmic stimulation, might bring about a loss of intracortical inhibitory mechanisms and so the cortical neurons stimulated directly entered a permanent state of excitability with enhancing effect upon other neurons. This led to the overexcitability of an increasing number of neurons and, the intracortical inhibitory mechanism being less efficient, to the occurrence of the after-discharge.

(4) The influence of the cerebellar (anterior and ansa-paramedian lobes) and diffuse thalamic systems on the various phases of the cortical epileptic discharges induced by topical application of penicillin to different neocortical areas was studied in the unanaesthetized curarized cat (Steriade, 1960). The small focal spikes marking the onset of the penicillin seizure were only inhibited by cerebellar stimulation. In a more advanced stage, when the spikes became larger and spread out, stimulation of the same cerebellar structures with the same parameters during the same experiment resulted, on the contrary, in a clear-cut facilitation, both in frequency and amplitude, of the penicillin spikes (Fig. 3).

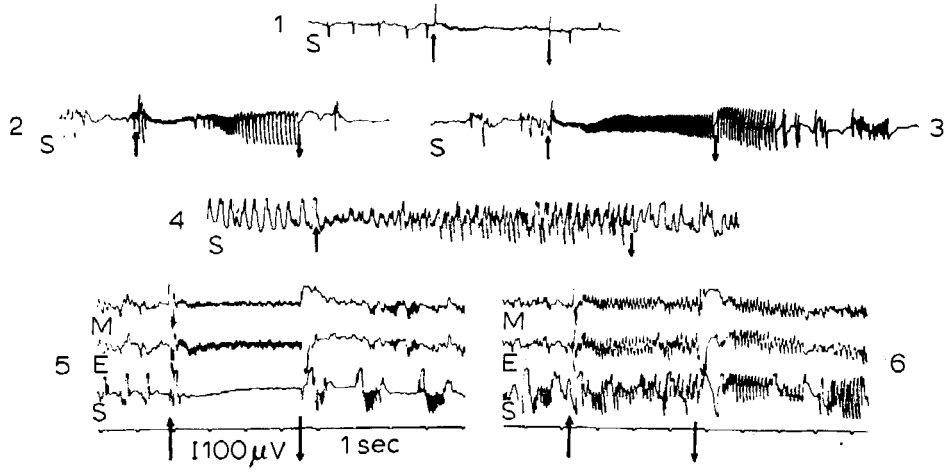

Fig. 3. Effects of stimulation of diffuse thalamic system on penicillin cortical spikes. (1) Small amplitude spikes inhibited by high rate stimulation of the left 'centre médian' nucleus (0.35 mA). (2) The same animal in a later phase; the spikes reaching greater amplitude are facilitated during stimulation at the same intensity (0.35 mA) of the same thalamic point. (3) The same animal; a stronger stimulation (0.5 mA) of the 'centre médian' nucleus induced the facilitation with a clear-cut after-effect. (4) The same animal; the generalized cortical seizure (graph reproduces only the record from sigmoid gyrus) is modified, showing an increase in frequency and amplitude during stimulation of the same thalamic nucleus (0.35 mA). (5) Another animal; note the inhibition of penicillin spikes in the sigmoid gyrus during stimulation of the left nucleus reuniens (0.3 mA) and the rebound which includes both the area to which the penicillin crystal had initially been applied (sigmoid (S) gyrus) and remote cortical areas (marginal (M) and ectosylvian (E) gyri). (6) The same animal as in (5); when in a more advanced phase the penicillin spikes came to assume the aspect of a well-organized seizure, the stimulation at the same intensity (0.3 mA) of nucleus reuniens induced a marked enhancement of the seizure with generalization in the leads from marginal and ectosylvian gyri. (After Steriade, 1960.)

Topical application of mescaline to a neocortical area resulted in the appearance of a discharge of biphasic spikes. The spike discharges were localized over 20–30 min (Crighel and Stoica, 1961). During the first 10–15 min, the frequency of the spikes was lower when the EEG traces were showing a desynchronization. Later, there was an enhancement of the epileptic focus during the neocortical desynchronization elicited by electrical stimulation of the mesencephalic reticular formation. The spikes became grouped and their aspect was modified (polyphasic or polyspike-wave). The trans-

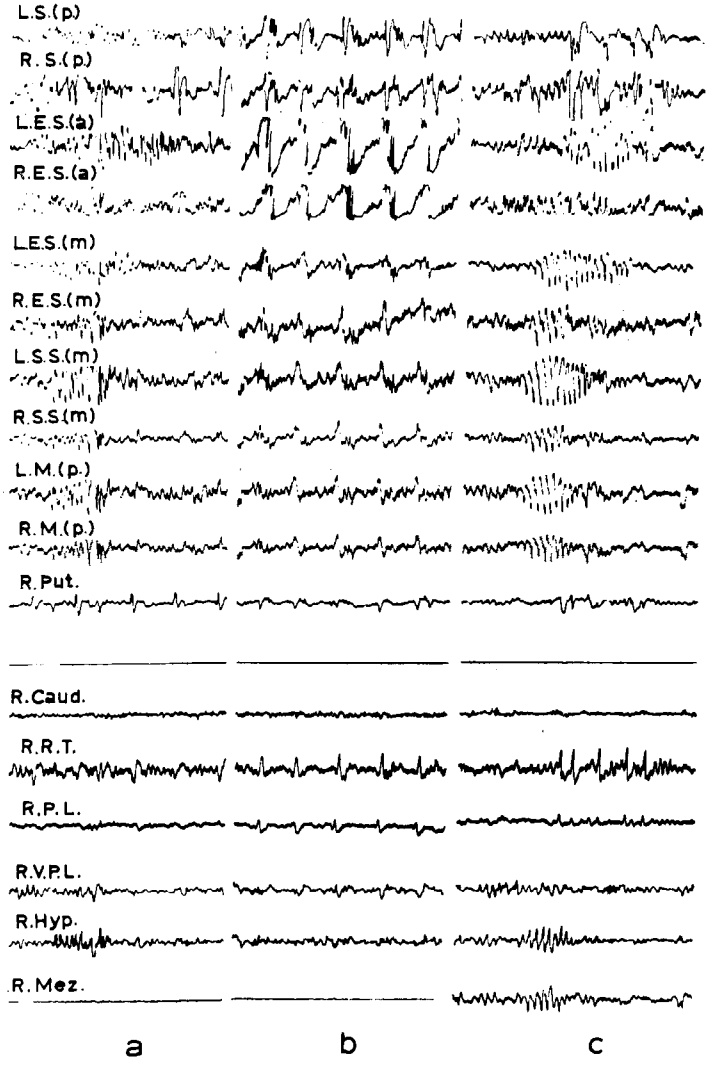

Fig. 4. (a) Before reticular stimulation; an epileptic focus induced by topical application of mescaline 3% on the right posterior sigmoid gyrus; spikes are apparent in right putamen and reticular nucleus of the thalamus, induced by the cortical focus. (b) Immediately after the mesencephalic reticular stimulation; spikes are generalized to all structures recorded. (c) After a generalized epileptic seizure. (After Crighel and Stoica, 1961.)

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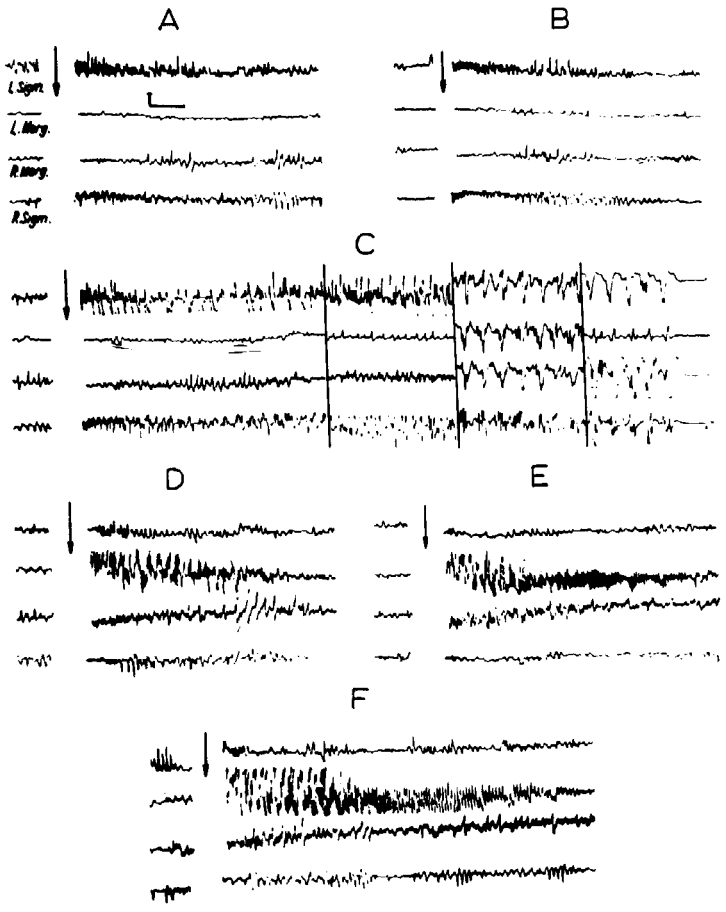

Fig. 5. Effects of VA nucleus stimulation on focal cortical after-discharges. (A) test AD induced by stimulating left sigmoid gyrus. (B) lack of any effect by associating a high rate stimulation of the dorsolateral area in VA nucleus with test cortical stimulation. (C) facilitation of test AD by associating a stimulation of ventromedial area in the VA nucleus, with the same parameters as in (B). (D) test AD induced by stimulating the left marginal gyrus. (E) lack of any effect by associating a high-rate stimulation of the ventromedial area in the VA nucleus with test cortical stimulation. (F) facilitation of test AD by associating a stimulation of the dorsolateral area in VA nucleus, with the same parameters as in (E). (After Kreindler *et al.*, 1960, unpublished graph.)

callosal and deep propagation was also facilitated by reticular stimulation (Crighel and Stoica, 1962; Fig. 4).

(5) In contrast to the diffuse facilitation obtained by stimulating the reticular formation or the thalamic midline nuclei, stimulation of the ventral anterior thalamic nucleus (VA) exerted characteristic effects on cortical AD. These effects were localized topographically and they depended on whether a ventral or a dorsal area of the nucleus had been stimulated (Kreindler *et al.*, 1960). Facilitation of the AD in the sigmoid gyrus was obtained by stimulating the ventromedial area of the VA, while facilitation of the AD in the marginal gyrus occurred when the dorsolateral area of the same nucleus was stimulated (Fig. 5). Such localized effects were only obtainable by liminal

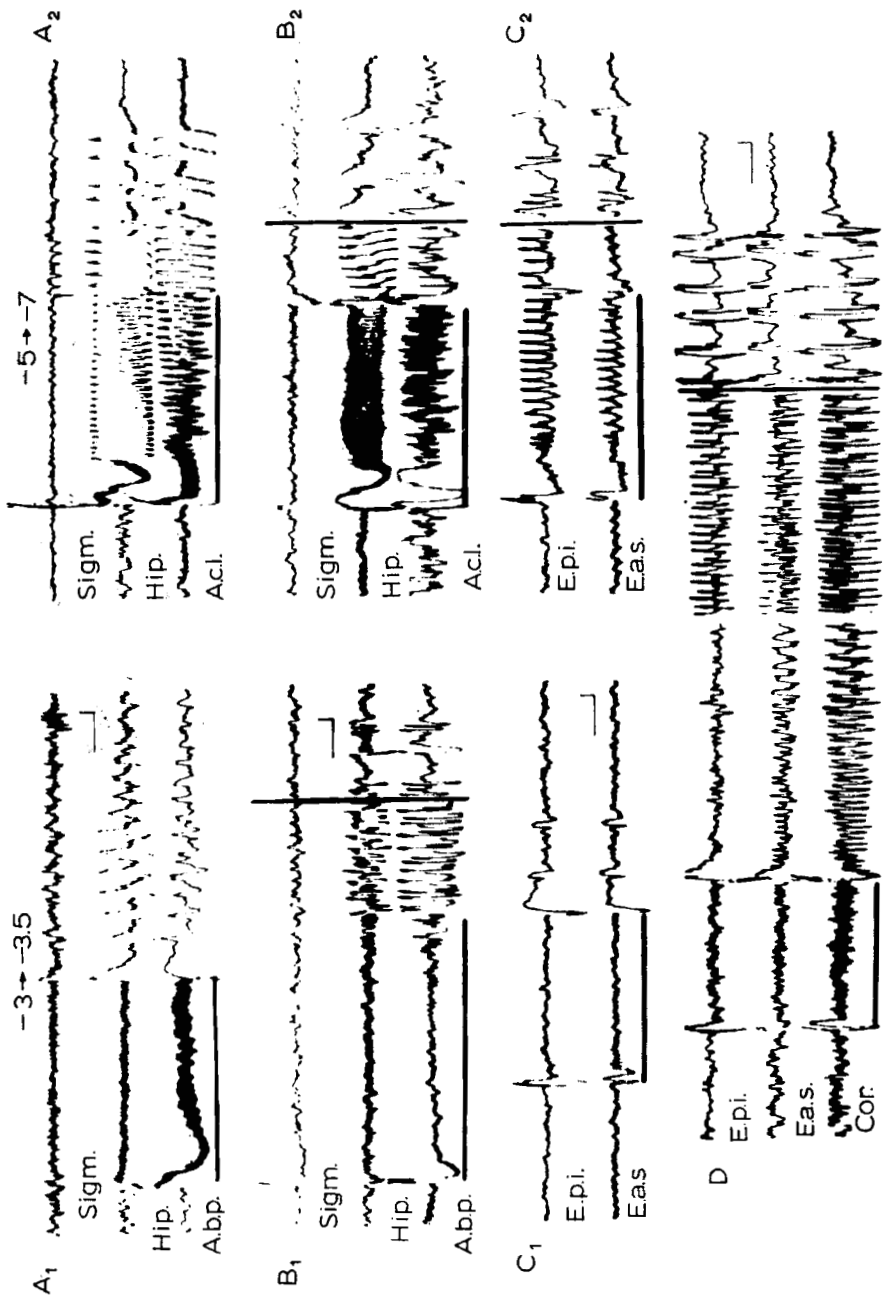

Fig. 6. Organization of intra- and post-stimulatory discharges elicited by high-rate (50–200/sec) stimulation of various amygdaloid levels. (A)–(D) four different experiments. (A), (B) and (C) stimulation with the same parameters of dorsal (left of figure) and ventral (right of figure) amygdaloid nuclei. In A_2–A_2, stimulation at 50/sec; in B_2–B_2 and C_1–C_2, stimulation at 200/sec; in D, 100/sec. Note that epileptic discharges induced by stimulating the dorsal amygdaloid levels (D. —3 to —3.5; A_1, B_1, C_1 and D) only appeared after cessation of stimulation, while by stimulating the ventral levels with the same parameters (D. —5 to —7; A_2, B_2 and C_2) discharges were obtained from the first seconds of stimulation. Sigm. = g. sigmoideus; Cor. = g. coronaris; E.a.s. = g. ectosylvius, anterosuperior; E.p.i. = g. ectosylvius, posteroinferior; Hip. = hippocampus; A.c.l. = nucl. amygdal. centralis lateralis; A.b.p. = nucl. amygdal. basalis parvocellularis. Vertical bars in this figure indicate 5 sec intervals (after Kreindler and Steriade, 1963).

voltage and they appeared more in the 'cerveau isolé' than in the 'encéphale isolé' preparation.

(6) Certain mechanisms of self-facilitation and self-suppression of epileptic paroxysms induced by stimulating allocortical structures (amygdaloid complex and hippocampus) were studied in another series of experiments.

In the framework of the basolateral amygdala of the cat, we found (Kreindler and Steriade, 1964) two mechanisms controlling cerebral electrogenesis. A high-rate stimulation of the dorsal amygdaloid structures (central lateral amygdaloid nucleus (A.c.l.) and dorsal areas of the anterior (A.a.) and lateral (A.l.) amygdaloid nuclei) resulted in a reticular-like desynchronizing reaction ('arousal' of the cortical rhythms), while stimulation of ventral amygdaloid structures (basal parvocellular amygdaloid nucleus (A.b.p.) and ventral portions of A.a. and A.l. nuclei) with the same parameters resulted in a synchronization of neocortical activity ('sleep' patterns). Such reactions depended on neither desynchronizing nor synchronizing structures of the lower brain stem as they had been found to persist in the 'cerveau isolé' preparation.

Investigating the dorsal and ventral portions of the basolateral amygdaloid complex in the same experiment, we found that the capacity of organizing epileptic waves during (D) and after (AD) electric stimulation differed at each level (Kreindler and Steriade, 1963). No epileptic-type discharges occurred during the desynchronizing reaction to stimulation of the dorsal amygdaloid nuclei; we only noticed an AD after cessation of the epileptogenic electric stimulation. In contrast to the dorsal amygdaloid nuclei, the A.p.b. nucleus, when stimulated with the same parameters, caused the epileptic discharges to become organized from about the first second of stimulation (Fig. 6).

The appearance of the AD only after cessation of the dorsal amygdaloid stimulation might be due to the suppression of the organizing epileptic discharges by stimulation. This suppressing effect can be explained by the fact that the stimulation evoking the epileptic paroxysm affects a structure desynchronizing *per se* the epileptogenic effects and therefore inducing their self-arrest. On the other hand, the greater epileptogenic capacity of the A.b.p. nucleus compared with the A.C.l. nucleus (Steriade, 1964) and the possibility of the ventral amygdaloid areas of organizing an epileptic paroxysm from the first second of electric stimulation are perhaps due to the synchronizing effects exerted by the stimulation of such areas on spontaneous cortical rhythms. The facilitating effects of the hypersynchronizing states (*e.g.* sleep) on the course of the epileptic paroxysm is thus well known.

SUMMARY

Experiments performed on unanaesthetized ('encéphale isolé') cats enabled the authors to infer a functional systematization of the amygdaloid complex according to the effects exerted by fast repetitive stimulation (150 per sec) on neocortical electrogenesis.

Stimulation of the dorsal amygdaloid regions induced an arousal reaction (low voltage fast activity) in neocortical areas; it was confined to ectosylvian areas when the

stimulation was liminal. When stimulation with the same parameters was applied to the ventral amygdaloid areas, a 'sleep' pattern (spindles and slow waves) appeared throughout the experiment, the hypersynchrony being particularly marked in the ectosylvian cortex. Both the arousal reaction and the hypersynchrony induced by stimulation of the amygdala at both levels persisted after intercollicular section ('cerveau isolé'), thus being dependent neither on activating structures nor on synchronized ones located in the brain stem.

The different effects exerted by dorsal and ventral amygdaloid levels on cerebral electrogenesis may be correlated with the different modalities with which these levels organize seizure after-discharge (AD). Fast repetitive supraliminal stimulation of the dorsal amygdaloid level brought about epileptic AD both in allocortical (hippocampal) and neocortical (especially ectosylvian) structures, but only after cessation of electrical stimulation. The occlusive effect of the desynchronizing amygdaloid stimulation on seizure AD is demonstrated by experimental evidence. On the other hand, it has been shown that electrical stimulation of ventral amygdaloid structures (with synchronizing effect) produces an epileptic AD from the first seconds of stimulation. Amygdaloid systems of self-regulation, that is seizure AD self-suppressing (dorsal levels) and self-facilitating (ventral levels) systems are discussed.

Fast repetitive stimulation of the cerebellum had a desynchronizing effect on cortical electrogenesis as well as on focal cortical AD but the latter effect varied according to the extent of paroxysmal hypersynchrony. For instance, AD with small amplitude (elicited by liminal faradic stimuli or occurring in the first moments of the focal penicillin seizure) were occluded by cerebellar stimulation, while the same cerebellar stimulation enhanced the paroxysmal hypersynchrony if the latter exceeded a certain amplitude. When the mesencephalic reticular formation was stimulated, only an enhancement occurred.

Local application of 3% mescaline solution to the neocortex produced a long-lasting epileptic focus. At the beginning of the discharges the frequency of spikes was lower during the periods of desynchronization of the EEG tracings. The epileptic focus was enhanced by stimulating the mesencephalic reticular formation: spikes became grouped while their transcallosal and deep propagations were facilitated.

The direct cortical response to a single stimulation of suprasylvian, ectosylvian or marginal gyri, either liminal or supraliminal, was little changed when the reticular formation was excited simultaneously. The most important changes obtained by stimulating the reticular formation simultaneously with the cortical stimulation were those of the rhythmic direct cortical response and of the AD. Fast reticular stimulation simultaneous with a rhythmic supraliminal direct cortical stimulation at a rate of 5–15 per sec amplified the positive phase of the direct cortical responses and brought on a second potential with a latency of 20–50 msec. This potential was positive–negative or only negative. When both the cortical and reticular stimulations were carried on steadily, the latency and the amplitude of the second potential gradually increased especially in its negative phase. Such changes sometimes preceded the AD. The same alterations were produced by the reticular stimulation in transcallosal rhythmic responses.

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The existence in the anterior ventral thalamic nucleus of two levels each having a different effect on focal AD in various neocortical areas has been demonstrated. For example, liminal stimulation of the dorsal part of the ventral anterior nucleus enhanced the AD elicited in posterior cortical areas while the same stimulation of the ventral part of that nucleus facilitated the AD evoked in rostral cortical areas.

The above studies show that self-regulation mechanisms are involved in the organization of paroxysmal activity at neo- and allocortical levels.

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The Role of the Meso-Diencephalic Activating System in Higher Nervous Activity: Its Role in Habituation, Learning Mechanisms and Conditioned Reflex Processes

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Abundant evidence accumulated over the past fifteen years shows that the nonspecific diffuse activating system of the brain stem, described by Moruzzi and Magoun (1949), plays an important role in the integration of behavioral and EEG arousal reactions, and in the formation of unlearned and learned behavioral processes. The ample connections of the brain stem with the diencephalon and the rhinencephalon as well as with the neocortex assure a dynamic complexity of facilitatory and inhibitory mechanisms, and through a cortical closure, these processes participate in the formation of attentive and orienting reactions as well as in the development of conditioned reflex activity (Gastaut, 1958; Morrell and Jasper, 1956; Anokhin, 1960; Livanov, 1960; Roitbak, 1960; Kreindler, 1960; etc.).

In our earlier experiments on walking cats and dogs it was demonstrated that stimulation of certain points within the brain stem and the diencephalon always facilitated those conditioned reflex reactions which appeared as dominant in a given environmental situation (Endröczi *et al.*, 1959). Such facilitation of a conditioned reflex reaction elicited by electrical stimulation of the subcortex in the absence of a positive signal may be regarded as being due to an artificially evoked driving force, which is the principal element of motivation. The analysis of conditioned reflex activity in the early phase of its development showed that drive played a fundamental role in forming temporary connections, and, as suggested by us several years ago, that the spontaneous goal-directed motor activity appearing in the intersignal intervals between two conditional signals could be regarded as an objective index of the intensity of motivation. In accord with the drive-reduction theory postulated by experimental psychology it was found by us that spontaneous goal-directed motor activity corresponded to the somatomotor manifestation of the driving force the intensity of which became reduced in the course of conditioning. Such reduction of drive should be related to the function of discriminative internal inhibitory processes as described in detail elsewhere (Endröczi and Lissák, 1962; Lissák and Endröczi, 1962; Endröczi, 1965). Starting with this assumption our attention was focused on the role of the mesencephalon and diencephalon in the integration of attentive behavior, orienting

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reaction and habituation as well as their participation in the organization of drive and learning processes.

In the present paper, dealing with the neuroanatomical basis of attentive behavior, habituation and conditioning, we present evidence that the brain stem reticular core, in close functional relationship with the basal forebrain structure, takes part in these processes. The investigations presented here were carried out on walking cats and rats bearing chronically implanted electrodes or lesions in different parts of the subcortex. The experiments were performed in a soundproof and electrically insulated room. The details of alimentary and avoiding conditioned reflex techniques used in these investigations have been described elsewhere (Endröczi and Lissák, 1962, Endröczi *et al.*, 1963, Korányi *et al.*, 1963). The first part of this paper deals with the EEG correlated with the neuroanatomical basis of attentive behavior and habituation. The second part is concerned with the role of drive in conditioning and with some special features of long-term somatomotor memory.

THE ROLE OF THE MESO-DIENCEPHALIC ACTIVATING SYSTEM IN THE INTEGRATION OF EEG AROUSAL, ATTENTIVE BEHAVIOR AND HABITUATION

The resting EEG activity of a nonmotivated cat placed in a soundproof room shows sequences of periodic changes. Each period starts with a desynchronized electrical activity of the neocortex featuring low voltage fast records, which are gradually

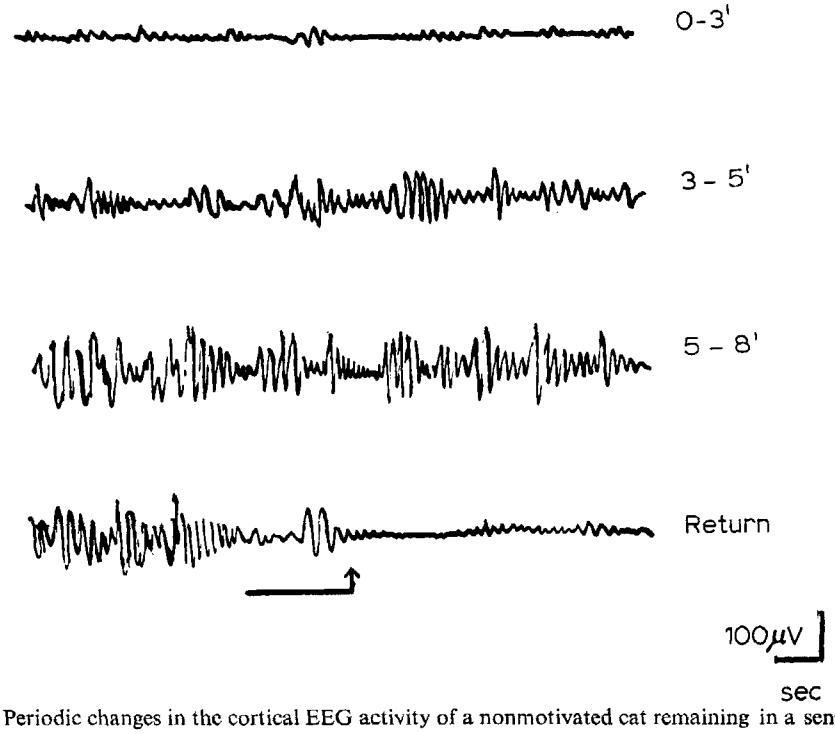

Fig. 1. Periodic changes in the cortical EEG activity of a nonmotivated cat remaining in a sensorily isolated condition.

replaced by high voltage slow waves of 4 c/s. Each period lasts for 5–8 min, depending on the individual animal. The highly synchronized EEG activity showing spindles even in the different subcortical structures (septum, midline thalamic nuclei, caudate nuclei) is suddenly turned into a desynchronized state as seen at the beginning. Any change in the environment which induces attentive behavior can block this periodicity and results in a desynchronized EEG activity of varying duration (Figs. 1 and 2).

The periodic changes in the cortical electrical activity raise the question whether or not the attentive reactivity of the animal can show parallel alterations. To answer this question we studied the habituation of a novel stimulus in the experimental circumstances mentioned above — usually the sound of a bell or a tone of 700 c/s was used — which was given for 8 sec repeatedly at 80 sec intervals. Approximately the first 20–30 trials resulted in an EEG and behavioral arousal lasting for 15–30 sec in each stage of resting EEG periodicity in all 12 cats used in these investigations. In a later phase of habituation the sound induced EEG arousal only in the first two-thirds of each period but not in the remaining highly synchronized one-third. In the later progressive phase of habituation the test stimulus failed to change the resting periodicity of EEG activity, and it frequently resulted in the facilitation of high amplitude slow waves. These findings show that attentive behavior cannot be regarded as the sole product of the extrinsic environment but that it has a characteristic intrinsic aspect which could be due to the periodic changes of facilitatory and inhibitory processes within the central nervous system.

Further experiments were conducted to study the role of certain basal forebrain structures in the organization of EEG and behavioral arousal, attentive behavior and learning processes. With the aid of a stereotaxic apparatus bilateral electrocoagulations were placed in the diencephalon and striopallidal region under Nembutal anesthesia. The EEG arousal induced by various environmental stimuli was compared in operated and in shamoperated animals in the postoperative two weeks. All details of methods used for recordings of electrical activity and stimulation of the subcortical structures have been described in our earlier paper (Endröczi *et al.*, 1963).

Bilateral lesions in the basal septum and/or in the anterolateral hypothalamus destroying the preoptic area and the medial forebrain bundle resulted in a lack of EEG response to different environmental stimuli. The cortical EEG records of such animals displayed high voltage slow waves often accompanied by a state of hypersomnolence. The whole picture of such lesioned animals was highly characteristic of cats bearing extensive lesions in the brain stem reticular core or in the midline thalamic nuclei (Lindsley *et al.*, 1949). A similar impairment of EEG and behavioral arousal was observed when the lesions destroyed the ventromedial part of the globus pallidus including the inferior peduncle of the thalamus. In contrast with the lesions mentioned above, bilateral electrocoagulations in the rostral septum including the descending fornices, in the amygdaloid complex of nuclei and lesions damaging the ventrolateral part of the thalamus and/or the greater part of the caudate nuclei failed to impair EEG arousal and attentive behavior (Figs. 3 and 4).

Fig. 3 shows the sites of the lesions which produced heavy impairment of EEG arousal and attentive behavior as well as lack of the functions of self-preservation.

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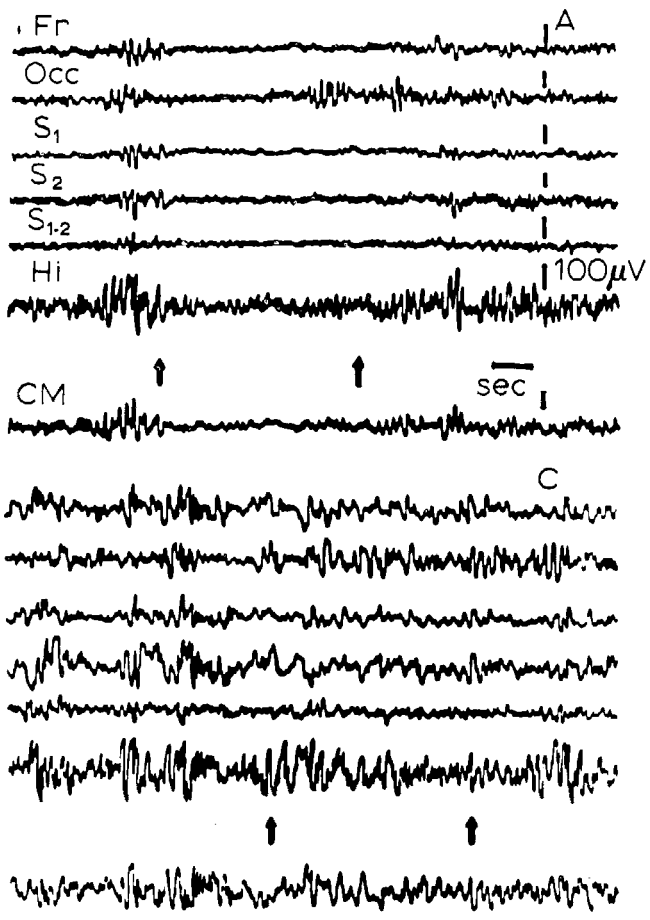

Because of the impaired food intake, such animals had a short survival time, approximately a week. Some of the animals walked around the cage without avoiding any harmful situation, although they did not show any impairment of coordinated locomotor activity. The general view concerning these behavioral changes is that the animal having effective lesions in the basal forebrain structures cannot decode the biological meaning of environmental stimuli, in other words the EEG arousal and attentive behavior involve the whole complexity of the meso-diencephalic activating system. In addition to the classic nonspecific diffuse activating system of the brain stem the foregoing experimental results suggest that certain basal forebrain structures also play an important role in these events.

Fig. 5 shows a schematic representation of neuroanatomical structures playing a role in the nonspecific activation of the cortex caused by environmental stimuli. Afferent impulses coming through specific pathways induce an excitatory state of the meso-diencephalic activating circuit, the frame of which is formed by the mesencephalic reticular formation, the thalamic midline nuclei, the striopallidal connections *via* the inferior peduncle of the thalamic basal septal area including Broca's diagonal band,

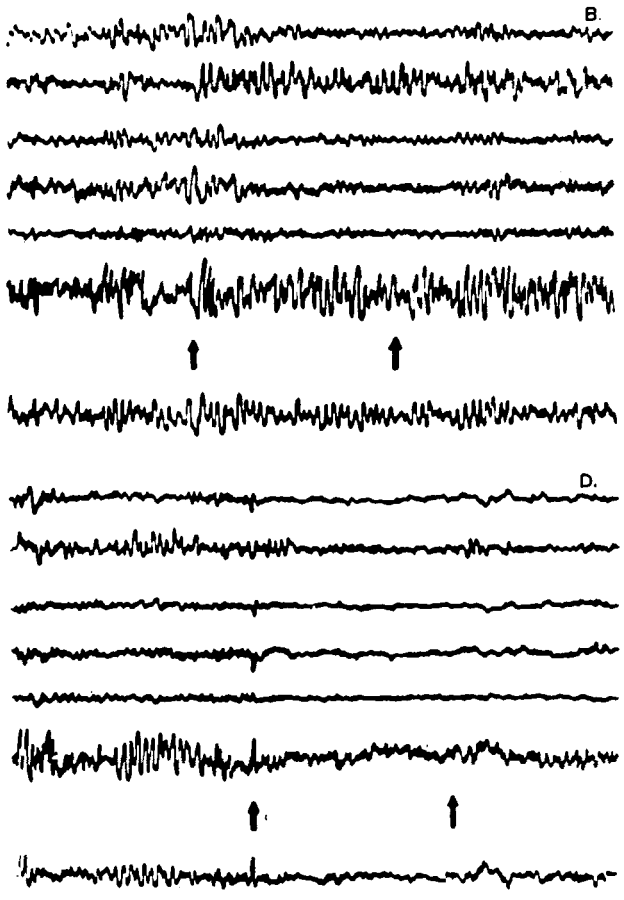

Fig. 2. The effect of a tone of 700 c/s on the EEG activity in the different stages of a resting EEG period in the midstage of habituation. Test stimulus was given between the arrows in consecutive trials.

the medial forebrain bundle, and finally as the starting point as well as the closure of the activating circuit, again the brain stem reticular core. The ample connections between such an assumed activating circuit and the limbic structures make possible extensive facilitatory and inhibitory influences in both directions. On the other hand, there is no doubt that the neocortex can inhibit or facilitate this meso-diencephalic activating system through its diffuse corticofugal and corticopetal projections. The excitatory state of the meso-diencephalic activating circuit assumed by us has its most rostral closure morphologically at the level of the basal septal region, although a great number of posterior closures within this circuit predispose it to discrete participation in the integration of a large scale of somatic and visceral processes as well as to the development and maintenance of cortical arousal. The participation of basal forebrain structures in the maintenance of consciousness and attentive behavior has also been reported in human subjects with lesions or tumors in the basal septum (Scoville, 1957; Jefferson, 1958). The absence of any damage to the brain stem reticular core in these

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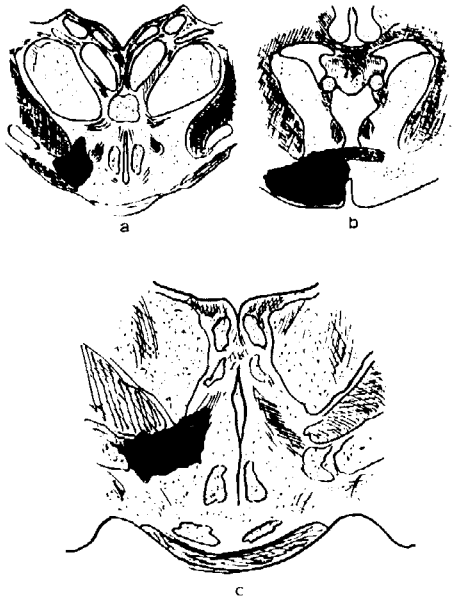

Fig. 3. The situations of lesions destroying the basal forebrain structures in cats. Half-side schematical reconstruction of the bilateral lesions in the medial forebrain bundle (a), in the basal septal region (b) and in the subthalamus (c) destroying the inferior peduncle of the thalamus and the medial part of the globus pallidus.

cases suggests the role of basal forebrain mechanisms in the development of the conscious state in the human too.

The assumption that cortical EEG arousal requires the excitatory state of the whole meso-diencephalic circuit, involving rostral forebrain areas, has been confirmed by us in further experiments with stimulation of the mesencephalic reticular formation in cats bearing extensive lesions in the basal septum and medial forebrain bundle. Stimulation of the reticular formation (5–15 V, 0.5 msec, 60 H) failed to induce EEG or behavioral arousal reactions in lesioned cats, although the animals exhibited some somatic manifestations during the electrical stimulation. This observation shows that interruption of the meso-diencephalic activating circuit, even at other points than the brain stem reticular core, prevents the development of an excitatory state which is fundamental to the integration of EEG and behavioral arousal of the neocortex and archicortical structures.

It is well known that macroelectrode records of cortical electrical activity give no information about many features of brain function. Thus, it was observed that in a habituated state where the test stimulus (sound) did not change the resting activity, the administration of a differential tone resulted not only in temporary EEG arousal but produced a deshabituation of the test stimulus lasting throughout several trials, in spite of resting EEG activity during the intervals. Similar deshabituation of a previously habituated sound stimulus was obseved in response to electrical stimulation of the reticular formation for 15 sec, which elicited a moderate temporary desynchronization of the cortical EEG activity. Such findings show that the subcortical

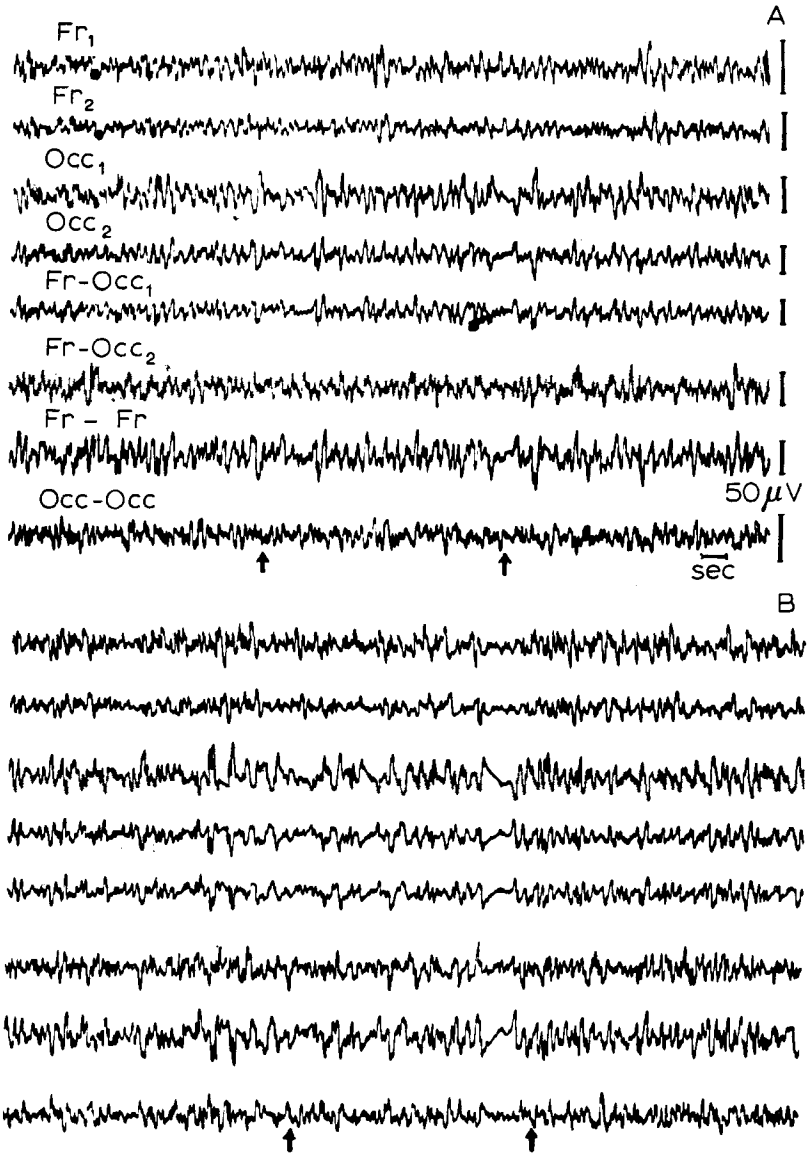

Fig. 4. The lack of EEG arousal reaction in cat lesioned in the medial forebrain bundle. The figure shows the ineffectiveness of a strong sound (A) and of a painful stimulation of the hindlegs (B). Stimuli were given between the arrows.

structures have the property of maintaining latent excitatory states caused by environmental stimuli or electrical stimulation of the brain stem reticular core, without any obvious manifestation in the resting cortical EEG activity in the poststimulatory period of 5–10 min duration. Such latent excitatory state may also be considered as due to postsynaptic excitatory processes within the cortex, although the macro-

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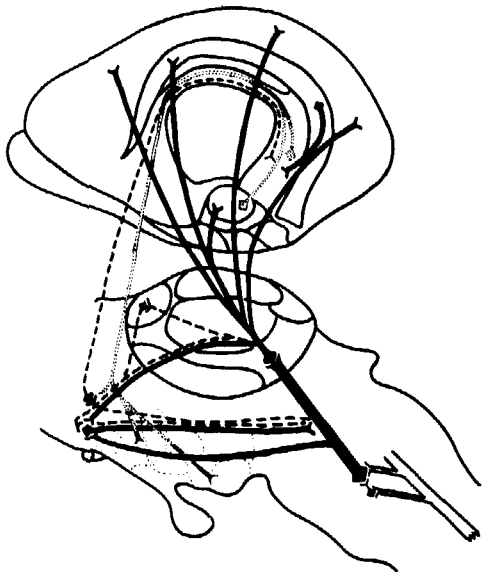

Fig. 5. Schematic representation of neuroanatomical structures playing a role in the 'non-specific' activation of cortex. (Details in the text.)

electrode recordings used in these investigations did not make the discovery of such changes possible (Fig. 6).

Concerning the general functions of the meso-diencephalic activating system in the integration of EEG arousal and attentive behavior, it is understandable that the interruption of this activating system, even at other points than the brain stem reticular formation, results in a complete loss of learned processes. Thus, the avoiding conditioned reflex response, in rats bearing bilateral lesions in the basal septum or in the medial forebrain bundle as well as in the striopallidal region, was completely abolished. The animals failed to show any response to the conditional stimulus, and reacted to the painful electric shock used as unconditional signal with nongoal-directed somatic reactions, but did not perform the previously established conditioned response. The lack of conditioned reflex activity as well as the failure of food intake and other self-preservative functions in such operated cats and rats may be regarded as the consequence of the inability to decode and transform into adequate reactions the meaning of environmental stimuli.

DRIVE-REDUCTION. DISCRIMINATIVE INTERNAL INHIBITION AND CONDITIONED REFLEX BEHAVIOR. THE ROLE OF SOMATOMOTOR MEMORY IN LEARNING PROCESSES

In alimentary conditioned reflex experiments the cats were trained to jump on to a bench 1½ feet high to obtain a piece of meat when a light was switched on for 10 sec above the feeder. The experimental compartment contained three similar feeders, spaced at a distance of 25 cm, each feeder having a light signal of its own. All details

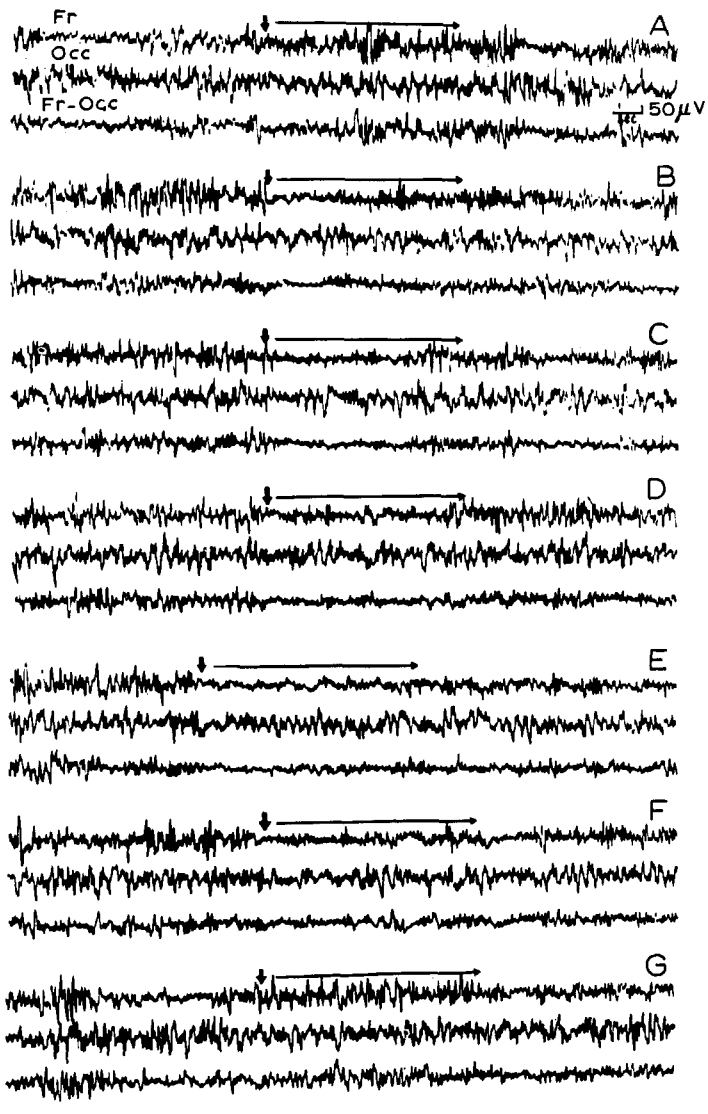

Fig. 6. The deshabituating effect of a differential tone given between the administration of two test stimuli (in the interval between A and B records). Deshabituation lasted for five consecutively given test stimuli employed at 80 sec intervals.

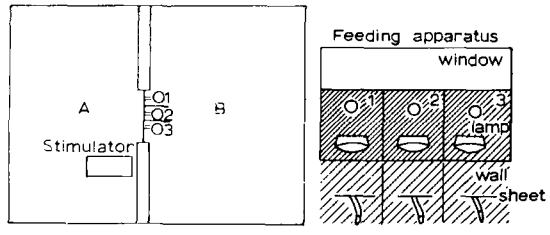

Fig. 7. Arrangement of the compartment used for the establishment of alimentary conditioned reflex in cats. A, observation room; B contained three equally equipped feeders shown on the right.

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of methods used in this investigation have been described elsewhere (Endröczi and Lissák, 1962) (Fig. 7).

The first moment in the learning process was closely related to memorization of the somatomotor pattern. After 8–12 trials, the animals had learned to jump on to the bench, although the conditional signal only triggered off the somatomotor reaction and did not result in discriminative space orientation. After the cats had been trained by several trials at feeder 1, and then a positive signal was given at feeder 3, they ran to feeder 1. This observation shows that the positive signal only served as an inductive factor of somatomotor response but had nothing to do with space discrimination. The administration of a positive signal above feeder 3 induced the cat to run to the original feeder 1 — where it had been originally slightly trained — and only afterwards did look for the food in feeders 2 and 3. The administration of at least 40–50 trials was necessary to establish the conditioned reaction straight to feeder 3, which is many times more than was required for the conditioned reaction at the first feeder. The fixation of the route and the environmental situation respectively to the conditioned reflex situation may be considered as the first moment of conditioning. In the first stage of conditioning the signal only triggers off the somatomotor pattern. The imprinting of the somatomotor pattern is a very rapid and permanent one and could practically never be removed but only inhibited during the extinction. This problem was studied in rats in the avoiding conditioned reflex situation where the animals jumped on to the bench to avoid a painful electric shock given through the grill of the floor. A bell sound of 10 sec duration was used as a positive signal, and the electric shock of short duration was given in the last 3 sec (Figs. 8, 9). Methodological

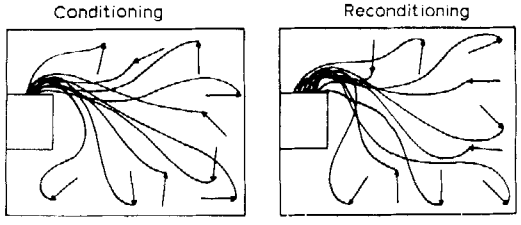

Fig. 8. Routes of a rat in the course of the first and the second conditioned reflex performance. Reconditioning means a new series of reinforcements following the extinction of previously established temporary connections.

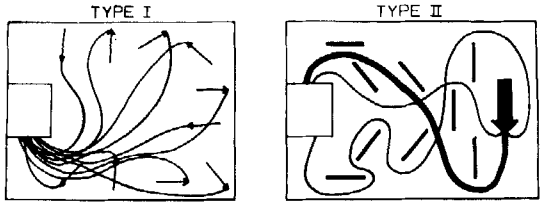

Fig. 9. Routes of a rat approaching the bench in the course of avoiding conditioned reflex activity in a nonmaze (type I) and the maze jumping box (type II). The arrows show the placing of the animal during the intertrial intervals. In type II experimental circumstances the rat followed the routes shown by thin lines at the first two approaches to the bench, but later it followed the route of the thick line in the course of a hundred more trials.

details of these observations have been described elsewhere (Korányi *et al.*, 1963). The rats were put in a particular place in the box and the route they ran was recorded during each trial. It was found that each animal followed the route it had run during the first conditioned reactions even in the time of highly stabilized conditioned reflex activity. Each animal approached the bench on its own route, even after it had been put in different places in the box, far from the original starting point. A week after the extinction of the conditioned reflex caused by the administration of nonreinforced trials the animals were reconditioned and their new somatomotor patterns accompanying the conditioned reflex performance were registered. Except for a very slight modification with some of the animals there was no difference between the route patterns of the first and the second conditioned reflex activity. This kind of experiment shows that somatomotor learning is not only the first moment of conditioning, but also that the acquired somatomotor pattern persists after complete extinction of the conditioned reflex response. The so-called 'extinction' used widely in the study of pavlovian physiology and recently in experimental psychology may be regarded as a technical word which does not mean anything but inhibition of a temporary connection. The observations described above show that the somatomotor component of a conditioned reflex becomes imprinted on the brain mechanisms so strongly that it cannot be extinguished by a short lasting experience of ontogenetic life. Such an assumption of the primary fixation of a pattern in the motor behavior shows a close relation with the 'incidental memory' and it is in good agreement with Pavlov's original concept of the nature of the conditioned reflex: 'When a conditioned reflex has been established it cannot be removed'.

There is a feature common to all kinds of conditioned reflex activity manifestating itself in locomotor performance, namely, that the animals show a number of spontaneous goal-directed motor reactions in the intersignal period. In alimentary conditioned reflex experiments on cats we have studied the characteristics of spontaneous goal-directed motor reactions and have come to the conclusion that such a motor activity corresponds to the somatic manifestation of a driving force in a given conditioned reflex situation. The number of spontaneous goal-directed reactions in the early stage of conditioning is fairly high and is slowly reduced in the course of stabilization. Without going into the details of spontaneous goal-directed motor activity described in detail elsewhere (Endröczi and Lissák, 1961; Endröczi, 1963), we would like to summarize our findings as follows.

(1) Spontaneous goal-directed motor activity may be considered as a somatic component of the drive, and plays an important role in the fixation of the somatomotor pattern associated with the conditioned reflex reaction.

(2) During the stabilization of a conditioned reflex the number of spontaneous motor responses decreases. Such a drive reduction may be regarded as due to the function of discriminative internal inhibition. In the absence of a differential signal the number of responses failed to drop to zero in our experimental conditions in cats, but the introduction of a differential signal, requiring a higher intensity of discriminative internal inhibitory processes, resulted in complete abolition of spontaneous goal-directed motor responses.

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(3) During the extinction of conditioned reflex responses caused by nonreinforced trials, the resting level of spontaneous motor reactions will be decreased much earlier than conditioned reflex responses have been suppressed. The reduction of spontaneous goal-directed motor activity during extinction suggests that internal inhibition takes effect primarily by the final suppression of drive, and is followed by the elimination of conditioned reflex performance (Fig. 10).

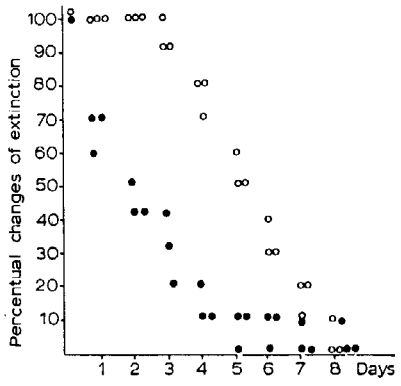

Fig. 10. Representation of the preceding extinction of spontaneous goal-directed motor activity during the extinction of an alimentary conditioned reflex activity in cats. Filled circles correspond to the spontaneous motor reactions, open circles show the conditioned reflex responses. The figure demonstrates the extinction values of three cats.

When dealing with the neuroanatomical basis of spontaneous goal-directed motor activity, the different parts of the mesencephalic and diencephalic structures were stimulated in alimentary conditioned reflex experiments with cats. Bipolar electrical stimulations with the appropriate parameters (0.2–0.5 V, 0.5 msec, 13–30 H), which usually induced moderate but obvious changes in attentive behavior or orienting reactions, elicited the inhibition or facilitation of spontaneous goal-directed motor activity, according to the placing of electrodes, without any impairment of the conditioned reflex performance. Stimulation of the septum, the anterolateral part of the hypothalamus including the medial forebrain bundle, resulted in depression of spontaneous motor responses, whereas stimulation of the posterior part of the hypothalamus, the ventral tegmental region and the midline thalamic nuclei, produced an increase in spontaneous motor responses. Such facilitation was observed even during extinction, in a period when the positive signal failed to elicit conditioned responses in a high percentage of tests. The electrical stimulations of positive points for 15–30 sec not only increased the spontaneous intersignal activity but also restored the effectiveness of the positive signal in inducing conditioned reactions throughout several trials (Fig. 11).

The depression of spontaneous goal-directed motor activity by stimulation of the rostral structures in the forebrain raised the question of the physiological meaning of this phenomenon. It is well-known from the literature that stimulation at rather low frequency of some of the diencephalic structures, including the rostral forebrain

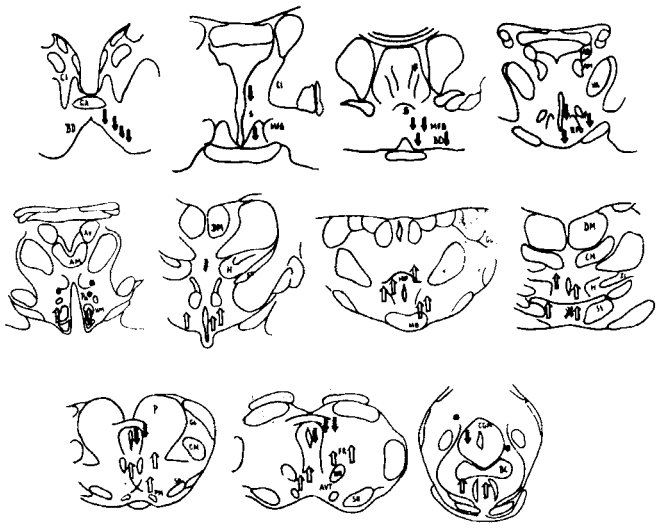

Fig. 11. Schematic diagram of electrode placements in the diencephalon and mesencephalon of the cat. The arrows show the depressive or eliciting effects of electrical stimulations on the spontaneous goal-directed motor activity. Black points show bi-directional responses.

area, results in a sleep-like condition (Hess, 1954; Clemente *et al.*, 1964; Hernández-Peón, 1962).

From the results obtained in the present experiments with conditioned reflex behavior and spontaneous goal-directed motor activity, we came to the conclusion that virtually different behavioral phenomena elicited by stimulation of some of the rostral forebrain structures might be due to the different manifestations of internal inhibition. It was found that habituation of a novel sound stimulus in soundproof circumstances was significantly enhanced by simultaneous stimulation of the basal septum or the medial forebrain bundle of the lateral hypothalamic area. However, when the stimulus had become ineffective in inducing EEG arousal the administration of a differential sound, though much weaker in intensity, did result in normal EEG and behavioral arousal. In contrast to this finding it was found that stimulation of the rostral forebrain structures following habituation to the test stimulus induced a temporary deshabituation lasting for several trials given at intervals of 80 sec. Such contradiction between the two types of experiment may be explained by the finding that stimulation in a habituated period results in EEG arousal and an increase in the discriminative internal inhibitory processes. Simultaneous and repetitive stimulation of the rostral structures of the forebrain with the administration of a test stimulus will enhance habituation by increasing the intensity of the discriminative capacity. The effect of a subcortical stimulation is highly dependent on the actual situation: the drowsy, sleepy state of the cats caused by stimulation of some of the rostral structures of the forebrain should be regarded as due to enhanced habituation, as a consequence of increased internal inhibitory processes. On the other hand in conditioned reflex experiments, stimulation of the rostral area of the forebrain resulted in a decrease in spontaneous intersignal goal-directed motor activity, which was due to the

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facilitation of discriminative internal inhibition producing drive reduction without impairment of the conditioned reflex activity. These experimental findings have led us to the conclusion that drive reduction and discriminative internal inhibition during the early phase of learning take place as one and the same function on a common neuroanatomical basis. The structures playing a role in these events overlap the meso-diencephalic activating system, and, on the basis of abundant experimental evidence, it may be assumed that the integration of driving forces as well as the spreading of internal inhibitory processes occur within this complex neuroanatomical unit.

SUMMARY

Some new functional and neuroanatomic features of the nonspecific diffuse activating system described by Moruzzi and Magoun have been observed in cats bearing chronically implanted electrodes and lesions. Disconnection of the meso-diencephalic activating system in different regions by means of electrocoagulation (*e.g.* lesions of the nonspecific thalamic nuclei, the inferior thalamic peduncle, the striopallidal system, the basal septal area, the medial forebrain bundle) showed that the integration of EEG and behavioral arousal by environmental stimuli through the mesencephalic ascending diffuse activating system requires the presence of the diencephalic structures mentioned above. Stimulation of the basal septal area, the medial forebrain bundle and the thalamostriopallidal connections with different parameters induced enhanced habituation to novel stimuli and increased discriminative activity in a conditioned reflex situation in waking cats. These events fit well into the concept of the meso-diencephalic activating circuit (mesencephalic reticular formation → thalamic non-specific nuclei → thalamostriopallidal connections → basal septal area → medial forebrain bundle → tegmentum) which plays a basic role in the integration of attentive behavior, in orienting reactions and in discrimination of situation signals (decoding function), and also forms the neuroanatomical basis of the driving force of motivation. The ample reciprocal connections with the rhinencephalic structures ensure facilitatory as well as inhibitory influences on the meso-diencephalic activating system.

Bilateral electrocoagulation in various links of the meso-diencephalic activating circuit blocked conditioned activity and the basic functions of self-preservation. Analysis of the neuroanatomical basis of the behavioral manifestations has led us to the conclusion that the loss of self-preservative functions and conditioned activity in learned and unlearned functions following an interruption of the meso-diencephalic activating circuit may be due to an inability to decode environmental signals rather than to impairment of the neural elements playing a role in the specific integration of processes of self-preservation. The meso-diencephalic activating circuit forms an inseparable unity with the cortex at a level where integrative closure of all behavioral reactions takes place, and corticofugal inhibitory and facilitatory influences can radically modify its excitatory state.

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Cortical Modulation of Transmission of the Afferent Volley through the Lateral Geniculate Body

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The discovery of the brain-stem reticular formation (Moruzzi and Magoun, 1949) helped to establish the important fact that sensory stimulation may cause a confrontation of 2 afferent sources in the brain cortex, *i.e.* a direct volley transmitted along the specific pathway, and unspecific influences from the reticular formation. There is some ground for considering the latter as the result of integration of the information arriving into the organism along all the sensory channels. Thus, while a specific volley brings the information on the quality of a given stimulus, impulsation from the reticular formation 'informs' on the correlation of this stimulus with other stimuli and with the general state of the organism.

On the other hand, the reticular formation, by means of centrifugal connections, influences the transmission of the afferent volley along specific pathways (Hernández-Peón, 1961). Activation of the reticular formation reduces the amplitude of evoked potentials in all the links of the specific afferent pathways (Hernández-Peón, 1955).

However, it still remains to be ascertained whether unspecific structures of the brain can effect a selective facilitation of the transmission in one of the analyzers, while blocking the rest. When the diffuse nature of unspecific influences is taken into consideration it appears highly doubtful. The whole concept of Hernández-Peón about the purely reticular mechanism of the control on the transmission of the afferent volley, independently of the cortex, evokes doubts. As is known, not only is the cortex subject to influences from the reticular formation, but, in its turn, it can influence the latter's activity (Bonvallet, 1959), thus controlling indirectly, through the reticular formation, the transmission of the afferent volley.

A certain role in the regulation of the afferent volley is played by the cortico-thalamic system which connects the cortical projection areas with corresponding specific nuclei of the thalamus.

This system has not been much dwelt on in the surveys concerned with centrifugal influences (Granit, 1955; Livingston, 1959; Narikashvili, 1962), and is not mentioned in the scheme of control of the afferent volley introduced by Hernández-Peón (1961). At the same time the cortico-thalamic feedback system localized within the bounds of the specific projectional pathways is of interest for comprehension of neurophysiologi-

cal mechanisms of regulation of the ascending inflow of sensory information, being capable of exerting a simultaneous but differentiated influence on different sensory systems.

There is no unanimous opinion on the role of corticofugal connections from cortical projection areas to specific nuclei of the thalamus. Some authors deny the existence of such connections at all (Weiss and Fifková, 1961; Buser *et al.*, 1963), others ascribe to corticofugal influences an inhibitory nature (Head and Holmes, 1911; Narikashvili and Kadjaya, 1963), a third group supposes that they transmit excitatory influences (Dusser De Barenne and McCulloch, 1938; Niemer and Jimenez-Castellanos, 1950). In the opinion of still other authors, corticofugal influences may effect both an inhibitory and excitory function (Ogden, 1960; Iwama and Yamamoto, 1961; Widén and Ajmone-Marsan, 1961).

Neither is there any unanimous opinion on the localization of corticofugal neurons. Some observations show that they are localized in the first projection areas of the cortex (Shkolnik-Yarrosóv, 1958), according to other observations they are situated in the second areas (Nauta and Busher, 1954; Szentágothai, 1962).

Such a contradiction in the literature, and insufficient elucidation of the functional significance of corticothalamic connections and their role in the accomplishment of the main functions of the nervous system, make it desirable that this question be the subject of further investigation.

In the series of experiments carried out together with Drs. G. D. Smirnov, V. M. Okudjava, V. M. Fyodorov, O. I. Merkulova, G. I. Yermakova, V. P. Guseva and P. P. Gustson, certain aspects of the functional organization of the cortico-thalamic system of the visual analyzer were studied. The experiments were performed on rabbits, cats and frogs.

Modification of responses of the lateral geniculate body (LGB) caused by changes in the functional state of neurons of the visual cortex

To increase excitation of neurons of the visual cortex local application of strychnine or penicillin solutions of different concentrations were used. For suppression of cortical activity KCl was applied, which causes a spreading depression (SD).

A brief application of weak solutions of strychnine (0.1–0.25%) to the visual cortex of a rabbit brings about facilitation of the primary responses both in the cortex and the LGB to a flash (Fig. 1A). Thereupon a certain parallelism is recorded in the degree of facilitation of responses for these 2 parts of the visual analyzer, *i.e.* the bigger the amplitude of cortical responses, the bigger the amplitude of LGB responses. Application of stronger concentrations of strychnine (0.5–1%) to the visual cortex of the rabbit does not evoke any changes in LGB primary response; however, it facilitates later components of the evoked potential.

Fig. 1B, 1 illustrates a full multi-component LGB response. The average frequency for emergence of different components in LGB response is demonstrated in Table I (Meschersky *et al.*, 1963).

Fig. 1B and Table I show that heavy strychninization of the visual cortex facilitates

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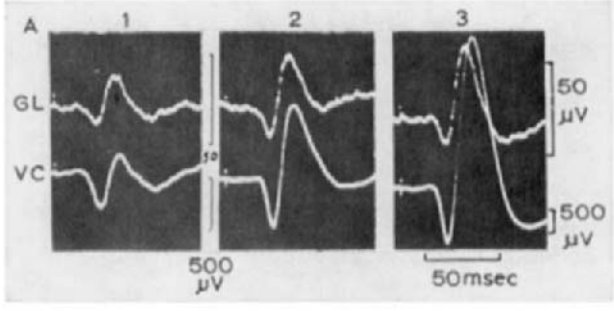
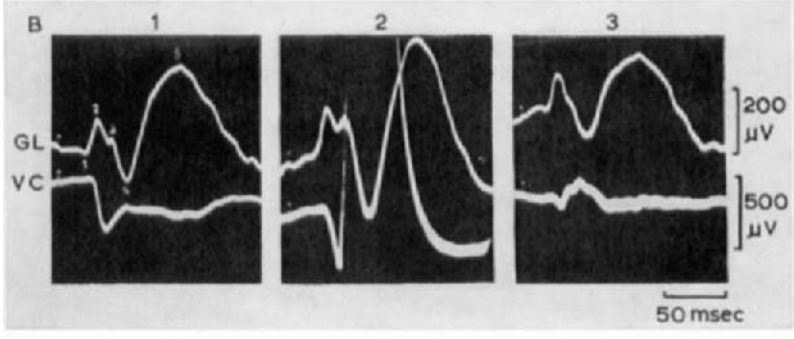
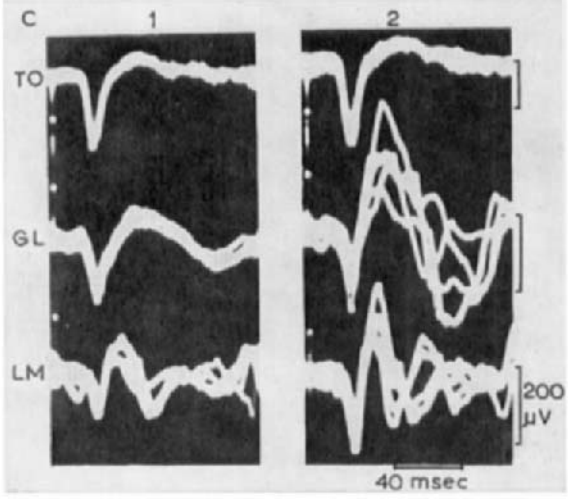
and increases the frequency of emergence of late positive (*4*) and negative (*5*) components of the LGB response. These changes are statistically significant.

Fig. 2 gives a graph of changes (average for 3 experiments) in latency and amplitude of components of the LGB response under strong strychninization of the visual cortex. Incomplete restoration of the initial level of the LGB response after strychnine had been washed away may be accounted for by partial diffusion of strychnine into the thickness of the cortex. It is also confirmed by the increased amplitude of the negative phase of cortical primary responses to photic stimuli. Subsequent application of a 1 %







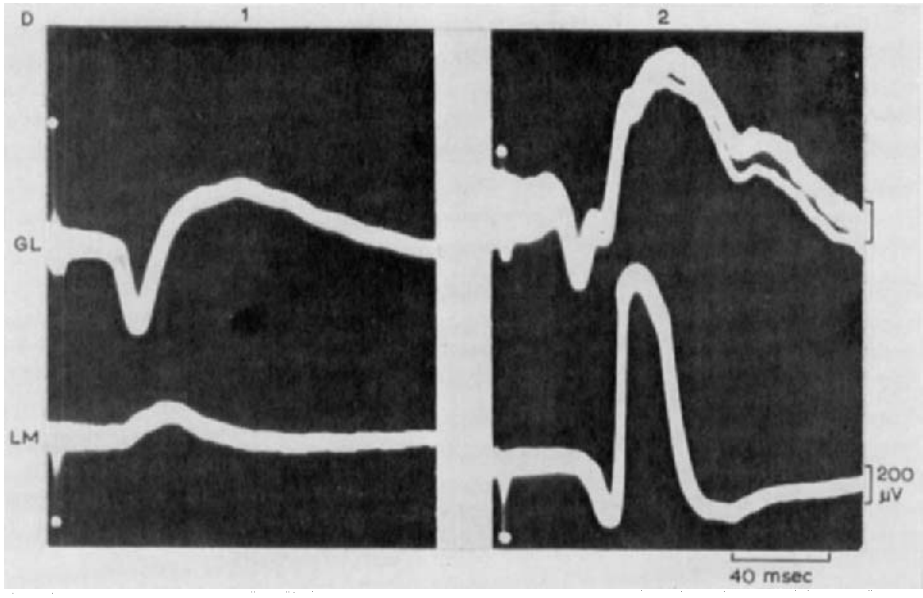


Fig. 1. Changes in LGB responses on application of strychnine to the visual cortex. A, The effect on LGB responses of weak strychninization of the visual cortex of rabbit. 1, Control; 2–3, after application of 0.25% strychnine solution. Rabbit under diplacine. B, The effect on LGB responses of intensive strychninization of the visual cortex of the rabbit. 1, Control; 2, after application of 1% strychnine solution; 3, destrychninized cortex. Rabbit under diplacine (Meschersky *et al.*, 1963). C, Facilitation of LGB responses of cat upon strychninization of the first visual area. 1, Control; 2, after application of strychnine on to LM (0.5%, 15 sec). Superposition of 5 responses. Nembutal, 30 mg/kg. D, Persistence of corticofugal facilitation of LGB responses in cat after coagulation of the second visual cortex. 1, Control, gyrus suprasylvius is coagulated; 2, after application of strychnine (1%, 10 sec) on LM. Encéphale isolé under nembutal, 30 mg/kg. GL, lateral geniculate body; VC, visual cortex (rabbit); TO, optic tract; LM, middle part of gyrus lateralis (cat). Artifacts of photic stimulation are marked by dots. Calibration: msec, μV.

TABLE I

FREQUENCY OF EMERGENCE (IN %) OF DIFFERENT COMPONENTS OF LGB RESPONSE TO A FLASH BEFORE AND AFTER STRYCHNINIZATION OF THE VISUAL CORTEX OF THE RABBIT

(Numeration of the components of LGB response is given in accordance with Fig. 1B, 1).

	Components of LGB response				
	1	*2*	*3*	*4*	*5*
Control	36	100	87	33	18
After strychninization	34	100	53	86	42

solution of γ-amino-butyric acid (GABA) to the visual cortex suppresses the negative component of the cortical response and restores LGB responses to their initial level.

Somewhat different results were obtained in our experiments on cats. Here facilitation of LGB responses was as well recorded after application of strong concentrations of strychnine to the visual cortex.

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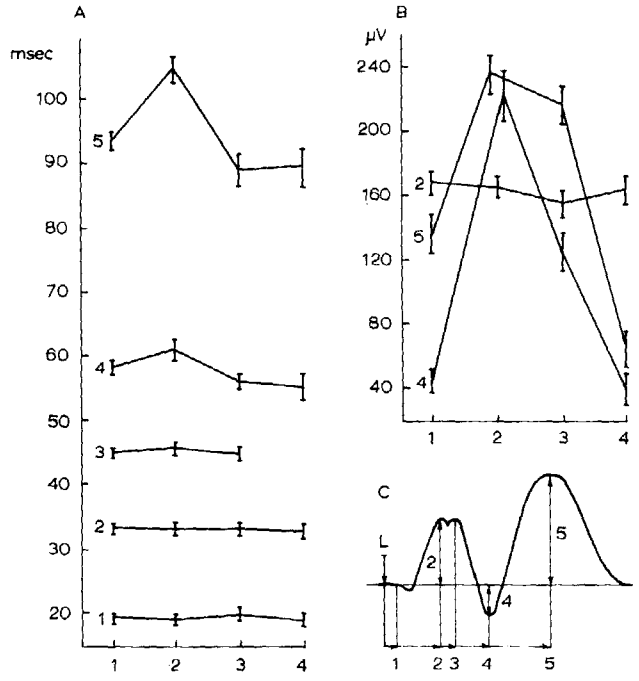
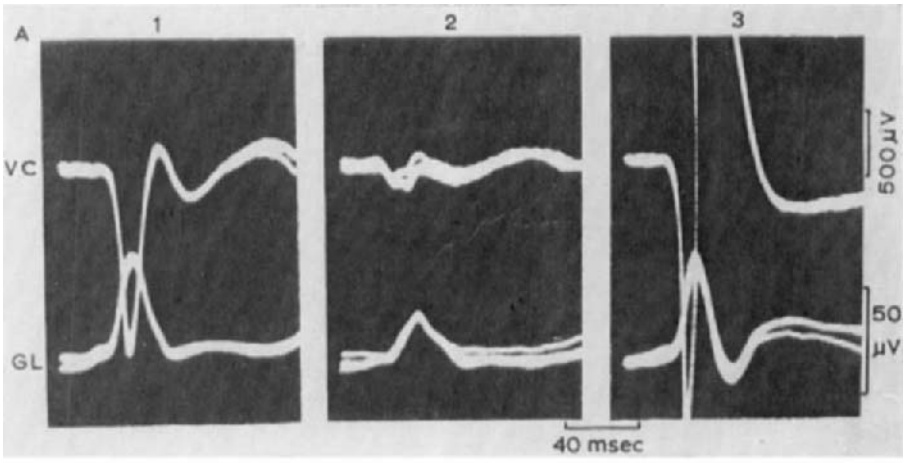


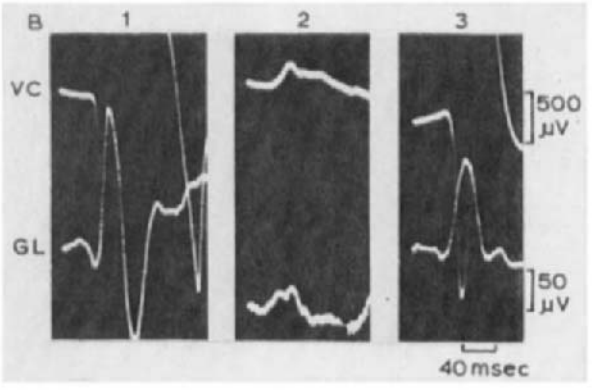
Fig. 2. Modification of the components of LGB responses of rabbit upon cortical application of 1% strychnine solution and GABA. A, Time parameters; B, amplitude parameters; C, the scheme for measuring the components of LGB responses. On the ordinate: A, time (in msec); B, amplitude (in μV). On the abscissa: 1, control; 2, after strychninization of the visual cortex; 3, after destrychninization; 4, after application of GABA. Vertical lines on A and B, R_1M_1S-errors. L (on C), moment of photic stimulation.

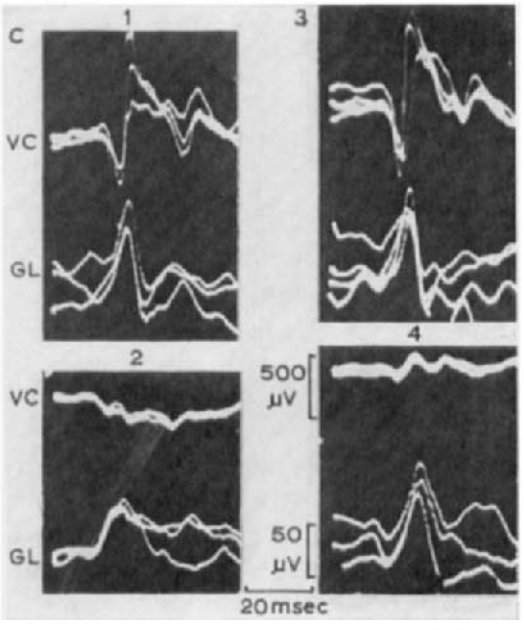
Fig. 1C demonstrates the responses from the optic tract, the LGB and the middle part of the gyrus lateralis before and after strychninization of the latter. Strychnine brought about faciliation of both cortical and LGB responses. At the same time the responses of the optic tract did not undergo any change, which excludes the participation of retinal processes in this phenomenon.

In cats with well-differentiated first and second visual cortices we studied the peculiarities of their corticofugal effects on the LGB. Coagulation or spreading depression of the second visual area do not interfere with the occurrence of corticofugal effects from the first visual area (Fig. 1D), but rather facilitate them. Activation of the second visual area brings forth no changes of LGB activity (Fig. 6C).

Fig. 3. Reduction of LGB responses during depression of the activity of visual cortex in rabbit. A, Modification of LGB responses upon application of KCl and strychnine to the visual cortex. 1, Control; 2, after application of KCl; 3, after application of 1% strychnine. Nembutal, 35 mg/kg; superpositions of 5 responses. B, Changes in LGB response after blocking of cortical convulsive activity with KCl. 1, Control; 1% strychnine applied on the visual cortex; 2, after application of KCl; 3, restoration of cortical and LGB responses. Nembutal, 35 mg/kg. C, Modification of LGB responses during spreading depression in ipsilateral and contralateral visual cortices. 1 and 3, Control; 2, after application of KCl to the ipsilateral visual cortex; 4, after application of KCl to the contralateral visual cortex. Calibrations: msec, μV.







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Application of KCl to the visual cortex of a rabbit, causing waves of spreading depression or a shutting-off of the cortical activity in some other way, as a rule, suppresses the LGB response (Fig. 3).

Application of KCl to the cortex produces temporary suppression of LGB responses which coincides in time with propagation of a wave of spreading depression through the visual cortex. A subsequent application of strychnine to the cortex completely restores the amplitude of the LGB primary response, and results in facilitation or appearance of the late positive wave, *i.e.* the 4th component (Fig. 3A, 3). Application of KCl on to the strychninized cortex blocks the LGB response (Fig. 3B). Thereupon the fourth component completely disappears and is restored much later than the primary response (Fig. 3B, 2 and 3).

Corticofugal influences from the visual cortex under strychnine and KCl manifest themselves both in cats and rabbits, but only in the ipsilateral LGB. Fig. 3C shows that depression of the visual cortex clearly blocks the response of the ipsilateral LGB and does not noticeably affect the response of the contralateral LGB. Application of strychnine to other cortical areas of the ipsilateral hemisphere does not affect the LGB either.

TABLE II

MODIFICATION OF THE LGB RESPONSE AFTER APPLICATION OF STRYCHNINE OR KCl TO THE FIRST OR SECOND VISUAL AREAS

Nature of influence	*No. of experiments*	*Mode of change*		
		Facilitation	*No changes*	*Inhibition*
Strychnine applied to the first visual area	13	7	4	2
Strychnine to the second area	7	1	3	3
KCl to the first visual area	7	—	1	6
KCl to the second area	7	3	3	1

In cats with the cortex treated with KCl, regular suppression of the LGB was observed only when KCl was applied to the first visual cortex (Fig. 4C). Application of KCl to the second visual area did not change the LGB responses (Fig. 4B). In some experiments, influences exerted on the second visual area entailed less pronounced changes in LGB responses, contrary in nature to those evoked by the same influence on the first visual area. It may be assumed that the influences from the second visual area upon LGB are indirect, being transmitted through the first visual area. Interrelations between the first and second visual areas are reciprocal.

Table II illustrates the changes in LGB responses in the cat under different influences on the visual cortex.

During pharmacological action on the ipsilateral visual cortex, changes of LGB responses occur both in waking animals (paralyzed with diplacine, encéphale isolé)

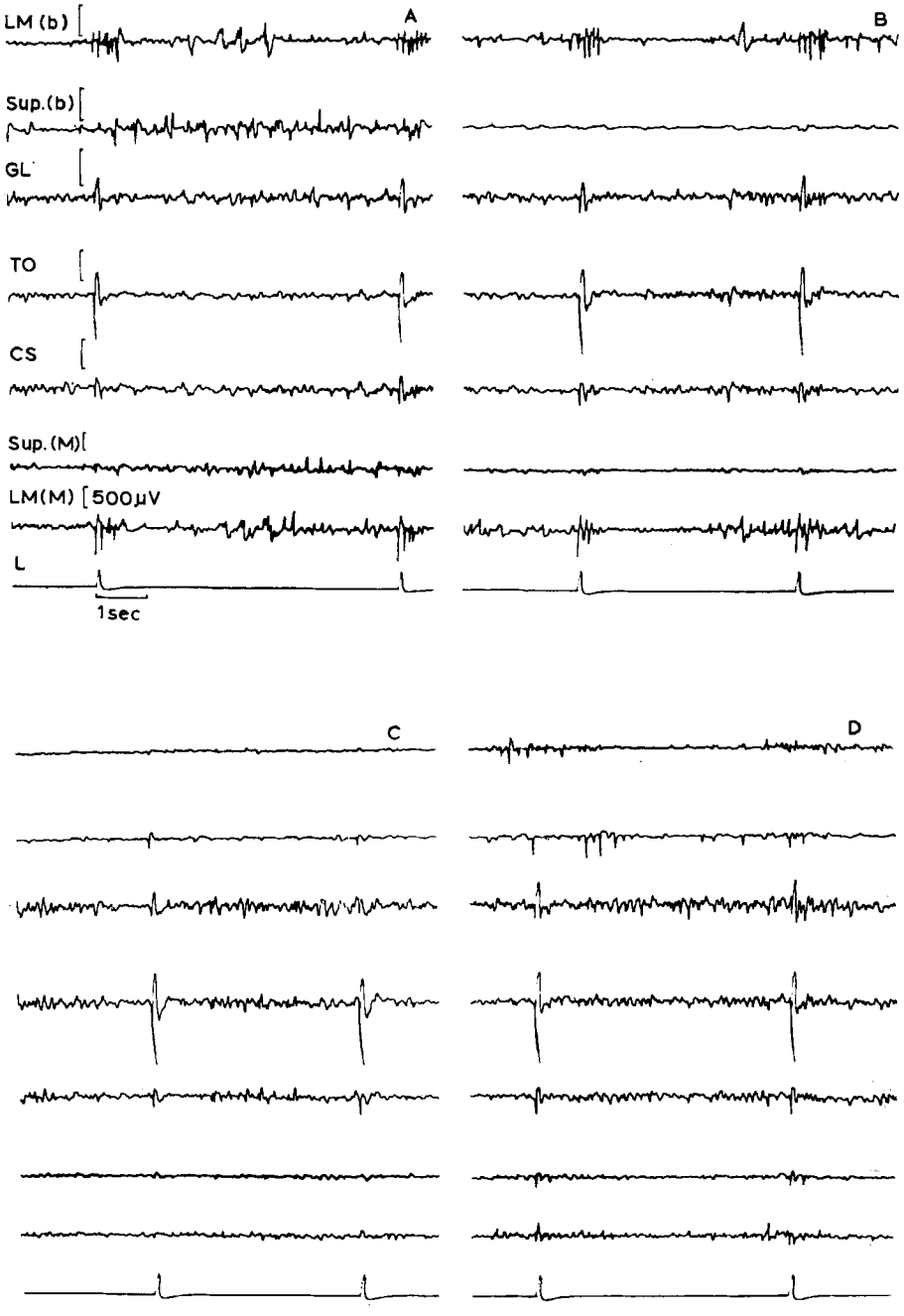

Fig. 4. Differentiated influence of the first and second visual areas of the cortex on LGB responses of cat. A, Control; B, after application of KCl to the second visual area; C, KCl washed out from the cortex; KCl applied to the first visual area; D, KCl washed out. Nembutal, 35 mg/kg. Sup, Gyrus suprasylvius; CS, Superior colliculus; (b), bipolar lead; (m), monopolar lead; L, photic stimulation mark. Other symbols see Fig. 1.

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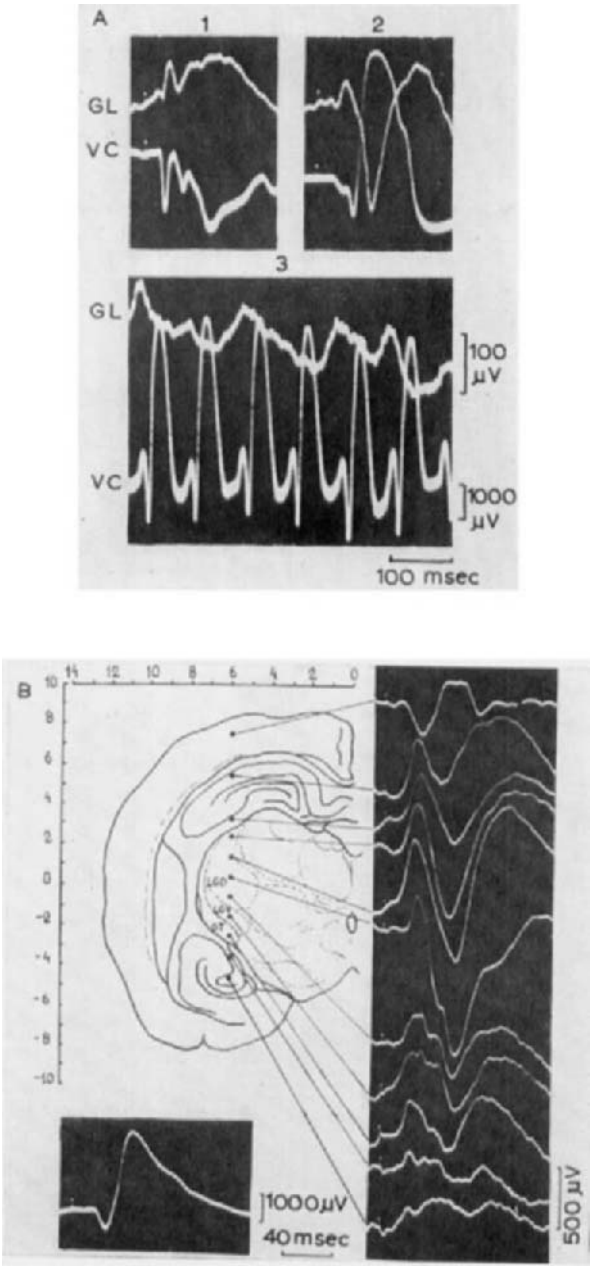

Fig. 5. Dependence of the fourth component of LGB response of rabbit evoked by photic stimulation on the state of cortical activity. A, Disappearance of the fourth component during high frequency strychnine discharge. 1, Control; 2, after application of 1% strychnine to the visual cortex; 3, during spontaneous convulsive discharge in the cortex. B, Localization of structures responsible for generation of the fourth component of LGB response. Recordings are obtained after application of 1% strychnine to the visual cortex. At the bottom to the left — cortical responses to photic stimulation. Co-ordinate net for the brain map is given in mm. LGD, dorsal nucleus; LGV, ventral nucleus; OT, optic tract. Calibrations: msec, μV.

and under deep nembutal narcosis. These results confirm the view that the transmission of corticofugal impulses goes past the reticular formation, since its influences upon the specific projection system are blocked by nembutal narcosis (Hernández-Peón, 1961).

Our control experiments showed that the fourth component of the LGB response (Fig. 1B), being so-to-say the mirror image of the strychnine spike in the cortex, and reaching, in a number of cases, a fairly great amplitude (up to 500 μV), is not an artifact caused by recording of high-amplitude cortical discharges by means of a subcortical electrode. This component appears fully after strychninization of the cortex, and is usually recorded as single or rare photic stimuli (Fig. 5A, 2).

Similarly, we recorded well pronounced LGB responses upon single spontaneous strychnine discharges in the visual cortex (Fig. 9A, 2; B, 2), their time relations with the strychnine cortical spike being the same as those of the fourth component. However, the fourth component completely disappears upon emergence of a high-frequency convulsive cortical discharge (Fig. 5A, 3). This evidence leads us to the conclusion that the fourth component is connected with transsynaptic transmission of impulses blocked by the high frequency discharges. With the help of subcortical electrodes inserted to gradually increasing depths it is possible to ascertain the area from which the fourth component is led off. It is situated in the LGB dorsal nucleus (Fig. 5B). This observation confirms the physiological nature of the fourth component.

LGB responses to transcallosal stimulation of the visual cortex and to its spontaneous convulsive discharges

Stimulation of the first visual cortex of the cat in the symmetrical point of the contralateral hemisphere produces a transcallosal response (Curtis, 1940), in our experiments with a latency of 2.5–3 msec. The transcallosal response of the cortex was sometimes followed by the LGB response (Fig. 6A), with a latency of about 4 msec. The difference of 1–1.5 msec points to the presence of 1 or 2 synapses on the way of the corticofugal volley.

More regular LGB responses to transcallosal stimulation occur after application of strychnine or penicillin to the cortex, which leads to a more generalized pattern of the appearance of transcallosal responses (Meschersky and Okudjava, 1963). Coagulation of the second visual cortex does not block LGB responses to transcallosal stimulation of the first visual cortex (Fig. 6B, 1). However, coagulation of the first visual cortex blocks them fully (Fig. 6B, 2). Transcallosal stimulation of the second visual cortex does not produce LGB responses (Fig. 6C, 1).

This is the evidence that LGB responses are conditioned by activation of the neurons of the ipsilateral first visual cortex, are not connected with the antidromic excitation of some ascending fibres in the contralateral hemisphere, and do not depend on the excitation of the brain reticular structures, which may be involved in the transmission of impulses to transcallosal cortical stimulation from one hemisphere to the other (Routledge and Kennedy, 1960).

On the appearance of strychnine spikes in the first visual cortex, we recorded LGB

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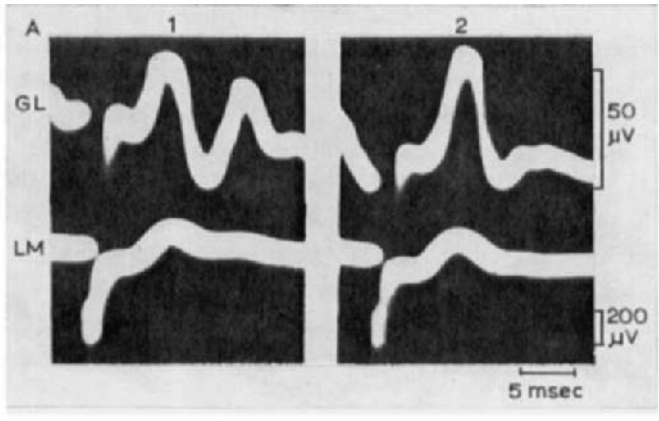
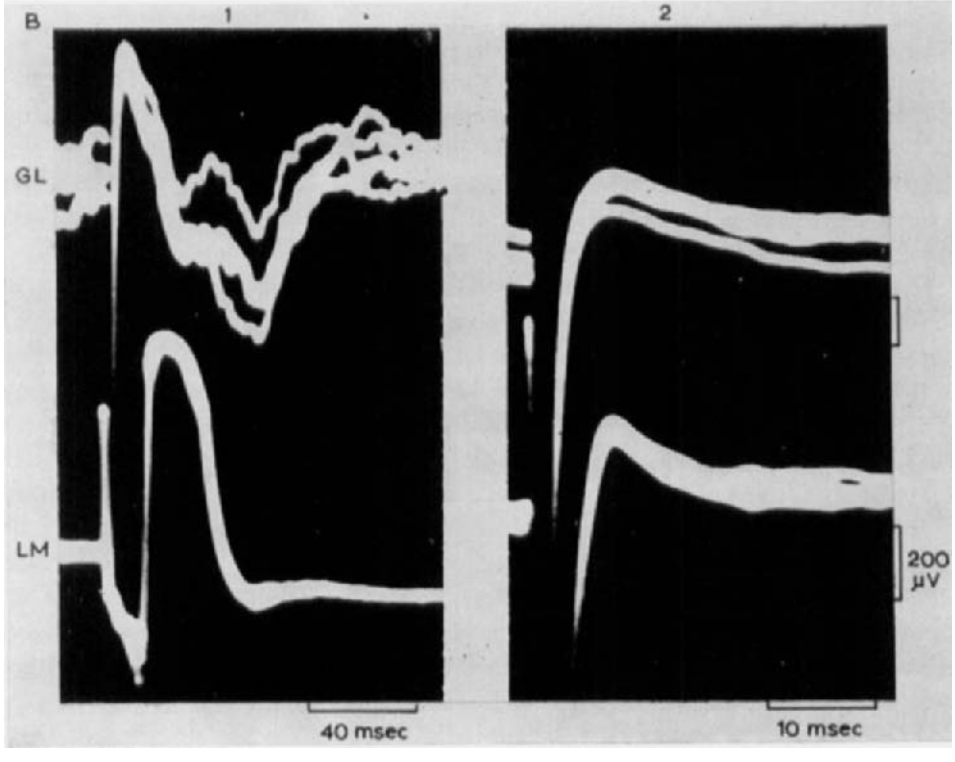
responses in the form of single (Fig. 7A), or sometimes multiple (Fig. 7B) discharges. LGB responses to spontaneous spikes resemble, to a great extent, LGB responses to transcallosal stimulation of the cortex. Strychnine discharges in the second visual cortex produce no effect in LGB (Fig. 6C, 3).

LGB responses to convulsive corticofugal neuronal discharges may have various latencies which presupposes, under these conditions, the existence of several multisynaptic pathways of transmission of corticofugal impulses.

Some of our experiments clearly showed the 'two-way' paths of transmission of





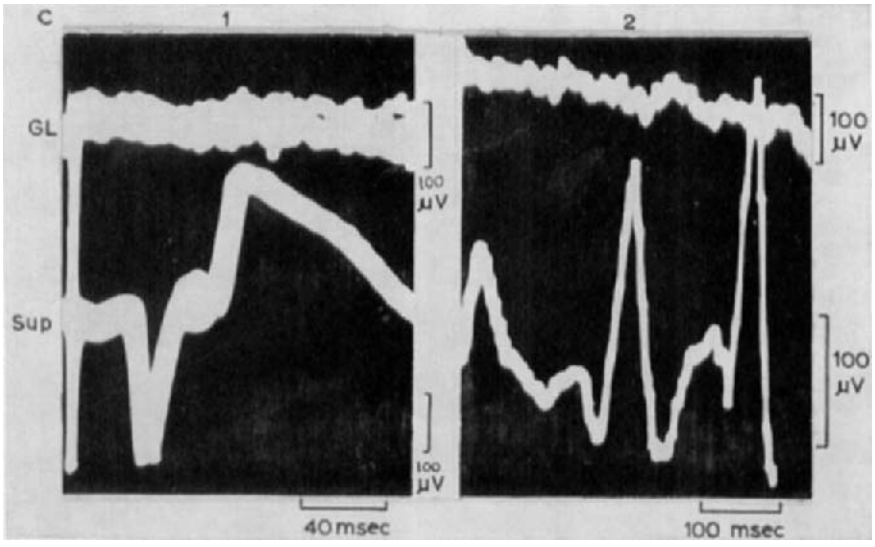


Fig. 6. Transcallosal responses of the visual cortex and of the LGB of cat. A, Transmission of transcallosal response from cortex to LGB. Two types of LGB response to transcallosal excitation of the cortex. Encéphale isolé. B, Disappearance of LGB responses to transcallosal stimulation of the visual cortex after coagulation of gyrus lateralis. 1, Control, 1% strychnine applied to the first visual area, the second visual area is coagulated; 2, after coagulation of the first visual area. Superpositions of 5 responses. Nembutal, 30 mg/kg. C, Absence of LGB responses during activation of the second visual area. 1, Transcallosal stimulation of the second visual area after application of penicillin to it; 2, spontaneous discharges in the second visual area. Nembutal 40 mg/kg.

impulses between the thalamic relay and the cortical projection area (Fig. 7C, D). The development of rhythmic convulsive activity in the visual cortex involves the LGB in the process. The dynamics of such changes of potentials is illustrated in Fig. 8: the character of LGB responses depends on the pattern and frequency of convulsive discharges in the cortex.

Corticofugal component of LGB response to a flash

It has already been said that the visual cortex may exert a corticofugal tonic facilitating or inhibitory influence, modulating the amplitude and altering the pattern of LGB evoked potentials. A synchronous discharge of cortical neurons is followed by a corticofugal volley and then by a postsynaptic LGB response.

The results cited allow us to assume the fourth component of the LGB response to photic stimulation of a rabbit to be, if not fully, at least in its greater part, not the element of the LGB response to an afferent volley arriving along the fibres of the optic tract, but the LGB response to a feedback discharge of corticofugal neurons (Fig. 1B; 9A, 1). LGB responses to spontaneous strychnine spikes in the cortex resemble in their pattern and latency the fourth component of the LGB response to photic stimulation (Fig. 9A). Analogous results were obtained in the experiments on cats. Fig. 9B gives the comparison of LGB evoked potentials to photic stimulation and to strych-

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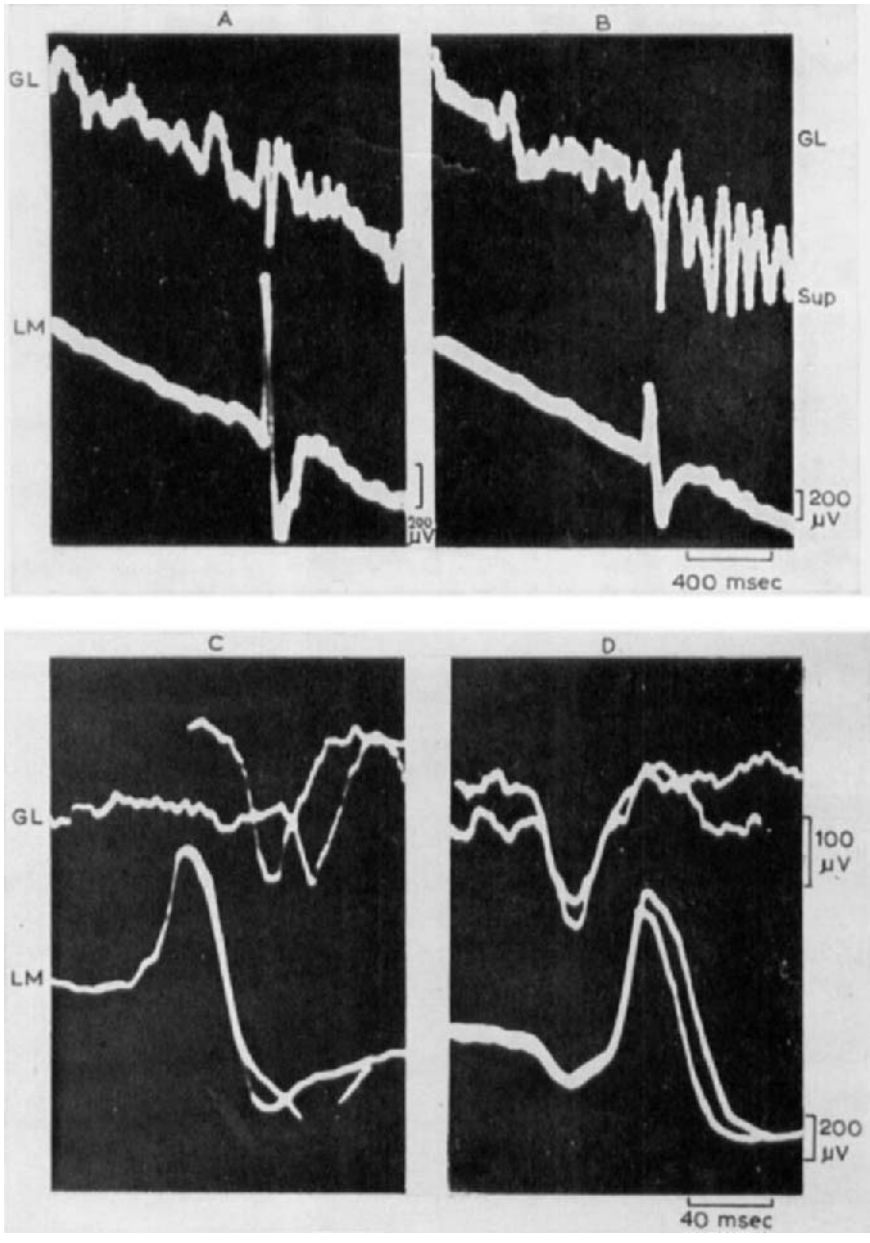

Fig. 7. LGB responses to spontaneous strychnine discharges of the visual cortex of cat. A, B, after application of 0.5% strychnine on the visual cortex. Two types of LGB response; C, the same, but with a greater speed of sweep; D, response of the visual cortex to a 'spontaneous' discharge of LGB; C and D, superposition of 2 reactions. Encéphale isolé.

nine discharge in the cortex. Genetically related components in the response of the cortex and LGB are linked by arrows.

Positive polarity of the fourth component of LGB responses to photic stimulation and to strychnine cortical spike, which is best revealed in the experiments on rabbits

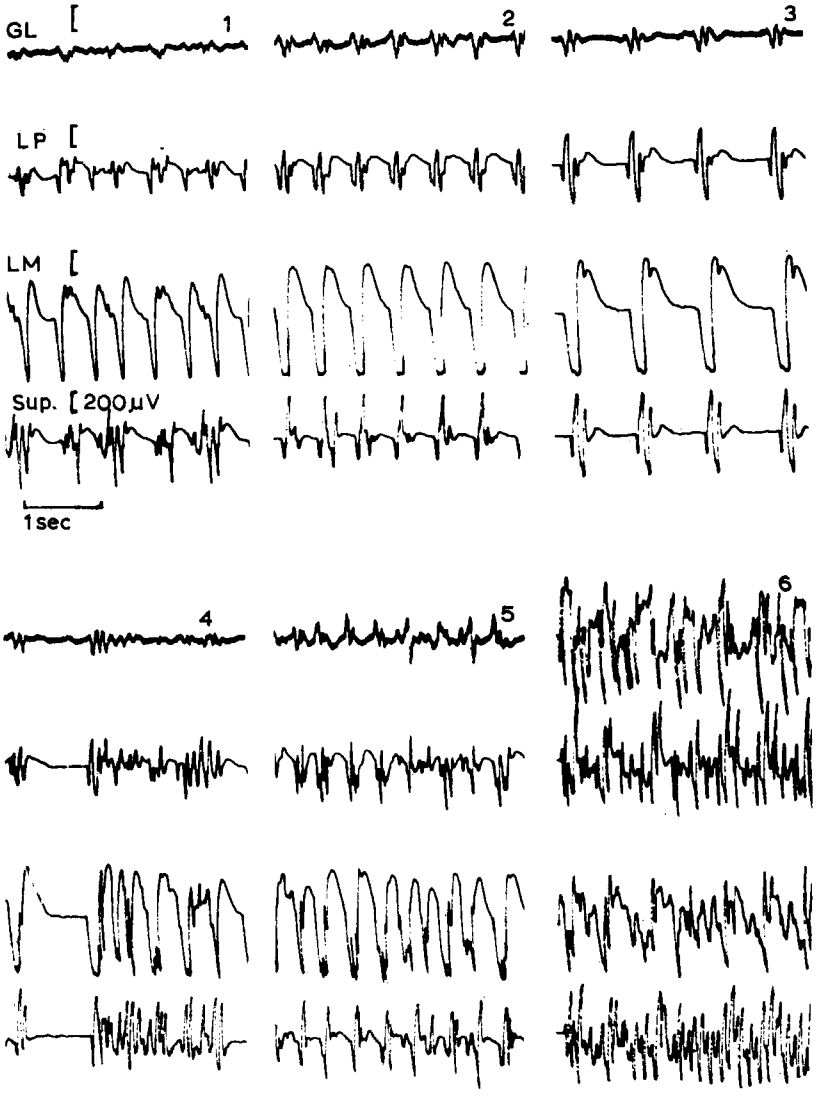

Fig. 8. Propagation of penicillin spikes and seizure activity from the visual cortex to LGB. (1–6), 3, 8, 9, 11, 11.5 and 12 min respectively after application of penicillin to the gyrus lateralis. LP, posterior part of gyrus lateralis. Cat under succinylcholine.

that have a diffuse structure of the LGB dorsal nucleus, cannot be explained by the recording of depolarization of cellular bodies of corticothalamic neurons through volume conductor, because the development of the strychnine spike in the cortex is completed before the peak of the fourth component of the LGB response. The fourth component may, in fact, be a post-synaptic hyperpolarization potential and consequently have an inhibitory nature.

Fig. 10 illustrates the origin of different components of the LGB response to a flash. The first positive component is caused, most probably, by excitation of presynaptic

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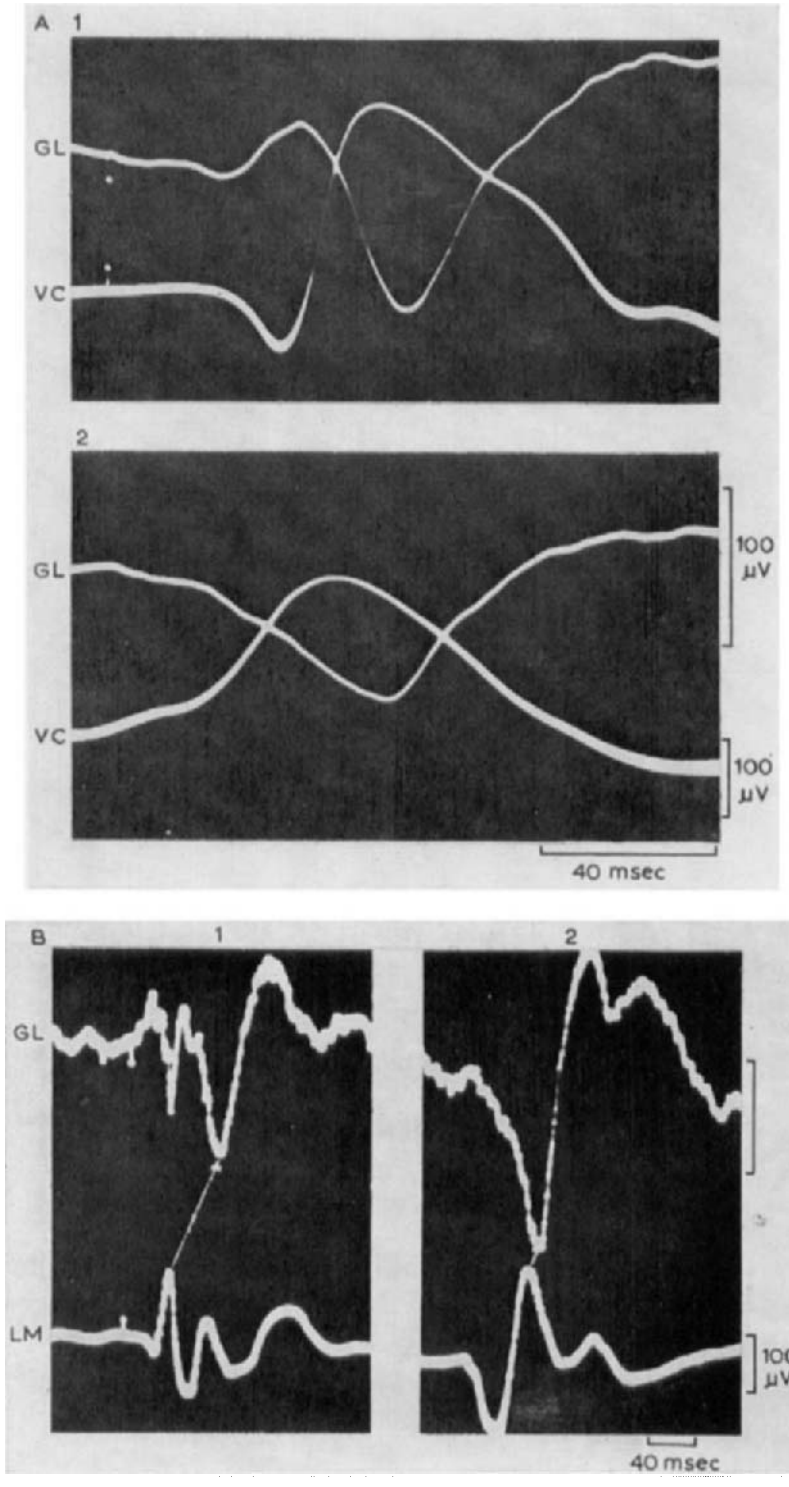

Fig. 9. LGB responses to strychnine discharges of the visual cortex. A, Experiment on rabbit; B, experiment on cat. 1, Cortical and LGB responses to a flash; 2, LGB response to spontaneous strychnine spike in the visual cortex.

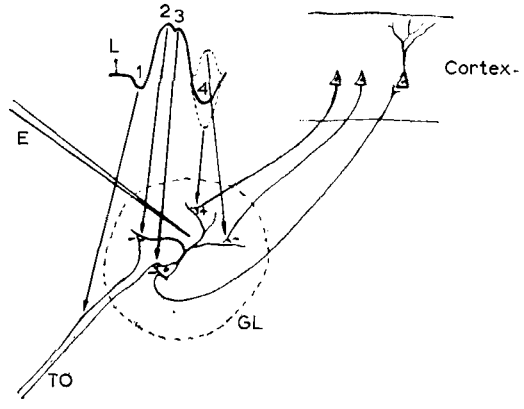

Fig. 10. Appearance of different components of the LGB response to a flash. E, electrode; L, photic stimulation; TO, optic tract.

fibres of the optic tract. The second and third components, being the primary response, are due to depolarization of soma and dendrites of LGB neurons. Localization of the recording electrode in the area of excited elements accounts for the negative polarity of these components. Lastly, the fourth component reflects the post-synaptic changes of LGB neurons caused by arrival of the feedback corticofugal volley. The positive polarity of this component bespeaks its predominantly hyperpolarized nature.

In a number of experiments the amplitude of the fourth corticofugal component considerably exceeded the amplitude of the primary LGB response (Fig. 5A, 2), the latter caused by the arrival of the afferent volley. A marked corticofugal effect was also observed in the experiments with spreading depression where the LGB response to photic stimulation may be practically fully suppressed (Fig. 3B, 2).

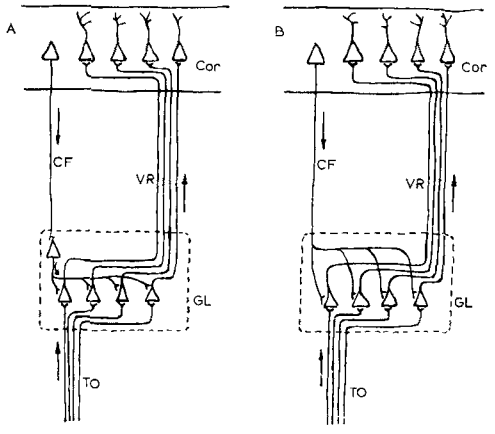

Fig. 11. Possible mechanisms of amplification of the corticofugal volley in LGB. A, Multiplication by means of interneuron; B, multiplication by means of axon's end branchings. Cor, cortex; CF, corticofugal fibre; VR, visual radiation; GL, lateral geniculate body; TO, optic tract.

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Such a pronounced corticofugal influence from the visual cortex on the LGB, presumably based on the existence of a developed system of corticofugal connections, is somewhat unexpected, since the number of corticofugal fibres is rather insignificant (one or two orders less than the number of afferent fibres of visual radiation). One may assume that the LGB has an effective mechanism of multiplication of corticofugal impulses, its morphological basis being either axon branches, terminating several LGB neurons, or intracellular interneurons also connected with a number of LGB neurons (Fig. 11).

The scheme of centrifugal control of the afferent volley

Our experimental results suggest that corticofugal influences from cortical projection areas on relay nuclei of the thalamus are not exclusively inhibitory in nature (Narikashvili and Kadjaya, 1963), but in a number of cases appear to be excitatory, causing facilitation of the transmission of the afferent volley through thalamic relay. Our experiments did not corroborate the observations of Weiss and Fifková (1961) on the absence of changes in LGB responses during spreading depression in the cortex.

In both rabbits and cats we observed that spreading depression in the visual cortex suppressed the LGB response to photic stimulation.

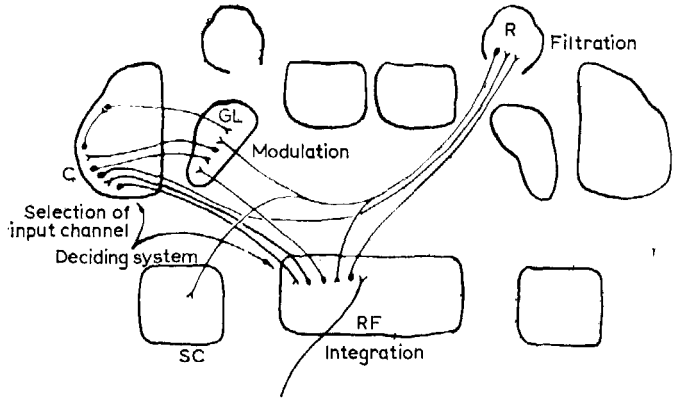

Fig. 12. Regulation of afferent volley in visual analyzer. C, cortex; SC, superior colliculus; RF, reticular formation; R, retina.

Our experimental results agree with the morphological data of Shkolnik-Yarrosóv (1958) about the existence of afferent connections between area 17 of the cortex and the LGB dorsal nucleus. However, we failed to observe any changes in LGB potentials to influences exerted on the second visual area, even though according to the data of Nauta and Busher (1960) and Szentágothai (1963) corticofugal fibres running towards the LGB dorsal nucleus originate, in the main, in the peristriate cortex.

On the basis of literature and our own data the following scheme of control of the afferent volley may be drawn (Fig. 12). At the level of the retina, due to centrifugal,

predominantly reticular, but maybe also to cortical influences, the primary filtration of the afferent impulses is performed. The nature of the filtration depends largely on the state of the organism and the degree of attention paid to the stimuli of this or that modality. It may be assumed that one of the most important functions of the reticular formation is to diminish the total amount of information arriving at the analytical centres of the cortex. Such reduction is effected mainly by means of blocking of all the sensory inputs. There should then exist an effective, most probably cortical, mechanism of deblocking of the sensory input, which receives the information important for the organism in the given circumstances. Such a deblocking of the 'attention' input may be the result of centrifugal influences in the reticular formation, or, which is more probable, the result of blocking of reticular impulses directly on the receptor apparatus.

Reticular influences are not likely to ensure differentiation between significant and nonsignificant information received by one analyzer. During sensory attention to visual stimuli, extraordinary and conditioned stimuli may be considered as significant information, whereas nonsignificant information will be the more or less stationary background and the habituated stimuli.

Differentiation of significant and nonsignificant information inside the same analyzer is evidently carried out by the thalamic relay nuclei, among them by the LGB.

In the LGB, corticofugal modulation of the afferent volley takes place. Thereupon, owing to the positive feedback connection from the cortex inside the given projection pathway, the impulses of the modality in which the organism is interested are increased, whereas the impulses of modalities, nonsignificant in the given circumstances, are blocked owing to the negative feedback connection. Positive feedback connection to the thalamic relay nuclei, among them to the LGB, develops when the corresponding projection area of the cortex (or part of this area) is in a state of excitation. When it is inhibited, the negative feedback connection takes place.

Inconsiderable 'resolving power' of the corticofugal system due to a small number of reverse fibres as compared with the afferent ones lays the ground for the assumption that corticofugal influences exert a rather crude differentiation of ascending information. Quite possibly the corticofugal system at the LGB level contrasts the switch from the peripheral to central vision while the subject seeks and examines the necessary object.

According to the proposed scheme, cortical projection areas may be considered as cells for picking up the connection channel, selectively deblocking one of the sensory inputs and facilitating the transmission of impulses along a certain specific projecting pathway. Selection of the connection channel is accomplished by means of confrontation of messages from 2 inputs, direct and unspecific, and interpretation of the information received.

The functional combination 'cortex + reticular formation' makes a computer system which provides the co-ordinated activity of the filtering and modulating mechanisms on the basis of associative and synthetic brain mechanisms.

Co-ordinated activity of the system of control of the afferent volley may be based on afferent synthesis (Anokhin, 1961), *i.e.* the process of continuous treatment of circumstantial, extraordinary and starting afferentation, afferentation obtained during

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orienting-investigation reactions, all these afferent influences being confronted with traces of previous ones.

Thus, the system of centrifugal control of projection pathways has 2 different tasks. Firstly it provides the opening and blocking of sensory inputs, and secondly it performs the transformation of the afferent volley within the specific projection pathway by means of regulation circuits with feedback connections.

Reduction of the total amount of information arriving at the cortex (by means of filtration at the receptor level and blocking of impulse transmission in the thalamic relays), and the picking out of the 'useful' signal from the 'noise' (by means of a positive feedback connection) appears to be an indispensable condition for a successful analytico-synthetic activity of the brain.

This should be taken into consideration when the versatile activity of the nervous system is modelled. Modelling cannot use disorderly networks of interconnected elements with propagation of all the signals through it according to the principle of probability. In particular, certain limits should be introduced into the stochastic patterns of the functioning of nervous structures, similar to those given by Fessard (1960) and Kogan (1962).

One cannot deny that the structure of the nervous system itself can evidently provide a propagation of impulses from any afferent input to the majority of neurons. However, during normal functioning, the greater part of the synapses is blocked, and the system functions under considerable driving. The very existence of the specific afferent systems and the topic projection of the receptor elements in the projection areas of the cortex testifies against the universality of the principle of architectonic probability of the nervous system. Functional and morphological differentiation of cortical neurons, and the characteristic features of localization of synaptic endings on different portions of the neuron depending on the origin of arriving axons, point to the existence of definite connections in the central nervous system.

There is direct evidence for the existence of neurons reacting selectively only to a certain modality of the stimulus (Mkrtycheva, 1965). So a part of the nerve impulses from receptor cells goes along definite pathways to definite neurons of the nervous system. But even this directed flow of information is partially blocked when it approaches the higher analyzer apparatus of the brain. Such mechanisms evidently ensure the optimal activity of the brain. Side by side with these somewhat fixed pathways for transmission of the sensory information, there are stochastic systems, such as the reticular formation, to which the principle of probability of propagation of the nerve impulse is applicable. It should be emphasized that the scheme described above in a rather simplified way, touches only upon the control of the afferent volley on the side of cortical projection areas and the reticular formation. Quite possibly there are other structures participating in the control, such as associative areas, the so-called 'intrinsic system' (Pribram, 1958). The 'images' coded in the intrinsic system effect the efferent control of the sensory stimuli, providing the process of selective attention to constant significant characteristics of the situation. It can be said, therefore, that the system of control of the afferent volley is one of the neurophysiological mechanisms of those processes in the central nervous system that are postulated in the form of

'images', 'model of the nerve impulse' or other similar phenomena (Apelbaum *et al.*, 1960; Pribram, 1961; Voronin and Sokolov, 1962).

Filtration of afferent impulses may underlie the sensory attention when the propagation of information along all the channels that are secondary in the given circumstances is blocked by the reticular formation. Alongside this filtration, there takes place a cortical facilitation of the central transmission in the analyzer which receives the most important information.

The corticofugal system of control of the afferent volley may play a certain role in the manifestation of one of the main properties of a dominant, namely, to react to a formerly ineffective subliminal stimulus (Ukhtomsky, 1926). Positive connection from the cortex to the thalamic relay nucleus may amplify subliminal impulsation up to a necessary level. A similar mechanism must underlie such a phenomenon, as 'safeguarding point' of the brain, described by Pavlov in the following way: intensive stimuli of some kinds are not effective for waking a sleeping person, whereas a weak stimulus but with a certain signal meaning produces this effect.

Modelling of some regularities of centrifugal control of the afferent volley

Insufficient data on the structure and processes of transmission, maintenance and

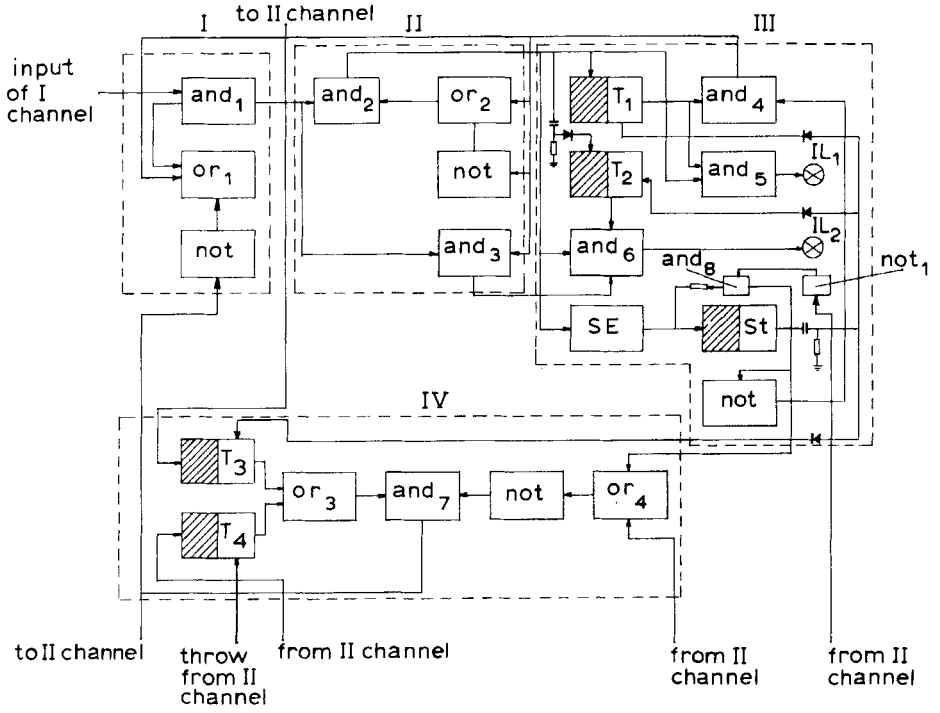

Fig. 13. Block diagram of model reproducing interrelations between 2 sensory channels and the reticular formation. I, receptor; II, thalamic relay nucleus; III, cortical projection areas; IV, reticular formation; SE, storage element: IL, indicator lamps; ST, Schmitt trigger; T, trigger; AND, coincidence circuit; OR, adding circuit; NOT, inverse circuit.

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treatment of information in the central nervous system limits the possibilities of their adequate modelling. For a neurophysiologist, however, this is the most interesting field of cybernetics. The model reproducing the architectonics of the central nervous system (on the basis of histological data) and its activity (on the basis of neurophysiological data) may sometimes serve as a reliable criterion of correctness of this or that hypothesis on the functional organization of the central nervous system.

Interrelations between 2 (or several) analyzers and the reticular formation, reproduced in Fig. 13, were modelled by V. P. Guseva on the circuit based on logic and memory elements. The arrival of the sensory signal to the inputs 'activates' the reticular formation which results in the negative feedback from 'and' gate 7, blocking the passage of the sensory impulses from receptors to relay nuclei. At the 'attention' input, reticular blocking is removed by the positive feedback from 'and' gate 4. The first sensory impulse switches trigger Tr1 and arrives through 'and' gate 5 at the indication lamp IL_1. Simultaneously the positive feedback from 'and' gate 4 is also sent to the relay nucleus. Memory trigger Tr1 provides the relay of the second and subsequent sensory impulses through 'and' gate 4 and 'and' gate 3 to the indication lamp IL_2, which is equivalent to the 'reaction of attention'. If, during the 'reaction of attention' at the I channel, a sensory impulse is sent to the input of the II channel, its relay is blocked by the reticular formation at the receptor.

A consecutive volley of impulses arriving at the input of 'attention' are added at the storage element (SE). Upon reaching the summation threshold the Schmitt trigger (ST) switches, which leads to removal of the positive feedback from 'and' gate 4 to the receptor and the relay nucleus. At the same time a signal is relayed to the reticular formation removing the unspecific negative feedback to the sensory inputs. After this, impulses continuing to arrive at the given sensory input will put into action only indication lamp IL_1, which is equivalent to the 'reaction of habituation', the latter persisting till the arrival of sensory signals discontinues. Some time later, when the capacity of the storage elements SE is discharged, the circuit comes back to its initial position.

If during the 'reaction of habituation' at the I channel, a sensory signal is sent to the II input, the passage of signals to IL_1 (at the I channel) is blocked while the II channel can reproduce the whole cycle of elaboration of 'reaction of habituation'.

This model, reproducing the connections between 3 parts of the projection systems of analyzers and the reticular formation, points to a considerable complexity of the processes, bringing into effect these intercentral connections, and sets new experimental tasks. Of particular interest is the problem concerned with participation of cortico-reticular connections in the mechanism of elimination of reticular blocking of sensory inputs during the development of habituation. In the circuit mentioned above, habituation is effected by means of purely cortical mechanisms, which contradicts Hernández-Peón's concept (1961), but agrees with the data of other authors (Mancia *et al.*, 1959; Altman, 1961; etc.). One of the conditions to ensure the work of the model is the existence of interanalyzer connections from the output of 'and' gate 1 of one channel to the input of 'not' circuit 1 of the other and *vice versa*. When the signal arrives at the input of one channel these connections are necessary to discontinue the influences of the other one on the reticular formation. These influences may take place if, after elaboration of

'reaction of habituation' at this channel, and discontinuation of stimulation, the Schmitt trigger has not yet switched to the initial position. Whether such connections with the analogous function are actually inherent in the brain remains the subject of experimental studies.

So, to match the physiological scheme of control of the afferent volley with its model, certain questions should be solved. Then necessary corrections could be introduced either into the scheme, or into the model.

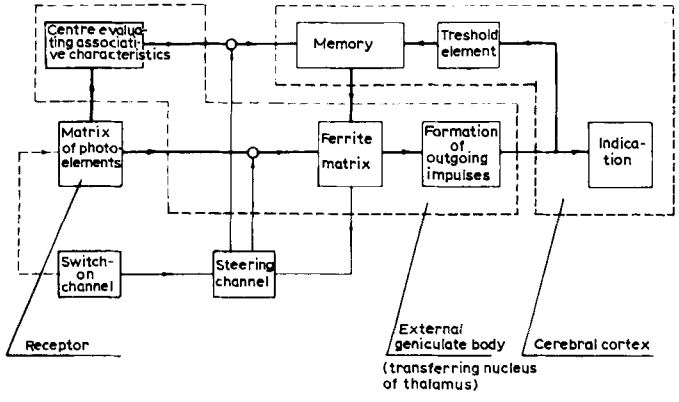

Fig. 14. Block diagram of model reproducing interrelations between the LGB and the visual cortex.

To model interrelations between the LGB and the visual cortex another logic circuit was offered by G. I. Yermakova. It consists of the following blocks (Fig. 14): photoelectric cell matrix (receptor), ferrite matrix, block of formation of output impulse and block of determination of the associative sign (LGB), memory, threshold element and indication field (cortex). The block of start and the control block are the technical elements of the model.

The work of the circuit (Fig. 15) is performed in the following way. One of the three images (Fig. 15, 1–3) is projected on to the photodiode matrix (PM). In the circuits of lighted photodiodes there appear signals passing over to the record windings of the corresponding ferrites. The control univibrator (CU) generates the reading impulse which results in the appearance of a voltage at the output windings of remagnetized ferrites, sufficient to shut the diodes of the corresponding coincidence circuit 'and' gate 2 and to light the indication lamps (IC). After 3 presentations of the image the capacity CH is charged to a certain level, and the corresponding memory trigger is switched.

After the model has 'memorized' the image it can be 'recognized' even when the photodiodes are not fully lighted (subliminal stimulation). Thereupon the signal from the photodiodes (insufficient for a full remagnetization of the cores) comes to the record windings of the ferrites corresponding to the lighted photodiodes.

Each of the 3 images which can be memorized and recognized by the model has its associative sign determining the totality of the 2 lighted photodiodes. With such

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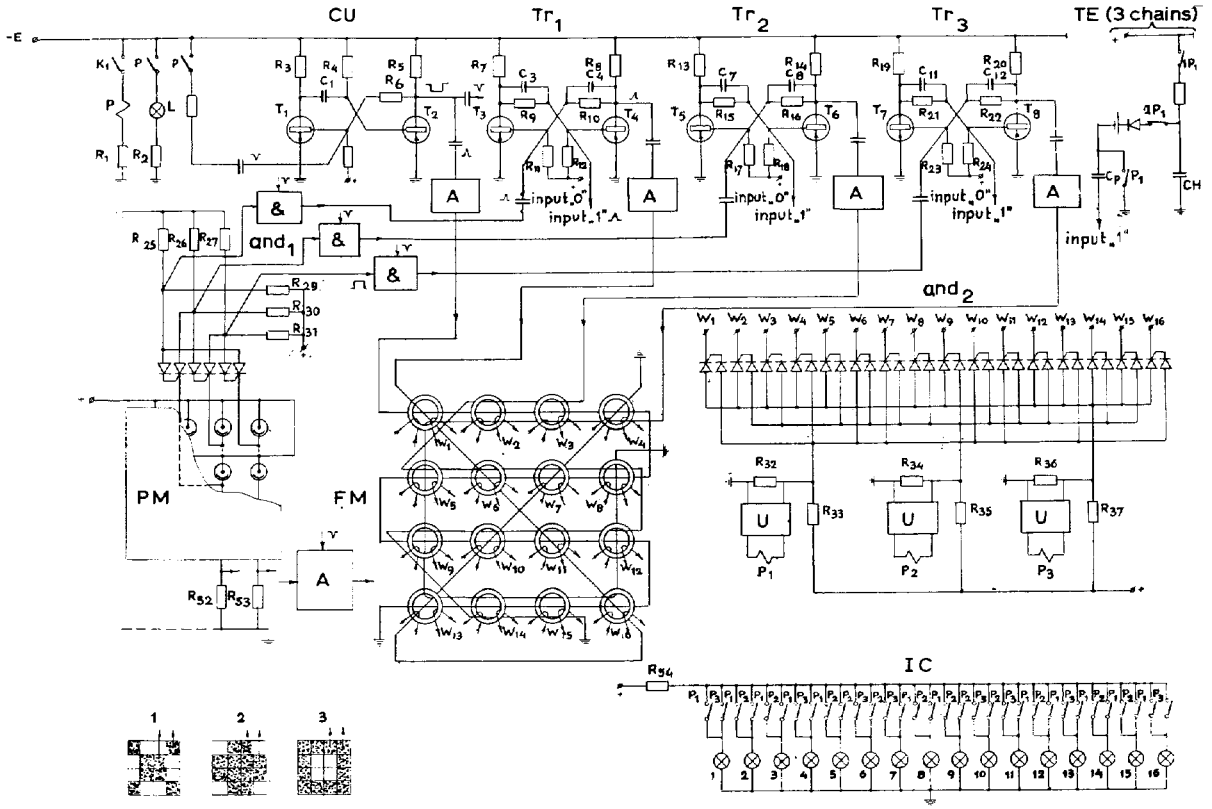

Fig. 15. Principle chart of the model reproducing interrelations between LGB and visual cortex. PM, Photoelectric cell matrix; FM, ferrite matrix; Tr_1–Tr_3, memory triggers; CU, control univibrator; TE, threshold element; AND 1, block of determination of associative sign; AND 2, coincidence circuit; IC, indication circuit; A, amplifier; U, univibrator; L, indicator lamp.

a sign inherent, a signal comes to the '0' input of the corresponding trigger from the block of determination of the associative sign ('and' gate 1).

If '1' is written in the trigger, it is read and a record impulse is sent into the windings of the corresponding combination of ferrites. Thus, the associative sign of the image calls forth from the memory the necessary totality of signs. The image is recognized if the totality of the signs coincides with the signs which arrived at the ferrite matrix FM from the receptors of the photoelectric cell matrix.

One of the important characteristics of the model is 'and' gate 1 and 'and' gate 2 which determine the associative signs of the image and compare the programmed image with the combination of excited photodiodes. Without those circuits modelling and comprehension of the process of recognition of the image would be rather difficult. However, there are no direct indications as to the existence of their physiological analogues in the central nervous system. The only reference available on this subject is the work of Sokolov (1963), who supposed participation of the mechanism of comparison in the realization of the orienting reflex.

It remains an experimental task to prove the existence of the comparators in the specific projection system.

Interrelations between the forebrain and midbrain tectum in amphibians

As is known, in amphibians all the visual information arrives without any synaptic relay into the midbrain tectum (TO), which is not only the higher visual, but also the co-ordinating, centre. Reptiles have a fairly well-developed retino-geniculate pathway. In mammals this pathway is fully developed, and the neocortex becomes the centre of perception of all the visual information. The superior colliculus of mammals, homologous to amphibian TO, loses its visual function.

In lower mammals (marsupial, rodent) tectal and geniculate fractions of the visual nerve contain an approximately equal number of fibres. In primates with well-developed and differentiated cortex the greater part of the fibres of the visual nerve terminate in the LGB.

However, it is not yet clear why the flow of visual information is switched from the TO to the forebrain. As a hypothesis it may be assumed that the existence of regulatory circuits in the forebrain specific projection systems is one of the factors that determined the progressive evolutionary development of thalamo-cortical pathways and finally the analyzer apparatus of the brain cortex. The morphological basis for formation of regulatory circles is the existence of stations of synaptic relay, *i.e.* thalamic specific nuclei. Evidently there is no control on the part of the forebrain over the TO activity.

Application of KCl and strychnine solution to the frog forebrain does not alter in any appreciable way the TO responses to photic stimuli (Meschersky and Merkulova, 1963). Fig. 16A shows that strychninization of the forebrain produces no changes in TO-evoked potentials. When strychnine evokes convulsive potentials in the forebrain they do not spread to TO (Fig. 16B). Electric stimulation of the forebrain or TO gives no TO or forebrain responses respectively (Fig. 16C, 2).

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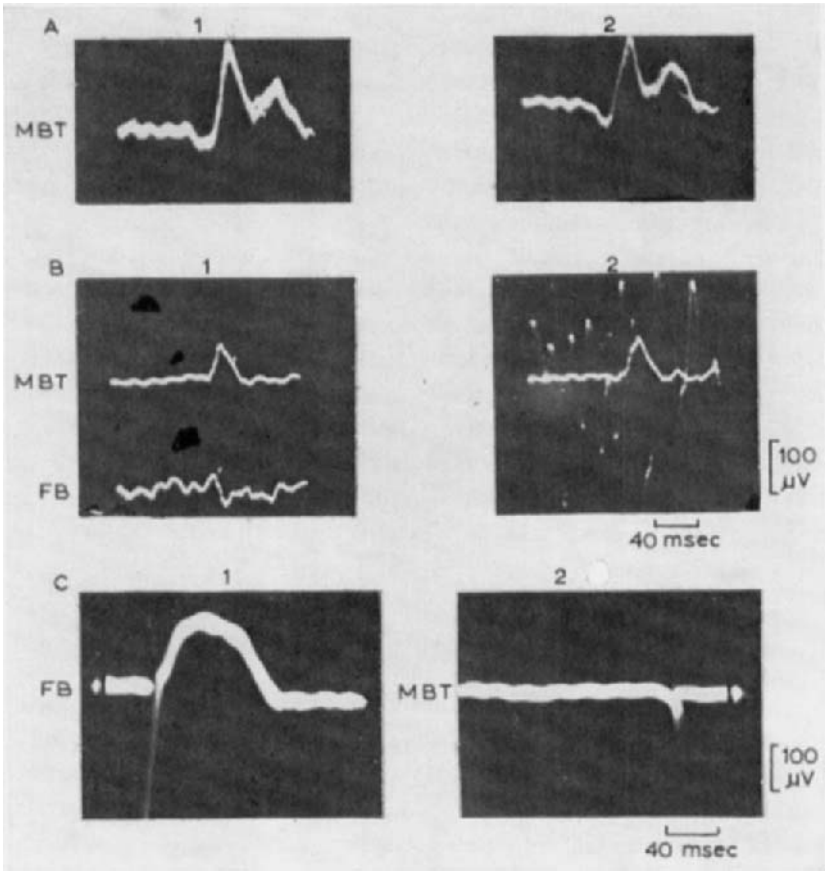

Fig. 16. Absence of changes in frog's midbrain tectum responses during pharmacological treatment of the forebrain and electrical stimulation of the latter. A, Midbrain tectum responses to a flash upon application of strychnine to the forebrain. 1, Control; 2, after application of strychnine. B, Eliciting of convulsive discharge in the forebrain with strychnine. 1, Control; 2, after application of strychnine to the forebrain. C, Electrical stimulation of the forebrain. 1, Responses of contralateral hemisphere to stimulation of the forebrain; 2, absence of changes in TO responses during stimulation of the forebrain. On A and C, superpositions of 5 responses. MBT, midbrain tectum; FB, forebrain.

Stimulation of the mammalian visual cortex provokes no responses in the superior colliculus, and stimulation of the latter entails no response in the visual cortex (Altman and Malis, 1962). It should be emphasized that, despite the well-developed anatomical connections from the cortex to the superior colliculus, the latter exerts no evident cortical control of the evoked potentials.

Thus, corticofugal control on transmission of the afferent volley through the LGB, essentially improving the possibility of selection, differentiation and analysis of the arriving information, might be an important factor which determined the progressive phylogenetic development of the given projection pathway, and, as a result of this, the regressive evolution of the retinotectal system, displayed in the loss of visual function.

SUMMARY

Investigation of the corticofugal effect on the conduction of sensory information and its primary analysis on the subcortical level is of importance for the interpretation of neurophysiological mechanisms underlying such processes as sensory attention, dominant and differentiation of stimuli.

There are different opinions on the existence and functional significance of reverse corticofugal connections from the projection areas of the cortex to specific relay nuclei of the thalamus. Some authors deny the existence of such connections at all (Weiss and Fifková, 1961); others ascribe to corticofugal influences an inhibitory nature (Head and Holmes, 1911; Narikashvili and Kadjaya, 1963); the third suppose that these connections conduct excitatory influences (Dusser De Barenne and McCulloch, 1938; Niemer and Jimenez-Castellanos, 1950). In the opinion of still other authors corticofugal influences may effect simultaneously both inhibitory and excitatory functions (Ogden, 1960; Iwama and Yamamoto, 1961; Widén and Ajmone-Marsan, 1961). Neither is there any unanimous opinion on the localization of corticofugal neurons. Some observations show that they are localized in the first projection areas of the cortex (Shkolnik-Yarrosóv, 1958); according to other observations they are situated in the second areas (Nauta and Busher, 1954; Szentágothai, 1962).

In our experiments carried out on rabbits, cats, and frogs, the functional significance and organization of the corticofugal system were studied on the example of the visual analyzer.

Application of strychnine at a low concentration (0.1–0.25%) to the primary visual cortex produces a facilitation of responses of ipsilateral LGB to a flash. The reactions of the contralateral LGB do not undergo any significant changes. Upon application of strychnine at a higher concentration (1%) or penicillin solution no facilitation of LGB responses is observed. However, they acquire a new component, namely a late postivite wave due to a reverse volley to LGB, occurring as a result of a synchronous discharge of corticofugal neurons provoked by a flash.

Spontaneous strychnine discharges in the visual cortex or epileptic discharges provoked by transcallosal stimulation may also be followed by LGB responses. LGB responses can be obtained only by activation of the first projection visual area (in cats) and not of the second area.

Application of strychnine to the white matter after the extirpation of the visual cortex does not evoke any coticofugal effects in the LGB.

Spreading depression of the visual cortex or its extirpation brings about a decrease in the LGB responses to flashes.

The data obtained lead to the assumption that the cortical projection areas modulate the transmission of afferent volleys through relay nuclei of the thalamus. Excitation of the cortex facilitates thalamic responses, and its inhibition provokes a partial blocking of the transmission of the afferent volley through the relay nucleus. Along with the control of the sensory inflow on the level of the receptors, there takes place in the relay nuclei of the thalamus a further selection of the information which is significant for the organism in the given circumstances. The inflow of nonsignificant information

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weakens and does not reach the analyzer apparatus of the cortex, whereas the significant information is amplified.

The decrease in the total amount of information coming to the cortex and the selection of the 'useful signal' from the 'noise' appears to be an indispensable condition for further successful analytico-synthetic activity of the brain. Co-ordinated activity of the system of the afferent volley control may be based on the afferent synthesis (Anokhin, 1961).

In amphibians the telencephalon exerts no influence on the tectum opticum. This fact allows us to suppose that the corticofugal control of transmission of the afferent volley through the LGB which improves the possibility of selection, differentiation and analysis of the inflowing information, may be an important factor determining the progressive phylogenetic development of the retino-geniculo-cortical projection system and consequently the regressive evolution of the retino-tectal system displayed in its loss of visual function.

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Cortical Inhibition of Thalamic Relay Nuclei

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In previous papers we have shown a considerable inhibition of thalamic non-specific (recruiting response) and specific (augmenting response) reactions during electrical stimulation of different cortical areas. Cortical inhibitory influence on non-specific thalamic nuclei is accomplished mainly through the activation of the brain stem reticular formation (RF) (Narikashvili *et al.*, 1960), whereas inhibition of specific relay nuclei is mainly carried out through direct cortico-thalamic fibres (Narikashvili *et al.*, 1961). As it was necessary to study in detail the character of cortical action on thalamic nuclei, the present experiments were made on the influence of cortical electrical stimulation upon the responses of thalamic relay nuclei.

METHODS

The experiments were carried out on 30 cats, immobilized by *d*-tubocurarine. After opening the skull of one or both of the hemispheres under ether anaesthesia the dura mater was removed and potentials were recorded from different areas of the cortical surface with bipolar electrodes. For subcortical (mainly lateral geniculate body and optic tract) recordings (monopolarly, indifferent electrode in frontal bone or in neck muscles), a steel or constantan electrode (diameter 100 μ) was used. The visual system was stimulated by light flashes (duration 1 msec). The cortex was stimulated by rectangular impulses (1–3 msec, 10–100/sec, 5–10 V, during 2–15 sec) mainly through the electrodes being far from each other. One of the electrodes was always located on the middle or posterior lateral or suprasylvian gyrus and the other on different cortical regions. In the text below only the position of the second electrode is noted. So if we mention in the text that the sensorimotor cortex is stimulated, this means that one of the electrodes is in this area and the other is in the visual cortex. However, different cortical areas were also stimulated locally with 2–3 mm distance between the electrodes. Potentials were registered on the multichannel Alvar electroencephalograph and on a cathode ray oscillograph.

RESULTS

Depending on strength, frequency and duration of cortical stimulation as well as on the general state of the preparation after cessation of cortical stimulation, three

different states of the afferent system, as judged by changes in spontaneous and evoked activity, were observed. These changes coincided more or less with those described by Sloan and Jasper (1950) for different cortical areas. Changes in spontaneous activity (SA) arose simultaneously in different cortical regions as well as in subcortical structures when stimulation ceased. They lasted for a certain period of time, gradually returning to the initial state. These changes are the following.

(1) After cessation of comparatively weak and short-lasting cortical stimulation, which does not elicit convulsive after-discharges, the SA of the cortex and that of the thalamic relay nucleus does not change markedly or is depressed.

(2) Sometimes, under the same conditions, but often after cessation of stronger and longer-lasting cortical stimulation (moderate intensity of stimulation) just the contrary is observed, namely, some increase in amplitude and frequency of SA potentials, which however does not grow into a convulsive discharge. Apparently we have here a general increment of excitability of cortical and thalamic neurons, for an inconsiderable strengthening of stimulation (or lengthening of its duration) already eaused convulsive activity. This state may be called the preconvulsive increase in cxcitability. Such a state of increased excitability is also observed after cessation of short-lasting convulsive activity evoked by cortical stimulation. This effect can be called the postconvulsive increment of excitability.

(3) After comparatively intensive cortical stimulation, a long-lasting convulsive discharge is followed by depression of cortical and thalamic SA (the latter often to a less degree). Frequently, depression develops only in the cortex with no marked changes of thalamic SA.

Depending on the state of SA that develops after cortical stimulation, responses of the cortex and thalamic nucleus undergo various changes. As the experiments were carried out mainly on the visual system — the reaction of other afferent systems was tested on three preparations — further description will concern the responses of the visual cortex and those of the lateral geniculate body (GL).

(1) Changes in responses of the visual system after cortical stimulation not resulting in convulsive discharge. With a decrease in slow SA of the visual cortex suppression of cortical responses (more considerable) as well as of GL responses, arising at slow rhythmical light flashes (Fig. 1), is observed.

(2) Changes in responses of the visual system after cortical stimulation producing preconvulsive or postconvulsive increase in excitability. More or less similar facilitation, *i.e.* increase in amplitude of cortical and thalamic responses, is usually obtained. Fig. 2 represents one such example. It is clear that after short convulsive stimulation (artifacts) followed by short convulsive activity (upper tracing), responses in all the exploring areas markedly increase in amplitude (lower tracing). Further, they are regular and have the same amplitude (with the exception of the optic tract).

(3) Changes in responses of the visual system after cortical stimulation causing postconvulsive depression. Depression of SA (as well as convulsive discharge), after intensive cortical stimulation, usually involves different cortical areas and subcortical structures simultaneously but to a different degree. Depression seems more considerable in the cortex, as judged by the amplitude of responses arising on light flashes. Some-

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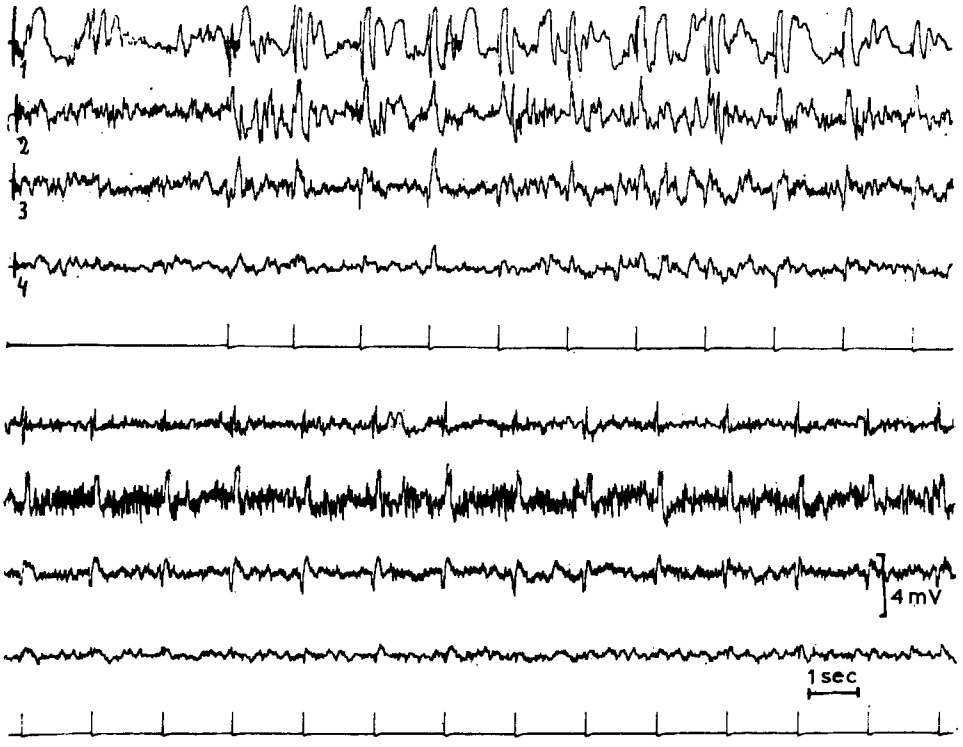

Fig. 1. Depressive action of cortical stimulation on the responses of the visual system. In each tracing from top to bottom, potentials are recorded bipolarly from: middle lateral gyrus (1), posterior suprasylvian gyrus (2), lateral geniculate body (3) and optic tract (4). Signal line indicates light flashes. Upper tracing, before cortical stimulation; lower one, immediately after cessation of stimulation of sensorimotor cortex (5 V, 10/sec, 2 msec, in the course of 3 sec).

times after generalized convulsive activity, evoked by stimulation of different cortical areas depression of SA often develops only in the cortex not involving the exploring subcortical structures (Narikashvili and Kadjaia, 1962, 1963; Kadjaia and Narikashvili, 1962). Postconvulsive depression then represents suitable conditions for revealing dependence of evoked activity of the thalamic relay nucleus upon the functional state of cortical neurons. In the course of a well-pronounced depression, the SA of the cortex disappears altogether, and the responses are either very much decreased or entirely missing: this points to a severe decrease in the activity of cortical neurons. Such a state continues for a certain time, and then the excitability of cortical neurons (as judged by spontaneous and evoked activity) is gradually restored. Cessation for a short time of the activity of cortical neurons with its subsequent restoration can be repeated for tens of times without a marked damage of stimulated cortical regions (provided the interval between repeated stimulations is great enough): this reveals the influence of the cerebral cortex upon evoked activity of thalamic relay nucleus.

Fig. 3 shows the result of one such experiment. After cortical stimulation (A) and cessation of convulsive activity (B) the SA of the visual cortex is wholly depressed (C),

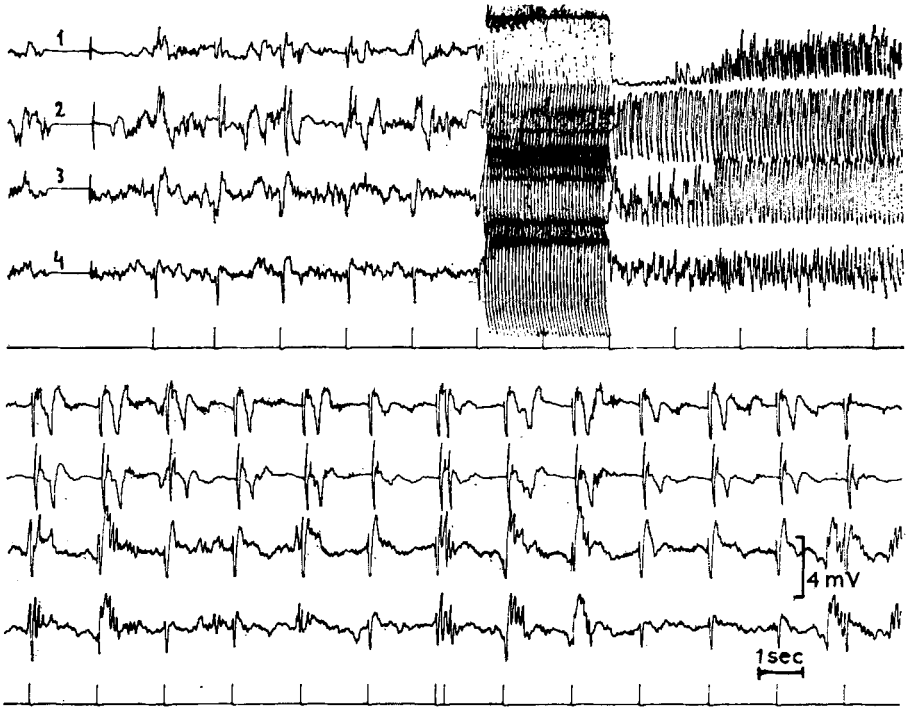

Fig. 2. Facilitatory action of cortical stimulation on the responses of the visual system. Potentials are recorded of the same regions as in Fig. 1. Suprasylvian gyrus is stimulated (8 V, 10/sec, 1 msec, during 5 sec) in front of recording electrodes. Lower tracing is a continuation of the upper one. After short-lasting convulsive activity responses at all levels of the visual system are equally increased in amplitude.

and evoked activity also markedly weakens. The SA is also depressed in the geniculate body and optic tract, but to a far less extent. The amplitude of GL responses now markedly increases, whereas the responses of the optic tract do not change or may even be depressed (C). With elapse of time the SA of the cortex, *i.e.* excitability of its neurons, is gradually restored: this is seen as a progressive increment in amplitude of cortical responses and after-effects of such stimulation (D, E). A gradual decrease in amplitude of GL responses takes place, and finally they reach the level observed before cortical stimulation (E). In the course of convulsive discharges GL responses do not change or are depressed, and their increase begins only immediately after cessation of the convulsive discharge and the onset of cortical depression. During intensive convulsive activity it is naturally difficult to distinguish responses from convulsive discharges, but at the end of convulsive activity they can be distinguished if convulsive activity ceases in the GL sooner than in the cortex. This is clearly seen in the right of the tracing B during fading of the convulsive activity. It is clear that GL responses do not change in amplitude.

Since postconvulsive depression involves not only the cortex but often also the GL (it is generalized), then it can be that depression of SA in the latter has a certain value in the increase in GL responses. But firstly, such depression and removal of SA do not

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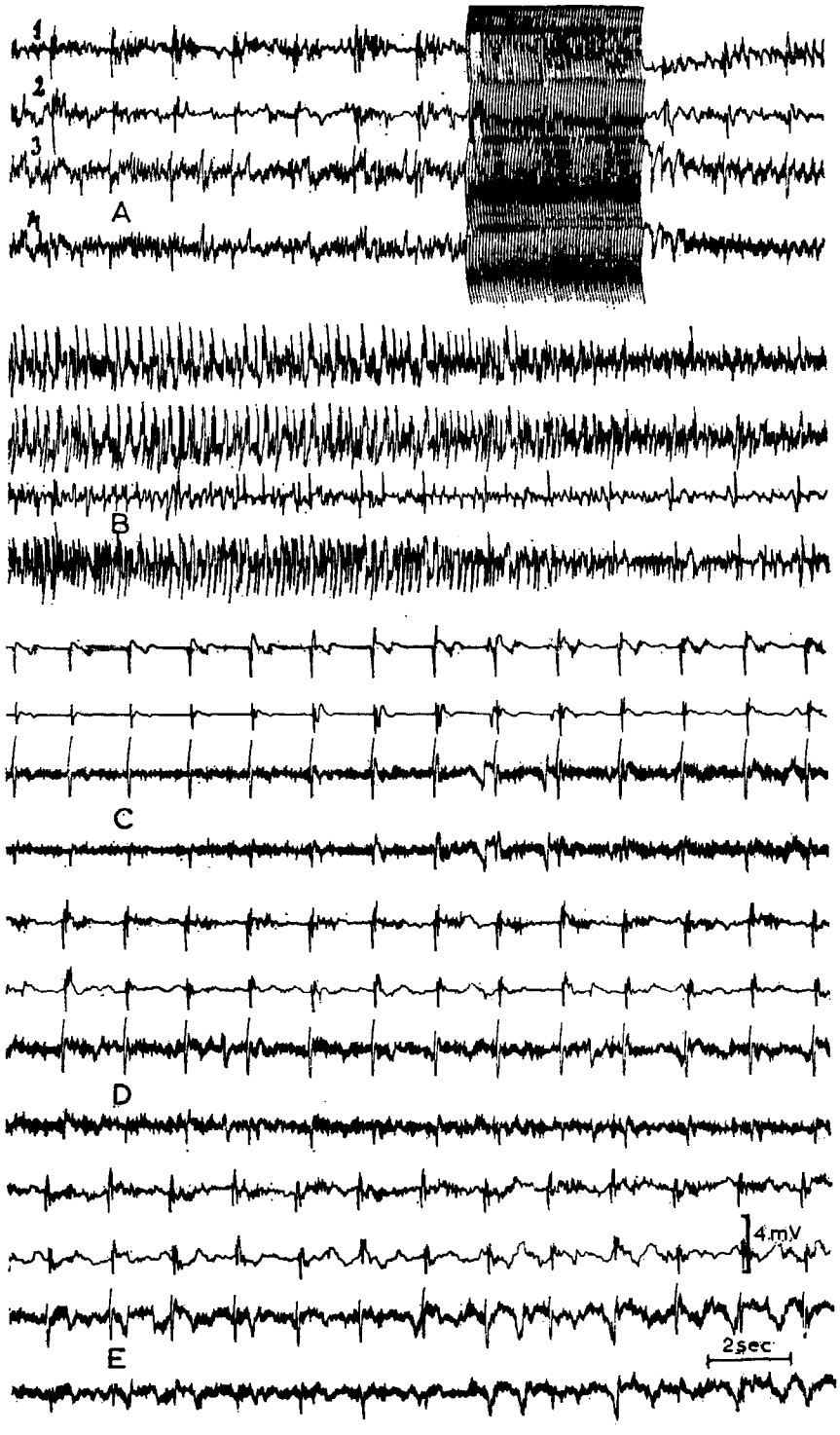

Fig. 3. Facilitation of geniculate response during postconvulsive depression of cortical activity. The same recordings as in Fig. 1. Sensorimotor cortex is stimulated (10 V, 10/sec, 1 msec, in the course of 4 sec), seen by the artifacts in tracing A. B, final part of long-lasting (up to 30 sec) generalized convulsive activity. C, just after cessation of convulsive discharges and the onset of depression of cortical activity. D and E, continuation. Indication of light flashes is removed in order to save space, but times of light flashes are indicated by responses.

bring about the increase in responses in the cortex, but depress them, and secondly, there are occasions when, at full depression of the cortical SA, the activity of the GL does not change at all. Under these conditions during depression of cortical activity the same picture of increase in GL responses is observed. Thus, the increase in GL responses during cortical postconvulsive depression appears independently of the character of its SA.

Because in previous work the reticular formation (RF) has been shown to play a significant role in the exertion of cortical influence on thalamic non-specific nuclei (Narikashvili *et al.*, 1960), it was necessary to find whether the RF plays any role in this phenomenon. First, we investigated how reticular responses arising on light flashes were changed during cortical postconvulsive depression. It was found that with the increase in the amplitude of GL responses the amplitude of reticular responses usually also increases, but to a significantly less extent (Fig. 4). However, reticular responses often do not change at all or even lessen when thalamic responses are

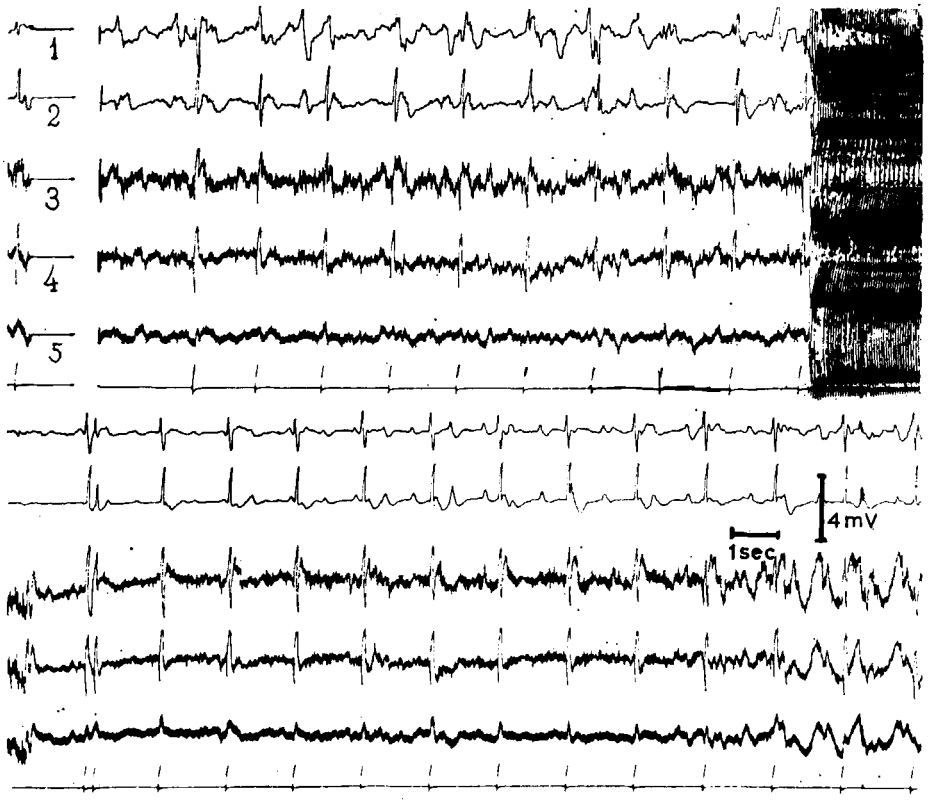

Fig. 4. Changes in reticular responses during postconvulsive cortical depression. Potentials of the same regions as in the previous figures are recorded. Lower (5) curve, potentials of mesencephalic reticular formation at the collicular level. Upper tracing, before stimulation; lower one, after stimulation of anterior suprasylvian gyrus (10 V, 20/sec, 2 msec, in the course of 10 sec) and just after cessation of long-lasting convulsive discharge. Some depression of spontaneous activity of the cortex before its stimulation (upper tracing) is conditioned by previous repeated stimulation of the cortex.

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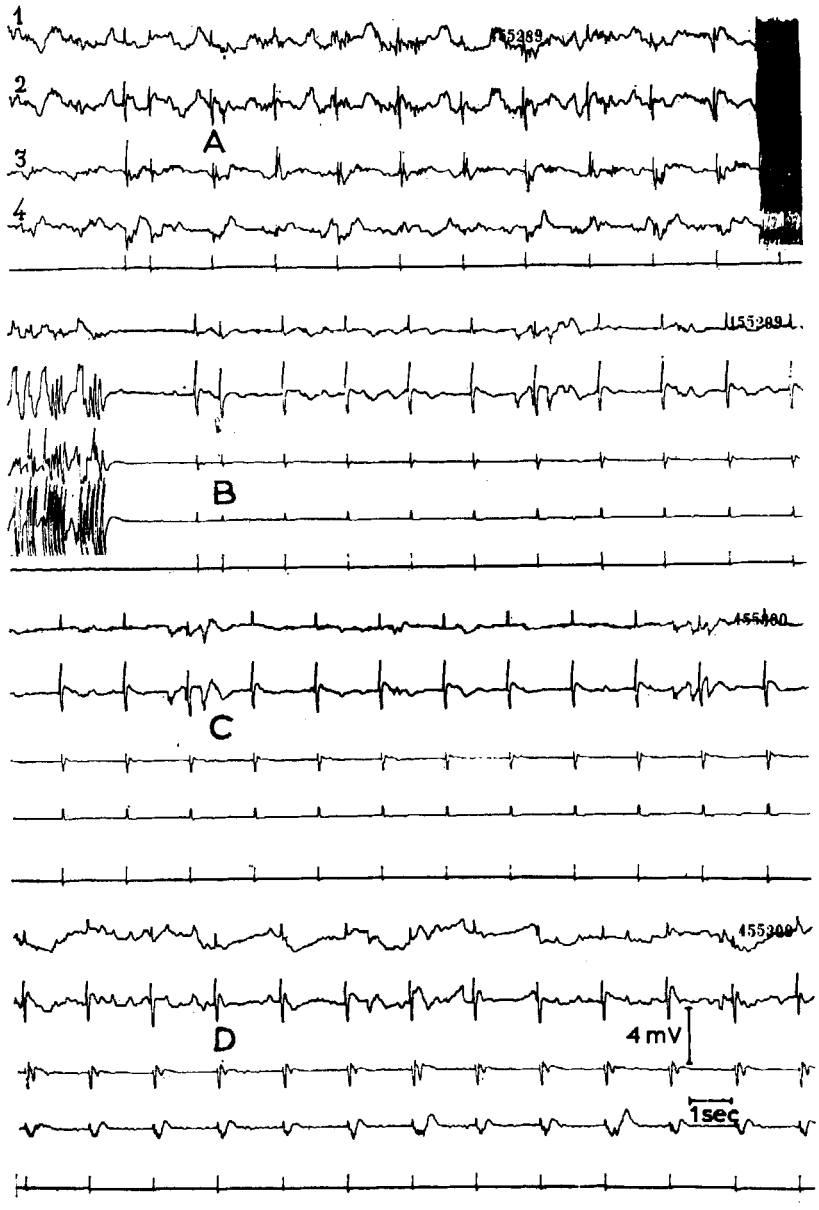

Fig. 5. Changes in geniculate responses during postconvulsive cortical depression in the preparation with electrolytic damage of brain stem. In each tracing from top to bottom, potentials are recorded from optic tract (1), lateral geniculate body (2), middle lateral gyrus (3) and posterior suprasylvian gyrus (4). Experiment carried out after 1.5 h of bilateral electrolytic damage of reticular formation at collicular level. A, before cortical stimulation; B, after cessation of homolateral sensorimotor cortical stimulation (6 V, 20/sec, 1 msec, in the course of 10 sec) and long-lasting convulsive discharge, which ceased simultaneously in all recorded structures, C, continuation of B. D, after 1.5 min of C.

increased (see also Bureš *et al.*, 1961). This shows that the increase in GL responses during cortical postconvulsive depression is not connected with the activity of RF and

with the cortical influence upon it, as the increase in thalamic responses occurs independently of whether reticular responses are increased, depressed or unchanged. The increase in GL responses is also obtained in the preparation after damage of the connection between RF and the thalamus. So, if RF is entirely lesioned electrolytically at the collicular level, then after a certain time this phenomenon might be repeated without a marked difference from that observed before in the preparation with an intact nervous system (Fig. 5).

Finally, the category of such phenomena also include the cases in which, after brain stem transection, the cortical functional state sharply worsens up to the cessation of its SA because of disturbances in cortical circulation. If GL responses to light flashes were previously irregular and of low amplitude, then after brain stem transection their amplitude and regularity increased (Fig. 6). The same is often observed on cooling the cortex: if the cortex (partially or wholly) is cooled to such extent that full depression of SA and a significant depression of evoked potentials take place, then the responses of GL evoked by light flashes markedly increase in amplitude and arise regularly. Such an increase may also be obtained after thermocauterization of pial

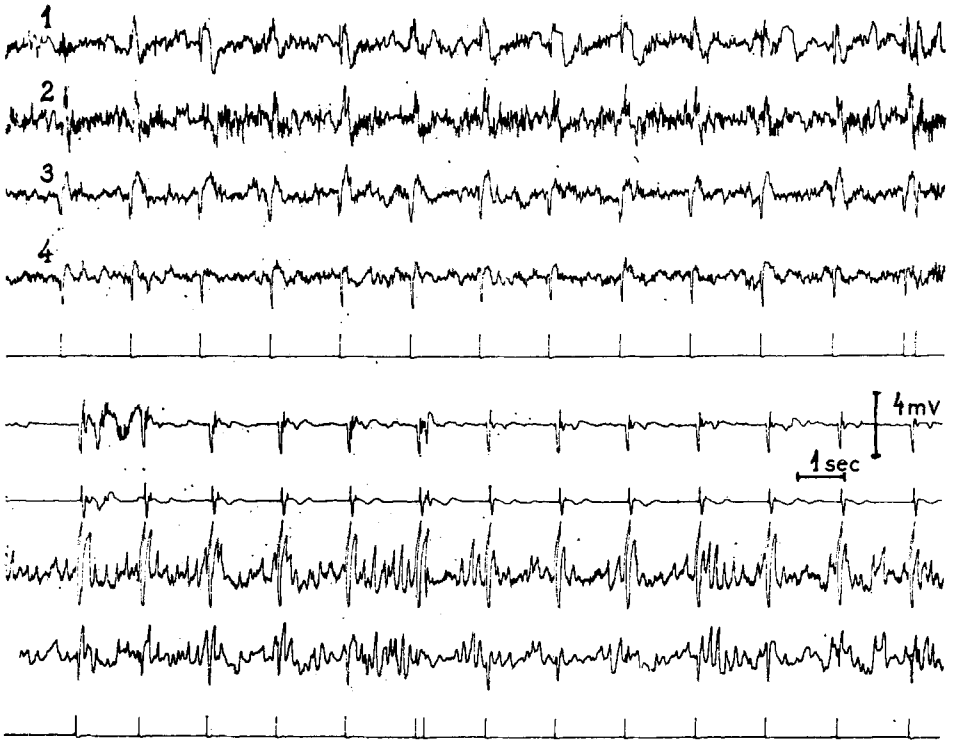

Fig. 6. Influence of worsening of the functional state of the cortex following brain stem transection upon geniculate responses. From top to bottom, potentials are recorded from suprasylvian gyrus (1), middle lateral gyrus (2), lateral geniculate body (3) and optic chiasma (4). Upper tracing, before surgical transection of the brain stem; lower one, 15 min after transection of the brain stem at the intercollicular level.

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vessels, *i.e.* through any means of depression of the functional state or lesion of the cortex.

Thus, with every suppression of cortical activity (caused either by electrical stimulation, cooling or disturbances in pial circulation), the increase in the amplitude of GL responses arising at an adequate stimulation of a receptor is observed. Further, the configuration of responses also changes and the duration of responses shortens; both phases increase but the positive increases more than the negative (Fig. 7). Sometimes only the positive phase is increased.

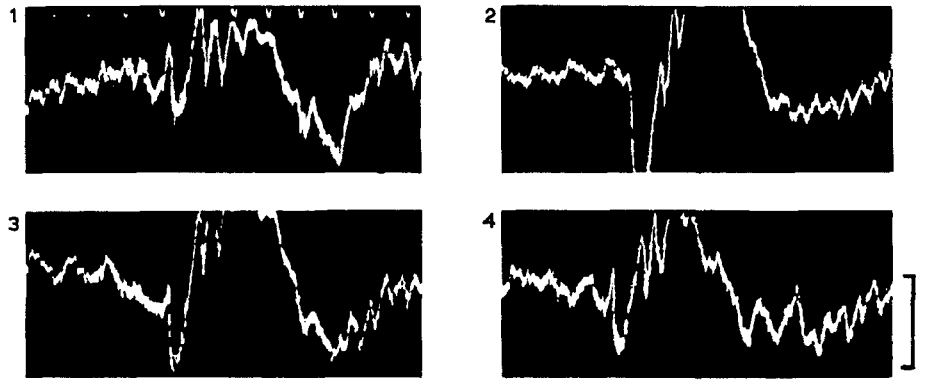

Fig. 7. Changes in geniculate response configuration during postconvulsive depression of cortical activity. 1, before cortical stimulation; 2, at the beginning of depression induced by local stimulation of sensorimotor cortex (8 V, 20/sec, 2 msec, during 10 sec); 3, after 10 sec from the onset of cortical depression; 4, after 25 sec, when cortical spontaneous activity is almost entirely restored. Voltage calibration, 0.4 mV; time, 20 msec.

DISCUSSION

Thus, geniculate responses, arising on light stimulation of the eye, significantly increase in amplitude during cortical postconvulsive depression. Such effect of cortical postconvulsive depression is not always observed. First, postconvulsive depression cannot be obtained after every strong cortical stimulation. Postconvulsive depression generally develops after an intensive and long-lasting convulsive discharge, but a well-expressed postconvulsive depression develops when convulsive activity is extinguished at once in all the structures studied; it may, however, also develop after gradual fading of the convulsive discharge. This last event can be considered as a result of the continuation of asynchronous irregular weak convulsive activity in cortical neurons, which in spite of depression of SA maintains its inhibitory action on geniculate neurons. If convulsive activity attenuates for a time and then revives, it now lasts for a long time and is not usually succeeded by a well-pronounced depression: the irregular weak convulsive discharges continue and they behave as if summed with the state of SA depression.

The phenomenon of the increase in geniculate responses is more complicated. Sometimes, during more or less pronounced postconvulsive depression, thalamic responses are unchanged in amplitude or they are even depressed. If, with a given preparation we were to succeed in finding the appropriate parameters of stimulation eliciting well-pronounced cortical or generalized depression with the effect of increase in thalamic responses, then this phenomenon could be further evoked dozens of times. In other words, it is evidently necessary to lower the level of the activity of cortical neurons to a certain extent, after which a descending inhibitory action of cortical neurons on the transmission of impulses in thalamic relay nucleus (Ogden, 1960; Widén and Ajmone Marsan, 1960, 1961) ceases to occur. The level of the activity of cortical neurons and their descending inhibitory action depending upon many external and internal conditions may fluctuate, as if within normal limits. It apparently conditions the fluctuation of amplitude and the irregularity of geniculate responses that are frequently observed. When cortical activity lowers to a certain level during postconvulsive depression, the cortical inhibitory influence upon GL decreases or disappears altogether, and this results in equal excitation of all geniculate neurons by the impulses arriving through the optic tract. This conditions, first, the increase in amplitude of responses, followed by their exclusive regularity and equality in amplitude. This inhibitory influence of the cortex is apparently carried out by direct cortico-thalamic fibres, for the phenomenon described above is as well observed before as after electrolytic lesion of the brain stem at the collicular level.

The above-described generalized convulsive activity and depression may be elicited by stimulation of any cortical region. It is a somewhat unspecific, general reaction of the whole cortex. However, the phenomenon of increase of thalamic responses is better shown when convulsive activity and the subsequent cortical depression are induced by stimulation of the corresponding cortical projection area.

A thalamic effect of cortical depression, as has been mentioned, depends to a considerable degree on the functional state of the preparation, and especially on that of the cortex. If because of the operative trauma, bleeding or some other reasons cortical activity becomes worse, then cortical depression may not bring about an increase in amplitude of thalamic responses. It is then better to judge the functional state of the cortex not only by spontaneous and evoked activity of the cortex but, primarily, by the amplitude and regularity of thalamic responses. When the cortex is in a good state thalamic responses have a comparatively low and alternating amplitude. If before cortical stimulation thalamic responses have high and equal amplitudes and they arise entirely regularly this points to a poor state of the cortex, at which its descending inhibitory influence upon thalamic neurons is weakened or is entirely missing. It is then clear that depression of cortical activity will not bring about any changes in thalamic responses, since what could be removed by depression of cortical activity (descending inhibitory influence) is already removed by the worsening of the cortical functional state.

What kind of cortical depression is it? Is it a spreading cortical depression (SD) of Leão? To say nothing of the difficulties in obtaining this phenomenon in cats, a number of observations are against it, in particular the following. (*1*) In our experi-

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ments convulsive activity and depression arose simultaneously in all the cortical areas, being far from each other (such as frontal and occipital areas), whereas SD spreads with a certain low velocity from one area to the other (Marshall, 1959). (*2*) In contradistinction to SD at which responses do not change in specific relay structures of the midbrain and thalamus (Weiss and Fifková, 1961a, b, c), we have mostly observed the increase or sometimes the suppression of their responses. (*3*) SD caused by unilateral cortical stimulation does not usually spread over the subcortical structures and on the other hemisphere. But convulsive activity and depression were mostly of a generalized character and bilateral.

Whatever this phenomenon might represent, its generalized character is not conditioned by participation of RF, since it is observed in preparations with a transected brain stem. In contradistinction to the SD, the effect of postconvulsive depression on thalamic responses should indicate the decrease of the cortical depression in the first case (which is, apparently, manifested not only by the level of SA lowering) or the fact that in the first case it develops more slowly than in the second. The fact that in some of our experiments geniculate responses were not changed during postconvulsive depression is probably due to insufficient depth of cortical depression. During postconvulsive depression, abolition of SA of the cortex and its descending inhibitory influence probably take place at different levels of lowering of cortical activity. SA of the cortex is abolished at weaker depression than its descending inhibitory influence. This is evident, above all, from the observation that geniculate responses are sometimes not changed during the cortical SA depression, and second, at postconvulsive depression cortical responses may vary in amplitude (being sometimes high, sometimes low), though in both cases the SA is suppressed in equal degree. The depth of depression of cortical activity is, apparently, better judged by the degree of decrease in amplitude of cortical responses but not by the lowering or abolition of the SA.

As to the mechanism of the increase in amplitude of thalamic responses during cortical postconvulsive depression it might be as follows. At strong electrical stimulation of the cortex, causing long-lasting convulsive activity, excitability can be increased in the cortex as well as in the subcortical structures. That is shown by our experiments too in which a preconvulsive or postconvulsive generalized increase in excitability was observed, manifested by an equal increase in amplitude of responses at all levels of the afferent system. It may be that, after cessation of convulsive discharges this heightened excitability can sometimes be lowered earlier in the cortex than in thalamic nucleus. Then during cortical depression some raised excitability of the thalamic nucleus can still continue (as a result of convulsive activity); therefore its responses can be of high amplitude for a certain time. But the following observations detract from this assumption. (*1*) An increase in amplitude of thalamic responses is observed both when postconvulsive depression develops in the cortex as well as in the thalamic nucleus, and when it evolves only in the former, *i.e.* when cortical stimulation and convulsive activity do not change (judged by spontaneous activity) the excitability of thalamic neurons. (*2*) When, during cortical postconvulsive depression, thalamic responses are increased they are usually depressed during convulsive activity. (*3*) Increase in thalamic responses is also observed during inactivation of the cortex by

cooling or disturbances in circulation, *i.e.* when there are no conditions for artificial increase of excitability of thalamic neurons.

Furthermore detailed study of the influence of cortical depression upon GL evoked activity by using other methods of local inactivation of the cortex, as well as studying it in relation with the activity of other relay structures, will show how much this phenomenon has the character of general regularity. If it is confirmed for other examples of cortical inactivation and in regard to different afferent systems as well as at microelectrode recording of the activity of thalamic neurons, then inactivation of one of the structures in the chain with interrelated formations will serve as a good physiological method to establish the presence of feedback connections.

Finally, if the experiments with thalamic single unit recording during cortical post-convulsive depression confirm the consideration stated above then Sechenov's idea on the inhibitory influence of higher centres of the brain on lower ones will take on the status of a universal law, characteristic for the whole central nervous activity. Obviously, with the development and formation of some new structures of the brain in evolution, in connection with transition of function to fine and more perfect mechanisms of the anterior brain, these new structures acquire regulatory influences upon the function of lower-lying structures manifested primarily in their tonic inhibition due to which corticopetal impulses are limited or filtered.

SUMMARY

(1) On weak or short-lasting electrical stimulation of the appropriate cortical regions, not resulting in convulsive activity or inducing short-lasting convulsive after-discharges, generalized (at all the levels of afferent system) depression of evoked potentials (arising at an adequate stimulation of a receptor) as well as their facilitation on account of pre- or postconvulsive increase of excitability of neurons of afferent system is observed.

(2) During generalized depression, obtained after intensive electrical stimulation of any cortical region and convulsive discharges, responses of the thalamic relay nucleus to light stimulation are markedly facilitated, expressed by increases in the amplitude and regularity. This phenomenon is observed in the preparations with intact nervous system as well as after electrolytic transection of the brain stem at the collicular level.

(3) With gradual restoration of cortical spontaneous and evoked activity, responses of the thalamic relay nucleus return to their original form and amplitude.

(4) The same phenomena sometimes can be observed on cooling of the cortex as well as on thermocoagulation of pial vessels or surgical transection of the brain stem, when on account of disturbances in circulation there develops in the cortex a pronounced depression of spontaneous and evoked activity.

(5) During postconvulsive depression occurring simultaneously with a considerable increase of thalamic responses, optic tract responses do not increase considerably and are sometimes even depressed. This excludes any decisive significance of changes in retina in this phenomenon.

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(6) This phenomenon is considered to be a result of the removal of the inhibitory influence of the cortex upon transmission of impulses in the thalamic relay nucleus.

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Mécanismes d'Autorégulation dans les Corrélations Cortico-Souscorticales

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Depuis le siècle précédent on connaît déjà des mécanismes de régulation cortico-souscorticale, mais c'est seulement dans des études plus récentes qu'une analyse plus détaillée en fut faite. Nous ferons ici un bref exposé de nos recherches dans ce problème.

Chez les animaux à néocortex extirpé des deux cotés (expérience chronique), nos expériences (Sager *et al.*, 1957a) ont démontré la présence d'une hypotonie et hyperkinésie de l'estomac et de l'intestin (donc des effets différents sur la tonus et la kinésie); sur l'électrocardiogramme on a noté l'inversion de l'onde T et l'apparition fréquente d'une onde Pardee (modifications qui peuvent disparaître plus tard); la tolérance au glucose était considérablement diminuée et la phase d'hypoglycémie après l'injection d'insuline n'était pas suivie d'une phase de normalisation de la glycémie; la réponse aux injections d'acétylcholine, éphédrine et pilocarpine se modifiait quantitativement aussi bien que qualitativement, les effets pouvant en être tout à fait contraires à ceux obtenus chez l'animal normal.

La trophicité de la peau chez l'animal décortiqué d'un seul côté est fortement diminuée (exagération du phénomène de Shwartzmann-Sanarelli dans la peau contralatérale).

L'extirpation du néocortex provoque, en dehors de la libération des centres sous-corticaux, une activité chaotique de ces centres due au fait qu'ils ne sont plus contrôlés et réglés par le cortex surtout lorsque l'organisme doit s'adapter aux nouvelles conditions du milieu externe et interne.

L'influence du cortex sur les formations sous-corticales s'exerce par des voies cortico-fugales d'inhibition et de facilitation dont l'activité est modulée en fonction des informations que le cortex lui-même reçoit de la périphérie par des voies spécifiques et non-spécifiques.

Sechenov (1863) a décrit pour la première fois l'existence d'une inhibition d'origine centrale sur les réflexes médullaires.

Rosenberg et Sager (1931) dans une série d'expériences sur la grenouille ont contrôlé si les excitations parties des lobes optiques pourraient influencer l'excitabilité des centres moteurs médullaires. Ces expériences se sont limitées à déterminer la vitesse de conduction dans le nerf périphérique chez la grenouille (fortement illuminée)

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avant et après la section pratiquée derrière les lobes optiques (tenant compte de l'existence d'une relation directe entre l'excitabilité du neurone moteur et la vitesse de conduction). Ils ont déterminé la vitesse de conduction au niveau du nerf sciatique à differents intervalles de temps avant et après la section pratiquée derrière les lobes optiques (chez la grenouille antérieurement fortement illuminée). La vitesse de conduction augmente de 12.4% au maximum après la section (on a eu soin de ce que la température de l'animal restât toujours la même et que le courant d'action ne changeât pas de forme).

L'action inhibitrice du cortex cérébral sur les centres sous-corticaux a pu être démontrée aussi par la méthode électroencéphalographique (Sager, O., Florea-Ciocoiu, V., et Nestianu, V., travail non publié).

L'injection de chloralose (80 mg/kg) chez l'animal à néocortex extirpé d'un seul côté provoque une importante augmentation du potentiel électrique évoqué par une stimulation lumineuse unique (S.L.U.) dans l'hypothalamus du côté décortiqué. Chez l'animal à néocortex extirpé des deux côtés, l'injection de chloralose provoque une augmentation de l'amplitude du potentiel évoqué dans l'hypothalamus des deux côtés (Fig. 1).

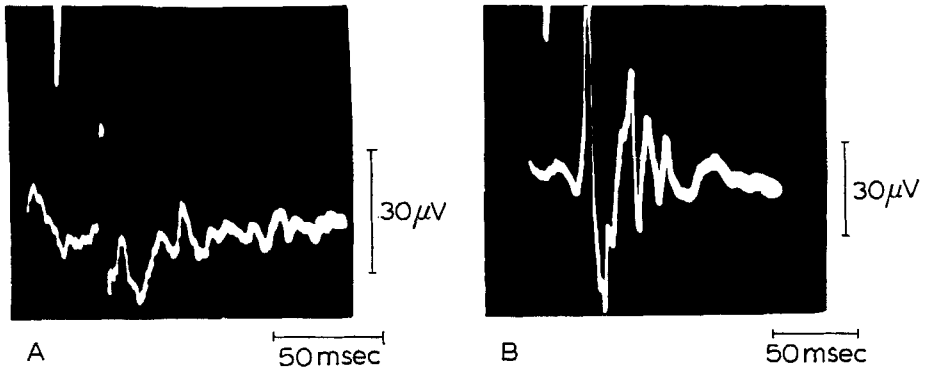

Fig. 1. Potentiel électrique évoqué par stimulation lumineuse unique (S.L.U.) dans l'hypothalamus chez le chat décortiqué des deux côtés. A, avant l'injection intraveineuse de chloralose; B, après l'injection intraveineuse de chloralose.

Les expériences ci-dessus montrent qu'il existe non seulement des fibres néocortico-fugales, mais aussi des fibres paléocortico-fugales inhibitrices actionnant sur les formations sous-corticales et que leurs effets peuvent être supprimés par l'injection de chloralose. Meulders *et al.* (1963) ont mis en évidence seulement des fibres néocortico-fugales inhibitrices.

On a observé chez l'animal normal une amplification du potentiel électrique évoqué par la S.L.U. dans le gyrus sigmoïde antérieur à la suite de l'injection de chloralose. L'effet de la chloralose est moins constant sur le potentiel électrique évoqué par la S.L.U. dans le gyrus marginal postérieur ou dans le ganglion genouillé externe (Fig. 2).

Ces données paraissent confirmer l'opinion des auteurs que l'action de la chloralose

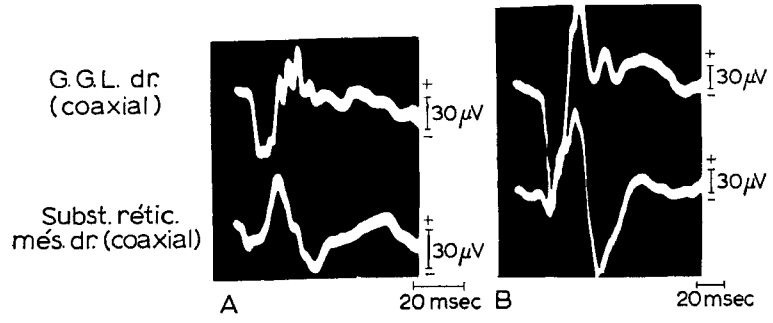

Fig. 2. Potentiel électrique évoqué par la S.L.U. dans le ganglion genouillé latéral (G.G.L.) et dans la substance réticulée chez le chat tranquillisé par flaxédil. A, avant l'injection intraveineuse de chloralose; B, après l'injection intraveineuse de chloralose.

sur les formations souscorticales s'exerce par la suppression de l'activité inhibitrice des fibres cortico-fugales sur la formation réticulée du tronc cérébral.

Le cortex cérébral a aussi une action de facilitation sur les formations souscorticales. Les expériences de Bremer et Terzuolo (1954) ont démontré que les excitations à point de départ dans les zones sensorielles amplifient le potentiel évoqué par la même excitation sensorielle dans la formation réticulée du tronc cérébral.

Nos recherches ont pu démontrer que le réflexe sino-carotidien est fortement diminué après la décortication bilatérale (expérience chronique); d'autre part, on a pu démontrer aussi que les excitations à point de départ dans le gyrus sigmoïde antérieur ont une action facilitante sur les centres vasomoteurs de la corne latérale de la moëlle. Les excitations qui partent du cortex cérébral passent par le subthalamus, l'hypothalamus postérieur, la substance grise péri-apéductale, la partie ventro-latérale de la formation réticulée ponto-bulbaire, la partie antérieure du fascicule pyramidal croisé et aboutissent à la corne latérale de la moëlle épinière (Sager *et al.*, 1957a).

Donc, le cortex cérébral est capable d'influencer les formations souscorticales dans les deux sens. L'activité des formations sous-corticales est réglée par le cortex cérébral en fonction des informations que celui-ci reçoit de la périphérie.

Nous nous sommes attachés à démontrer les voies extra-genouillées capables de transmettre les excitations lumineuses au cortex cérébral (Sager *et al.*, 1955, 1957b).

Chez un animal à néocortex extirpé des deux côtés, avec le lobe frontal conservé d'un seul côté (expérience chronique), nous avons pu démontrer que le rythme de la stimulation lumineuse intermittente (S.L.I.) est approprié par le lobe frontal intacte jusqu'à 10 c/s (notons que chez cet animal les ganglions genouillés externes étaient complètement dégénérés), démontrant que les excitations visuelles se sont transmises au lobe frontal par des voies extra-genouillées, par une collatérale de la voie optique.

Nous avons alors émis l'hypothèse que cette collatérale optique se terminerait dans l'hypothalamus antérieur et que d'ici les excitations visuelles iraient par des voies non-spécifiques au cortex cérébral frontal.

Nos expériences ont été continuées sur des animaux décortiqués des deux côtés (expérience chronique), chez lesquels on a appliqué la S.L.U. et S.L.I. Les résultats montrent que le temps de latence du potentiel évoqué dans l'hypothalamus antérieur

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par la S.L.U. est plus court si on le compare à celui du potentiel évoqué par la S.L.U. dans l'hypothalamus postérieur ou dans la formation réticulée mésencéphalique, et que le rythme de la S.L.I. est approprié par l'hypothalamus antérieur jusqu'à 15 c/s ou même au-dessus de cette fréquence, tandis que pour les autres formations l'appropriation du rythme de la S.L.I. se fait seulement jusqu'à 4–6 c/s. Ces données confirment notre hypothèse que les excitations visuelles peuvent se transmettre à l'hypothalamus antérieur par une voie extragenouillée et d'ici à l'écorce frontale par des voies non-spécifiques diencéphaliques.

Krieg (1928) décrit une collatérale du chiasma optique qui se termine dans l'hypothalamus antérieur. Knoche (1957a,b) met en évidence une collatérale du chiasma optique envoyant des fibres à l'hypothalamus antérieur, à l'infundibulum et à l'hypophyse postérieure. Cette collatérale a une fonction végétativo-endocrine. Nos expériences déjà mentionnées ont démontré que la collatérale extra-genouillée qui arrive à l'hypothalamus antérieur a aussi une fonction visuelle.

Le cortex cérébral est influencé dans son activité par de nombreux systèmes sous-corticaux dont la description est de date assez récente: le système réticulé activateur ascendant de recrutement (Dempsey et Morison, 1943), le système bulbo-réticulé de synchronisation (Moruzzi, 1960, 1963), le système inhibiteur ponto-limbique (Jouvet, 1962), le système caudé (Buchwald *et al.*, 1961). L'activité de ces systèmes qui peuvent avoir différents effets est certainement déterminante pour le degré d'excitabilité du cortex cérébral.

Les expériences que nous avons effectuées sur la fonction *sommeil–veille* ont montré que la destruction de la substance grise péri-apéductale et périventriculaire, ainsi que de certains noyaux de la lamina medullaris interna produit une importante perturbation de la fonction sommeil–veille (l'animal dormait 22 h sur 24, se réveillant seulement pour manger). La fonction du sommeil se rétablissait 2½ mois plus tard, restant normale pour toute la durée de la survie de l'animal. Donc, quoique les zones dynamogène et somnogène du diencéphale (Hess, 1954) ou le bout diencéphalique du système réticulé activateur ascendant (d'après Jasper) eussent été supprimés, ainsi que l'origine du système de recrutement (Dempsey et Morison, 1943), il y a eu quand même un rétablissement de la fonction sommeil–veille (Sager et Mareş, 1955).

L'apparition du sommeil à la suite des lésions de la substance grise péri-apéductale et périventriculaire est moins en relation avec la qualité des noyaux lésés qu'avec l'étendue des lésions, c'est à dire avec la quantité de noyaux dont on a supprimé la fonction; on supprime ainsi les excitations qui du milieu externe et interne se transmettent par ces noyaux à l'écorce cérébrale et servent à en entretenir le tonus d'excitabilité; cependant le fait que la fonction sommeil–veille peut se rétablir après quelque temps montre que les excitations transmises par les systèmes non-spécifiques ne sont pas les seules à entretenir le tonus d'excitabilité du cortex cérébral, mais qu'il est également entretenu par les excitations empruntant des voies spécifiques et peut-être même non-spécifiques, qui ont été éliminées du point de vue fonctionnel de manière transitoire. Ce sommeil par déafférentation explique nos résultats chez les animaux à néocortex extirpé des deux côtés et décérébellés chez lesquels dès la troisième semaine

après l'opération on pouvait provoquer facilement un état de sommeil après l'alimentation, en fermant leurs yeux. On explique de la même manière les états de somnolence et de sommeil notés par nous dans un cas de tumeur kystique du troisième ventricule (Drăgănescu et Sager, 1935), la distension du kyste provoquant périodiquement de la somnolence et du sommeil. Les cas de mutisme akinétique que nous avons poursuivis (Sager et Mares, 1961), où il existait des lésions au niveau de certains noyaux non-spécifiques diencéphaliques s'étendant aussi à la formation réticulée mésencéphalique, se caractérisaient par l'impossibilité du malade de prendre contact avec le monde extérieur même à l'état de veille (déglutition normale, possibilité de suivre ce qui se passait autour de lui mais impossibilité de parler ou d'exécuter ce qu'on lui commandait). Ceci montre une fois de plus l'importance de cette région diencéphalo-mésencéphalique pour l'entretien du tonus d'excitabilité du cortex cérébral (Marinescu *et al.*, 1929a,b) et la corrélation entre l'intensité des symptomes observés et l'étendue des lésions.

Nos expériences complétant certaines recherches antérieures (Marinescu *et al.*, 1929a, b) montrent que l'injection de $CaCl_2$ (quelques gouttes 2%, pH 7.3) dans le troisième ventricule provoque le sommeil avec synchronisation de l'activité électrique corticale et une légère diminution de la tension artérielle. L'injection de KCl (quelques gouttes en solution 2%, pH 7.3) produit un état d'agitation (chez l'animal non-curarisé des accès d'épilepsie) et, du point de vue EEG, l'apparition de complexes pointe–onde, de nombreuses pointes, ondes bifides très amples ainsi qu'une importante élévation de la pression artérielle. Ces modifications EEG apparaissent également chez l'animal 'encéphale isolé' (en présence d'une tension artérielle normale) (Sager, 1960).

Des expériences relativement récentes de Hess (1954) chez le chat et de Jung (1957) chez l'homme en excitant le thalamus médian ont provoqué soit le réveil, soit la somnolence, la réponse dépendant de l'intensité et la fréquence de la stimulation électrique (sommeil par stimulus électrique à basse fréquence ou à petite intensité, réveil par stimuli à haute fréquence et à grande intensité). Le sommeil provoqué par l'excitation électrique du thalamus moyen utilisant des stimuli électriques à basse fréquence ou à petite intensité peut s'expliquer dans le sens que les stimuli à petite intensité répétés produisent l'inhibition de certains groupes de cellules corticales où sont arrivés ces stimuli, inhibition dont l'extension sur le cortex cérébral provoque l'apparition du sommeil (d'après Pavlov, 1926). Donc, il n'existe pas de centres spéciaux au niveau du diencéphale pour la fonction de la veille ou pour celle du sommeil, mais seulement des groupes cellulaires recevant les excitations du milieu interne et externe qui sont ensuite transmises au cortex cérébral pour en entretenir le tonus d'excitabilité. Leur suppression favorise l'apparition du sommeil.

Le sommeil normal s'accompagne d'une diminution du tonus d'excitabilité corticale, provoquée soit par la fatigue des cellules corticales qui se transmet aux formations souscorticales (Bremer, 1954), soit par l'inhibition envahissant d'abord le cortex cérébral et puis les centres souscorticaux (Pavlov, 1926). Cette diminution du tonus d'excitabilité corticale est pour la plupart uniforme dès le début, permettant ainsi la décharge synchrone d'un grand nombre de cellules corticales. Plus tard, soit par l'accentuation des processus ci-devant mentionnés, menant à l'apparition du sommeil,

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soit par l'intervention du système inhibiteur de Jouvet (système ponto-limbique), l'activité des différents groupes cellulaires se désorganise, les uns étant inhibés plus et les autres moins; quelques cellules peuvent même augmenter leur excitabilité à cause de la perte de leurs connexions synaptiques avec d'autres groupes de cellules réglant leur activité. C'est ainsi qu'apparaît le sommeil caractérisé par la désynchronisation de l'activité électrique corticale (sommeil profond à rythme rapide). Rappelons que le sommeil à ondes rapides (le sommeil avec désynchronisation de l'activité électrique corticale) n'apparaît jamais dès le début; il se présente après le sommeil à ondes lentes (synchronisation EEG). Il reste encore à démontrer si entre la période du sommeil à ondes lentes (sommeil passif) et celle du sommeil à ondes rapides (sommeil actif) interviendrait le système synchronisant de Moruzzi (1960).

Les études sur les relations cortico-souscorticales ont porté aussi sur l'élaboration des réflexes conditionnés chez les animaux à néocortex extirpé des deux côtés. Les animaux à néocortex extirpé peuvent encore discriminer la lumière de l'obscurité (Sager, 1960; Sager *et al.*, 1958).

On a également conditionné chez l'animal à néocortex extirpé la réaction végétative-affective lors de l'établissement d'un réflexe de défense. On a conditionné la sécrétion salivaire (Sager et Cincă, 1962) et, chez un animal à néocortex extirpé des deux cotés et à petit estomac Pavlov, la sécrétion gastrique qui présentait les qualités spécifiques comme pour l'excitant adéquat (l'injection d'histamine, respectivement l'alimentation de l'animal avec du pain et du lait) auquel était associé l'excitant conditionnel (Sager *et al.*, 1961). Le réflexe conditionné s'éteignait très vite chez tous les animaux décortiqués sans permettre d'obtenir une différentiation fine.

Chez tous ces animaux, le contrôle anatomique a montré l'extirpation du néocortex entier, avec dégénération rétrograde — entre autres, des ganglions genouillés externes et internes aussi bien que des noyaux spécifiques du thalamus.

Chez l'animal à néocortex extirpé et rhinencéphale lésé des deux côtés, l'élaboration même de ces réflexes élémentaires était impossible. D'autre part, la lésion bilatérale seulement du rhinencéphale provoquait, en dehors de l'hyperoralité (syndrome de Klüver–Bucy) et la boulimie, une impossibilité de former de nouveaux réflexes conditionnés; il y avait aussi une profonde altération des réflexes qu'on avait conditionnés avant l'extirpation, les animaux ne pouvant plus différencier les excitants positifs de ceux négatifs (Sager et Cincă, 1962).

Tenant compte de ces données, nous avons admis que lors de l'élaboration du réflexe conditionné, une première liaison temporaire se formerait au niveau du rhinencéphale; l'excitant conditionnel acquiert à ce niveau une importance biologique par la charge végétative-affective qu'il y reçoit au cours de son association avec l'excitant non-conditionné. L'extinction rapide de ce réflexe conditionné s'explique — semble-t-il — par le fait que les excitations se transmettent au rhinencéphale par des voies non-spécifiques, l'habituation s'installant ainsi rapidement.

La question qui se pose est de savoir par où se transmettent à l'hippocampe les excitations algiques, auditives et visuelles chez les animaux à néocortex extirpé des deux côtés, ayant en vue la dégénération des noyaux spécifiques du thalamus chez ces animaux.

Les études EEG (Sager et Butkhuzi, 1962) démontrent la possibilité d'évoquer un potentiel électrique dans l'hippocampe et l'amygdale par un stimulus électrique unique

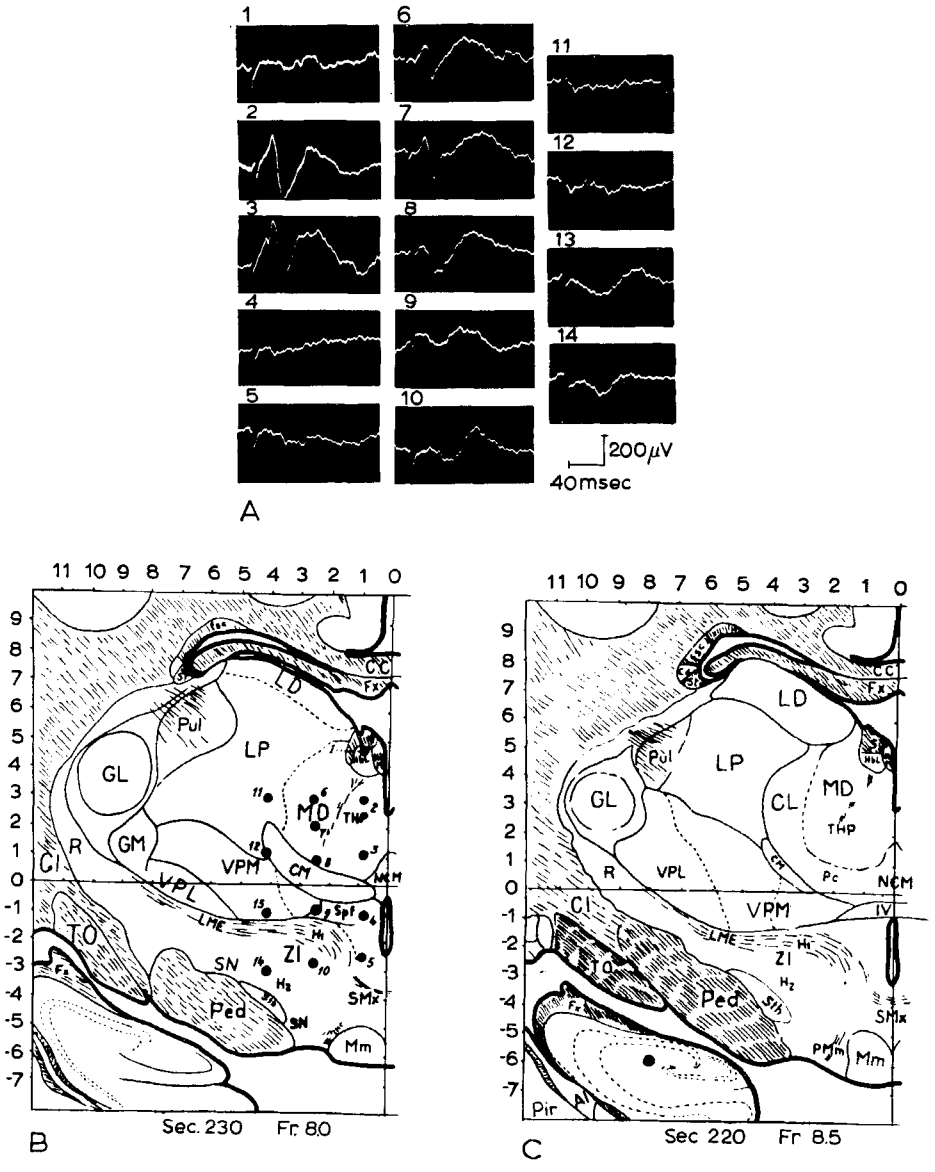

Fig. 3. A, les potentiels électriques dérivés de l'hippocampe après l'application d'un seul choc électrique (15 V; 0.5 msec) aux divers points du noyau dorsomédian et du diencéphale. On observe que l'amplitude des potentiels évoqués par l'excitation des points 2 et 3 (situés dans la partie interne du noyau dorsomédian) est plus grande que celle des potentiels évoqués par l'excitation des points 6, 7, 8 (situés dans la partie externe du noyau dorsomédian). L'excitation des autres points du diencéphale évoque des potentiels électriques à amplitude minime (à remarquer que la stimulation du point 9 a été faite avec 20 V; 0.5 msec). B, topographie des divers points stimulés (carte d'après Jasper et Ajmone Marsan, 1954). C, le point hippocampique d'où le potentiel électrique évoqué a été dérivé.

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appliqué sur la partie interne du noyau dorsomédian. Le temps de latence de ce potentiel est très petit (Fig. 3). D'autre part, nous avons obtenu (Sager et Mares, 1957) une dégénération rétrograde de la partie interne du noyau dorsomédian qui apparaît après l'extirpation de l'hippocampe. Ces données montrent ainsi qu'il existe une voie directe entre la partie interne du noyau dorsomédian et l'hippocampe et l'amygdale; c'est par cette voie que se réalise la transmission la plus rapide des excitations extéro- et intéroceptives de l'hypothalamus à l'hippocampe.

Chez deux animaux décortiqués des deux côtés, longtemps tenus en observation après l'opération, des accès de rage sont apparus après 24–28 mois. Ils se mordaient les membres et salivaient fort abondamment. Le contrôle anatomique a révélé chez ces animaux l'extirpation du néocortex des deux côtés et la lésion bilatérale du rhinencéphale, ainsi qu'une lésion de la partie caudale du noyau amygdalien (certaines lésions sont probablement apparues à la suite de l'oblitération vasculaire tardive). La question se pose si ces accès de 'sham rage' ne seraient pas dûs à l'irritation de la partie orale de l'amygdale.

Toutes ces données montrent qu'en dehors des mécanismes de régulation cortico-souscorticale, il existe une étroite liaison, une interdépendence, entre le néocortex et le paléocortex qui est absolument nécessaire pour un bon fonctionnement de l'activité nerveuse supérieure.

Il résulte encore de ces données que le cortex cérébral reçoit des informations des milieux externe et interne et qu'il envoie à son tour des stimuli aux formations sous-corticales, influençant et modifiant ainsi leur activité justement en rapport avec les informations reçues. C'est de cette manière que se maintient la constance du milieu interne dans les conditions variables des milieus interne et externe et c'est de la même façon que se réalise un certain tonus d'excitabilité du cortex cérébral nécessaire à l'analyse et à la synthèse des différentes excitations reçues, ainsi qu'à leur intégration dans une activité coordonnée, destinée à remplir certaines fonctions.

Les circuits réverbérants cortico-souscortico-corticaux, qui comprennent des voies de facilitation et d'inhibition dans les deux sens, ayant des points de relai dans les noyaux spécifiques et non-spécifiques corticaux et souscorticaux expliquent par des processus d'information et de réinformation la modalité d'action des mécanismes d'autorégulation dans les corrélations cortico-souscorticales ainsi que la physiopathologie de certaines manifestations cliniques.

RÉSUMÉ

Nos expériences effectuées sur des animaux décortiqués ont montré que le cortex cérébral joue un rôle essentiel dans le contrôle de l'activité des centres souscorticaux en relation avec l'innervation cardiaque, gastro-intestinale, avec certains aspects du métabolisme glucidique et la trophicité de la peau.

En absence du cortex cérébral, il apparaissent des modifications non seulement quantitatives mais aussi qualitatives de l'activité des centres souscorticaux qui sont particulièrement évidentes quand l'organisme doit s'adapter aux modifications du milieu externe ou interne.

Sechenov (1863) a montré que des stimuli inhibiteurs partant des lobes optiques de la grenouille augmentent le temps réflexe de Türck. Rosenberg et Sager (1931) ont démontré qu'une section derrière les lobes optiques d'une grenouille fortement illuminée auparavant fait augmenter (de 10% environ) la vitesse de conduction du nerf périphérique.

Le cortex cérébral exerce une influence inhibitrice sur les potentiels électriques évoqués par des stimuli visuels dans les noyaux non-spécifiques diencéphaliques, dans la formation réticulée et le cervelet. L'influence du cortex cérébral sur les centres souscorticaux est non seulement inhibitrice mais aussi facilitatrice. Le réflexe presseur du sinus carotidien diminue fortement après la décortication bilatérale (expérience chronique); au surplus, le gyrus sigmoïde antérieur peut faciliter la fonction des centres vasomoteurs souscorticaux et nous avons pu suivre les voies depuis ce gyrus jusqu'à la corne latérale de la moelle thoracique.

Nos données expérimentales et anatomo-cliniques ont montré que la fonction sommeil–veille n'a pas de centres spéciaux; cette fonction est maintenue par beaucoup de circuits réverbérants à valeur fonctionnelle diverse ayant des relais dans les noyaux spécifiques aussi bien que non-spécifiques des structures souscorticales et du cortex cérébral.

Les stimuli visuels peuvent arriver à l'hypothalamus même dans les conditions d'une complète dégénération des corps genouillés. Chez les animaux décortiqués bilatéralement avec le lobe frontal conservé d'un côté, le rythme de la stimulation lumineuse intermittente est approprié (jusqu'à 8–10 par sec) dans le lobe frontal intact à la même fréquence que dans l'hypothalamus antérieur. La transmission des stimuli visuels à l'hypothalamus antérieur explique aussi l'effet de la lumière sur les processus endocrino-végétatifs.

Nous avons pu obtenir des réflexes conditionnés visuels, gastriques, de défense et salivaires (très élémentaires) chez les animaux décortiqués bilatéralement avec paléocortex intact. Par des études anatomiques et électroencéphalographiques, nous avons montré que les différentes excitations sensitives peuvent arriver à l'hippocampe par la partie interne du noyau dorsomédian.

Les circuits réverbérants cortico-souscortico-corticaux (comprenant des voies inhibitrices et facilitatrices en deux directions par des mécanismes d'information et réinformation peuvent expliquer les processus d'autorégulation des relations cortico-souscorticales ainsi que la physiopathologie de certaines manifestations cliniques.

SUMMARY

Mechanisms of selfregulation in the cortical–subcortical correlations

Our experiments on decorticated animals have shown that the cerebral cortex plays an essential role in the control of the activity of subcortical centres with gastro-intestinal and cardiac innervation, as well as with some aspects of glucose metabolism and skin trophics.

In absence of the cerebral cortex, there appear not only quantitative but also

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qualitative changes in the activity of the subcortical centres, which are particularly conspicuous when the organism must adapt itself to changes in the external or internal milieu.

Sechenov (1863) showed the existence of inhibitory stimuli from the frog's optic lobes increasing the 'Türck reflex time'. Rosenberg and Sager (1931) demonstrated that a section behind the optic lobes of a previously strongly illuminated frog causes an increase (about 10%) in conduction velocity in the peripheral nerve.

The cerebral cortex exerts an inhibitory action on electrical potentials evoked by visual stimuli in the diencephalic unspecific nuclei, in the reticular formation and cerebellum. The effect of cerebral cortex upon subcortical centres is not only inhibitory but also facilitatory. The carotid sinus pressor reflex strongly decreases after bilateral decortication; moreover, the anterior sigmoid gyrus can facilitate the function of subcortical vasomotor centres. We have been able to demonstrate the pathways from the anterior sigmoid gyrus to the lateral horn of the thoracic spinal cord.

Our experimental and anatomo-clinical findings showed that there are no special centres for the sleep–wakefulness mechanism; this function is maintained by numerous reverberating circuits of different functional value, having relay stations in both unspecific and specific nuclei of subcortical structures and in the cerebral cortex.

Visual stimuli can reach the hypothalamus even when the external geniculate bodies are fully degenerated. In bilaterally decorticated animals with unilaterally preserved frontal lobe a photic driving occurs (up to 8–10 per sec) in the frontal lobe and in the anterior hypothalamus. The transmission of visual stimuli to the anterior hypothalamus explains the effect of light on vegetative-endocrine processes.

We have been able to obtain salivary, defensive, gastric and visual conditioned reflexes (very elementary) in bilaterally decorticated animals with preserved paleocortex. In this connection, we have shown anatomically and electroencephalographically that the various stimuli can reach the hippocampus *via* the inner part of the dorsomedian nucleus.

The cortico-subcortico-cortical reverberating circuits (including two-way inhibitory and facilitatory pathways) through information and re-information mechanisms may explain the self-regulation processes in the cortico-subcortical relationships as well as the pathophysiology of certain clinical manifestations.

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The Contingent Negative Variation: An Electro-Cortical Sign of Sensori-Motor Reflex Association in Man

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As previously reported (Walter, 1963), the electric responses evoked in nonspecific cortex by significantly associated sensory stimuli interact so that the vertex-negative component of the conditional responses is augmented while that of the unconditional responses is attenuated. Until recently, the records showing these effects were taken with amplifiers using rather short coupling time constants (0.3 sec). In most of these records a secondary negative wave was seen between the conditional and unconditional

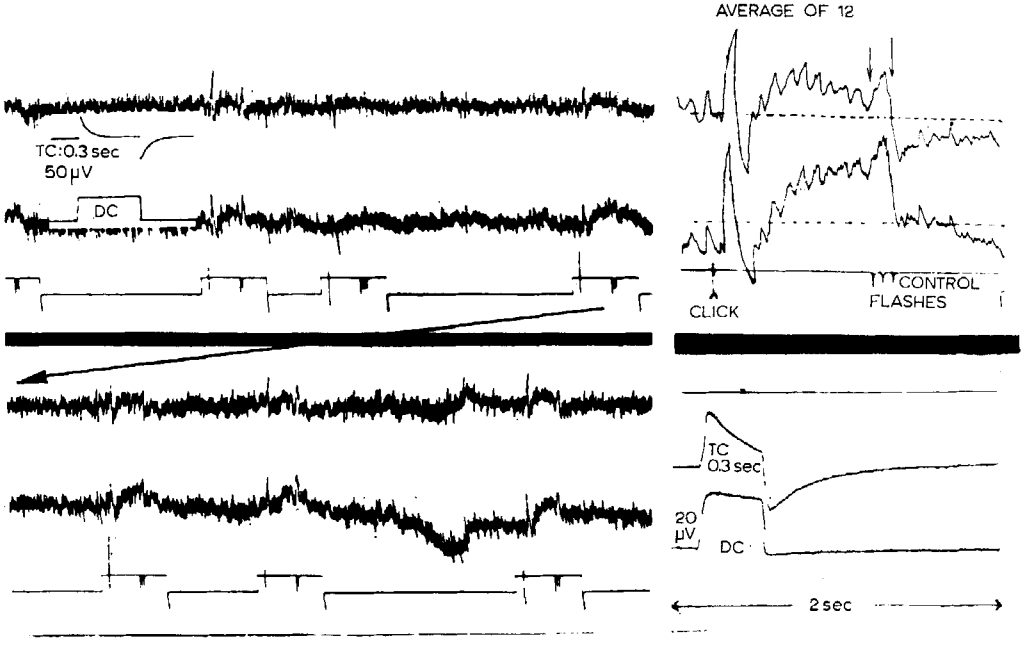

Fig. 1. Primary records, with corresponding averages, showing the attenuation and distortion of the Contingent Negative Variation with a coupling time constant (TC) of 0.3 sec (channel 1), compared with direct coupling (DC channel 2). Seven of the twelve trials incorporated in the average are illustrated. Both channels were connected between vertex and mastoid electrodes, an upward deflection indicating negativity at the vertex.

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responses; this negative component developed so slowly and lasted so long that it was decided to use direct coupling (DC) or very long time constants, in order to obtain a better reproduction of the time course of the slowest changes (Fig. 1). Specially selected and prepared silver–silver chloride electrodes were used to reduce steady electrode potential differences and baseline drift. Satisfactory conditions for DC recording can be maintained for several hours while the subjects undergo a form of conditioning. The intrinsic brain activity is analysed automatically 'on-line' with a two-channel wave-analyser, and the evoked responses are extracted with a two-channel barrier-grid averager (Cooper and Warren, 1961). Six, 12 or 24 samples of the evoked responses are stored and the averages written out directly on the original record obtained with a 16-channel Offner Type T.C. instrument. Other channels record stimuli, pulse rate, respiration, palmar resistance and the EMG of the operant muscles used for the motor responses by the subject.

With this arrangement the interaction between the conditional and unconditional responses has been found to depend on the development of the slow negative potential which follows the conditional response. This has been designated the Contingent Negative Variation (CNV) because its appearance and amplitude reflect only and

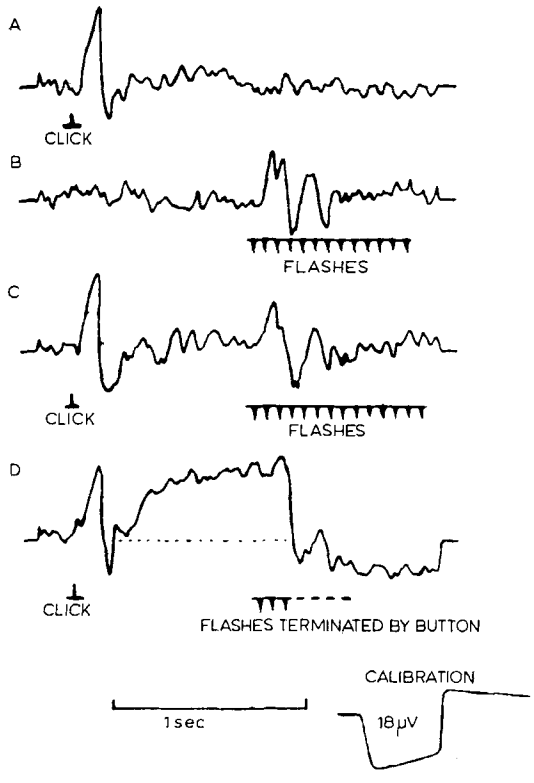

Fig. 2. Development of the Contingent Negative Variation (CNV). Averaged responses to 12 presentations of stimuli indicated: (A) Single clicks. (B) Flashes at 15 per sec. (C) Clicks associated with flashes. (D) Clicks associated with flashes which the subject could terminate by pressing a button. The Contingent Negative Variation appears only with operant control following instruction. An upward deflection indicates electronegativity of the vertex with respect to the mastoid process.

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precisely the significance of the association between stimuli over a wide range of intensities, and the attitude of the subject to the association.

The development of the CNV and its interaction with the unconditional responses are illustrated in Fig. 2 which is from a normal adult subject. The averages of 12 responses to isolated clicks (Fig. 2, A) consist of a small positive wave almost obscured by a much larger negative component. The responses to isolated trains of flashes (Fig. 2, B) show several brief negative components, also superimposed on a small positive deflection. When the clicks are paired with the flicker about one second later (Fig. 2, C) both responses are reproduced with little alteration. When the subject is instructed to press a button in response to the second stimulus the addition of an operant response (Fig. 2, D) is accompanied by a pronounced change in the conditional response to the clicks; a large CNV appears, rising to the peak amplitude of the

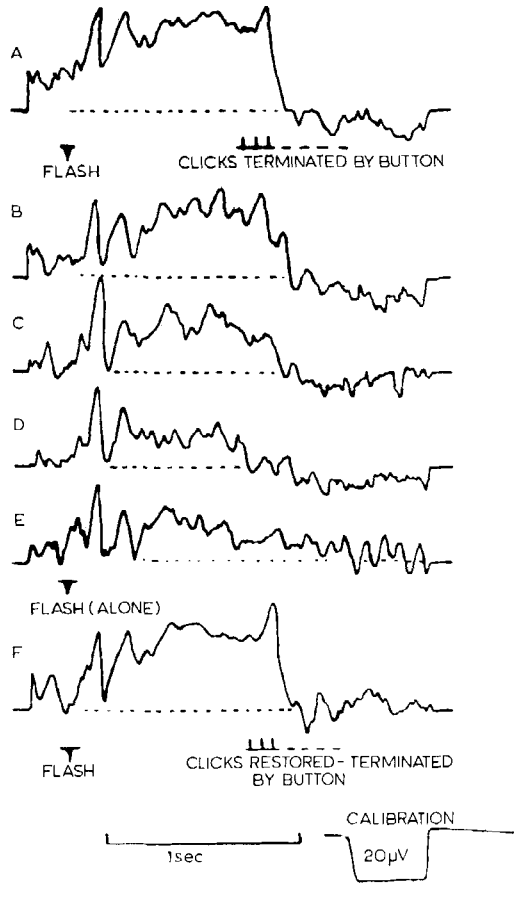

Fig. 3. Extinction of the CNV. Averaged responses to 6 presentations. (A) Flashes associated with clicks terminated by the subject pressing the button. The CNV is fully developed and falls with the operant response. (B) The average of the first 6 presentations of unreinforced flashes. The CNV is reduced and there is still a 'conditioned' response one second after the flash. (C–E) The progressive decline in the CNV as extinction proceeds. (F) Re-appearance of the CNV with restoration of the unconditional stimulus and operant response.

unconditional response during the interval between them. The unconditional response itself is now seen merely as a sharp drop in potential difference to the base line. This pattern is maintained indefinitely as long as the subject is attentive and presses the button promptly. It is the same whatever the modalities of the first and second stimuli (Fig. 3, A). However, when the unconditional stimulus is withdrawn, the responses to the reinforced conditional stimuli show a progressive deflation of the CNV which disappears almost entirely after 30 unreinforced trials (Fig. 3, A–E). Restoration of the unconditional auditory stimulus re-establishes the CNV after about 12 trials (Fig. 3, F). The dependence of the CNV on the contingency of the association between the two stimuli is shown most clearly when their relation is made equivocal. After a long series of regular associations of flashes with clicks (Fig. 4, A) the probability of significance was 'diluted' by presentation of 27 unreinforced flashes

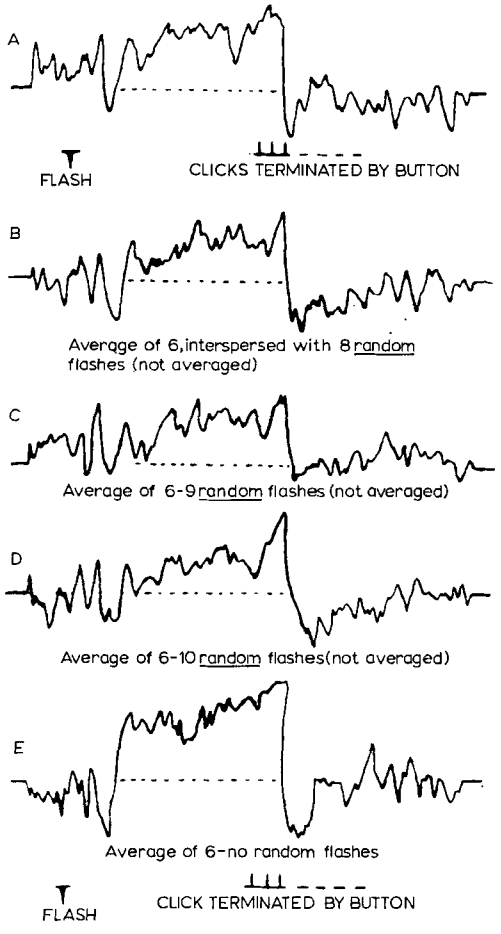

Fig. 4. Attenuation of the CNV by equivocation. Averaged responses to 6 presentations. (A) The CNV after repeated association of flashes with clicks and operant responses. (B) Attenuation of the CNV and re-appearance of the negative response to the clicks after presentation of 14 flashes only 6 of which were followed by clicks. (C–D) Progressive attenuation by continued equivocation (48 flashes of which 21 were reinforced). (E) Restoration of CNV by 6 unequivocal presentations.

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at random between 20 associated pairs, providing only 21 out of 48 reinforcements. Averages taken during this series of equivocal presentations show that the CNV is reduced to about half its previous amplitude, while the unconditional response re-appears as a negative wave (Fig. 4, B–D). In most normal adult subjects the CNV shows signs of attenuation when the probability of occurrence of the association drops below 0.7 over 12 or more trials, but both the total number of trials and the critical proportion of reinforcements needed for suppression of the CNV vary considerably from subject to subject. Unequivocal restoration of the unconditional stimulus once again brings about a rapid re-establishment of the CNV and absorption of the unconditional negative wave (Fig. 4, E).

Another factor which influences the form of the CNV is the attitude of the subject. During one experiment the subject was told that during a long series of unequivocal presentations of clicks followed by flashes he could decide not to press the button for the operant response. The average of 6 of these trials showed almost complete absence of a CNV (Fig. 5, A), but this re-appeared immediately when the subject decided to resume the operant response. The CNV is also susceptible to social stimuli in the form of verbal statements. When the unconditional stimulus was withdrawn without notice or warning (Fig. 5, B–E) the change in the pattern of physiological stimuli ultimately suppressed the CNV, but only after about 30 trials. In contrast,

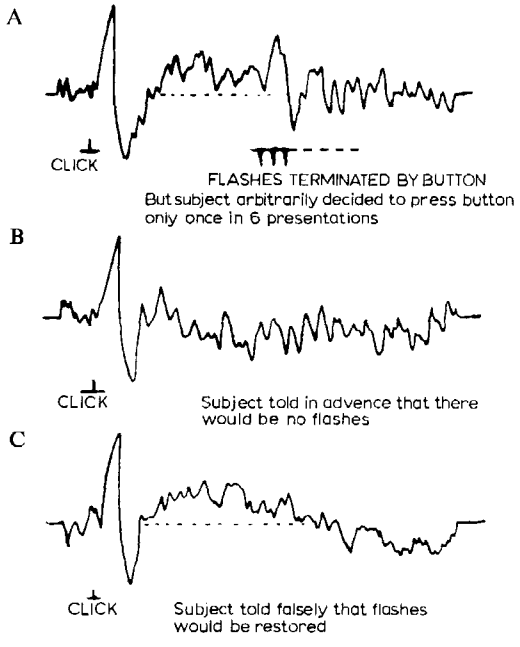

Fig. 5. The effects of attitude and verbal extinction. During a long series of clicks followed by flashes and operant responses, the subject decided not to press the button; the CNV disappeared immediately (compare with Fig. 1, C). (B) The subject was told that there would be no more flashes following the clicks. The CNV disappeared at once, and was replaced by a small *positive* variation. (Compare with Fig. 2, B). (C) The subject was told that the flashes would be restored but this was not done; the first three presentations showed a CNV, the last three none, and the average indicates this proportion.

when the subject was told: 'There will be no more flashes', the CNV disappeared immediately (Fig. 5, B). In this objective sense therefore, progressive changes in subjective expectancy produced by repetitive physiological experience can be matched against those established by a single phrase. There may be a qualitative as well as a quantitative difference between the effects of physiological and social suppression. In Fig. 3, B the first average after withdrawal of the visual unconditional stimulus, the CNV is slightly smaller than during reinforcement, but there is a small negative wave followed by an abrupt change in potential difference exactly one second after the conditional stimulus, that is at the instant when the unconditional stimuli would have been expected. Bearing in mind that this is the average of 6 trials and that the second, third and fourth sets of unreinforced trials show steadily diminishing signs of this effect, it may be regarded as a true 'conditioned response' induced by the long experience of unequivocal presentations and extinguished as the probability of association declined when the unconditional stimuli were withheld. There was no sign of this conditioned response when the subject was told beforehand that there would be no reinforcement (Fig. 5, B); in fact, with this 'social extinction' there was a slight slow *positive* variation following the conditional response. Before the next series the subject was told that the flashes would be restored, but this was not done; the first 3 trials showed an appreciable CNV but the second 3 showed none, and the average consequently contained only a small CNV (Fig. 5, C). These features reflected accurately the subjective reports of fluctuations in expectancy induced by the conflict between the verbal and physiological experiences.

The contingent interaction between conditional and unconditional responses is further illustrated by the effect of changes in intensity of the unconditional stimulus. In one set of experiments in which flashes were used as conditional and loud repetitive clicks as unconditional stimuli the interaction pattern was quite similar to that with the relations reversed (Fig. 6, A), but when the intensity of the unconditional clicks was reduced by 47 dB to a barely audible level the amplitude of the CNV decreased by only about 50% and the unconditional response was still represented only by the sharp termination of the negative variation (Fig. 6, B). The subject stated that the buzzing noises alerted him, but the physiological effect was similar to that of equivocation by unreinforced conditional stimuli. However, continuation of the series of interfering (and of course irrelevant) stimuli had progressively less and less effect on the CNV until, after 53 presentations with 35 interspersed buzzes, the unconditional response was again represented only by a sharp drop in potential of the CNV (Fig. 6, E). In general, irrelevant distracting stimuli have progressively less effect, whereas equivocal stimuli identical with the conditional ones attenuate the CNV more and more as the contingent significance declines, provided that there is no discernible pattern (such as alternation) in the distribution of reinforced and unreinforced stimuli by which the subject can learn to predict the likelihood of reinforcement.

The effect of an irrelevant stimulus interposed between the conditional and unconditional stimuli has also been studied. Fig. 6, F is the average of 6 presentations in all of which a buzz was interposed about halfway between the conditional and unconditional stimuli. The effect of this was to drop the CNV to about half its peak

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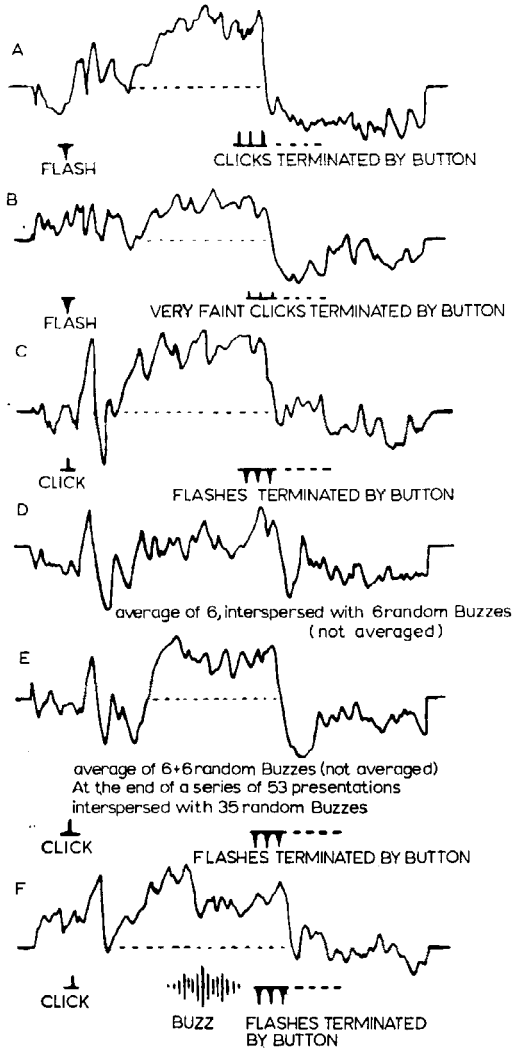

Fig. 6. The effect of signal attenuation and distraction. (A) The CNV with flashes associated with loud clicks and operant responses. (B) The CNV diminishes only slightly when the intensity of the clicks is reduced by 47 dB. (C) The CNV when clicks are associated with flashes and operant responses. (D) The first 6 presentations of clicks and flashes with 6 buzzes interspersed between them; the CNV is reduced as during equivocation (Fig. 3, B). (E) After 53 presentations with 35 random buzzes the distraction effect has faded and the CNV is restored. (Compare Fig. 3, D). (F) When the buzzes are interspersed between the conditional and unconditional stimuli the CNV is reduced by about half.

value and thus to disclose the negative component of the unconditional visual response.

The relation between the development of the CNV and the operant response is quite subtle in these experiments; the first responses are made in compliance with a personal request, but the circuit is set so that pressing the button has no detectable effect on the situation, while later the responses are arranged to terminate the unconditional stimulus (which is rather disagreeable but not painful). In these latter

conditions pressing the button could be considered an avoidance response rather than a purely social one. Later still, when the subject has realised that anticipation is useless, the situation develops a more challenging aspect and many subjects try very hard to shorten their reaction times by using the conditional stimulus as a warning but without 'jumping the gun'. As a result the normal reaction times to auditory stimuli of about 180 msec often fall to about 100 msec or even less. It was assumed at first that in such cases the subjects were merely learning to respond at the correct interval after the conditional warning, but it was noticed that when the conditional stimulus was given alone, either in an extinction or an equivocation series, the subject very seldom made even one false response as indicated by the electromyogram of the operant muscles. Measurement of the reaction times in associated presentations showed that they were not distributed normally around the target time; the distribution was very skew with a peak near the shorter durations. These observations indicate that

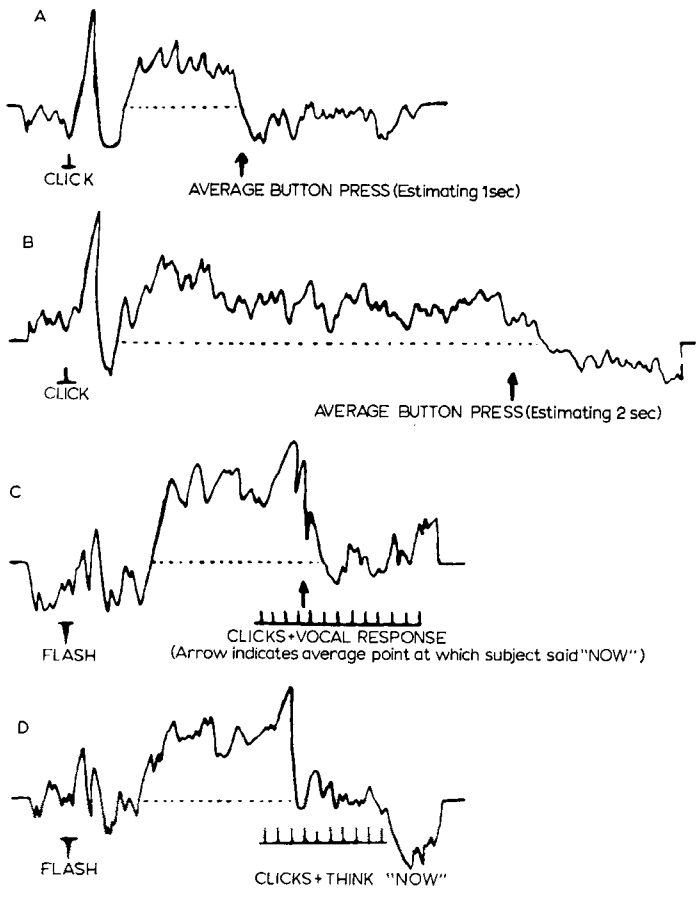

Fig. 7. Termination of CNV by subjective time estimations, vocal responses and mental responses. (Average of 6 presentations). (A) Pressing button at estimated 1 sec after the click. (B) Pressing button at estimated 2 sec after the click. (C) Subject instructed to say 'Now' in response to clicks preceded by flash. (D) Subject instructed to *think* 'Now' in response to clicks preceded by flash.

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in this situation the conditional stimulus actually abbreviated the reaction time to the unconditional stimulus. The close correlation between attentiveness, contingent significance, operant response and the amplitude of the CNV suggests that this feature may be the electric sign of cortical 'priming', whereby the motor response to the associated stimuli is accelerated and synchronised, the final detonation being indicated by the abrupt decline in the CNV about 120 msec after the unconditional stimulus, corresponding with the positive component of the isolated unconditional response.

The termination of the CNV is certainly not an 'off-effect', since it occurs even when the effector response does not abbreviate the unconditional stimulus, as in the preliminary phases of this procedure.

A further pointer to the direct association of the CNV with the making of an operant response has been found in its occurrence when the conditional stimulus is not followed by the unconditional stimulus, provided that the subject is instructed to estimate a short time interval, for example 1 sec, before pressing the button (Fig. 7, A, B). Once the CNV is established it can be elicited even when an alternative motor response is substituted. For example, in one experiment, in which the CNV had been associated with a situation involving a single flash followed by clicks to which a button had to be pressed, the subject was told to say 'Now' to the unconditional stimulus instead of pressing the button (Fig. 7, C). Later in the same experiment the subject was told to think, but *not* say, 'Now' (Fig. 7, D). In this last situation the CNV was found to decline slightly with successive trials, but the rate of decline was considerably slower than that seen in other series in which the unconditional stimulus had been withdrawn without comment (Fig. 3) or in which the subject had been told simply to stop making the operant response. Lengthening the interval between the conditional and unconditional stimuli in a situation requiring an operant response to the unconditional stimulus brought about an equivalent lengthening of the CNV (Figs. 7, B and 8, C). For the first presentations at the longer interval the CNV continued to reach its peak at the end of the previously 'conditioned' shorter interval. But as the new interval became established the CNV rose steadily to reach its peak roughly coincident with the onset of the unconditional stimulus.

The discovery that the substitution of a verbal warning, 'Ready', for the conditional flash or click would produce a CNV (Fig. 8, D), led to the substitution of verbal stimuli, in the form of the experimenter saying 'Ready ..... Now', for both the conditional and unconditional stimuli. Once again the CNV was found to occur provided that an operant response had to be made to the unconditional 'Now'. (Fig. 8, E).

The mechanisms underlying this remarkable feature can be inferred from other sources. Records from electrodes implanted in frontal cortex (Crow *et al.*, 1963) show similar effects, but smaller in proportion to the other components of the sensory response. As far as can be ascertained the electric field of the CNV involves a very large area in the frontal regions, suggesting the participation of a small but widely distributed proportion of cortical elements. Comparison with subdural recordings suggests that the negative potential spreads from the anterior frontal cortex back to the pre-motor zone in about 0.5 sec (Fig. 9). The largest potential difference

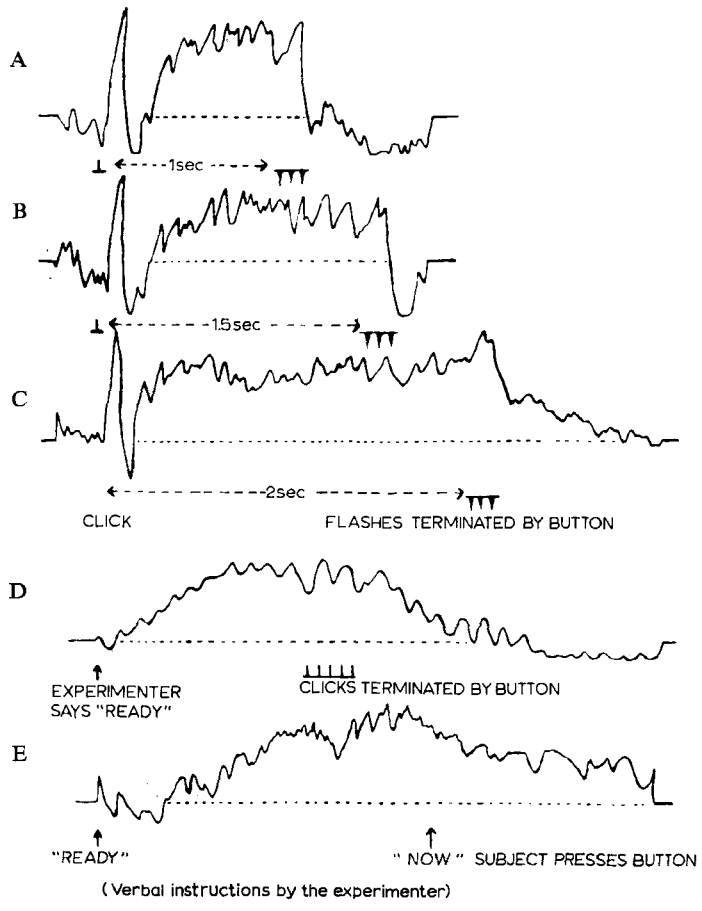

Fig. 8. Extension of CNV to longer intervals and CNV evoked by verbal signals. (Average of 6 presentations). (A) 1 sec interval between stimuli. (B) 1.5 sec interval. (C) 2 sec interval. (Compare with Fig. 6, B). (D) Conditional warning given by experimenter saying 'Ready', unconditional stimulus clicks 1.2 sec later. (E) Conditional and unconditional verbal stimuli given by experimenter.

recorded so far on the scalp is about 40 μV between vertex and mastoid process and in some subjects this has been maintained for 10 sec or more in early extinction trials when the conditional stimuli have been given alone after long association. The CNV is often quite visible in the primary records, though obscured by the intrinsic rhythms. The possibility of the effect being due to an extra-cerebral source, such as eye or scalp movements or changes in skin resistance, has been eliminated by the usual control experiments and the most likely explanation is that the potential change is due to widespread depolarisation of the apical dendrites in the feltwork of the upper layers of the frontal cortex. The participation of neuroglial as well as neurodendritic processes in the development of the CNV cannot of course be excluded. It is rather surprising that so large and extensive an effect in the brain should be associated with the trivial act of deciding when to press a button, but the amplitude, both in scalp and intra-cerebral recordings, indicates that only a small proportion — perhaps only

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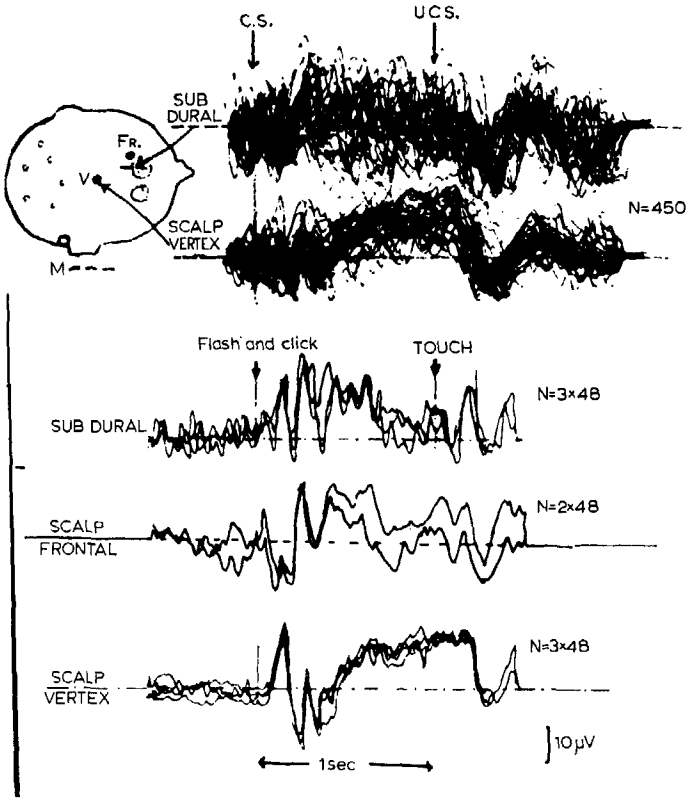

Fig. 9. Comparison of CNV from cortex and scalp. Anterior frontal subdural and scalp derivations show CNV starting earlier than at vertical electrode, suggesting a process moving antero-posteriorly.

about 1% — of the available neuronic elements is involved, so that there would be an ample reserve for more complex tasks.

Although comparisons between human and other species are notoriously deceptive, it is encouraging to note that Rowland and Goldstone (1963) have observed very similar effects in cats with implanted cortical electrodes during conditioning; they interpret these as 'extension of an electrical arousal effect in terms of activation of cortical structures based on relation to drive'. Rusinov (1960) also described slow negative waves recorded by Shvets (1958) which appeared in the cortex of rabbits simultaneously with the first appearance of conditioned movements and vanished during extinction. As would be expected from the Pavlovian description of conditioning, the slow waves described by Rusinov were 'generalised' in the early stages of association and 'concentrated' later as the conditioned responses were consolidated. This effect has not been seen in the human subjects of the experiments reported here, but it should certainly be looked for in more elaborate situations providing higher spatial resolution. More recently, Roitbak (1963) has described slow surface negative potentials which are larger following the first of two paired stimuli, and this is probably the same phenomenon.

As already mentioned, the electric field of the CNV is generally limited to the anterior

and central parts of the head, including frontal association, motor and somatosensory zones, but in some subjects it extends much further back. There is some indication that variations in the spatial extent of the CNV in different people are related to its contingent responsiveness. Subjects in whom the CNV spreads to the parieto-occipital regions tend to retain a negative variation longer during equivocation or extinction than do those in whom the field is more restricted. This relation may provide a link between the functional anatomy of the brain and the lability or versatility of the associational mechanisms in different people, and as affected by mood or medication.

The clinical application of these methods to the study of neuropsychiatric disorders has already shown surprisingly close correspondence between the contingent interaction of responses to associated stimuli and the mental state of disturbed patients.

The relation of the CNV to the cortical processes of excitation and inhibition is clearly illustrated in these records and is entirely predictable when the statistical relation between the stimuli and the attitude of the subject are known. The response of the subject in most of these experiments has been a 'positive' one, pressing a button, but the CNV develops in the same way when the action is 'negative', that is when the subject is told to 'stop' pressing the button rhythmically or continuously; it is necessary only that the subject should make some mental effort in response to the second stimulus (Fig. 10). Even when the stimulus is purely intellectual, such as solving a mathematical problem, a CNV can be detected by synchronising the problem with the averaging epoch.

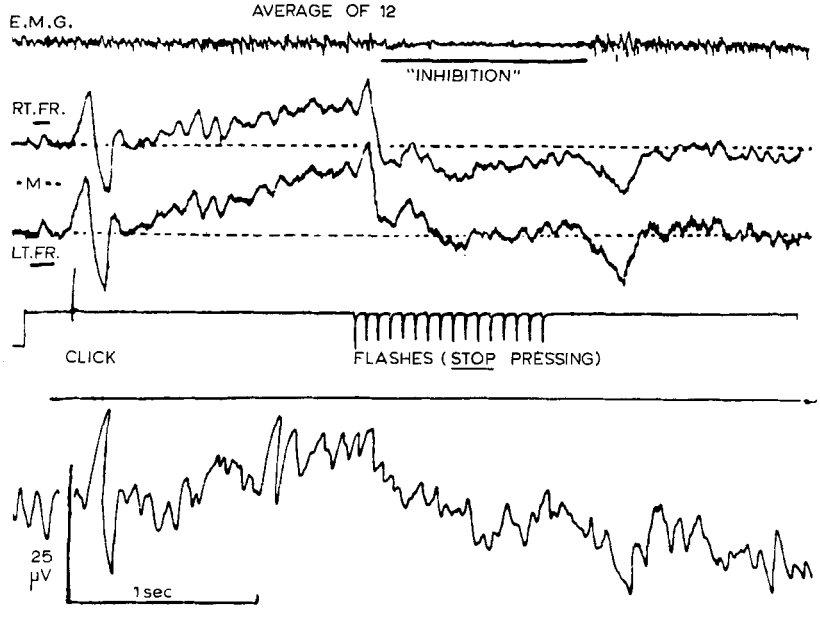

Fig. 10. CNV during performance of an 'inhibitory' action — releasing a button in the left hand in response to an unconditional stimulus. The resumption of the pressure is accompanied by a second decline in negativity. This record also shows the cortical symmetry of the CNV in spite of the unilateral movement.

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Whether the CNV should be regarded as indicating a slowly *increasing* conditional probability of *excitation*, or a slowly *decreasing* probability of *inhibition* is really a matter of verbal convenience. These concepts, which were introduced to describe the behaviour of single one-way systems in the spinal cord and periphery, are probably too naive for application to statistically determined processes in the richly interconnected mechanisms of higher nervous activity. Clearly the CNV could act as a short-term timing or memory mechanism. In fact it resembles remarkably the 'sawtooth' voltages generated by the time-base circuits in an oscilloscope, even to the way in which it extends at a smaller angle and longer duration when the interval between stimuli is lengthened. In a sense, such a process is 'inhibitory' since the final action is restrained or deferred until a certain time elapses, but this is not the sense in which the term is generally used. An alarm-clock may be regarded as an inhibitory device since it prevents the sleeper from awakening too early. The amplitude of the CNV in the situations described here is often of the same order as that of the intrinsic rhythms and it is possible that the slower rhythms and irregular shifts of potential seen in most EEG records are actually negative variations contingent on events outside the control of the experimenter. As would be expected, the CNV is most prominent in records with little intrinsic slow delta activity, and is usually imperceptible in those with larger low frequency components (for example young children and drowsy adults). This would suggest that the diminished functional competence associated with delta activity in sleep or organic disturbance is due to occlusion of the cortical mechanisms responsible for the Contingent Negative Variation. This effect may provide the long-sought operational links between the classical discoveries of Sechenov and Pavlov on the one hand and the electrical brain phenomena on the other.

SUMMARY

(1) When paired stimuli separated by about 1 sec, in any combination of modalities, are presented to a human subject who intends to act in some way in response to the second, a slow surface-negative wave appears in frontal cortex.

(2) This effect has been designated the Contingent Negative Variation (CNV); it reflects the probability of association of the stimuli and the intention to respond on the part of the subject.

(3) Extinction by withdrawal of the unconditional or 'imperative' stimulus results in a slow decline of the CNV to zero over about 20 trials.

(4) When the subject is warned beforehand that the imperative stimuli are to be withdrawn the CNV disappears at once.

(5) Dilution of the probability of association by partial reinforcement ('equivocation') produces a decline in the CNV which follows the subjective probability as estimated by the subject.

(6) If the subject decides not to act on the imperative stimulus the CNV disappears at once.

(7) A purely mental response such as estimating a time interval or making a

decision is enough to establish a CNV provided that the subject is interested and involved.

(8) A 'negative' stimulus such as the cessation of a tone and a 'negative' response such as stopping pressure on a button are also adequate for the development of a CNV.

(9) The CNV is considered as the major outward sign of frontal dendritic depolarisation in any situation in which some cerebral action can be accelerated and simplified by conditional learning.

ACKNOWLEDGEMENT

These observations were made during researches sponsored in part by the Mental Health Research Fund and the Parapsychology Foundation of New York.

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Spreading Depression and Cortico-Subcortical Interrelations in the Mechanism of Conditioned Reflexes

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Although Sechenov's work was published a centenary ago it is still valid not only owing to its philosophical content but also owing to its methodological approach. An attempt to influence the behaviour of a frog by a reversible interference in its brain can be considered as the prediction of modern trends and methods of investigation using electrical or chemical stimulation of the central nervous system with the purpose of analysing higher nervous functions. Spreading cortical depression (SD) — a specific cortical reaction of the brain (and some other formations) to directly influencing chemical and physical stimuli (Leão, 1944) — especially resembles Sechenov's initial experiments.

The results of numerous analytical works (see Marshall, 1959; Ochs, 1962; Brinley, 1963; Zachar and Zacharová, 1963; Bureš, 1962) showed that SD is a reaction transmitted by a humoral means. If a sufficient number of neurons is depolarized in the

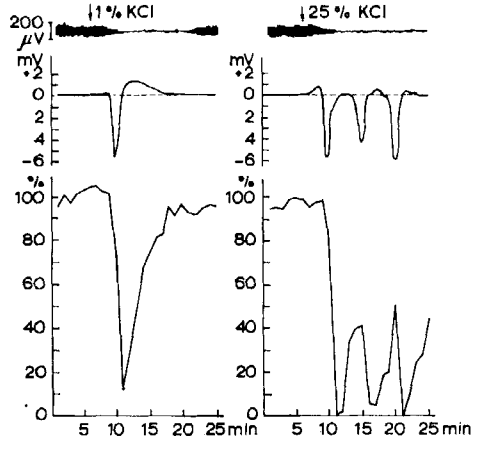

Fig. 1. Main electrophysiological manifestations of spreading depression (SD). From top to bottom: electroencephalogram; steady potential of the cortical surface; spontaneous activity of cortical neurons. The left part of the figure shows changes caused by a single depression wave, in the right part of the figure repeated SD waves after application of 25% KCl.

zone of stimulation a local increase in the concentration of potassium ions in the extracellular space gives rise to the depolarization of neighbour neurons, potassium ions leak out of the latter again, depolarizing further elements so that the reaction spreads in the form of a concentric wave moving in all directions at a speed of 3 mm/min. SD is characterized by marked electrophysiological manifestations (Fig. 1): depression of spontaneous and induced EEG activity (hence the term 'SD'), disappearance of the activity of single neurons and a pronounced variation in a slow potential (negative wave reaching an amplitude of about 10 mV and lasting 1–2 min). The full restoration of EEG activity is reached only 10–15 min later after one SD wave. Since the refractory period of the slow change of a potential lasts about 5 min (Zachar and Zacharová, 1962) repeated waves make it possible to obtain a prolonged depression of EEG activity suppressing normal functions of a respective zone during several hours. Series of repeated waves of the slow potential can be caused simply by application of super-threshold chemical stimuli (for example, 25% KCl solution) on the exposed surface of the brain cortex.

Functional consequences of SD consist not only in the intensity of a depression in certain parts of the brain cortex but also in the size of zones involved in the depression (Fig. 2). In rats SD involves all zones of neocortex and stops only in the cingular,

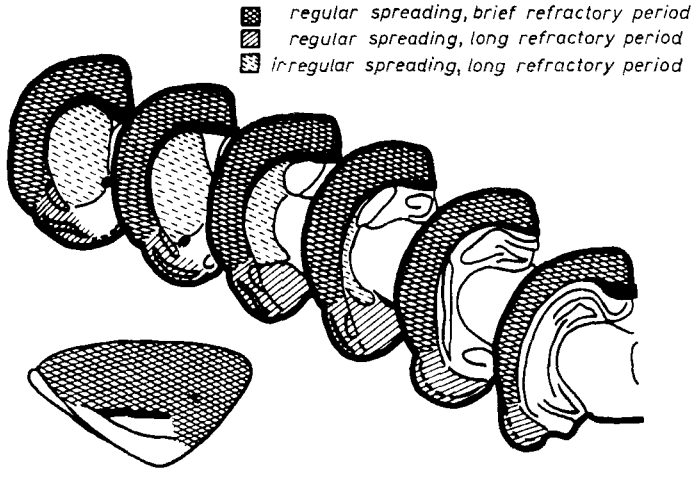

Fig. 2. Spread of cortical SD up to temporal zones of large hemispheres of the brain and up to the caudate in a rat. Probability of spreading is shown by the density of shading of the respective zones. The left lower part of the figure shows the limits of cortical SD spread after the cortex was cut parallel to the sulcus rhinalis.

pyriform and entorhinal cortex (Fikfová, 1963). SD of the neocortex never changes to the hippocampus; the first wave, however, as a rule enters emygdala and hence, in approximately 50% of tests, spreads over the caudate. The transition of SD from neocortex to amygdala or striatum is characterized, however, by a prolonged refractory period. From an uninterrupted series of SD waves only every sixth reaches the caudatum so that the function of the striatum proves to be much less disturbed

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than that of neocortex. The spread of SD to amygdala and caudate can be prevented by a cut 5 mm long and 2 mm deep made over the fissura rhinalis.

Changes in the behaviour caused by bilateral cortical SD in non-narcotized freely moving rats have been described more than once (Burešová, 1956; Bureš and Burešová, 1956, 1960; Burešová *et al.*, 1958; Bureš, 1959; Larsson, 1962; Tapp, 1962; Travis and Sparks, 1963; Olds, 1962). Although the animals maintain almost normal mobility, conditioned reflexes worked out beforehand disappear and the formation of new conditioned reflexes is markedly disordered. A good correlation was found between the duration of electrophysiological changes and the disturbance of the behaviour. The disturbance of conditioned reflex activity in rats is caused in the first place by switching off cortical functions since the effect of 25% KCl solution remains the same even when the transition of SD to amygdala is prevented by a cut.

As a reversible functional decortication SD makes it possible to check the results provoked by anatomical injuries of different zones of the brain. In certain cases, however, SD can be used to solve problems that cannot be solved with the help of the method of irreversible injury. As a rule, the experimental removal of different zones of the brain answers the question of whether the reaction elaborated by the intact brain will be preserved even after the removal of some of its zones. The method of functional decortication enables us to put an opposite question: will the reaction elaborated during SD be preserved even after the restoration of the suppressed zones? Travis and Sparks (1963) could not obtain a conditioned defensive reaction in rats in the course or bilateral SD. When they made a new attempt to work out the same conditioned reaction after the normal function of the cortex was restored the process of training was much slower than in control (naive) animals. Thus training during the functional decortication had an unfavourable effect upon the further elaboration of the same reaction by the normal brain. The authors, in trying to explain this phenomenon, suggest that in the course of SD the conditioned reaction of fear was elaborated which later on hampered the development of an active defensive reaction after the cortical functions had become normal.

By using a simpler variation of the experiment we succeeded in showing that functionally decorticated rats are able to elaborate simple conditioned defensive reactions (Bureš, 1959). Now we can cite additional observations. Rats with bilateral cortical SD were trained to pass from an electric platform in one half of the square apparatus to a wooden platform in its other half. When the reaction was not performed during 5 sec the animals were given an electric shock until they ran to the other half of the apparatus. The reduction of latent periods of running-away reaction showed that the conditioned reaction was usually elaborated after 10–20 combinations. After 24 h the retention was tested in the rats completely restored from the functional decortication. The rats were placed in the apparatus for 5 min and the period of time during which the animals stayed in the electric half of the platform was measured. The results are presented in Fig. 3; the columns show the mean time (in sec) of the animals' stay on the electric platform out of the total 300 sec of the test. While the animals placed in the apparatus for the first time prefer a metallic platform, the animals stimulated by an electrical current during functional decor-

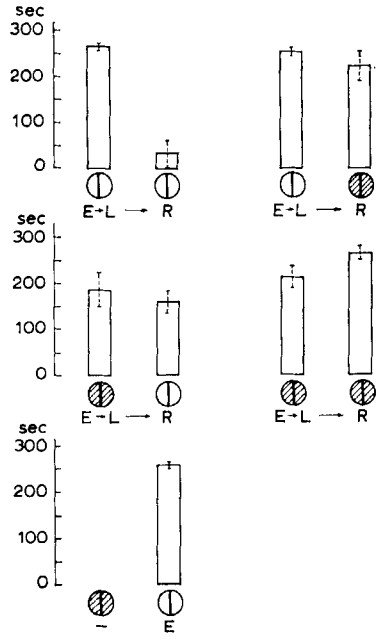

Fig. 3. Maintenance of a conditioned defensive reaction elaborated in normal or functionally decorticated animals. The columns denote the time spent by animals in the part of the apparatus with an electric grid either in the course of the primary exploration (E) or in the course of the retention test (R). L corresponds to the elaboration of an avoidance conditioned reaction immediately after the primary exploration. The diagram of the brain under the columns; SD is denoted by shading.

tication (shown by shading) spent a shorter, statistically significant, period of time in the given part of the apparatus during the test. If the escape reaction was elaborated in normal rats, the retention test repeated in normal conditions showed almost 100% preference for the wooden platform. This passive defensive reaction, however, completely disappeared during the bilateral cortical SD. Trained rats than preferred the electrical platform as well as decorticated control (naive) animals.

Hence, it seems probable that at least part of the information received during functional decortication can be retrieved again by the normal brain. Of course this does not explain what zones of the brain present the main substrate of the formation of a conditioned reflex after the brain cortex is switched off. Therefore, in further experiments we tried to check up directly with the help of electrophysiology the elaboration of conditioned reflex reactions of similar character. With this aim in view we made use of the procedure for the elaboration of conditioned reflex changes in the activity of individual reticular neurons in cats described by Yoshii and Ogura (1960). Unlike these authors, as the expression of reactions of certain neurons, we used the technique of post-stimulation histograms obtained with the help of a simple computer.

The experiments were carried out on 44 white rats 3 months old. The animals were made immobile by D-tubocurarine, fixed in the stereotaxic apparatus, warmed (to the body temperature of 35°) after which artificial respiration was carried out (60/min). With the help of the hydraulic micro-manipulator, steel or glass microelectrodes

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were introduced into the pontobulbar reticular formation. Sound (conditioned) stimuli were delivered through a loudspeaker placed at a distance of 20 cm from the head of an animal. Electrical stimuli (= unconditioned stimulus) were delivered by means of a pair of silver electrodes applied to the exposed sciatic nerve. The efficiency of the sciatic nerve stimulation was measured by registering cortical primary responses from the contralateral somatomotor cortex. The activity of individual neurons was registered in one channel, and primary responses in the second channel of a double-ray oscillograph. When a well-isolated unit exceeding at least thrice the maximum noise was found in the reticular formation, its response to sound stimuli and stimulation of the sciatic nerve were tested with the help of a simple analyser of the activity of individual neurons (Tuma and Bureš, 1963).

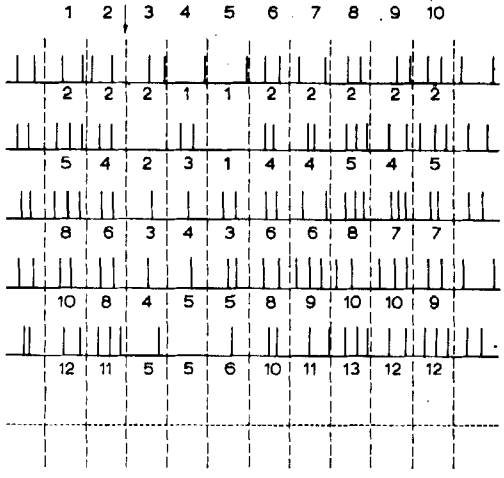

Fig. 4. Diagram showing the operation of the analyser of the activity of nerve units. In the intervals denoted by the vertical dotted lines nerve impulses are summed up in counters 1–10. Details can be found in the text.

The function of the apparatus is schematically shown in Fig. 4. A certain period of neuron activity is divided into 10 equal successive intervals and action potentials in individual intervals are summed up separately in the respective counters. The operational cycle of the apparatus, *i.e.* successive switching on of individual counters, is synchronized with stimuli influencing the animal. Thus, for instance, a stimulus used always in the beginning of the 3rd time interval will cause a change (for example, a decrease in the activity of a neuron) in the third or also in a further interval. Such change is expressed the better the more cycles were repeated and summed up for the elaboration of the post-stimulation distribution of the activity of individual neurons, *i.e.* of the so-called post-stimulation histogram. The mathematical principle of this method elaborated in detail by Gerstein and Kiang (1960) consists in the fact that spontaneous activity is considered as a random process characterized by more or less constant statistical parameters. If a stimulus, synchronized with a certain analyser interval does not produce an effect on the activity of neurons, and if the number of summed-up cycles is big enough, action potentials will appear with equal probability

in all counters. In order to decide whether deviations from the average level are caused by external stimuli statistical methods should be applied.

In the experiments on the elaboration of conditioned reactions a 2-sec analyser interval was used. A sound stimulus (tone of 200 or 2000 c/s) was delivered during the 3rd interval, and a single electrical stimulation of the sciatic nerve in the beginning of the 4th interval. Average values of the activity of individual neurons were determined by summing up 10 cycles repeated at 45-sec intervals. For the elaboration of conditioned reactions only those neurons were used which showed a pronounced response to an unconditioned stimulus but did not respond to sound stimuli. As a rule in the course of the elaboration of conditioned reactions up to 100 combinations of conditioned and unconditioned stimuli were applied.

A typical experiment is presented in Fig. 5. Histogram A shows the efficiency of an

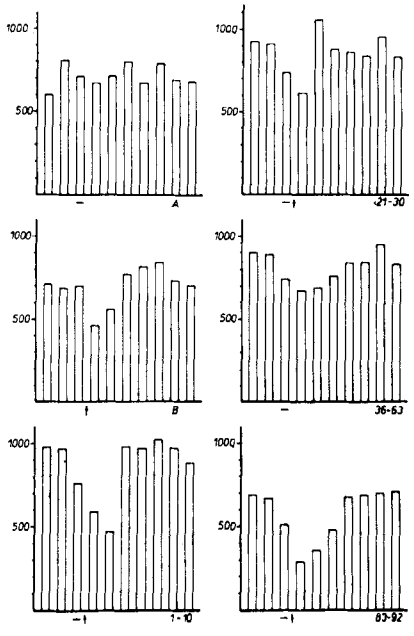

Fig. 5. Post-stimulation histograms of a reticular unit activity during the elaboration of a conditioned reflex reaction. The use of sound stimulation is denoted by a horizontal strip in the third interval, use of electric stimulation of the sciatic nerve by an arrow between the third and the fourth intervals.

auditory stimulus while stimulation of the sciatic nerve caused a marked inhibitory reaction (B).

During the first 10 combinations a sound stimulus began to cause an inhibitory reaction which was also well pronounced in 21–30 and 83–92 combinations. Thus, as a result of the formation of a conditioned connection a given neuron began to respond to an initially indifferent stimulus. A conditioned reaction can be demonstrated still more convincingly if the unconditioned stimulus is omitted. To avoid the extinction here only every third unconditioned stimulus was used. The histogram obtained by summing up 10 reactions to an isolated conditioned stimulus in the 36th–63rd combinations

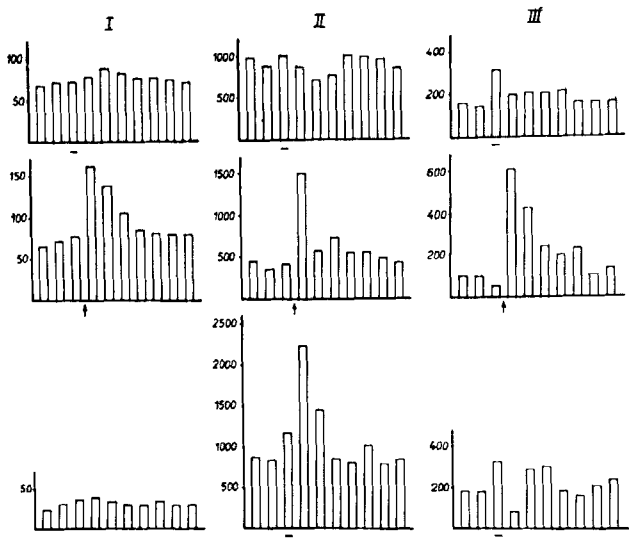

Fig. 6. Post-stimulation histograms of reticular neuron activity during the elaboration of a conditioned reflex reaction. From top to bottom: reaction to a conditioned and unconditioned stimulus, reaction to the non-reinforced use of a conditioned stimulus after 50 combinations of conditioned and unconditioned stimuli.

provoked changes similar to those caused by a combined use of conditioned and unconditioned stimuli.

Conditioned reactions were usually similar to responses to unconditioned stimuli. In some cases, however, elaboration of a conditioned reaction resulted in an opposite change. Fig. 6 shows 3 main types of results obtained by means of an isolated conditioned stimulus after 50 reinforced combinations. In all these experiments an unconditioned stimulus provoked an excitative action but a conditioned stimulus caused either a usual imitating reaction in the 4th interval (type 2), remained without effect (type 1) or even caused a response of opposite character (type 3). In the latter a conditioned stimulus seemed to cause an activity that acted against the effect of an unconditioned stimulus on a given neuron. This compensatory activity can be revealed only by the omission of the unconditioned stimulus.

Fig. 7 presents a survey of results obtained in 22 individual neurons. Statistically significant conditioned reactions were found in more than 50% of reticular neurons. This result is in good agreement with the data obtained by Yoshii and Ogura (1960) who found conditioned reactions in 40 out of 73 neurons. These authors found, as we did, that the first manifestations of the formation of a conditioned connection often as early as in the first 10 combinations, but at later stages of the experiment conditioned reflex reactions, disappeared despite a further reinforcement.

In another group of rats a conditioned reaction was elaborated similarly during a bilateral functional decortication. The suppression of cortical activity was judged by the disappearance of the primary response to an unconditioned stimulus (stimulation of the sciatic nerve). Despite the disturbance of cortical functions the formation of a conditioned reaction in reticular neurons occurred in similar manner

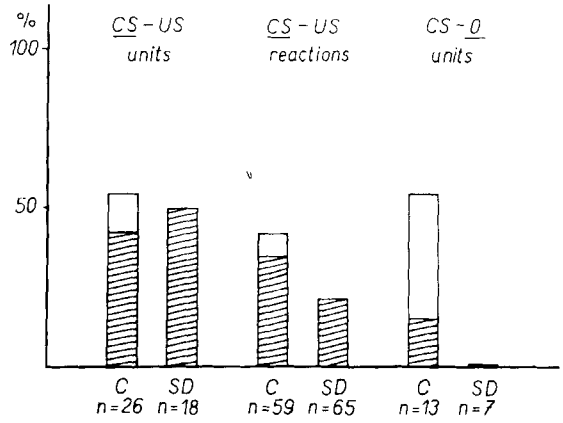

Fig. 7. Correlation of conditioned reflex changes in reticular unit activity in normal (C) and functionally decorticated rats. The columns correspond to the percentages of cases in which either imitating (hatched part) or inverse (white part) reactions were observed in the third interval during a conditioned stimulus (CS–US), or in the fourth interval at the place of the omitted unconditioned stimulus (CS–O). The first part of the figure shows the units in which at least one statistically significant conditioned reflex reaction appeared; the columns in the middle part of the figure were obtained through the treatment of all histograms from the first 50 combinations; the last part of the figure illustrates the behaviour of reticular units in the experiments with the isolated use of conditioned stimulation after 50 preliminary combinations.

to that in control rats. Some differences in conditioned responses of normal and functionally decorticated rats can be understood from the survey presented in Fig. 7. Functionally decorticated animals did not show inverse reactions, and all conditioned reactions occurred within a shorter period of time than in control rats which is testified by the presence of a smaller number of positive reactions in the first 50 reinforced combinations. The difference between the normal and functionally decorticated animals was most pronounced in the experiments with an unconditioned stimulus. In these conditions functionally decorticated animals did not show any positive reaction though in normal animals over 50% of neurons had conditioned reactions.

An explanation of the above data is not simple. In the first place it is necessary to exclude the possibility of 'pseudo-conditioning'. In this case a newly formed reaction to a sound stimulus would present only the result of a general rise in the excitability of the reticular formation under the influence of a pain stimulus. This explanation is not plausible since it was established in several experiments that a reaction to an auditory stimulus remains unchanged also after a series of nociceptive shocks. Reactions observed in the 4th interval after the release of an unconditioned stimulus can also hardly be explained by the pseudo-conditioning mechanism. Therefore it is reasonable to suggest that changes in responses of individual nerve cells found in 50% of reticular neurons are the result of the formation of a real conditioned connection. It is true that the results obtained never demonstrate that plastic changes have occurred only in those neurons whose activity was determined. The observed reactions seem to be a component of the reticular activation which accompanied early stages of the elaboration of classical conditioned reflexes.

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Indications that at later stages of training the reticular activation diminishes are also in accord with the fact that the greatest number of conditioned reactions of individual neurons was found during the first 20 combinations, gradually decreasing in further combinations. Therefore the phenomena described in our experiments can be regarded as a cellular manifestation of a conditioned arousal reaction. Neurons displaying conditioned reactions are links of complex nerve chains normally activated by an unconditioned stimulus, and — as a result of the formation of a conditioned reflex — by a conditioned stimulus as well. Since a plastic change may occur in input elements of such a chain which can be placed at a considerable distance from the recorded neuron it should be noted that this method is hardly applicable for revealing neurons initially taking part in the process of forming new temporary connections. To accomplish this, localized conditioned or unconditioned stimuli had to be used that influence as far as possible only a recorded neuron.

SUMMARY

Elaboration of simple conditioned reflexes was studied in normal rats and in rats with bilateral functional decortication elicited by cortical spreading depression (SD). In the first series of experiments naive rats learned to avoid the grid floor and to stay on the wooden floor of a two-compartment box. The rats were able to learn this task even under bilateral SD and retained the habit after recovery from the functional decortication. On the contrary the experience acquired with normal brain could not be retrieved during decortication.

In the second series of experiments classical conditioning was studied in 44 reticular neurons of unanaesthetized curarized rats using computer plotting of post-stimulation histograms. Only units were used that originally did not react to the sound (conditioned stimulus, 2 sec, 2000 c/s) but that were markedly excited or inhbited by the sciatic nerve stimulation (unconditioned stimulus). Sound induced responses appeared in more than 50% neurons after the conditioned stimulus was repeatedly reinforced by the unconditioned stimulus (up to 100 times with 30–60 sec intertrial interval). The conditioned reactions were most frequent in the first 20 trials; later their incidence declined in spite of continuing reinforcement. Results obtained in reticular neurons of normal and functionally decorticated rats were essentially similar. Unit conditioning of this type seems to be related to the conditioned arousal reaction. Similar neurophysiological mechanisms may play an important role in the learning described in the first part of this study.

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Peripheral and Central Modulation of Visual Input during 'Habituation'

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Many hypotheses have been proposed to explain the decrease of amplitude of evoked potentials during sensory habituation. The original hypothesis of an inhibitory action of the reticular formation to the first synapses of the sensory pathways (Hernández Peón *et al.*, 1956a) has not been proved. Moreover, there are many experiments which demonstrate that during habituation there is a progressive deactivation of the reticular formation (Mancia *et al.*, 1959a,b; Cavaggioni *et al.*, 1959; Fernández-Guardiola *et al.*, 1960) with slowing down of the cortical rhythms.

The role played by the 'para-receptors' *i.e.*, the pupils in the visual pathway or the intrinsic ear muscles in the auditory one, was neglected in the early electrophysiological studies on habituation. Nevertheless many authors had proved the influence of these structures on the process of sensory transmission and habituation (Hugelin *et al.*, 1959; Naquet *et al.*, 1960; Guzmán-Flores *et al.*, 1960; Fernández-Guardiola *et al.*, 1960, 1963; Alcaraz *et al.*, 1961, 1962).

The experiments on habituation have been performed using single repetitive stimuli of brief duration (flashes or clicks). In the present work based on experiments previously performed in humans (Fernández-Guardiola *et al.*, 1963) a type of photic stimulation in 'trains' has been used. The intrinsic frequency of these 'trains' modifies the process of habituation as well as the basic rhythms of the EEG. This method has proved itself useful to demonstrate some dynamic events occurring during habituation such as the evolution of an interaction of the evoked responses in the cerebral cortex and the lateral geniculate body and the oscillatory nature of pupillary changes during habituation.

The experiments were done in intact curarized cats and 'encéphale isolé' preparations (Bremer, 1936). In order to habituate the dark adapted animal, 'trains' of flashes were used, applying 1 every 15 sec. The intrinsic frequency in each 'train' as well as its duration could be controlled. A combination of 'trains' and single flashes was obtained connecting the synchronization input of a Grass photostimulator to the output of an electronic mixer which was in turn connected to the outputs of two Grass stimulators coupled as modulation and stimulation units.

In order to dehabituate the animal, different procedures were used: (a) suppression

of stimuli; (b) change of intrinsic frequency of the 'trains'; (c) stimulation by other sensory modalities; (d) direct electrical stimulation of mesencephalic reticular formation; (e) intravenous injection of nor-adrenaline; (f) intravenous injection or topic eye application of atropine sulphate.

The course of habituation in these preparations was variant and no definite rate was found. The habituation was evaluated by the following signs (Figs. 1, 2 and 3):

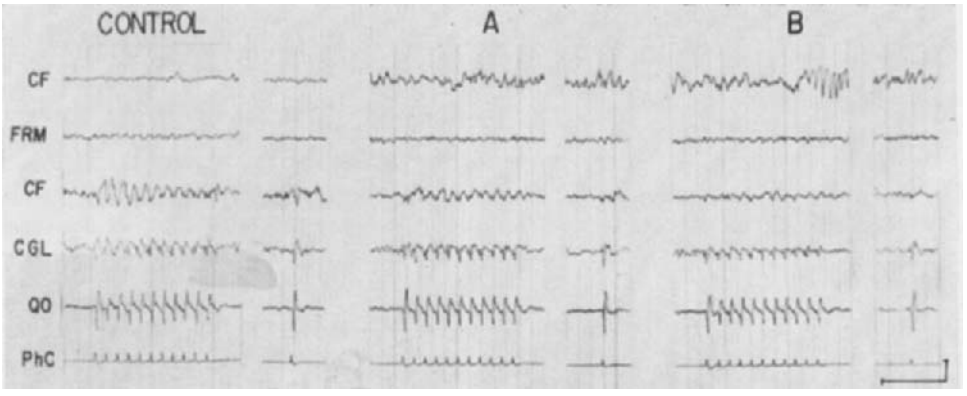

Fig. 1. Intact curarized cat. CF, frontal cortex; FRM, mesencephalic reticular formation; CGL, lateral geniculate body; QO, optic chiasm; PhC, photo-electric cell. Habituation to 'trains' of flashes followed by a single flash. Progressive stages of photic habituation. Calibration: 1 sec; 100 μV.

(1) Slowing down of the frontal and visual cortex electrical activities; (2) Decrease of amplitude in the cortical evoked potentials; (3) Disappearance of evoked responses in the mesencephalic reticular formation; (4) Progressive miosis with decrease of the chiasmatic responses.

It was often necessary in the course of the experiments to distinguish between ha-

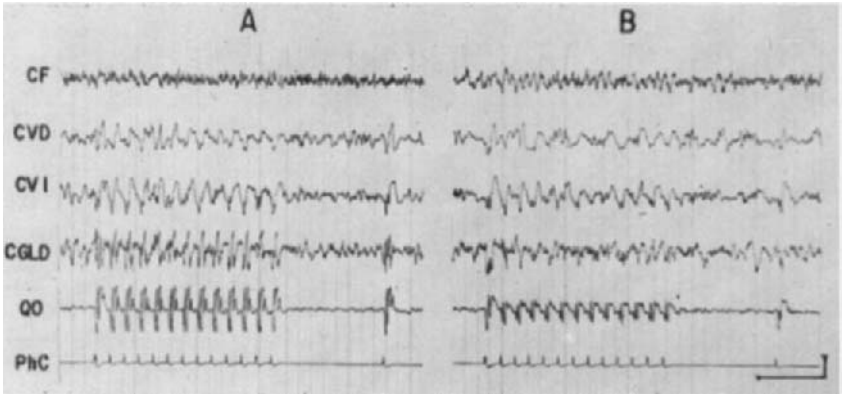

Fig. 2. Intact curarized cat. CF, frontal cortex; CVD, right visual cortex; CVI, left visual cortex; CGLD, right lateral geniculate body; QO, optic chiasm; PhC, photo-electric cell. Habituation to 'trains' of flashes (5/sec for 3 sec) followed by a single flash. A, control; B, habituation, miosis and slowing of cortical rhythms. At this moment the depression of evoked potentials at the optic chiasm and lateral geniculate body are noticeable. Calibration: 1 sec; 100 μV.

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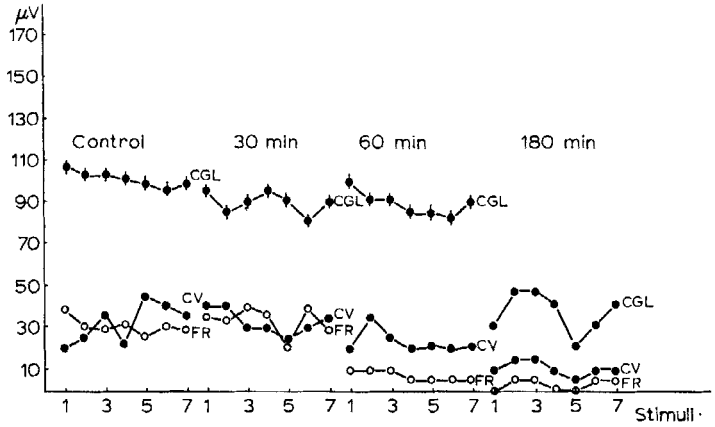

Fig. 3. Intact curarized cat. Habituation to repetitive single flashes (0.6/sec). Progressive decrease of the photic evoked potentials in visual cortex (CV), lateral geniculate body (CGL) and reticular formation (FR).

bituation and actual sleep which appears as the final outcome of the habituation process. Both phenomena present different characteristics (Fig. 4). In sleep there is a noticeable decrease of the chiasmatic responses produced by the concomitant fissurated miosis, while the response in the visual cortex may be of great amplitude; the EEG presents the usual slow waves and 'spindles'. The threshold for sensory stimulation is increased.

On the other hand during habituation the amplitude of evoked potentials decreases progressively in all structures along the visual pathway, the decrease beginning at the

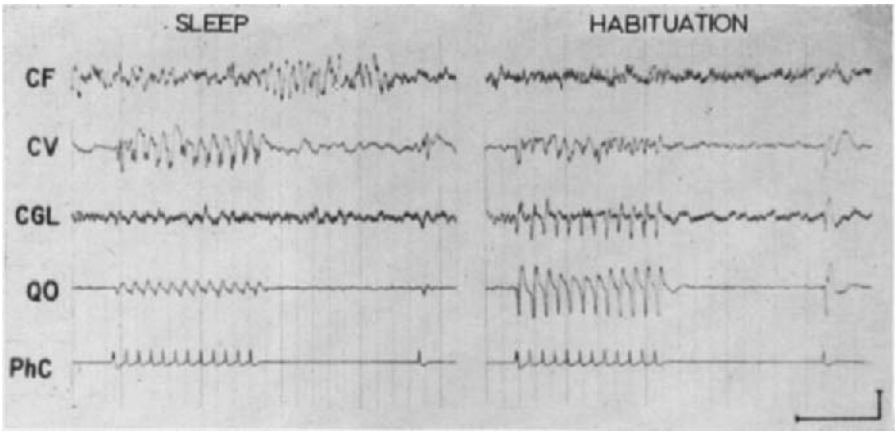

Fig. 4. Intact curarized cat. CF, frontal cortex; CV, visual cortex; CGL, lateral geniculate body; QO, optic chiasm; PhC, photo-electric cell. Habituation to 'trains' of flashes followed by a single flash. Electrocorticographic differences between sleep and early habituation. Notice that during sleep and accentuated miosis the evoked potentials in visual cortex are of great amplitude despite the fact that the optic chiasm and lateral geniculate body responses are depressed. At the beginning of habituation the depression of evoked potentials at the visual cortex is noticeable while no depression is observable in the recorded responses from lateral geniculate body and optic chiasm. Calibration: 1 sec; 100 μV.

cerebral cortex. The EEG also slows down but in a less noticeable manner than during sleep, reaching only the θ band.

Through the habituation test the pupils showed oscillatory movements (pupillary unrest, Fig. 5) which were coincident with the variations of the chiasmatic responses

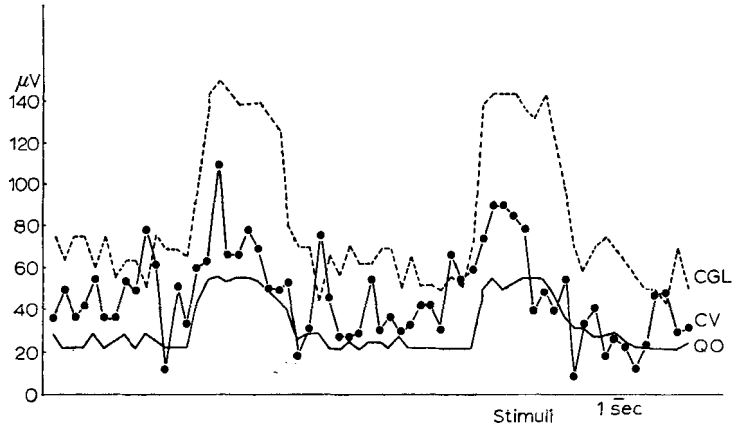

Fig. 5. Encéphale isolé. Habituation to single flashes (2/sec). Evolution of the optic responses to 63 consecutive stimuli in visual cortex (CV), lateral geniculate body (CGL) and optic chiasm (QO). These changes are coincident with spontaneous changes in pupillary area.

(Fig. 6). At the early stages of the habituation process these variations were accentuated showing a tendency to miosis, and were more noticeable in the 'encéphale isolé' than in intact curarized cats (Fig. 7).

The topic eye application or the intravenous injection of atropine sulphate abolished the pupillary variations (Fig. 8).

Cortico-geniculate relationships

In the course of habituation and simultaneously with the slowing of the EEG an

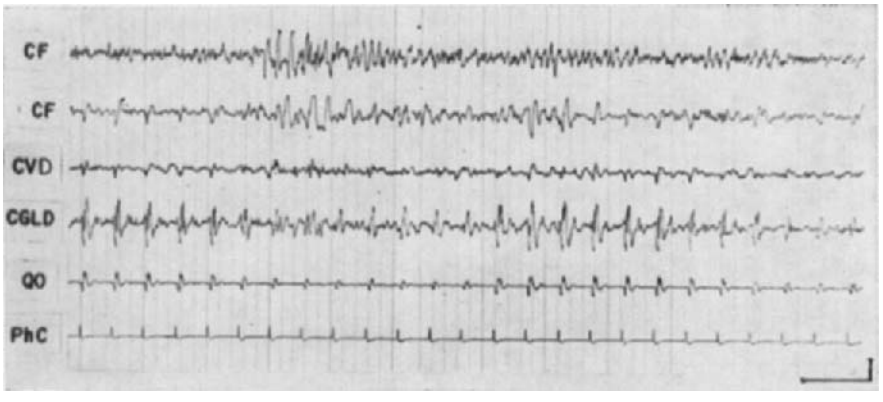

Fig. 6. Encéphale isolé. CF, frontal cortex; CVD, right visual cortex; CGLD, right lateral geniculate body; QO, optic chiasm; PhC, photo-electric cell. Habituation to single flashes. Spontaneous variations of pupillary area. Notice that during progressive habituation the frontal activity is slowing down, presenting bursts of 'spindles' during miosis. Calibration: 1 sec; 100 μV.

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inverse relation appeared between the amplitudes of the responses recorded from the cerebral cortex and the lateral geniculate body. This phenomenon was independent of the magnitude of the evoked responses in the optic chiasm (Figs. 9, 10 and 11). This inverse relationship of the cortical and geniculate evoked potentials was not

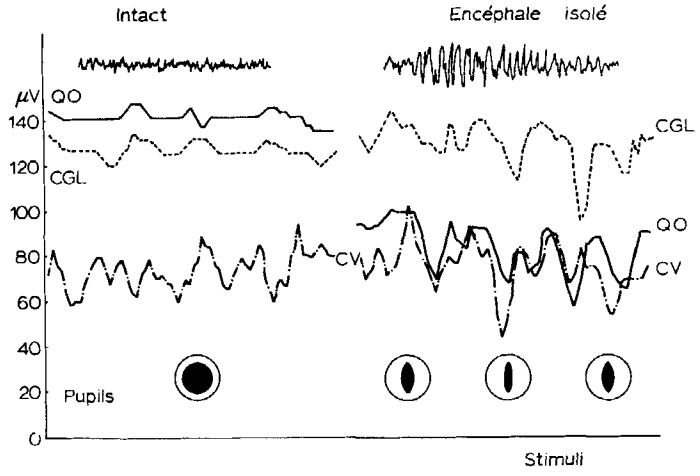

Fig. 7. Photic evoked responses recorded from an initially intact curarized and later encéphale isolé preparation. After isolation of the brain the variations of photic responses are prominent coinciding with an EEG deactivation and an increase of pupillary oscillations. CGL, lateral geniculate body; QO, optic chiasm; CV, visual cortex. Frequency of stimuli: 2 flashes per sec.

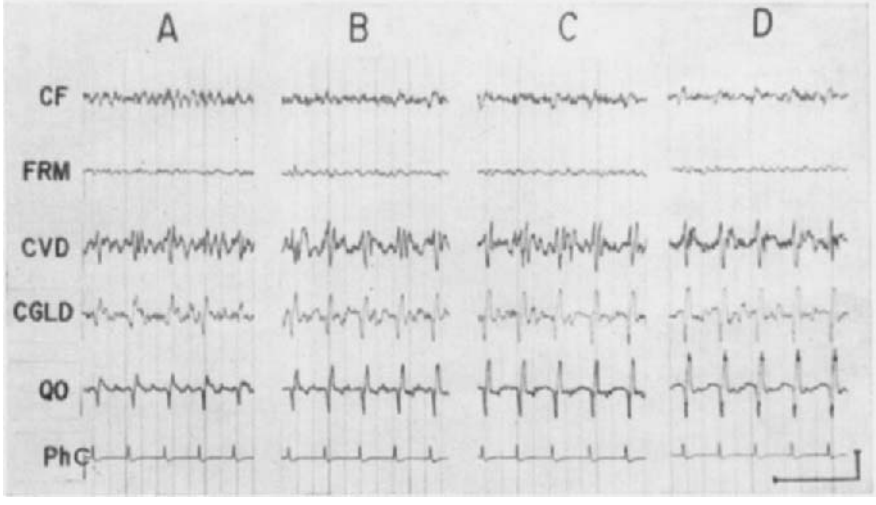

Fig. 8. Encéphale isolé. CF, frontal cortex; FRM, mesencephalic reticular formation; CVD, right visual cortex; CGLD, right lateral geniculate body; QO, optic chiasm; PhC, photo-electric cell. Habituation to single flashes (3/sec). A, recorded at the end of the habituation with preserved pupillary motility; B, 15 sec after 0.25 mg atropine sulphate intravenous injection; C, after 25 sec; D, after 1 min. Notice that the evoked responses increased progressively in all structures and that the pupillary oscillations disappeared. Calibration: 1 sec; 100 μV.

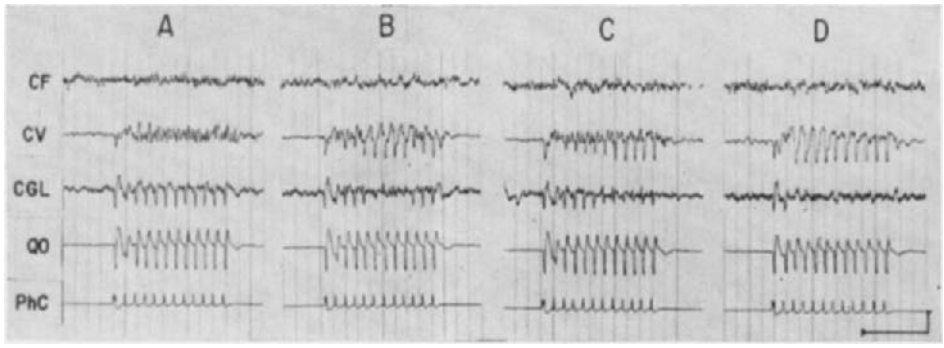

Fig. 9. Intact curarized cat. CF, frontal cortex; CV, visual cortex; CGL, lateral geniculate body; QO, optic chiasm; PhC, photo-electric cell. Habituation to 'trains' of flashes. Notice the inverse amplitude relationship between evoked responses in visual cortex and lateral geniculate body. This phenomenon occurs independently of the variations of the evoked responses of optic chiasm. A, B, C, D, sequence of photic habituation. Notice that the amplitude relation in A is reverted in D. B and C are examples of intermediate states. The first evoked responses to each 'train' do not participate of this relation. Calibration: 1 sec; 100 μV.

present during photic stimulation with single flashes at low frequency. 'Trains' of 6–8 flashes per sec were found optimal for provoking this effect.

Dehabituation

An increase in amplitude of the chiasmatic evoked potentials was always found as a result of the electrical stimulation of the mesencephalic reticular formation in habituated animals. The maximum increase was attained 2–3 sec after the onset of stimulation. In the post-stimulatory period the evoked potentials decreased progressively following the same course as the pupillary areas (Fig. 12).

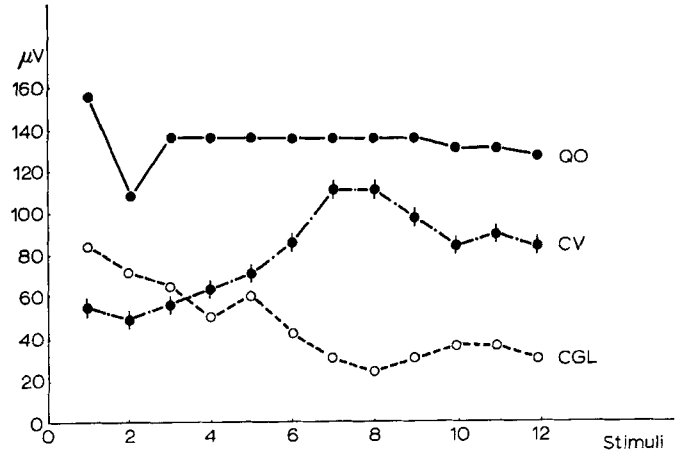

Fig. 10. Intact curarized cat. Responses to trains of flashes (6/sec); QO, optic chiasm; CV, visual cortex; CGL, lateral geniculate body. Notice the inverse relationship between amplitudes of the visual cortex and lateral geniculate body potentials independently of the variations of the evoked potentials of the optic chiasm.

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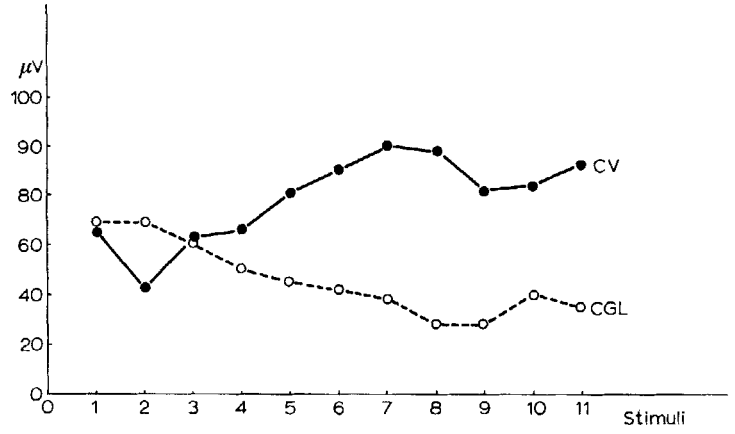

Fig. 11. Average of the responses of the visual cortex and the lateral geniculate body evoked by 'trains' of photic stimuli (6/sec); CV, visual cortex; CGL, lateral geniculate body.

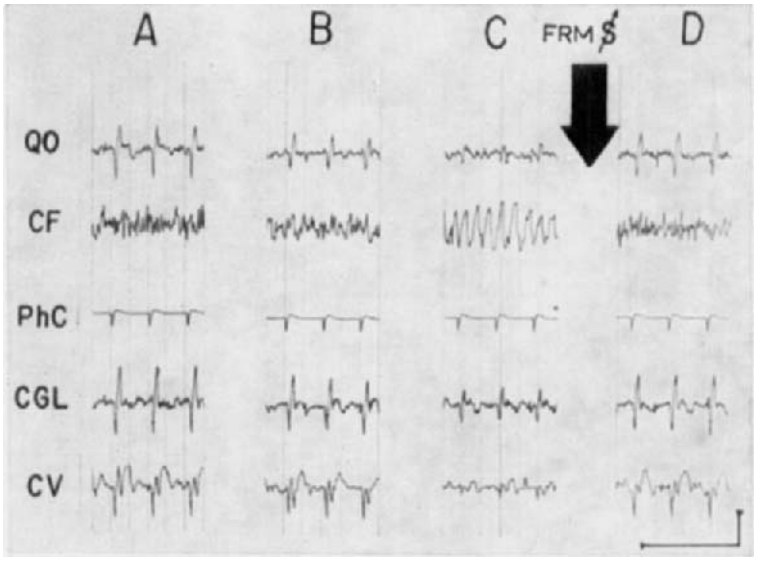

Fig. 12. Encéphale isolé. QO, optic chiasm; CF, frontal cortex; PhC, photo-electric cell; CGL, lateral geniculate body; CV, visual cortex. Habituation to single flashes (3/sec). Dehabituation by electrical stimulation (arrow) of the mesencephalic reticular formation (6 V, 1 msec, 300 c/s). A, control; B and C, 15 and 17 sec after iterative photic stimulation; D, immediately after the reticular stimulation. Notice the arousal reaction of the frontal cortex and the increase of the evoked responses. Calibration: 1 sec; 100 μV.

These variations provoked by reticular stimulation were also observed in the responses recorded from the lateral geniculate body and the visual cortex, but with longer latencies (Fig. 13).

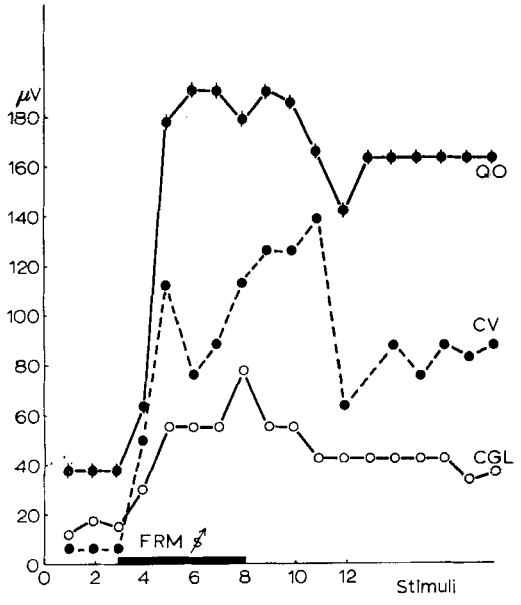

Fig. 13. Encéphale isolé. Dehabituation by direct reticular stimulation. QO, optic chiasm; CV, visual cortex; CGL, lateral geniculate body. Habituation to single flashes (1/sec). Notice the increase of the evoked potentials during and after reticular stimulation.

A brief suppression of photic iterative stimuli in animals habituated to single flashes (0.5/sec) produced a remarkable dehabituation. The evoked potentials increased progressively until they reached the maximum amplitude 10–18 sec after the stimulus suppression (Fig. 14).

Moreover, the same dehabituation effect was obtained by increasing the frequency in each 'train' of flashes without altering the interval between 'trains' or their duration (Fig. 15).

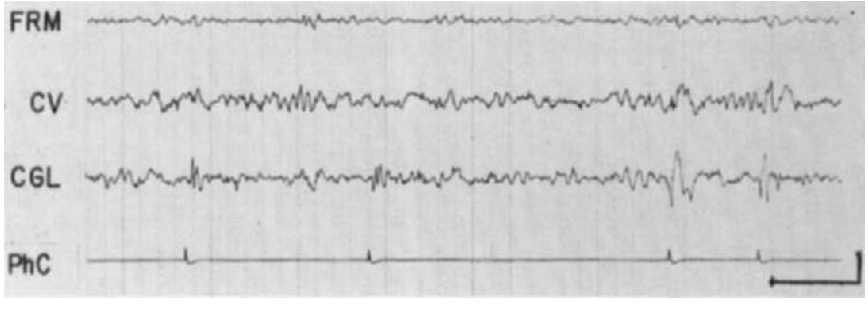

Fig. 14. Encéphale isolé. FRM, mesencephalic reticular formation; CV, visual cortex; CGL, lateral geniculate body; PhC, photo-electric cell. Habituation to single flashes (0.5/sec). Dehabituation by brief suppression of stimuli. Notice the reappearance of evoked responses in the reticular formation. Calibration: 1 sec; 100 μV.

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At the beginning of habituation the pupils react with the usual photomotor response. During the dehabituation provoked by an increase in frequency this response reverted showing a 'paradoxical' course, *i.e.*, more light per unit time produced mydriasis instead of miosis (Fig. 16).

Fig. 17 shows the dehabituation obtained by auditory stimulation. In this case the pupils also reacted by progressive dilation.

Intravenous administration of nor-adrenaline provoked dehabituation in doses as

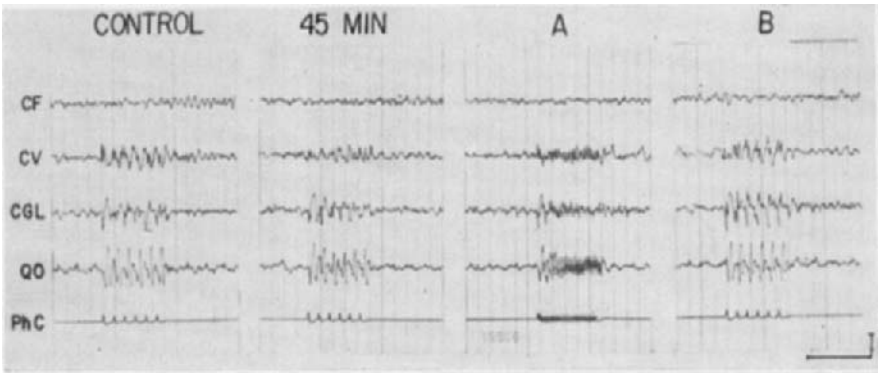

Fig. 15. Intact curarized cat. CF, frontal cortex; CV, visual cortex; CGL, lateral geniculate body; QO, optic chiasm; PhC, photo-electric cell. Habituation to 'trains' of flashes (6/sec) of 1 sec of duration and with an interval of 10 sec between each 'train'. Preserved pupillary motility. A, changes in frequency of photic stimulation (21 flashes/sec); B, dehabituation effect provoked by this change. Calibration: 1 sec; 100 μV.

low as 2 μg. A bradycardia of about 20% was obtained with a latency of 4 sec. 8–10 sec after the injection the evoked potentials along the visual pathway began to increase showing oscillations. The arousal reaction appeared after 25 sec coinciding with the maximum amplitude of the photic evoked potentials and pupillary area. With doses of 5 μg these effects followed a different temporal course: the bradycardia and arousal reaction vanished in about 20 sec while the increase of evoked potentials and pupillary area persisted as long as the animal continued in its state of dehabituation.

All the habituation signs as well as the pupillary unrest disappeared when atropine sulphate was instillated in the eye or was injected intravenously. Subsequent photic iterative stimulation failed to provoke a diminution of the responses at the chiasmatic level (Fig. 8).

DISCUSSION

These results are showing the oscillatory nature with a characteristic temporal pattern of the habituation process. As measured by the evoked potentials along the visual pathway, the habituation seems to develop on the basis of two processes acting in the visual pathway at different levels but with a common origin.

One of these processes is the liberation of tonic inhibition acting on the pupillary motor centres which leads to oscillatory movements of the pupil and progressive

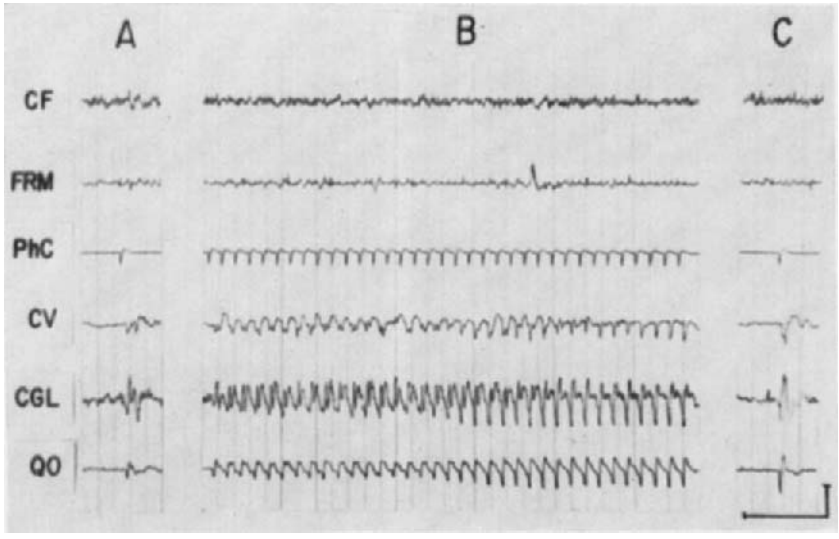

Fig. 16. Encéphale isolé. CF, frontal cortex; FRM, mesencephalic reticular formation; PhC, photoelectric cell; CV, visual cortex; CGL, lateral geniculate body; QO, optic chiasm. A, habituation to single flashes (0.5/sec); B, dehabituation provoked by changing up the frequency (6 flashes/sec); C, Response to single flash after dehabituation. Notice the progressive increase in amplitude of the responses coinciding with progressive mydriasis. Paradoxical response of pupil to the increased amount of light per unit time. Calibration: 1 sec; 100 μV.

miosis. The second process takes place at the level of cortico-thalamic circuits and provokes the depression of responses in both structures. While the oscillations of optic chiasm responses are related to the variations of pupillary area, the changes in visual cortex and lateral geniculate body responses occur independently and with characteristics that suggest a functional relationship developing during habituation in both structures.

The common origin of these processes could be a corticofugal inhibitory action developing as a consequence of the repetitive and monotonous arrival of impulses to the same cortical projection area (Harmony *et al.*, 1960). How is this corticofugal inhibitory discharge elicited? Several authors agree on the fact that in the first stages of habituation a 'waxing and waning' of the cortical evoked potentials can be observed, phenomenon which coincides with the slowing of the EEG and the appearance of 'spindles'. In this state it has been demonstrated that the corticofugal discharges increase in the pyramidal tract (Whitlock *et al.*, 1953). There is a great amount of anatomical evidence on the corticofugal fibres projecting on the thalamus and other subcortical structures (Macchi and Arduini, 1957). Sachs (1909) described fibres from the visual areas 17, 18 and 19 projecting on the lateral geniculate body. Similar observations were made by Ramon y Cajal (1911).

Thus, physiological as well as anatomical evidence exists which lends support to the hypothesis of a restricted corticofugal inhibitory action progressively developing during habituation. This leads to the depression of reticular mechanisms and puts into action corticothalamic inhibitory feedback circuits.

The pupillary modulation demonstrated in the cat might be different in other species,

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particularly in those who lack vision in darkness. The pupils of the cat, unlike other species, are capable of reaching such a degree of miosis that no photic evoked potentials can be recorded along the visual pathway.

Our results are pointing to the fact that central sensory structures are receiving a variable amount of stimulation even when very uniform and controlled external

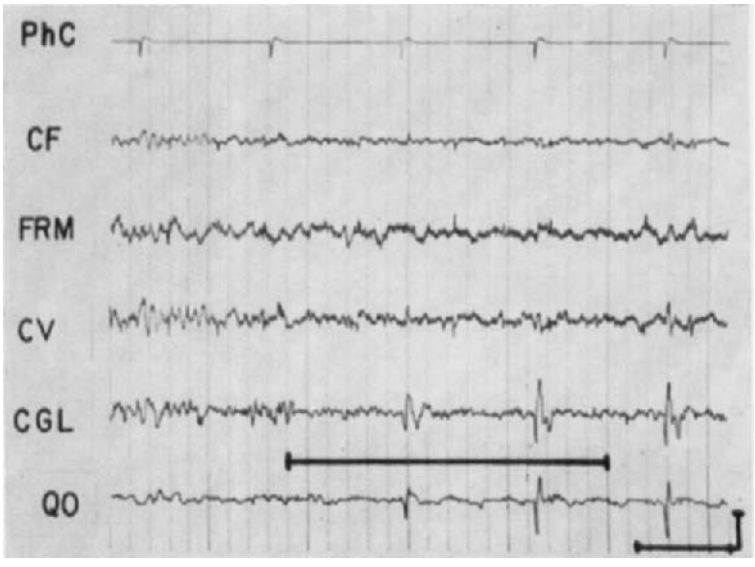

Fig. 17. Encéphale isolé. PhC, photo-electric cell; CF, frontal cortex; FRM, mesencephalic reticular formation; CV, visual cortex; CGL, lateral geniculate body; QO, optic chiasm. Habituation to single flashes; effect of dehabituation by acoustic stimulation. The heavy line marks the continuous application of one 1500 c/s tone. Notice that the increase in amplitude of the evoked responses is progressive and has a considerable latency. Calibration: 1 sec; 100 μV.

stimuli are used. These variations are provoked both by peripheral (para-receptors) and central (corticofugal) influences. It is necessary to emphasize that these influences could act through non-specific processes leading to the modulation of sensory input in relation to behavioural changes and different levels of consciousness.

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EVOLUTIONARY PHYSIOLOGY OF THE NERVOUS SYSTEM AND BRAIN ONTOGENESIS

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I. M. Sechenov and some Problems on Evolution of Nervous Activity

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Evolutionary ideas have been profoundly dealt with by the founders of Russian materialist philosophy and representatives of Soviet biology. For instance, Sechenov's teacher in the Moscow University, the well-known evolutionist Roulier was a direct predecessor of Darwinism in Russia. The evolutionary trend in Sechenov's ideas could be observed even when he was a University student, when he read his scientific paper 'On the Gradual Complication of Living Phenomena'.

In 1873 there appeared in Russia the first Russian translation (edited by Sechenov) of Darwin's outstanding work 'Origin of Man and Sexual Selection', a translation which is regarded the best there is even today. Sechenov expressed his high appraisal of Darwinism in the following words: 'The great teaching of Darwin, as is known, has raised the question of evolution or succession of the development of animal forms on such a tactile basis that today the great majority of naturalists hold this view. Thus the great majority of naturalists are logically put into a position, in which they are also compelled to recognize in principle the evolution of psychic activities' (Sechenov, 1935, p. 410).

In his public lecture in 1861 on 'the vegetative acts of animal life' Sechenov expressed some remarkable thoughts; thus, for example, for the first time in physiology he formulated the idea of the ties of organism with the existing environment, which affirms unity of organism and environment.

Later on Sechenov frequently reverted to these thoughts. Having advanced the problem of unity of organism and environment, he outlined the main lines of its development, which even today has not lost its significance, as a scientific task of major importance. We shall try to elucidate some problems outlined by Sechenov.

Touching on the question of the environment's influence on organisms Sechenov expressed the following thought.

'The comparative study of animals shows that progress of material organisation and life goes on not along direct lines, but in ramified ways, in details deviating to the sides. Here at these cross-roads of organization there is felt a tremendous force of the influence on the organism of that environment, in which they live, or to be more precise, conditions of their existence. Firstly, they give the possibility of defining life at all levels of its development, as an adaptation of the organism to conditions of existence; secondly, they prove that external influences are not only necessary in

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life, but that they represent at the same time factors capable of changing material organisation and character of living functions. Always and everywhere life is composed of co-operation of two factors — of definite but changing organization and of external influences' (Sechenov, 1935, p. 412).

These words of Sechenov may be regarded as the programme of further researches in this direction. From this point of view we ought to draw the reader's attention to Sechenov's words referring to 'ramified ways' and 'cross-roads' of evolutionary development.

Pavlov showed the essence of those physiological mechanisms, which ensure an organism's changeability, adjustable to external influences. Depending precisely on this factual and methodological basis of the Pavlov and Michurin teaching, the Soviet scientists successfully resolve the cardinal problem advanced by the Soviet creative Darwinism — the organism's directed variability.

Defining the substance of the interrelation between the organism and environment, Sechenov also stressed the other major part of the problem. He considered that variability, remaining within the confines of individual adjustability and not established hereditarily to become a link in the adaptable activity of the species on the whole, would prove to be devoid of any biological significance. Therefore, Sechenov concentrated on the question of hereditary establishment of acquired symptoms. 'Still another factor in the successive evolution of an animal organism', he pointed out 'is, as is known, hereditary, *i.e.* it possesses the ability to hand down by inheritance to posterity the changes acquired during its life' (Sechenov, 1935, p. 412).

In full agreement with Sechenov's concepts, Pavlov expressed his conviction in the possibility of fixing hereditarily some acquired forms of temporary connections. This thesis was also supported by numerous physiologists, such as Ukhtomsky, Orbeli, Bykov, Anokhin and others.

Without postulating this hereditary function, Pavlov's teaching of the adaptable significance of conditional reflexes loses all meaning. It would be impossible to comprehend how the evolution of species works out if the adaptability of temporary ties would spread only over the period of individual developments and would not be fixed phylogenetically.

In our laboratory we were able to obtain some forms of 'inextinguishable' temporary ties, which allowed us to classify them, as belonging to the transitory form of temporary ties from conditional to unconditional ones (Klimova, 1955).

Sechenov's ideas are very advanced, namely, the complication of environment and the character of the stimuli influencing the organism, the animal's organism itself, *i.e.*, its nervous system, sense organs, etc. also become more complicated. This results in the complication of reactions implemented by them. In correspondence with this, Sechenov defined a general concept of life, as the concordance of living necessities with the conditions of environment. In this respect the thoughts of Ukhtomsky are of special importance as regards the 'biologically interesting' stimuli, which become adequate as soon as they find themselves in 'biological affinity with the stimulated substrate' (Ukhtomsky, 1954). These thoughts harmonize with Michurin's idea about an organism's 'adjustability' to the environment of its existence.

Our laboratory research into the problem of ecological adequacy of stimuli and reactions resulted in extensive factual data dealing with this problem.

On numerous occasions we have enunciated the question of the importance of the quality of stimuli in the formation of higher nervous activity. We emphasized that it is wrong to consider it as correct, when physiologists regard the sphere of environment as something which does not differentiate and is monotonous, and appraise it more physically than physiologically. Ukhtomsky well expressed the essence of the problem when he said: 'The volume of perception and the degree of realisation of the surrounding environment in a Norwegian herring, in a rat or in a Libyan lion differ approximately in the same degree, as their behaviour ...'. In one and the same physical environment the tiger reacts in a tiger way, while the lion reacts as lions do. This proves that although an environment is physically the same, physiologically it is different for the diverse animal species living in it, and it is different as far as the type of its receptions are concerned.

Pavlov's teaching of conditioned reflexes vividly demonstrates the wonderful ability of cortical cells to maintain the conformity of stimulus, substrate and reaction in quantitative and in subtly qualitative relations.

We draw attention to the importance of the concept of ecological adequacy of conditioned and unconditioned stimuli for comparative physiology.

Such an attitude, of course, does not contradict one of the general principles of Pavlov's teaching, that any change in the external and internal environment may become a conditioned stimulus. Naturally, in the unlimited number of possible stimuli some of them may prove to be more and some less effective. That depends on how this or that stimulus is near to the natural environment and the animal's mode of life. Therefore, we say that in comparative physiological conditions the final effect of the action of a stimulus is determined not so much by its physical or chemical characteristic, as by its physiological or ecological significance.

The importance of the aspect advanced by us has been pointed out by Koshtoyants. He wrote: 'Inasmuch as the term adequate stimulus was also used by Pavlov and his pupils on other occasions, it is important to emphasize that Biryukov has in view the ecological adequacy of stimuli, which is of special importance for the comparative physiology of conditioned reflex activity.'

Today many research workers are engaged in questions dealing with ecological adequacy. Thus, Schneider (1954) studying differences in the field of vision of Amphibia, established their ties with ecological species. Weidman (1956) compares some peculiarities of the wild ducks' behaviour with their ecology. Kogan (1956, 1959) corroborated the dependence of the development of an analyzer's activity on ecological conditions, as described by us. Mason and Langenheim (1957) offered a theoretical analysis of the role of the environment's factors. Wohlfahrt (1957) demonstrated the ecological significance of natural stimuli on fish *(Phoxinus)*, Toporkova (1957) on cats, Promptov (1947) on birds and Getzova and Lozina-Lozinsky (1957) on insects.

Illustrations of ecological adequacy of stimuli received with the aid of electrophysiological methods are convincing. For instance, illustrations show the specific reaction of inhibition of the spontaneous electrical activity, which depends on the quality of

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stimuli and often on how near the stimulus is to the animal's ecology in its biological importance. Thus, for example, the reaction of inhibition of the spontaneous activity has been revealed with the utmost clarity in tactile and auditory irritations produced in cats (Rheinberger and Gasper, 1937; Travis and Milisen, 1936), in imitation of rat's squeaks among rats, during smell irritations of frogs (Gerard and Young, 1951) and in light and sound stimulation of birds and rabbits (Zagorulko, 1955; Bagriansky, 1958; Kogan, 1959).

If the problem of ecological adequacy of stimuli up to this time illuminated the qualitative effect of the phenomena, then the quantitative value of signalling remained unaccountable. The deficiency was filled in the form of cybernetic analysis through a statistical probability approach to the use of apparatus of the theory of information.

One of the conditions determining the difference in the effect of action of various 'neutral' or 'indifferent' factors of the external environment as conditioned stimuli may be the difference in the probability of the appearance of the given signals and in their correlation with the biologically active agents (unconditioned stimuli) of the environment of their habitat (Menitzky, 1960). It has been demonstrated, in experiments on fish and rats, that the nervous system may detect the difference in reinforcement of average probability, and that the rate of working out conditioned reflexes, as well as the rate at extinction or remaking of these reflexes, is proportional to the logarithm of the probability of reinforcement of conditioned stimulus with the unconditioned one during the period of formation and consolidation of the temporary tie. In Fig. 1 we see diagrams (each the average for 10 fish) of the dynamics of formation

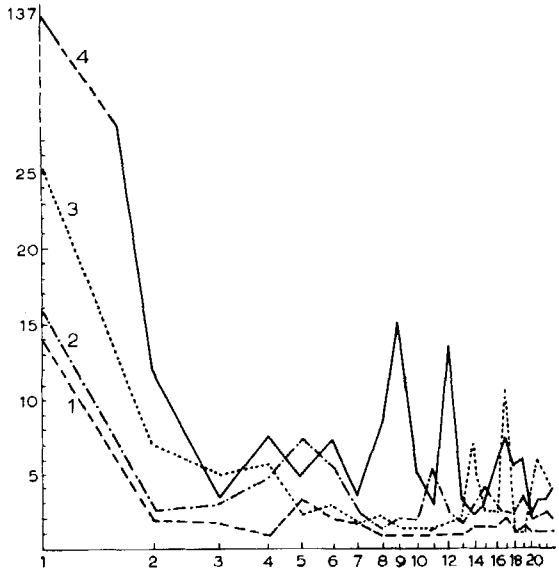

Fig. 1. Dynamics of formation of motor conditioned reflexes, worked out in various probabilities of reinforcement. On abscissa, numbers manifesting conditioned reflex. On ordinate, average significance of the number of applications of conditioned stimuli between two neighbouring manifestations of conditioned reflex. 1, 2, 3, 4, curved formations of conditioned reflexes, worked out at 1.0, 0.5, 0.25 and 0.1 probabilities of reinforcement respectively.

of kinetic protective conditioned reflexes, produced in fish (carp) at various probabilities of reinforcement. Manifestations of conditioned reflex are depicted horizontally and vertically the average meaning of the quantity of applications of conditioned stimulus between two consecutive applications of a conditioned reflex. As can be seen from these diagrams, the greater the probability of reinforcement, the sooner the line of intervals between the manifestations of conditioned reflex diminishes and comes to a stable level.

During the subsequent experiments on rats use was made of the T-shaped labyrinth for the study of differentiating appraisal of the probability of reinforcement. After a preliminary training of the animal through equal reinforcement on both sides with food, changes were made in correlations of probability of reinforcement, when the limit in the difference of probabilities proved to be a difference of 5–10%. After raising this correlation to 1 : 3 the animals started running mainly to one side of the labyrinth, which corresponds to 'the optimal strategy' of behaviour in the concept of play, as it is then that the largest number of reinforcements is secured.

TABLE I

CONTINUITY OF EXTINCTION OF KINETIC ALIMENTARY CONDITIONED REFLEXES IN A RAT, WORKED OUT THROUGH A PARTIALLY ACCIDENTAL AND PARTIALLY REGULATED (STEREOTYPE) REINFORCEMENT

No. of rat	*50% reinforcement*		*25% reinforcement*	
	Regulated	*Accidental*	*Regulated*	*Accidental*
1	15	17	16	28
2	18	28	36	46
3	15	23	32	46
4	27	34	36	54

The animals appeared to 'calculate' their behaviour. Sechenov said that the stimulus 'forewarns' the animal.

Let us cite additional data on the extinction of conditioned reflexes. Lovchikov and Menitzky (1963) made a study of the continuity of extinction of conditioned kinetic reflexes in partial, but accidental and stereotype, reinforcements. Altogether 60 applications of a conditioned stimulus were given (of these there were 30 reinforcements with 0.5 probability and 15 with 0.25 probability) after which a sharp intermittent extinction was made on the same day. Calculation was made of the number of times the animal approached the feeding trough after the start of extinction up to emergence of 5 consecutive negative results, which was taken as the characteristic of continuity of extinction of a temporary tie. On these same animals reflexes were worked out both during accidental and regular reinforcement in several series at 0.5 probability (and correspondingly reinforcement after 1 application) and 0.25 probability (use was made of each 4th application of the conditioned stimulus). The analysis of the results of experiments demonstrated that average continuity of ex-

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tinction of reflexes, elaborated during accidental reinforcement, is greater than in regular reinforcement, and this difference is statistically reliable.

Thus it was proved convincingly that the probable structure of the stimuli system has a vital importance for the conditioned reflex activity, which is usually taken into account: the stronger the correlation link between the appearance of a conditional signal and reinforcement the more additional (the so-called surplus) information is transmitted, which is used by the organism due to which the preparation of an adequate form of reaction (for example the formation and extinction of temporary tie) is speeded up.

Thus we come to a general conclusion that the alleviated creation of the more stable temporary ties on the ecologically adequate stimuli is a result of the more probable reaction on a more probable factor of the external environment. This corresponds to the statistical probability approach to the theory of conditioned reflexes, and coincides with the results of mathematical analysis of the alleviated formation of conditioned reflexes, which was made by Grey Walter in regard to the stimuli frequently met by the animal in his usual conditions.

In his 'Brain Reflexes' Sechenov gave much of his attention to the conditions of formation of the brain's reflex activity. As a rule, when these views of Sechenov are expressed, the role of external environment is stressed, while Sechenov himself wrote frequently in his works of many questions of diverse internal conditions of brain and organism, which determine to a large degree this or that level of the work of the central nervous system.

The problem of the tonus of the higher spheres of the central nervous system must first be included among these questions. Of special interest are ecological factors, which could be of decisive import in regard to the tonicity of the brain.

In this respect a very convenient subject was found in a domestic hen, which inherited from its ancestors an ecologically conditioned ability to fall into cataleptic constraint and remain motionless during twilight (hen's night blindness). This phenomenon was produced, as our experiments have shown (Karmanova, Blinkova and Semyonova), on the basis of developing central inhibition.

Comparing these results with the former researches, implemented in our laboratories regarding the influence of chromatic rays on the myogenic tonus of man's body and animals (Margolin) we supported, in developing further Markelov's theory of optical vegetative influences, an apportionment of a special group of phenomena, testifying to the correctness of the Sechenov tenet contending that from a visual nerve reflexes may stray to all muscles of the body. We designated this group of phenomena as optical or phototonic, having in view not the visual function of the retina, but its ability to alter the tonic condition of muscular apparatus depending only on the prevailing light. Sechenov distinguished the visual act, by which a child learns to distinguish only gradually from reaction to light. Obviously to this reaction also belongs the phototonic muscular reflex that we have isolated. At present we conduct the study of this phenomenon in a comparative physiological and ecological plan. This has made it possible to discover a number of peculiarities of the phototonical reflex. It has been established that, for instance, in gray wild mice, and in the white

mice used in laboratories, the bioelectric activity of skeletal muscle is sharply different, when animals are illumined at night time (Ler). This can be well seen in Fig. 2

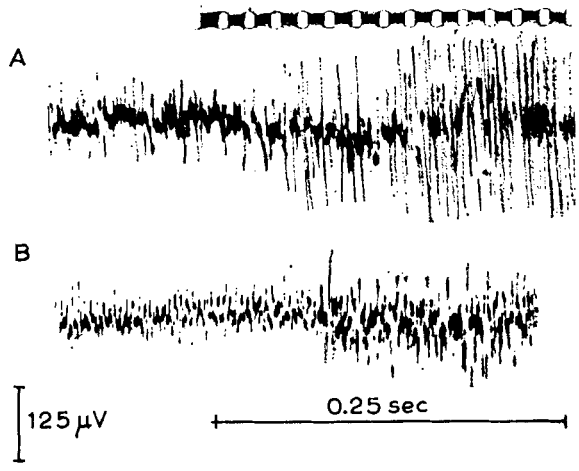

Fig. 2. Change in bioelectric activity of skeletal muscles of the thigh in wild gray and white laboratory mice at night (2 a.m.) under the influence of light. Black intermittent line, the moment of switching on the light stimulus. A, reaction of the skeletal muscles of the gray mouse's thigh at night. B, the same in a white mouse.

In the light of Sechenov's ideas the laboratories of Kupalov and Asratyan conducted researches of toning up influences on the higher levels of the central nervous system on the part of sense organs or 'analyzations' (this term was invented by Sechenov).

Our data make it possible to widen the results of these researches in a comparative and ecological manner. It was discovered, for example, that although the change in the visual regime of pigeons and rabbits (their lengthy stay in darkness or in light) reflects little on their conditioned reflex activity (heart or respiratory reflexes), in cats these factors produced sharp variations in the work of the brain. Very sharp differences were observed in the experiments with wild gray rats and laboratory white rats (Katinas and Popova).

These initial facts emphasize the urgent need of a study of the problem both in comparative and especially ecological aspects.

The basic mass of researches is related, for instance, to the factor of light. However, there is no doubt that sound stimuli, the sense of smell and others may produce a soothing influence on the work of large cerebral hemispheres. Moreover, one and the same stimulus, as we have shown, may have various influences, affecting representatives of diverse species.

The primary attention, justifiably, given to the soothing influences rendered by the external 'analyzators', must not exclude the interest to the problem of interoceptive and especially proprioceptive influences on the tonus of the higher sectors of the central nervous system.

In this respect, of much interest are the results of embryo-physiological researches conducted here on the hen's embryo. It was shown that up to the 13th day, despite

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complex kinetic acts testifying to well developed muscular receptions, the efferent impulse does not reach the central embryonic structures. Only on the 13th day does the impellent act result in the appearance of electrical discharges in central formations, which certifies to the fact that efferent impulses reach the central nervous system. From the 15th day of development, when there is a steady background electrical activity of the central nervous system, the shutting off of impulses from proprioreceptors due to the motionless embryo of curative-like substances results in a sharp inhibition of electrical activity (Bogdanov) (Figs. 3 and 4).

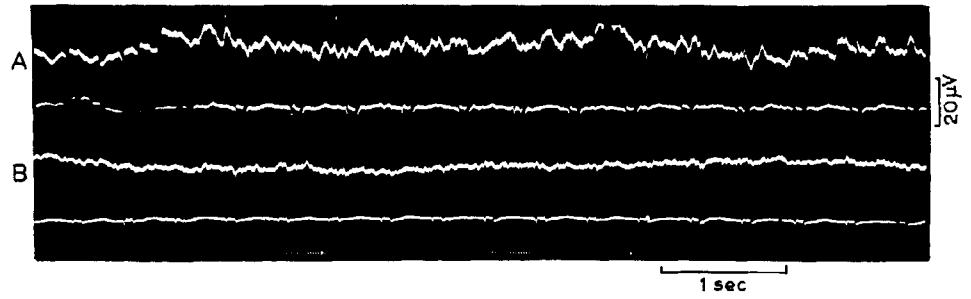

Fig. 3. Influence of the motionless state on electric activity of the brain in 15-day hen embryo. From top downwards: electroencephalography, electrocardiography. A, before, B, after introduction of procuran. Localization of electrode, in dorsomedial part of striatum.

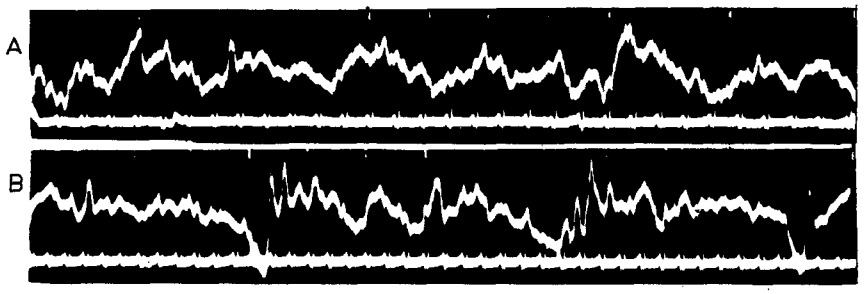

Fig. 4. Electroencephalography of 18-day hen embryo during motionless period. A, before, B, after introduction of procuran. From top downwards: electrocardiography, electroencephalography. Time mark, 1 sec.

Thus, it was established that at this age the leading role in maintaining the tonus of the central nervous system, whose expression lies in electrical activity, belongs to influences from proprioceptive apparatus. After the 17th day of embryonic development the leading role of proprioception in maintaining the tonic excitation of the central nervous system disappears, apparently due to the inclusion of the external 'analyzator' systems.

The problem of tonicity of higher spheres of the central nervous system must be regarded as one of importance, since its significance extends far beyond the limits of theoretical researches and leads to numerous practical applications and problems.

The basic fact is that for the effective work of a wide-awake brain it is necessary to have permanent stimulation reaching it from without.

The cybernetic analysis of the quality and quantity of stimuli also testifies to the fact that for maintaining the tonus of the central nervous system it is necessary to have some optimal average level and variety of incoming information.

Influenced by the ideas evoked by the problem of ecological adequacy of external environments worked out here, we wrote several years ago (1951) critically regarding the methodical ways of the study of man's higher nervous system, founded on a principle of its isolation from the stimuli of its usual external environment. It must be recognized that introduction into the methods of study of man's higher nervous activity on principles of isolating cells, which were used for the study of special questions dealing with the dogs' higher nervous activity is not permissible, since this deprives man from the influx of the necessary sensual information.

Some time ago Grey Walter developed the notion of the negative significance of the removal of sensorial influences ('sensorial deprivation').

Cosmic psychology and physiology which originated recently has proved convincingly that, for the normal behaviour of the nervous activity of cosmonauts during flight, the possibility of their contact with the earth and between themselves, various visual and auditory stimuli constitute an adequate background.

In this brief summary we wish to show that today too Sechenov's ideas suggest numerous new themes and problems for research.

SUMMARY

The investigation was concerned with the problem of biological and ecological adequacy of stimuli and the role of biological and ecological 'training' of the effector involved in conditioning. The facts obtained justify the hypothesis of a transitional form of response intermediate between unconditioned and conditioned reflexes.

A great number of observations made in our laboratory has recently gained theoretical support from analysis involving cybernetic experimentation. Animals have been shown, so to say, to 'pre-calculate' their behaviour (Sechenov spoke of a stimulus 'pre-informing' the animal). The rate of elaboration of a reflex has been shown to depend on probability of reinforcement. Animals also prove capable of discriminating even slight differences in the degree of probability.

The notion of ecologic adequacy of stimuli, of specific sensitivity of analyzers has been interpreted in terms of cybernetics. This conforms to the results of mathematical analysis of facilitated conditioning carried out by Grey Walter with respect to stimuli of most common occurrence under natural conditions of the animal. Sechenov wrote of environments 'capable' of maintaining the organism's existence.

A highly significant concept of Sechenov is that of tonus of higher brain parts being maintained by incessant stimulations, by 'impacts' from the environment. In this connection, the data obtained in our laboratory on the role of light, as a factor in the onset and maintenance of muscle tonus in animals are of considerable interest. The concept of non-visual phototonic function of the retina, displaying considerable

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variations, depending on the ecological significance of photic stimuli was elaborated. It was stated by Sechenov that stimulation of the optic nerve could elicit reflexes of every muscle of the body.

Cybernetic analysis of the significance of quality and quantity of stimuli for nervous activity supports our earlier statements on the necessity of a certain mean amount and variety of incoming information to ensure optimal activity of the nervous system.

The consideration of such tonus-promoting stimuli for the animal's relations with the outer world, discerned by Sechenov, should be extended to the variety of stimulations arising within the body. The effects of such internal stimuli have been shown in our laboratory to be active as early as the embryonic stage, particularly in studies of conditions under which the onset and patterning of electrical activity takes place in the higher parts of the embryonic brain.

Sechenov's contribution to the problem of central inhibition is of outstanding importance in connection with evolution of reflex activity. To a considerable amount of data on the evolution of inhibition (its phylogenesis and postnatal ontogenesis) accumulated in our laboratory, observations have recently been added on the occurrence of 'Sechenov inhibition' in the embryo. It has been shown that even at this early stage of development Sechenov's central inhibition displays its controlling influence in co-ordinating reflex activity.

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Electrophysiological Signs of Hippocampal Development in Ontogenesis

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After successful neurophysiological exploration of the functions of the reticular formation, attention of physiologists was directed to elucidation of the activity of other 'obscure areas' of the brain, especially of the archicortex, and particularly the hippocampus.

The interest of the hippocampus is not accidental. It is known that phylogenetically this structure occupies an intermediate place between the brain stem reticular formation, the thalamus and the neocortex. In early stages of phylogenesis, before formation of the neocortex, the hippocampus has well developed nervous connections with the hypothalamus. After formation of the neocortex these connections persist, and a special pathway develops which binds the temporal area of the cortex with the hippocampus (Adey and Meyer, 1952; Lorente de Nó, 1934).

On the other hand, the peculiar but relatively simple architectonics of the hippocampus facilitates the analysis of its electrical activity components. Comparison of the electrical effects recorded from different hippocampal areas with cortical effects may also help us to comprehend the mechanisms of the origin of neocortical electrical phenomena.

The results presented herein were obtained in the study of spontaneous electrical activity and evoked potentials of the hippocampus during its direct stimulation in rabbits of different ages. The results were obtained from the hippocampal area with the greatest number of small pyramids (area CA1 according to Lorente de Nó). Recording of electrical effects was done in conditions of acute experiments, mostly on unanaesthetized or curarized animals.

SPONTANEOUS ELECTRICAL ACTIVITY

As it is known, spontaneous electrical activity of the hippocampus of adult rabbits differs from the neocortical activity and is characterized by the predominance of a relatively slow rhythm of small amplitude. In the early postnatal period, however, spontaneous activity of the hippocampus hardly differs from the activity of the neocortex. A simultaneous recording of hippocampal and cortical activity displays a homogeneous pattern: predominance of low voltage irregular slow waves (Fig. 1, A).

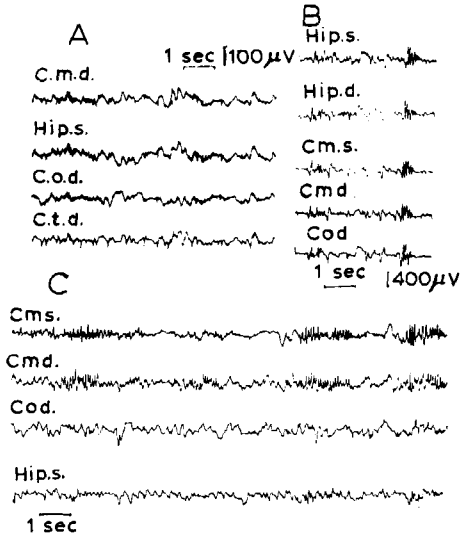

Fig. 1. Spontaneous electrical activity of rabbits of different ages. (A) 3-day-old unanaesthetized rabbit. (B) 9-day-old curarized rabbit. (C) curarized rabbit of the third period. Hip.s = left hippocampus; Hip.d = right hippocampus; Cm.s = left motor cortex; Cm.d = right motor cortex; C.o.d. = right visual cortex; C.t.d. = right temporal cortex.

Beginning on the 6–7th day of life the amplitude and frequency of oscillations increase. From this age on in the hippocampus, as well as in the neocortex, there occur groups of a periodic discharges — waxing and waning waves of spindle type, with a frequency of about 15 per sec. Such discharges appear in the group of 5–6 waves every 1–3 sec (Fig. 1, B).

Beginning from the third postnatal week, there develops a differentiation of the activity of the hippocampus and the neocortex. While the hippocampus is still characterized by low voltage activity of 4–6 per sec, the neocortex shows a higher frequency and larger amplitude of spontaneous brain potentials. Certain cortical areas then display an activity of 'individual' nature. From this age the groups of spindle-like discharges do not occur in the hippocampus; they appear only in the motor cortex every 3–4 sec, and last for 2–2,5 sec (Fig. 1, C).

The homogeneous patterns of the spontaneous electrical activity in the hippocampus and neocortex give us reason to believe that the neuronal structures of these formations are influenced by a common subcortical centre. In the early postnatal period, when structural development of neurons is far from complete, this common centre evidently exerts an equal influence on all these elements. Since the latter have not yet developed their individual properties, the activity in all the recorded areas is similar.

This assumption is also corroborated by the structural data on the ontogenesis of brain cortex neurons and their interconnections.

According to Noback and Purpura (1961), ontogenetic development of the electroencephalogram should be associated with enhancement of the number of cortical neu-

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ron synapses. These authors showed that in the postnatal development the main importance should be ascribed to a quick growth of cortical basal dendrites. On the other hand, on the basis of electron microscopic observations, it was concluded that in the establishment of axodendritic synaptic contacts dendritic spines appear to be specialized apparatuses (Gray, 1959; Purpura, 1961; Pappas and Purpura, 1961). Dendrite spines develop in kittens only by the end of the first postnatal week. According to Noback and Purpura (1961), well developed branchings of basal and apical dendrites are observed in an 8-day-old kitten, its pyramidal neuron dendrites being short and without branchings before this period. In a 14-day-old kitten basal dendrites and collaterals of apical dendrites develop further; dendritic spines at this age are well pronounced. In a 3-week-old kitten pyramidal neurons of the cortex are fully differentiated, and the quantity of basal dendrites and apical dendrite collaterals is actually the same as in an adult cat. All the dendrites have numerous spines, just as in the adult cat.

The morphological data on the ontogenetic development of cortical neurons in rabbit are not very rich. It may be that the development of the rabbit brain follows approximately the same pattern as in cat. According to the data of Schadé (1959), the first phase of morphological development of the rabbit cortex is complete during the first days of life. At this phase division of nerve cells takes place. The second phase lasts for 15–18 days. At this phase Schadé observed the growth of nerve cells, development and branching of axons and dendrites. By the end of the second phase the weight of the rabbit brain is 70% of the weight of the brain of an adult rabbit. During the third phase nerve cells stop growing, but the development of the dendrite plexus continues. Alongside it, myelinization of cortical neurons begins.

Proceeding from these observations it may be concluded that development of hippocampal and cortical neurons must take an approximately similar course. Morphological development of the rabbit hippocampus has been studied by Svanidze (1964) of our Institute. The results of the experiments, which are still in progress, seem to corroborate our assumption. According to Svanidze's observations, all the hippocampal layers are represented in the very first days after birth. In the first two postnatal weeks he observed changes in the depth of the hippocampal cortex and in the density of cellular elements and a considerable increase in the number of collaterals on the main branches of basal and apical dendrites in areas CA1 and CA3 which he studied. In structural development of the hippocampus, as well as of the neocortex, three main phases are manifest. In the first days of life the pyramidal layer in the area CA1 consists of relatively small cells arranged in five rows. Apical dendrites of these cells reach the molecular layer. The second phase is characterized by the growth of nerve cells. On the seventh day after birth pyramidal cells are more densely packed due to the enlargement of the soma of the cell, and branches of apical and basal dendrites become more abundant.

The greatest packing density of the pyramids is observed on the 17th day. Some pyramidal cells shift to the radial layer. At this stage of development, *i.e.* at the third phase, pyramidal cells have a well developed net of apical and basal dendrites. Apical dendrites reach the molecular layer, and axons go to the alveus.

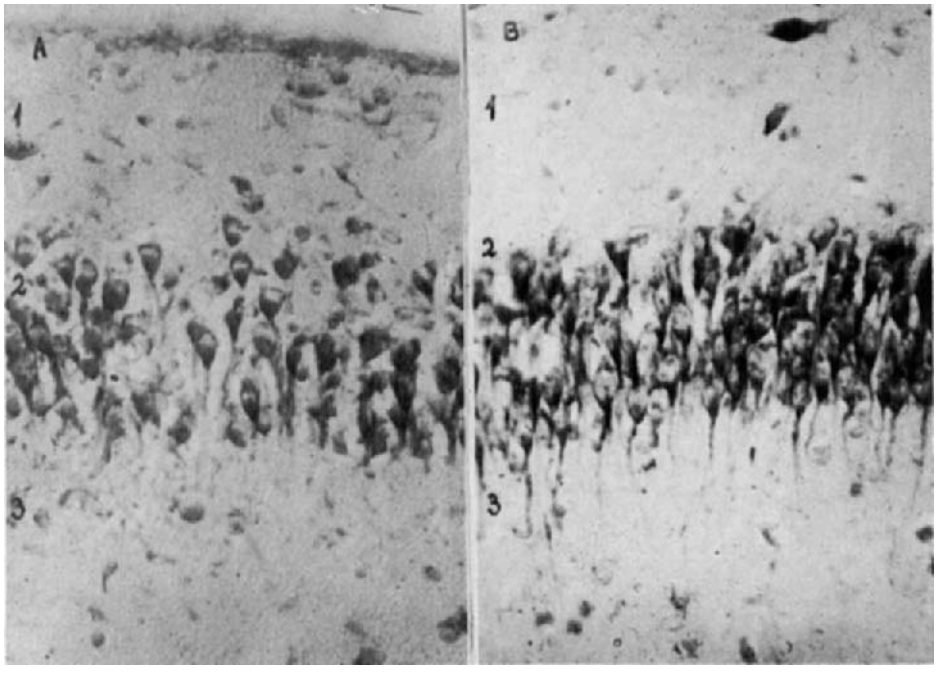

Fig. 2. Packing density of hippocampal pyramidal neurons (area CA1) in the first and second period of development. (A) hippocampus of 2-day-old rabbit. (B) hippocampus of 12-day-old rabbit. Staining by Nissl. Enlargement 400. 1 = alveus; 2 = layer of pyramidal cells; 3 = radial layer (data of Svanidze).

Distribution of pyramidal neurons at different stages of hippocampal development is shown in Fig. 2.

Modification of the character of spontaneous electrical activity of the hippocampus and neocortex is believed to be closely connected with morphological changes in the course of development of these structures.

The first period of spontaneous electrical activity, *i.e.* mainly low voltage and slow activity with almost no fast oscillations, evidently reflects the activity of the elements with immature soma, underdeveloped basal dendrites and apical dendrites with a very small number of collaterals. On the other hand, in the cortical cells (and probably in the hippocampal cells) axodendritic contacts through dendritic spines are absent. On the apical dendrites these contacts are established without dendritic spines as evidenced by electron microscopic studies (Purpura, 1961; Voeller *et al.*, 1963).

The development of dendritic spines results in an increase of amplitude and frequency of spontaneous electrical activity in the cortex and hippocampus. Evidently they initiate spindle-like discharges.

When the development of neuronal structures is complete, *i.e.* when branching is over, axodendritic synaptic contacts are finally established and axosomatic contacts are stabilized owing to myelinization of axons and maturation of axosomatic synapses, when the structural peculiarities of the hippocampus and of separate areas and fields of the neocortex are eventually formed, then the connections between different brain

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areas become well differentiated, and the influence exerted on all these structures by the common subcortical centre is not homogeneous it appears in the form of a differentiated activity of the hippocampus and different areas of the neocortex.

We have made electrophysiological observations which indicate that even at the earliest stages of postnatal ontogenesis there exists a close connection between the hippocampus and subcortical structures, the latter exerting a simultaneous influence on the neocortex (Dzidzishvili and Kvirkvelia, 1963). This connection is corroborated by phylogenetic development of the nervous connections of the hippocampus with other brain structures. For instance, phylogenetically, even before the development of the neocortex, the hippocampus shows close connections with the brain stem, hypothalamus, thalamic structures and corpus striatum (Herrick, 1948).

From the appearance of spindle type discharges, it may be concluded that this connection of the hippocampus with nonspecific thalamic structures appears well before the structural completion of the cortex and hippocampus itself. Moreover, the appearance of spindle type discharges in the spontaneous activity of the hippocampus and neocortex suggests that at the second stage of the postnatal period when dendritic spines appear, and, consequently, when morphological grounds for the formation of perfect axodendritic synaptic contacts are created, the degree of neuron development is sufficient to generate relatively fast waves.

Evoked potentials

In the very first days of life, stimulation of the hippocampal surface provokes response potentials on the side of stimulation, as well as in the contralateral hippocampus. If recordings are taken from the side of stimulation not far from the stimulating electrodes, a prolonged negative potential appears, sometimes preceded by a small positivity. In the contralateral hippocampus at the point symmetrical to the stimulated one, a more complex response appears: following a small positivity there comes a negative potential of high amplitude consisting of several components. The ascending phase of the response is characterized by a slow upward deflection followed by a quicker one. In the descending phase a slow component is recorded in the form of a 'tail'. This component drops to the initial level in a few scores of milliseconds, and for a few milliseconds transforms into a positive phase (Fig. 3, A). By this time the prolonged and relatively small negative potential on the stimulated side falls to the vanishing point.

In the second period, approximately from the 6th day of life, duration of the negative potential on the stimulated side is considerably reduced, and is often preceded by a small positive wave. In the opposite hippocampus, at the point symmetrical to the stimulated one, no slow upward deflection on the ascending part of the negative phase is recorded after the small positivity, as occurred in the first period. The length of the 'tail' at the descending part of the phase also changes its character, reduces in duration and returns to the initial level without transformation into positivity. Therefore the total duration of the transcommissural response is somewhat diminished (Fig. 3, B). In the third period, beginning at approximately the third postnatal week, evoked

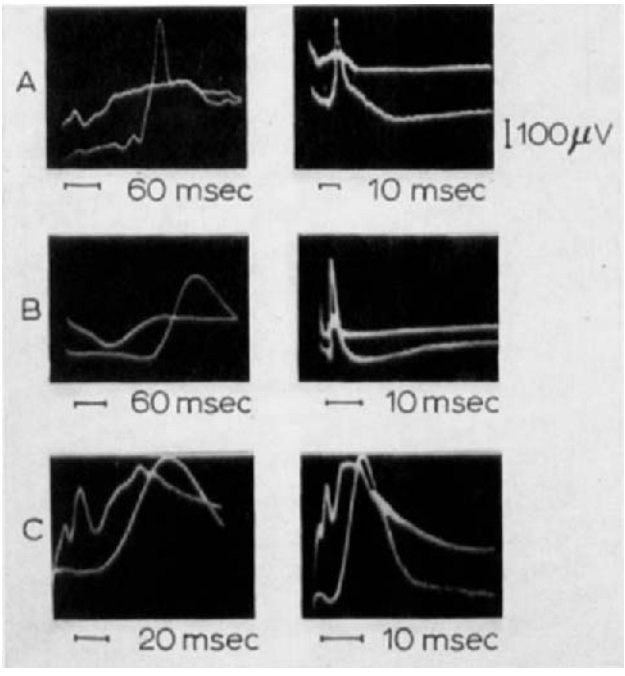

Fig. 3. Effect of hippocampal single stimulation. On all the oscillograms: upper line, recording from the surface of left hippocampus; bottom line, from the right. In all the experiments the left hippocampus was stimulated. Stimulus intensity in experiments (A) and (C): 3 V; in (B): 2 V. Duration of the stimulating impulse in msec. AC-amplifiers with time constant of about 1 sec. (A) I period; 3-day-old rabbit under urethane (1 g/kg.) (B) II period; 13-day-old rabbit under urethane. (C) III period; adult curarized rabbit.

potentials do not differ in their character from the hippocampal responses of the adult rabbit (Fig. 3, C): on the stimulated side there sometimes occur multiphasic responses with the predominance of prolonged negativity; while in the contralateral hippocampus, following a small positivity, there appears a high amplitude negative wave with a small 'tail' on its descending portion. In the second and third periods the configuration of the hippocampal evoked potential hardly differs from the neocortical evoked potential, and the commissural response hardly differs from the transcallosal. However, when cortical evoked potentials are recorded, the recording electrodes are situated over the apical dendrite layer, whereas hippocampal responses are recorded by the electrodes placed over the white matter, the alveus, that contains a layer of fibres. Basal dendrites are localized beneath this layer, they are shorter than apical dendrites, are not rich in branchings and do not approach the surface so closely as the apical dendrites of the neocortex do (Kandel *et al.*, 1961).

The negative phase of the hippocampal evoked potential of the adult rabbit is the result of activation of surface elements: according to Per Andersen (1959), the amplitude of the negative phase of the hippocampal evoked response gradually diminishes with further plunging of the recording electrode into the depth of the hippocampus, and falls to zero at the level of the pyramidal layer. At this layer the biphasic response

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becomes fully monophasic negative. In his later work (1960) Per Andersen considered the negative potential of the commissural response to be the result of postsynaptic activation of the shafts of apical dendrites of hippocampal pyramidal neurons. However, on the basis of their experiments with intracellular recordings, Kandel *et al.* (1961) came to the conclusion that stimulation of the hippocampal surface can hardly produce selective activation of the most deeply localized apical dendrites. Even if they are excited synaptically, these dendrites cannot be the only structures activated by stimulation of the alveus. Having discovered the effect of γ-amino-butyric acid on the evoked potentials of the hippocampus, they found that inversion of the potential under the influence of this agent took place long before it reached the layer of apical dendrites. This led them to the assumption that stimulation of the hippocampal surface may bring about activation of pyramidal cells as well as of basal dendrites and, finally, of the axons localized in the alveus.

The study of response potentials obtained in the first period suggests that the mechanism of origin of the response on the stimulated side is somewhat different from the mechanism of the origin of the contralateral commissural response of the hippocampus. The slow potential that appears in the hippocampus on the stimulated side is evidently a manifestation of the activity of its yet underdeveloped basal dendrites, their shafts and non-numerous collaterals. It is true that the fibres of the alveus, on whose surface stimulating electrodes are placed, are also activated, but the activity of these underdeveloped non-myelinated elements may also be the manifestation of the slow activity, summed with the activity of the dendrites. A feeble positivity, which occasionally precedes a homolateral negative wave, evidently reflects the activity of a small number of cell bodies. A more complex commissural response must reflect the postsynaptic activation of different cell bodies, then the activity of axons, and, finally, of dendrite shafts and branches, as evidenced by the initial ascent of the curve with a subsequent long 'tail' following the peak.

In the second period the commissural response spike potential begins without the slow component on the ascending portion of the curve, and the activity of dendrite branches finds its expression in the form of a long 'tail'. The positive phase of the homolateral response is now more pronounced, which points to an easier excitability of the cell. In the third period the hippocampal commissural response manifests the activity of the elements with 'slow' electrical characteristics (probably basal dendrites) and cell bodies. The homolateral response is complex and includes the responses of cell bodies and dendrite elements. Therefore the character of this response is largely dependent on the intensity of the stimulation current employed.

RESPONSE POTENTIALS DURING RHYTHMIC STIMULATION OF THE HIPPOCAMPUS

In the first period of animal development, approximately up to the 6–7th day of life, hippocampal stimulation, even at the low rate of 1–2 per sec, brings about a gradual decrease in the potential on the stimulated side. The commissural response undergoes sharp changes, too: the negative phase of the response gradually diminishes. Mean-

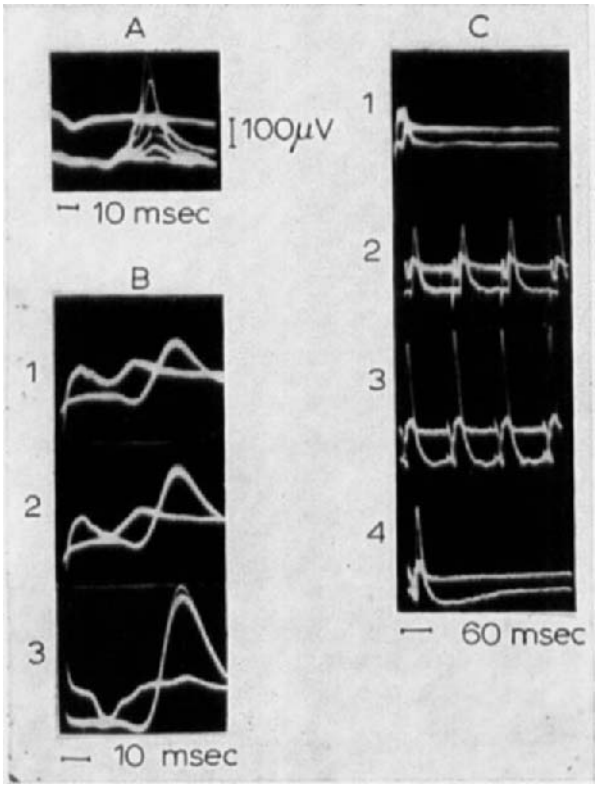

Fig. 4. Effect of rhythmic stimulation of hippocampus. (A) I period; 3-day-old unanaesthetized rabbit under urethane. Stimulation frequency: 6 per sec, 3 V. (B) II period; 13-day-old rabbit under urethane. 1 = beginning of stimulation; 2 = a few sec later; 3 = a few sec later still. (C) the same preparation. 1 = test of single stimulus before the beginning of tetanization; 2 = beginning of tetanization; 3 = a few sec later; 4 = test of a single stimulus upon cessation of tetanization and a few sec later. Stimulus intensity is always 2 V.

while the positive phase remains more or less unchanged (Fig. 4, A). Relative stability of the positive phase persists through successive periods. Thus, in the first period, the phenomenon of tetanic potentiation in the hippocampus does not occur. Moreover, a contrary process is observed. This circumstance evidently determines another peculiarity: absence of convulsive discharges in the hippocampus regardless of the intensity and frequency of stimulation.

We believe that such a fast decrease in hippocampal effects during its rhythmical stimulation is conditioned by the 'fatigue' of those elements which produce the negative phase of the response. (True, the term 'fatigue' offers little for the comprehension of the intimate mechanism of the decrease phenomenon during repetitive stimulation, but, nevertheless, it gives an impression of the small 'working capacity' of immature dendrite and axon formations.) In this respect pyramidal cells appear to be steadier, for the positive phase of the response at this time undergoes no particular changes. The decrease in response potentials cannot be accounted for by modifi-

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cation of synaptic transmission of impulses, as evidenced by the fact that the positive phase of the commissural response remains unchanged.

In the second period a contrary picture is observed: response potentials, produced by low frequency stimulation of the hippocampus, are gradually augmented or remain unchanged. Modification of the effects may be different in one and the same preparation. For instance, rhythmic stimulations may produce potentials of more or less equal amplitudes, exceeding that to a single stimulus, sometimes accompanied by the phenomenon of posttetanic potentiation. Rhythmic low frequency stimulation may evoke a gradual increase in the response (Fig. 4, B and C). These gradually increasing potentials attain a certain level at which, as a rule, they turn into convulsive discharges. Convulsive discharges appear most easily in the second period; they may be provoked by a stimulus of low intensity or even occur without any stimulus. They can last for minutes or scores of minutes followed by a prolonged phase of depression of activity. An increase in stimulus rate up to 25–30 per sec brings about a rapid depression of response potentials, but these seemingly 'depressing' stimuli easily generate convulsive discharges. The phenomenon of tetanic and posttetanic potentiation most often occurs with commissural response. This suggests that in the origin of the phenomenon a decisive role is played by facilitation of transsynaptic transmission, which appears even in the second period, *i.e.* during growth of the nervous cells and development of the dendrites. This period is supposed to be that of appearance of dendritic spines, which participate in the establishment of axodendritic synaptic contacts.

The third period is characterized by a gradual transition from the second period to the adult state. Eliciting of convulsive discharges in this period, as compared with the second period, becomes difficult. With a stimulation rate of up to 6 per sec, the amplitude of response potentials does not increase. With a stimulation frequency 8–12 per sec, response negative potentials in the hippocampus opposite to the stimulated one augment and attain a value approximately twice as big as the response to a single stimulus. A further increase in the stimulus frequency up to 16 per sec induces not an increase, but a decrease in the negative potentials of the commissural response. With a frequency of 30–40 per sec, these potentials diminish sharply, although they do not disappear completely. Cessation of fast rhythm stimulation (30–40 per sec) results in posttetanic potentiation of the activity.

The third period is also marked by inversion of evoked potentials to rhythmic stimulation, *i.e.* when low rhythm stimulation lasts for several seconds, the amplitude of negative potential, augmenting progressively, attains its maximum, then begins to

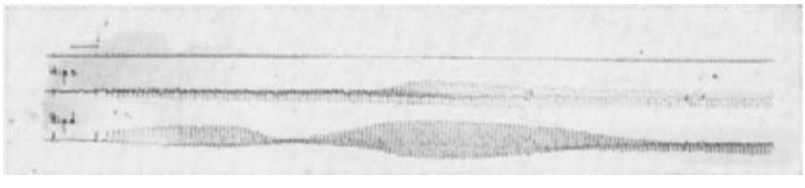

Fig. 5. Effect of rhythmic stimulation of hippocampal surface of adult rabbit. The mark at the top indicates the stimulation (3 V). Left hippocampus is stimulated as in previous experiments.

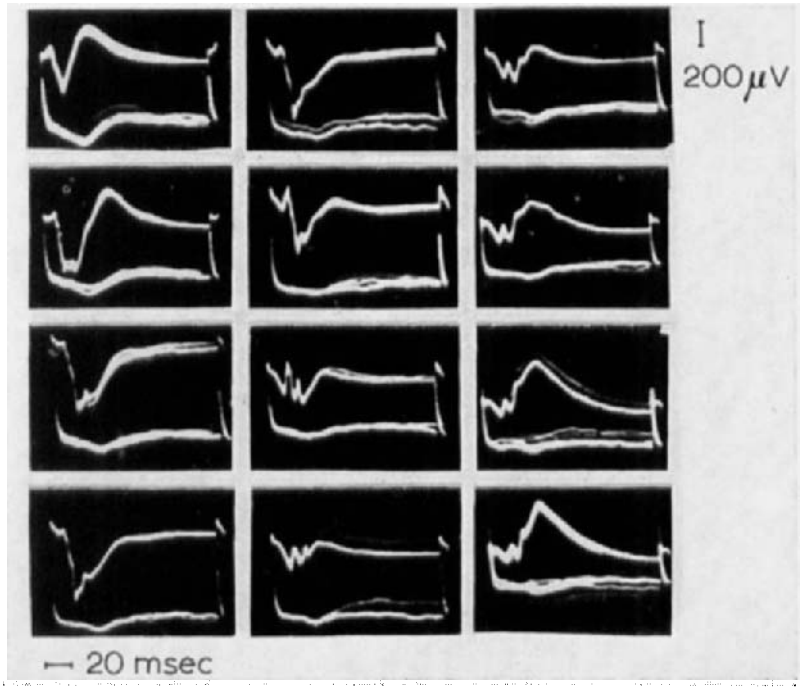

Fig. 6. Inversion of hippocampal evoked potentials during rhythmic stimulation. In the given experiment right hippocampus is stimulated (lower line); III period. Stimulation frequency 8 per sec, 2 V; succession of effects: from top downwards, the second row is an immediate continuation of the first, and the third of the second.

fall, and the potential subsequently disappears (Fig. 5). After that the positive phase develops, which, in its turn, having attained the maximal amplitude, decreases and having dropped to a minimum, gives way to the negative phase, which is again augmenting. Further, at the positive phase of the response several components are observed, which are more pronounced the lower the amplitude of the total positive potential (Fig. 6). Occurrence of inverted potential, characteristic of the adult state, is not recorded simultaneously in both the hippocampi. It is most pronounced in commissural response. If, however, it occurs in the stimulated hippocampus as well, some distance away from the stimulation point, then it is not recorded in the opposite one. Judging by the nature of the changes in commissural responses to stimulation 8–12 per sec, it may be concluded that the waxing and waning effect of the responses must have a common mechanism with the origin of the recruiting response during 8–12 per sec stimulation of nonspecific thalamic nuclei. It is interesting that, in response to such stimulation, Kandel *et al.* (1960) observed in rabbit hippocampus the appearance of a prolonged negative shift (d.c.-shift) of 20 sec and 2–6 mV. According to these authors, inversion of evoked potentials is in some way associated with the moment of the return of a slow negative potential to the initial zero level. In the opinion of Kandel *et al.*, this prolonged shift, with the inversion of hippocampal evoked responses accompanying it, differs from the potential which is accompanied by spreading depression.

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It is believed that the inversion of potentials, and somehow connected with it the prolonged negative potential, recorded by Kandel *et al.* (1960), takes place only after the development of hippocampal neurons is more or less complete. At this time, when the axon elements are myelinated and synaptic contacts are most developed, differentiated connections are formed and close interrelations with subcortical structures are established. All this creates most favourable conditions for manifestation of complex effects of the electrical activity, which we intend to subject to a detailed analysis in the work concerned with some peculiarities of the electrical activity of the hippocampus of the adult rabbit (Dzidzishvili and Kvirkvelia, 1965).

SUMMARY

The peculiar but relatively simple architectonics of the hippocampus allows us to study the genesis of the electrical activity components not only of the hippocampus but also of the neocortex. In this respect it is important to study the electrical activity of the hippocampus in ontogenesis.

The hippocampal electrical activity of rabbits was studied in the area CA1 (according to Lorente de Nó) containing small pyramids.

In the first postnatal days the spontaneous electrical activity of the hippocampus does not differ from that of the neocortex, and is characterized by low voltage potentials with the predominance of slow waves. With age the amplitude of the electrical activity increases and fast rhythms appear. Six to seven days after birth groups of periodic spindle type discharges, peculiar to sleeping adult animals, appear both in the hippocampus and neocortex. The homogeneous patterns of the spontaneous activities of the hippocampus and the neocortex suggest that there must be a common centre spreading its influence both on the hippocampus and the neocortex.

In the very first postnatal days stimulation of the hippocampal surface evokes potentials both in the stimulated and contralateral hippocampus, especially at the point symmetrical to the stimulated one (commissural response). During the first days these potentials have maximal latency and minimal amplitude. With age their latency diminishes, while their amplitude increases. Their duration is reduced. The evoked potential appears in the stimulated hippocampus as a slow surface negative wave; in the opposite hippocampus this wave is preceded by a small positivity.

During rhythmic stimulation (8–10 per sec) the evoked potentials of the hippocampus diminish gradually and after 4–5 stimuli they disappear. The surface negative phase of the commissural response diminishes too, although it does not disappear completely. The surface positive phase of the response does not undergo any significant change. Till about the 6th day after birth, no convulsive discharges are recorded in the hippocampus, though they appear very easily in a more adult rabbit. Apparently, this can be accounted for by the absence of the potentiation phenomenon at this age.

From the 6th day on, tetanic and posttetanic potentiation is observed. Upon rhythmic low rate stimulation (8–10 per sec) the evoked potentials of the hippocampus

augment progressively, and may turn into convulsive discharges with long-lasting afterdischarges.

From the 17th day on, when the hippocampus and neocortex spontaneous activity is distinctly differentiated, an inversion of the surface negative phase of the evoked potential takes place in the hippocampus during the potentiation. When rhythmic stimulation lasts for several seconds, the surface negative potential, after attaining its maximum, begins to fall and then passes into a positive potential which augments progressively. No appearance of simultaneous bilateral inverted potential has been observed. In general at this age the convulsive discharges in the hippocampus take place only under strong and long-lasting rhythmic stimulation. At the same time, more localized responses arise to both single and rhythmic stimulation.

Recruiting response of the neocortex, potentiation phenomenon and convulsive discharges in hippocampus during rhythmic stimulations may be based on common mechanisms.

During the first postnatal days the pyramidal layer of the area CA1 consists of relatively small cells with short apical dendrites. On the 7th day, as a result of the growth of soma, the pyramids are arranged more densely, and the number of the apical and basal dendrites is increased. On the 17th day, the pyramidal cells acquire a still more compact arrangement with a well developed net of dendritic branches.

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On the Evolution of the Integrative Activity of the Central Nervous System in the Phylogeny of Vertebrates

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In his classical work 'Elements of Thought' Sechenov formulated a well-balanced framework of views on the evolution of nervous activity. He wrote that the general aspect of evolution is the same at all stages of development. At the lowest level of the animal kingdom sensibility is evenly distributed throughout the body; with the progress of evolution, separate systems are formed out of this diffuse form of sensibility, and eventually, in higher mammals, specialized functions arise co-ordinating the intracorporal and total nervous activity of the organism with the conditions of the external environment. These ideas of Sechenov ensured the basis for the evolutionary physiological studies of Orbeli who, as early as 1923, expounding the evolutionary nature of Pavlov's teaching on the hereditarily fixed and individually acquired forms of nervous activity, wrote that 'the study of conditioned reflexes is a means of conceiving the paths traversed by the history of the origin of co-ordinations'.

Later, while summarizing the results of the many years of researches by his pupils, Orbeli (1961, 1958) stressed that the investigations into the fields of comparative and ontogenic physiology, zoophysiology, and experimental and clinical pathology enable evolutionary physiology to be singled out as an independent subject among the other biological sciences, constituting a new stage in the development of physiology.

According to Orbeli, the aim of this new subject is 'not only the ascertainment of the course of development, the pattern of development and the sequence of events, but also the establishment of their interrelations and the causative dependence of all transformations and changes upon the factors of the environment affecting the living being' and upon the factors 'originating in the organism itself as the interaction between its separate parts'.

Proceeding from the quite definitely stated views of Sechenov and Orbeli on the ways of formation of separate integration systems in the course of evolution of the animal kingdom, we have endeavoured, during the past years, to find out the peculiarities of the functional and structural organization of some divisions of the central nervous system in the members of the vertebrate classes constituting the main line of development of the nervous system — namely, in the Acrania, Cyclostomata, plagiostomes, amphibians, reptiles and lower mammals.

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Peculiarities of the activity of the central nervous system in the lancelets and lampreys

The nervous system of the lancelet, as is known, has no signs yet of cephalization (Zavarzin, 1941), and is in fact a diffuse nerve net. This indifferentiation of the central nervous system is of interest for the study of the peculiarities of the functioning of the central nervous system at the initial stage in the phylogeny of vertebrates. In order to discover these peculiarities, both simple reactions to external stimuli — such as photic, algesic and tactile — and the possibility of temporary connections being formed were studied. The tests conducted to this end by Sergeyev (1961, 1963) indicated that at the action of photic, algesic or/and tactile stimuli both on the head and tail portions of the nervous tube of a lancelet, the latter reacts to these to an equal extent and with an equal degree of intensity.

When the nerve tube is sectioned the above reactions remain seemingly unaffected; at least no discernible difference is observed between the reactions elicited from isolated sections of the rostral or caudal ends of the nerve tube.

Tests involving formation of positive conditioned reactions have indicated that, when indifferent stimuli are combined with unconditioned reinforcement, temporary connections are developed. The characteristic trait of these temporary connections is their singular instability. They are easily evoked after a few combinations of conditioned and unconditioned stimuli and disappear as easily after the reinforcement is discontinued. Tests involving disjunction of the nerve tube into several anatomically disunited segments indicated that temporary connections can be evoked in any isolated portion of the nerve tube if conditioned and unconditioned reflexes are addressed to them.

These observations have made it possible to draw the conclusion that at the earliest stages in the development of vertebrates, *e.g.* in the lancelet, the nervous system functions equipotentially with respect to all its constituents, both as regards the external stimuli and the formation of conditioned reactions.

Members of the following, higher class of vertebrates, particularly the lampreys, in spite of the formation and differentiation of various divisions of the central nervous system, still preserve some elements of the diffuse mode of functioning. Thus, it was ascertained in the earlier investigations of Baru (1955) that the establishment of temporary connections in lampreys proceeds as the formation of momentary unstable reactions of the summation reflex type. If the forebrain and/or the optic lobes are excised conditioned reactions for light and other stimuli will remain. In a different set of experimental conditions. Sergeyev (1964) managed to evoke stronger conditioned reactions in lampreys than those in lancelets, but even in these tests the formation of conditioned reactions had a generalized character.

To verify the results of these studies, testifying to the diffuse functioning of the central nervous system in cyclostomes, electrophysiological investigations were undertaken. These investigations (Veselkin, 1963) showed that when the retina was lighted or the optic nerve electrically stimulated, distinct evoked potentials were recorded in the tecti optici, medulla oblongata and the octavolateral complex. Further, on optic stimulation of the medulla oblongata and the spinal cord, rapid

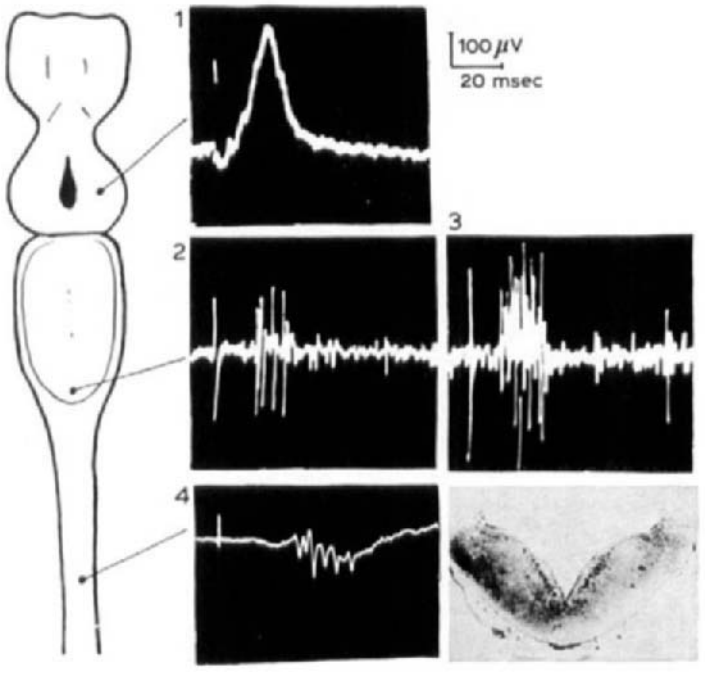

Fig. 1. Responses in a lamprey's brain evoked by electrical stimulation of the optic nerve. Left, schematic drawing of the lamprey brain. 1, Response in an optical tectum; 2 and 3, rapid discharges in the medulla oblongata before (2) and after (3) destruction of both tecti optici; 4, discharges in the spinal cord. Negativity in this and all other drawings, the ray deflecting upwards. The photomicrograph shows the frontal section of the mesencephalon following the coagulation of the tecti optici.

discharges of descending fibres were clearly observable, although they were absent in other species of animals under normal testing conditions. Control tests indicated that with the complete extirpation of the tecti optici or their coagulation electric reactions in response to a photic stimulus in the spinal cord and the medulla are preserved (Fig. 1).

The foregoing results of experimental studies have generally led us to believe that there exists a definite correlation between the structural organization of the central nervous system and its inherent function; at the earliest stages in the development of vertebrates — *i.e.* in lancelets and largely in lampreys — a generalized, less coordinated form of nervous activity is conditioned by the diffuse, unspecialized structure of the central nervous system.

The functional and structural organization of the central nervous system in plagiostomes and Osteichtyes

An important feature of the central nervous system at this level of the vertebrate development is the appearance of a powerful suprasegmental apparatus, the cerebellum, having abundant afferent and efferent connections with the optical (tecti optici), and auditory (ganglion isthmi, torus semicircularis) systems, with the reticular

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formation of the brain stem and the midbrain, with the hypothalamus, and with the nuclei of the oculomotor, facial and vagal nerves (Franz, 1911; Kappers *et al.*, 1936; Tuge, 1934; Zelikin, 1957). These evolutionary-structural changes governed by the mode of living of animals (development of the swimming function, active form of food procurement) bring about a number of changes in the forms of functioning of the central nervous system.

It has now been proved beyond doubt that more stable positive conditioned reflexes and all types of internal inhibition are developed in the fishes (Voronin, 1957). The absence of the pallial system in fish poses the question: in what structures are the temporary connections closed? It has been established that, with the extirpation of the forebrain, conditioned reflexes remain intact; after the extirpation of the optic lobes conditioned reflexes for light disappear, while those for a bell endure (Baru); and finally, after the extirpation of the cerebellum, according to some authors (Karamyan, 1956; Malyukina, 1955) conditioned reflexes disappear, and according to others (Bianki, 1962; Kholodov, 1958), conditioned reflexes in decerebellar fishes, though impaired, preserve the ability to form.

Electrophysiological studies have also yielded controversial results. Zagorulko (1959), Schadé and Weiler (1959), Enger (1957), in response to photic stimulation discovered distinct reactions in the cerebellum. Voronin and Guselnikov (1963) attribute these responses to the volumetric conductor properties.

On the basis of the earlier (Karamyan, 1956, 1958) physiological studies of the motor, compensatory, trophic and conditioned-reflex activity of the various divisions of the central nervous system of fishes, as well as on the basis of the results of morphological studies (Kappers, Zelikin and others), according to which efferents from all subdivisions of the diencephalon and mesencephalon — including those from the nuclei of the optic and auditory systems — are represented in the fish cerebellum, and efferent fibres run from the cerebellum to these systems, it was suggested that in the fish 'the principal system of the reflex adaptational activity, including the establishment of temporary connections, is the cerebellum together with the mesencephalic nerve formations, and not the forebrain hemispheres'. Bianki (1962) believes that the closure organ of the conditioned reflexes for visual stimuli is not the cerebellum but the tectum, while the cerebellum participates in the closure of interoceptive conditioned reflexes. According to Kholodov (1958), the conditioned reflexes closure organ in the fish is the thalamus. The important aspect of these assumptions is not so much the proof of the existence of some highly localized structures (nowadays such notions are too archaic to be considered seriously); the important part is that in fishes, for the first time in the phylogenic series of the vertebrates, the new, cerebellar-mesencephalic system is formed, which provides a highly-developed motor activity with both its conditioned and unconditioned components.

Of interest in this respect are the results of the experimental studies by Malyukova (1964) who, using the motor-alimentary conditioned-reflex technique of Prazdnikova (1953), showed that in golden carp a distinct differentiation of three simultaneously presented geometrical figures — a circle, a triangle and a cross — can be developed. It was further established that the electrical excitation of the basal nuclei of the forebrain

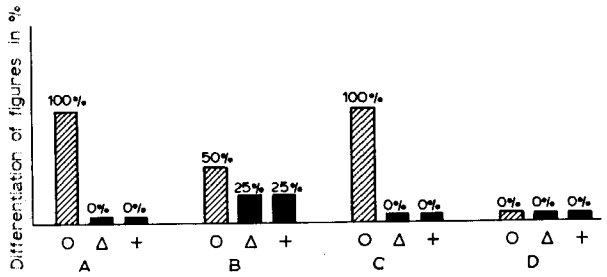

Fig. 2. Changes in conditioned-reflex activity in fish after a partial coagulation of the prosencephalon and the valvula cerebelli. A, differentiation of figures before the operation; B, differentiation of figures after partial coagulation of the prosencephalic basal nuclei; C, restoration of the differentiation of figures; D, disappearance of conditioned reflexes after unilateral coagulation of the valvula cerebelli. *Conventional signs.* On the X-axis, conditioned reflex values, in %; on the Y-axis, conditioned reflexes for geometrical figures. The hatched columns denote motor reaction for a positive stimulus (a circle); the black ones denote failure to move in response to inhibitory signals (a triangle and a cross).

brings about a misinterpretation of the figures presented, and when the valvula cerebelli is stimulated conditioned reflexes are observed to disappear with automatic motor reactions taking their place. The disturbance in the differentiation of the geometrical figures was discovered after a partial coagulation of the forebrain basal nuclei. After the subsequent unilateral coagulation of the valvula cerebelli the food-procuring motor conditioned reflexes disappeared (Fig. 2).

Therefore, the results of these investigations in recent years show that, in the formation of complex forms of conditioned reflexes in fish, not only the cerebellum but also the forebrain takes part.

The formation of a new type of integrative activity of the central nervous system in amphibians and reptiles

Following the transition of animals from the aqueous to the terrestrial mode of living, fundamental changes occur in the structure of their brain. The distance-receptor system is developed, sharp regressive changes in the structure of the cerebellum are observed, and lastly, the formation of the neothalamic and neocortical structures commences, with functional connections becoming established between them. This transition from the mesencephalic-cerebellar type of integration (in fish) to the diencephalic-cortical type leads to a considerable decline in the functions of the central nervous system.

It was suggested on the basis of our former studies (Karamyan, 1956) that at certain stages in vertebrate development, when one integration system undergoes regressive changes and another newly-formed one is in the rudimentary state, the functioning level is sharply reduced. Indeed, all workers studying conditioned reflexes have stressed the notion that members of this class of animals develop unstable conditioned reflexes. The imperfection of the conditioned-reflex activity of the amphibians was revealed in the experiments of Sergeyev (1964), who showed that in

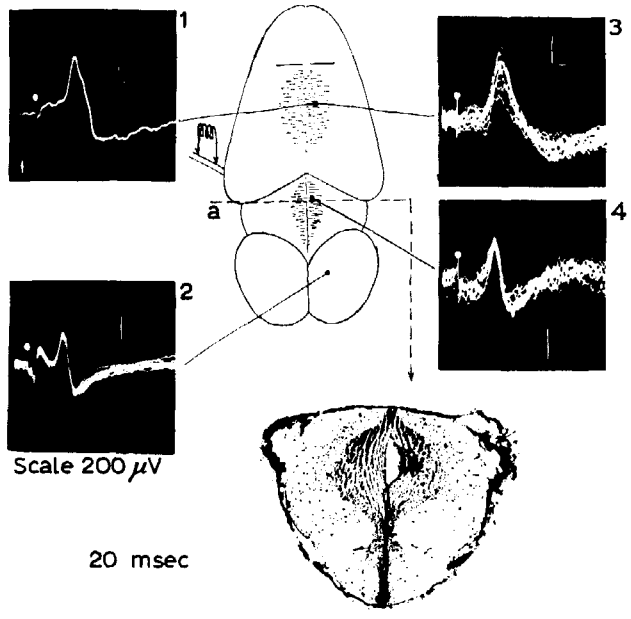

Fig. 3. Evoked responses in a frog's brain. 1, In the cerebral hemispheres; 2, in the tecti optici mesencephali; 4, in the periventricular portion of the dorsal thalamus at the excitation of the optic nerve; 3, response in the prosencephalon at the excitation of the periventricular portion of the dorsal thalamus. Response localization zones are hatched. In the section, the point of stimulation in the thalamus is marked.

toads, tritons and axolotls, conditioned connections between two indifferent stimuli do not develop. Moreover, in amphibians, the forebrain hemispheres participate, though to a smaller extent, in the formation of conditioned reflexes (Baru, 1955).

These results agree with the results of the electrophysiological investigations (Veselkin, 1963), according to which the dorsal surface of the amphibian hemispheres records distinct responses when the retina is illuminated, the optical nerve stimulated and the dorsal thalamic nuclei directly stimulated (Fig. 3). The subject of the thalamocortical interrelations has been more systematically studied on reptiles. According to the morphological studies of some authors (Kappers *et al.*, 1936; Papez, 1956), there are direct connections between the thalamus and the cerebral cortex in reptiles; other authors (Kruger and Berkowitz, 1960; Powell and Krüger, 1960) deny the existence of such connections.

On the basis of the study of evoked responses in the cerebral hemispheres to various afferent stimulations, Moore and Tschirgi (1962) and Orrego (1961) point out that, in reptiles, there exist connections between the thalamus and the general cortex, though of a non-specific character. At the same time Voronin and Guselnikov (1963) point to the specific nature of these connections.

Tests were staged involving electrical excitation of the inter-brain of varans in order to reveal the peculiarities of the thalamocortical interrelations in reptiles. The results of these studies (Belekhova, 1963) showed that at a rhythmic low-frequency stimulation of the dorsal thalamus (n. rotundus, n. dorsalis) the general and

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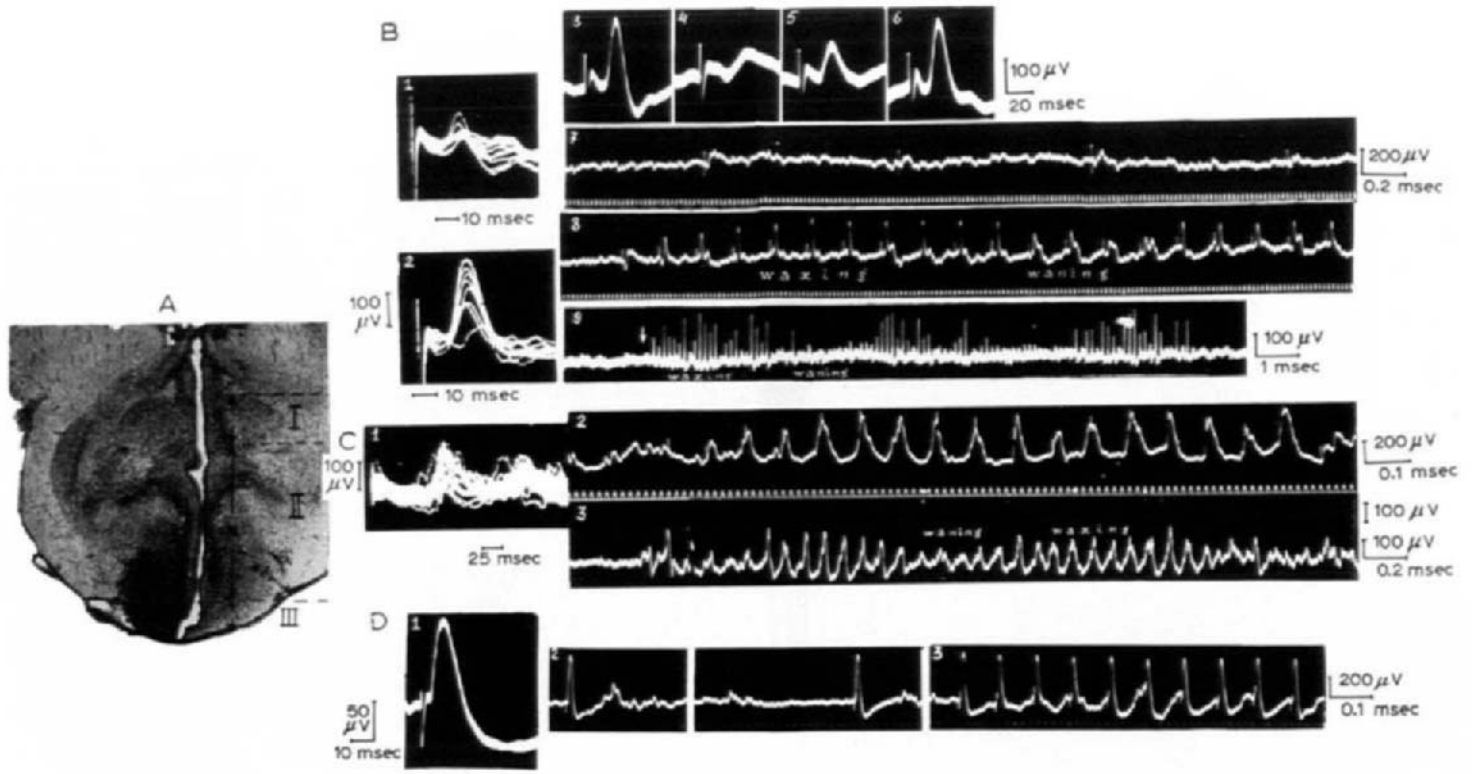

Fig. 4. Electrical reactions in a varan's cerebral cortex on stimulation of the inter-brain. A, microphotograph of a section of the varan inter-brain; I, dorsal thalamic level; II, ventral thalamic, subthalamic and hypothalamic levels; III, level of the basal portions of the hypothalamus. Vertical arrow indicates the path of the stimulating electrode. B, electrical reactions at the stimulation of the dorsal thalamus (level I). 1,7, Weak responses at a stimulation of 1/sec (1, eight leads applied); 2, increase in the response amplitude on stimulation of 7/sec (8 leads applied); 3–6, effect of a 0.1% solution of γ-aminobutyric acid on the recruiting reaction potential: 3, before the action; 4, in 15 sec; 5, in 8 min; 6, in 12 min after the commencement of the action (washing off begun after 2 min); 8, gradual increase in the reaction amplitude at a stimulation of 5/sec; 9, typical rising and falling phases of the recruitment reaction. C, electrical reactions at the stimulation of the ventral thalamus, subthalamus and hypothalamus (level II). 1, Application of 20 leads at a stimulation of 1/sec; 2, gradual increase in the reaction amplitude; 3, characteristic rising and falling phases. D, evoked potentials on stimulation of the basal portions of the hypothalamus (level III). 1, Response to a single stimulation; 2, at a stimulation of 1/sec; 3, at a stimulation of 9/sec.

hippocampal cortex register a response which by its character (arousal when stimulated at an optimum rhythm of 4 to 12/sec, gradual rise in the amplitude, the growth and decline in the course of stimulation), by the latent period (from 8 to 30 msec) of the surface-negative potentials and by the attitude to nembutal and to the local agency on the cortex of γ-aminobutyric acid (GABA), is analogous to the recruitment reaction of mammals, when phylogenically older, non-specific nuclei of the median thalamus are stimulated (Fig. 4, A, B).

When the ventral thalamus, subthalamus and dorsal hypothalamus structures (n. suprapeduncularis, n. periventricularis) are stimulated, the same portions of the varan's cerebral hemispheres record a synchronized reaction similar to the recruiting reaction with respect to the gradual development, increase and decline in the course of rhythmic stimulation (from 6 to 12/sec). However, the character of the potentials in this reaction is different; at the stimulation by single supraliminal stimuli a response is elicited consisting of from 1 to 3 surface-negative waves with a considerably longer

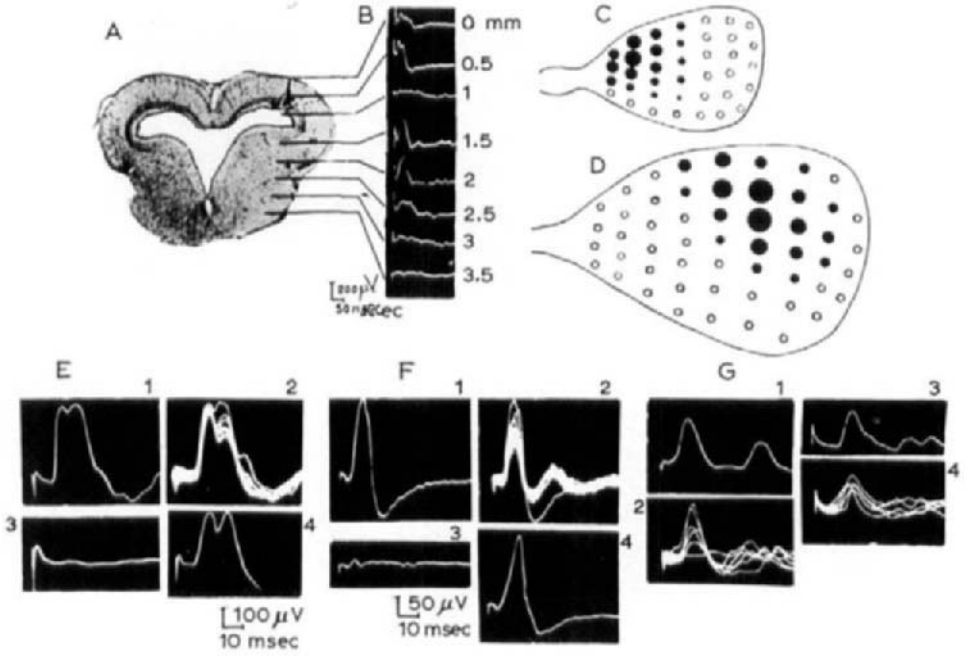

Fig. 5. Responses in the cerebral cortex of a turtle and a varan on stimulation of the tectal and tegmental structures of the mesencephalon. The micrograph of a section of the mesencephalon of a turtle (A) shows stimulation levels and responses in the cortex (B); diagram of response distribution on the dorsolateral surface of the hemisphere of a turtle (C) and that of a varan (D). E, responses evoked by the stimulation of the tecti optici of a turtle: 1, 2, normal for single stimulation (1) and at a stimulation rate of 1/10 sec (2, 10 leads applied); 3, 15 sec after the application to the brain of a 1% solution of GABA; 4, restoration of response after washing off. F, responses evoked by the stimulation of the tegmenti mesencephali in a turtle: 1, 2, normal for single stimulation (1) and at a stimulation rate of 1/sec (2, 10 leads applied); 3, 3 sec after the action of a 1% solution of GABA; 4, restoration of response after washing off. G, responses evoked by the stimulation of the tegmenti mesencephali in a varan: 1, 3, to single stimulation in the ipsilateral (1) and contralateral (3) hemispheres; 2, 4, at a stimulation rate of 1/sec in the ipsilateral (2) and contralateral (4) hemispheres; application of 6 leads.

latent period: 100 msec for the first wave (Fig. 4, A, C). Introduction of nembutal and application of GABA have the same effect on them as on the recruitment reaction potentials.

Only on stimulation of the basal portions of the hypothalamus, surface-negative short-latency (from 1.5 to 4 msec) responses are obtained in the cerebral cortex, testifying to the presence of direct ties between them (Fig. 4, A, D). Rhythmic low-frequency stimulation of the basal hypothalamus brings about in the cortex potentials in the same rhythm displaying no similarity to the recruitment reaction.

On stimulation of the tectal and tegmental structures of the midbrain (Karamyan

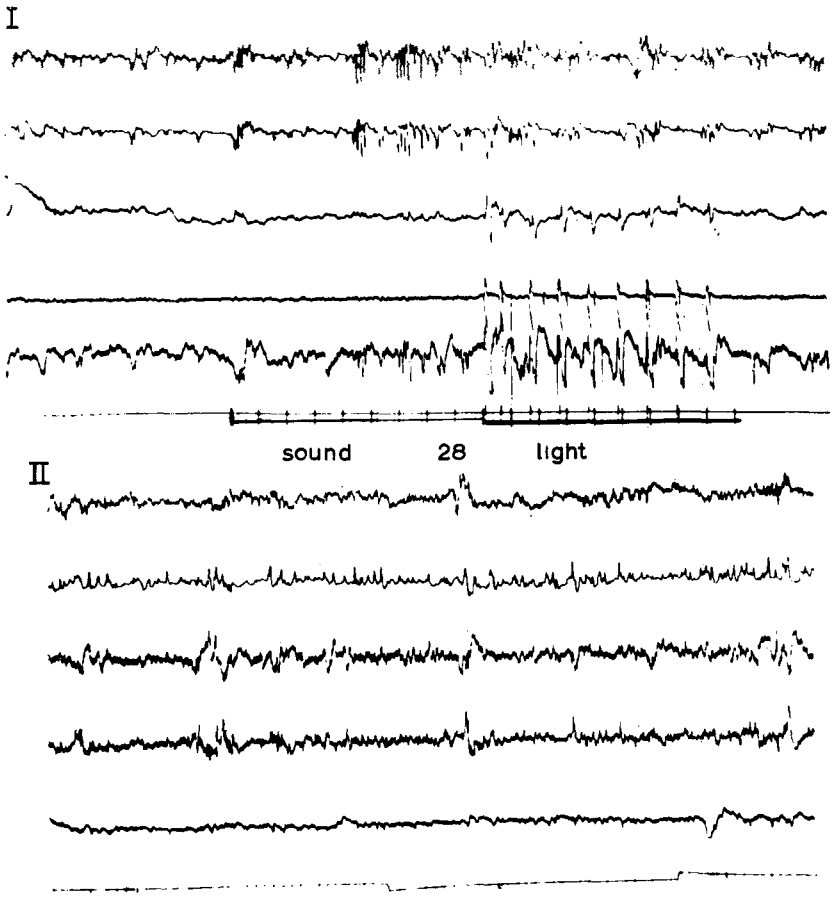

Fig. 6. Changes in the electrical activity of a varan's cerebrum with the development of associative conditioned reflexes. I, electrographic conditioned reactions after multiple combination of auditory and photic stimuli (28 combinations). The curves represent, from top to bottom: right-side view of the forebrain hemispheres, left-side view of the forebrain hemispheres, the striatal area, the tecti optici, the hypothalamic area, marks of the auditory and photic stimuli. II, lack of changes in the electrical activity of the brain on the application of a control auditory stimulus. The curves represent, from top to bottom: right-side view of the forebrain hemispheres, left-side view of the forebrain hemispheres, the striatal area, the hypothalamic area, the optic tecti, the control auditory stimulus mark.

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1964) of varans and turtles their cerebral hemispheres register long-latency (from 13.8 to 22.4 msec) surface-negative responses susceptible to nembutal and GABA (Fig. 5). It should be emphasized that the localization of these responses coincides with the localization of potentials evoked both by exteroceptive and electrical stimulation of the diencephalic structures. These researches have, therefore, led to the conclusion that in reptiles, between the dorsal thalamus and the cerebral cortex, definite connections are established which by their functional characteristics can be referred to the diffusely polysynaptic type of nervous activity.

The development of the structures of the dorsal thalamus and the cerebral cortex leads to the origin of a more elaborate form of conditioned-reflex activity. Thus, Sergeyev (1964) showed in his systematic studies on six species of different reptiles that the higher of them, namely swamp turtles and varans, develop associative temporary connections. These associative conditioned reflexes, however, differ from those developed in mammals in that the connections are set up only at a combination of one modality stimuli.

It should be underlined that, in reptiles, only positive character associative temporary connections are established.

During the recording of the electrical activity of the various structures of the brain (the cortex, the hippocampus, the reticular formation, the hypothalamus) in the course of formation of associative conditioned reflexes, the greatest changes are observed in the cortex rather than in the subcortical structures (Fig. 6).

The foregoing observations are not yet sufficient fully to characterize the new thalamocortical integration system, but there are grounds for believing that the coincidence of the possibility of setting up associative temporary connections with the formation of the thalamocortical integration system is not accidental, but related to the origin of a new level of functioning of the brain.

On some features of the development of the integrative activity of the brain in mammals

Modern neurophysiology and neuromorphology supply definite evidence indicating that the mammals have undergone a number of morpho-phylogenic progressive and regressive changes over all the forms of the previous classes of animals. As the neocortical, neocerebellar and neothalamic systems progress, the old and ancient brain structures and the old modes of co-ordination disappear or reorganize with the new conditions of functioning. In the thalamic system, specific nuclear formations are intensely developed, and direct connection is established between these and the projecting zones of the cortex. Phylogenically younger associative fields are set up in the structures of the thalamus and the cerebral cortex. And finally, the older non-specific thalamocortical system is differentiated and gains in complexity (Buser, 1957; Albe-Fessard, 1957; Bishop, 1958; Morillo, 1961; Meulders, 1962 and others).

Thus, in place of a single non-specific system (in reptiles) a complex superstructural apparatus is developed above the subcortical formations, from which qualitatively new modes of nervous activity arise.

One of the striking manifestations of this evolutionary process is the fact that in

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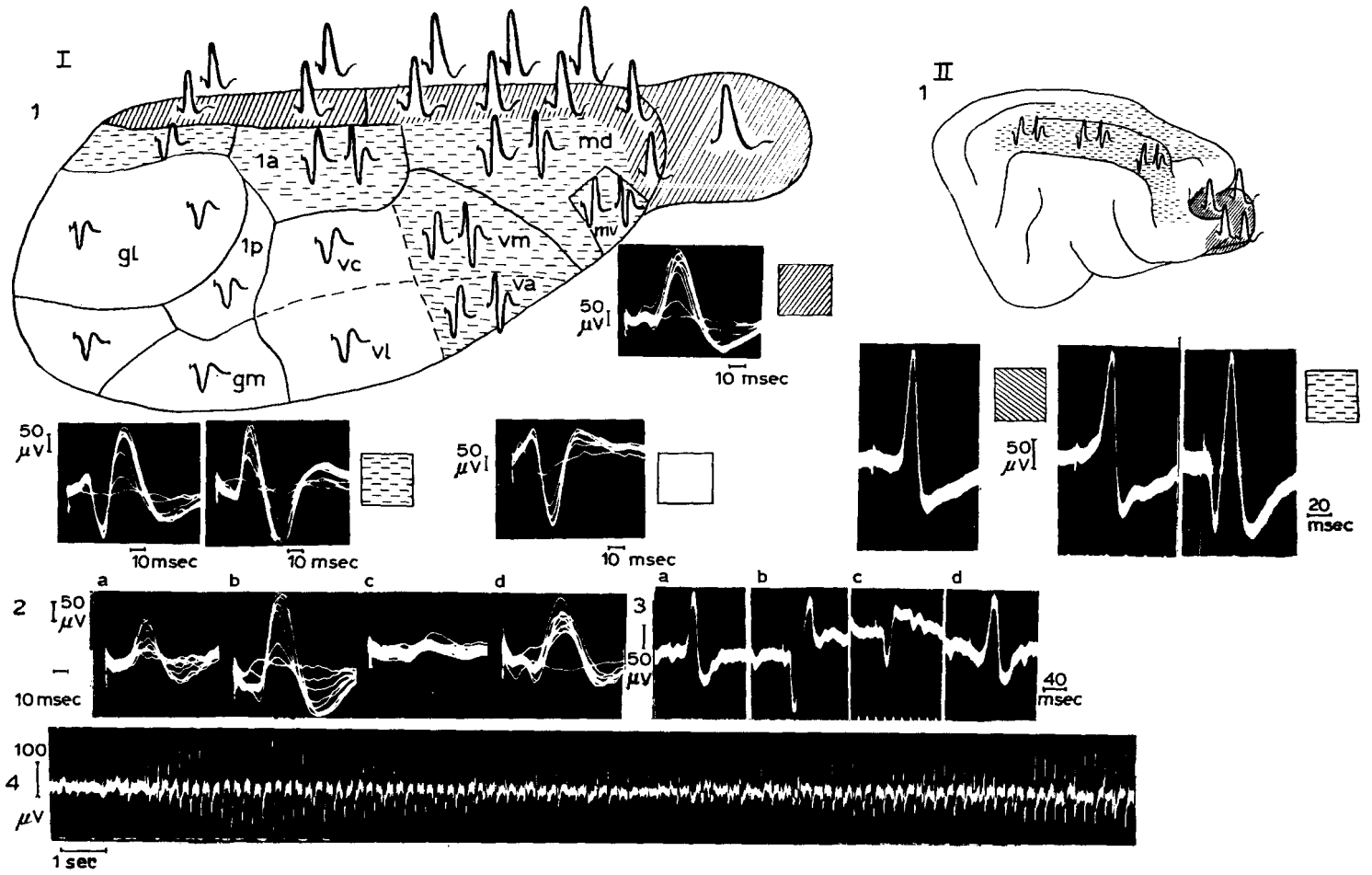

Fig. 7. The character and distribution of responses in the cerebral hemispheres evoked through the stimulation of non-specific thalamic nuclei: n. parafascicularis, n. centrum medianum, n. medioventralis, in rats (I) and n. centrum medianum in cats (II). In I and II: 1, localization diagram (slanting hatching, maximum recruitment responses zone; horizontal dotted line, transitory responses zone). For each zone typical responses are given. In I: 2, effect of nembutal on the recruitment reaction potentials in a rat; a, before the introduction; b, 3 min after the intravenous introduction of 3 mg/kg nembutal; c, 10 min after an additional introduction of 10 mg/kg nembutal; d, 36 min. 3, Influence of local application of a 1% solution of GABA: a, before the application; b, 15 sec; c, 5 min; d, 12 min after the application; 4, recruitment reaction in the recruiting zone of a rat.

the course of evolution of the mammals a new type of reflex activity is highly perfected, namely the associative conditioned-reflex activity. Systematic investigations carried out recently by Sergeyev, on insectivorous, cheiroptera and carnivorous rodents, indicate that, in mammals, rather complex and strong forms of associative temporary connections are developed: these forms of temporary connection are perfected in the ascending mammalian series. Unfortunately, we have insufficient data on the evolution of the interrelations between the three thalamocortical systems: non-specific, specific and associative.

Our experiments on rats and cats give some idea of the development only of non-specific systems. In Belekhova's experiments with low-frequency stimulation (from 4 to 12/sec) of the non-specific nuclei of a rat's thalamus (n. parafascicularis, n. centrum medianum, n. ventro-medialis, n. centralis medialis, n. medio-dorsalis) three types of potential were recorded in the cerebral cortex (Fig. 7, I). In the zone comprising the frontal pole of a hemisphere, the olfactory bulb, the cingular, and part of the motor and retrosplenial cortex, a typical recruiting reaction is recorded with all the traits described for the recruiting reaction in higher mammals. A typical response for this zone is a surface-negative or a negative-positive potential with a latent period of from 12 to 30 msec. Lateral of this zone is the transitory responses zone, including a large portion of the dorsal surface of the hemisphere; part of the frontal, the precentral, motor, parietal, and part of the retrosplenial areas. Typical of the transitory zone responses is the presence of a non-recruiting component in the shape of a short-latency positive or negative potential preceding the main surface-negative recruiting wave.

The recruitment reaction in this zone is less constant; its prominence depends more on the experimental conditions, especially the deepness of narcosis. In the other portions of the cortex, when the non-specific nuclei of the thalamus are stimulated, only positive oscillation is recorded.

The boundaries between the three zones are not clear-cut, are overlapping and will shift with experimental conditions. With the introduction of small doses of nembutal (up to 5 mg/kg intravenously) the recruitment reaction is strengthened; during deep nembutal narcosis the region of the recruiting responses contracts; on local application of GABA all differences between the zones disappear and the recruitment reaction is not recorded at all (Fig. 7, 1, 2, 3).

In cats, when low-frequency stimulation is applied to the n. centrum medianum, n. centralis medialis, and n. ventralis anterior, the recruiting reaction is recorded mainly in the associative and motor zones of the cortex. Three zones of evoked responses similar to those in rats (Fig. 7, II) can be singled out for separate nuclei. Thus, while the stimulation of the above non-specific thalamic nuclei in rats brings about an even distribution of recruiting responses in the cortex, in cats, though they overlap, maximal-recruitment zones arise, localized differently for separate nuclei.

A more diffuse organization of the non-specific thalamo-cortical system in rats is evidenced by the more pronounced dynamicity of the boundaries between the evoked response zones and by the greater facility of transition of the zones into each other with changes in the experimental conditions.

When the observations obtained on varans and rats are compared with the results of experiments on cats and primates (Starzl and Whitlock, 1952; Jasper *et al.*, 1955 and others) a definite impression is created of the development of thalamocortical interrelations. At the first stage of the formation of this system, *i.e.* in reptiles, the connection of the thalamus with the cortex is through a single diffuse non-specific system. In rodents, the area of the recruitment reaction is characterized by a broad representation zone, and finally, in higher mammals, a more limited representation of recruitment is observed, mainly in the frontal and parietal parts of the cortex. Thus, it may be stated with a fair degree of certainty that the general aspect of evolution from diffuse forms to discrete nervous activity is also revealed in the process of formation of the thalamocortical interrelations.

I will not dwell in this paper on the subject of the evolution of the cerebellar-cortical interrelations, as it has been discussed in a number of articles published elsewhere (Karamyan, 1958, 1959, 1963). It should only be emphasized that here again, the general aspect of evolution is the same, *i.e.* evolution proceeds from phylogenically ancient diffuse modes of nervous activity to more specialized young ones.

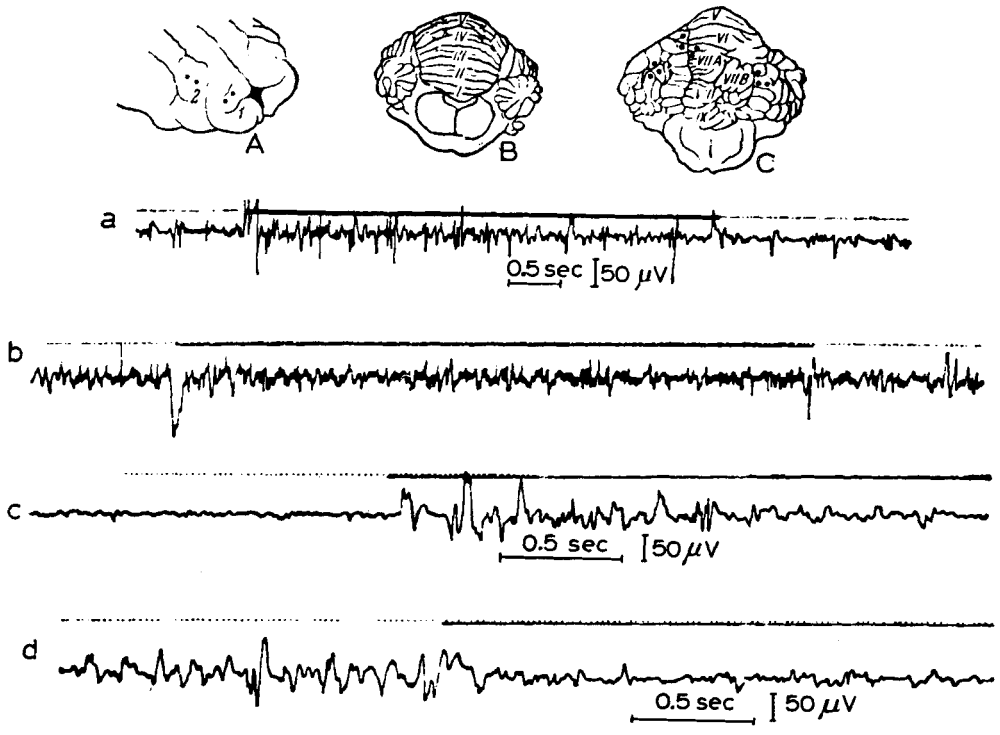

Fig. 8. Changes in the electrical activity of the contralateral sensomotor region on stimulation of the neocerebellar (a, c) and paleocerebellar (b, d) formations in a cat's cerebellum. A, points of derivation from the cerebral cortex; 1, motor cortex; 2, somatosensory cortex; B, stimulation points of the paleocerebellum (xx); C, stimulation points of the neocerebellum (.); I–IX, lobules according to Larsell's classification. The black solid line stands for the cerebellum polarization period; the broken line stands for the time marks by 20 msec.

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Thus, in cats and dogs, the extirpation of the paleocerebellum or the electrical destruction of the fastigial nuclei (Karamyan, 1959) is followed by a distinct, though brief, heightening of orientation reactions, increased intensity of positive conditioned reflexes, and disinhibition of conditioned inhibition. After the extirpation of the neocerebellum and the stereotaxic destruction of the dentate nuclei, reverse phenomena were discovered, *i.e.* more lasting disturbances in the direction of lower positive and stronger inhibitory conditioned reactions. These results accord with those obtained in the Moruzzi laboratory (Dow and Moruzzi, 1958), as well as in our laboratory by the electrophysiological technique (Grigoryan, 1961, 1962), according to which the excitation of the paleocerebellum brings about a generalized effect of desynchronization. The excitation of the neocerebellum, on the contrary, is attended by synchronized rhythms originating in certain zones of the cerebral cortex (Fig. 8)

These observations confirm our assumption, stated above, of the existence of two

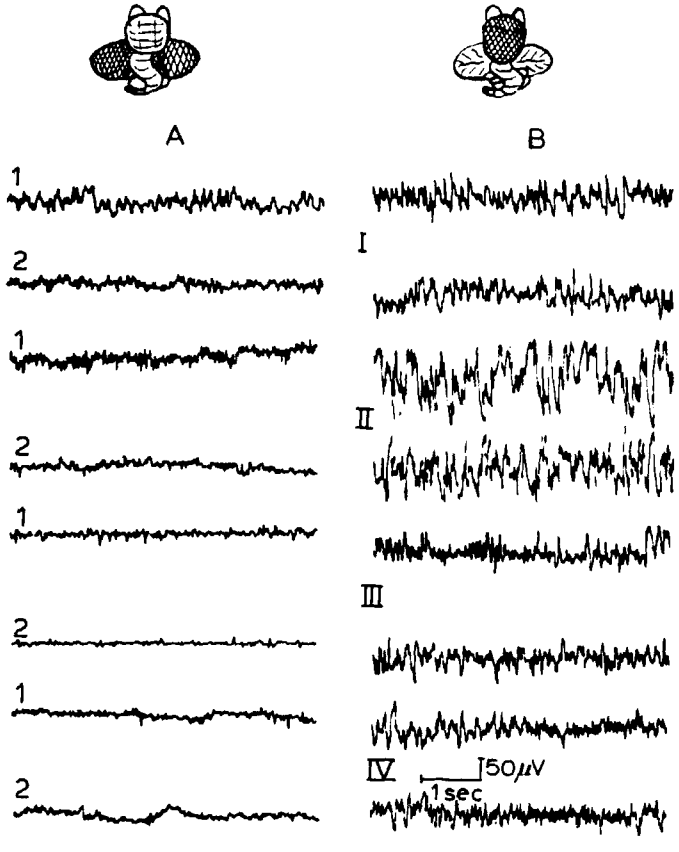

Fig. 9. Spontaneous electrical activity of a cat's cerebral cortex before (I) and after the excision of both hemispheres (A) and the frontal lobes of the cerebellum (B) at 10 (II), 20 (III) and 30 (IV) days. The lead is bipolar from the suprasylvian (1) and the ectosylvian (2) cortical gyres. On top of the cerebellum diagram the remote parts are hatched.

types of ascending influences of the cerebellum: a phylogenically older toning-up influence *via* the paleocerebello-reticulocortical system, and a phylogenically younger actuating influence *via* the neocerebello-thalamocortical system.

Electroencephalographic changes on the extirpation of phylogenically differing portions of the cerebellum also point to the differentiated significance of the paleocerebellum and neocerebellum for the formation of the cortical electrical activity (Fig. 9).

In neurohistological studies employing Nauta's technique and its modifications, on

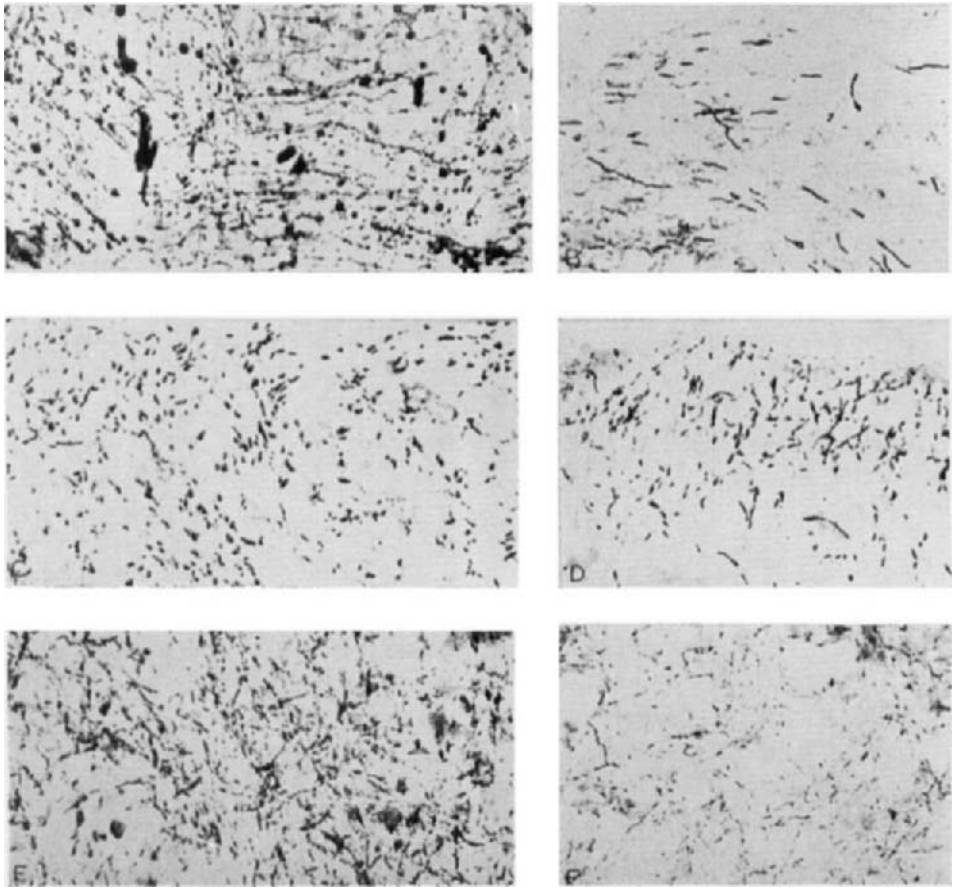

Fig. 10. Degeneration of the efferent fibres of the cerebellum following its extirpation in a toad and a varan, and the extirpation of the paleocerebellum in a cat. A, a toad. A great amount of nerve fibres of the cerebello-spinal tract in the process of disintegration into small rounded fragments. Impregnation after modified technique of Nauta. Paraffin filling. Magnification ×526. B, a toad. The forebrain. Absence of degenerative changes in fibres. Treatment and magnification as above. C, a varan. Disintegration of nerve fibres in the tr. thalamostriaticus. Treatment and magnification as above. D, a varan. Disintegration of nerve fibres in the paleocortex (tub. olfactorium). Treatment and magnification are as above. E, a cat. VPM thalamic nucleus. Wide-spread disintegration of nerve fibres. Frozen sections, impregnation after Nauta. Magnification ×526. F, a cat. Optic cortex. Disintegration of part of the nerve fibres into small rounded fragments. Impregnation after Nauta. Frozen sections. Magnification ×526.

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the extirpation of the cerebellum in amphibians (*Buffo buffo*) degenerative changes were observed in the efferent fibres to the cerebellum and the preterminals, principally in the caudal parts of the brain. In the rostral parts, these changes are either negligible or absent (Fig. 10, A, B). In reptiles (*Varanus griseus*), apart from the changes in the caudal portions and the brain stem, degenerative changes are already found in the rostral parts of the brain, specifically in the ancient structures (paleocortex) of the cerebral hemispheres (Fig. 10, C, D). No changes in the neocortical nerve fibres are observed in varans. As far as mammals are concerned, the investigations indicated that with the extirpation of the paleocerebellum or the stereotaxic destruction of the fastigial nuclei in cats, intensive degenerative changes are observed in the caudal regions of the brain, in the reticular formation of the brain stem, and in the non-specific nuclei of the thalamus (midline, intralaminar, and paralaminar nuclei). With the extirpation of the cerebellum or the destruction of a dentate nucleus, changes in the nerve fibres and preterminals are observed in the brain-stem structures, to a smaller degree, in the non-specific nuclei of the optic thalamus (VA, VM, CM, NR) and in the same specific nuclei as during paleocerebellar affection (VZ, VPM, VPZ); and also in the subthalamic area, in the corpus caudatus, and the cerebral cortex (Fig. 10, E, F).

Thus, conditioned-reflex, electrophysiological and neurohistological researches make it possible to assume that the paleocerebellar integration system is a superstructure over the phylogenically older structures of the brain stem and exerts a generalized tonic influence upon the cortical activity; while the cerebellar system, being a superstructure over the younger structures of the diencephalic area, exerts more differentiated influences upon higher nervous functions.

CONCLUSIONS

The foregoing factual material corroborates the thesis formulated by Sechenov — that the general aspect of evolution is the same at all stages of development and consists in 'the breaking up or differentiation of the fused into parts and their isolation into groups of different specialized functions' — and emphasizes that this is one of the major directions of functional evolution.

It should be mentioned that the principle of development from diffuse modes of functioning to more differentiated ones is also indicated by neuromorphologists (Zavarzin, 1941; Bishop, 1958; and others) who consider that the different structures of the brain, especially the cerebral cortex, develop by way of a common non-specific system breaking up into more specialized integration systems. The truth of these views is beyond doubt, and the aim of our explorations is not merely to corroborate this thesis. We find it more important to elucidate the problem of the correlation of the structural and functional evolution for the different levels of phylogenesis. In this respect our observations may be used to discuss one of the major problems of the present-day neurophysiology: whether the higher nervous functions are formed in the cortex or in the subcortical formations.

As is well known, some authors (Fessard, 1960; Gastaut and Roger, 1960; and others), arguing from new electrophysiological data, believe that the conditioned-

reflex activity develops primarily in the subcortical systems, while others uphold Pavlov's opinion on the cerebral cortex as the closure organ of temporary connections. The history of the functional and structural evolution of the brain and its separate integration systems indicates that the very statement of the question of where temporary connections are closed, does not reflect the present-day level of development of neurophysiology and neuromorphology. We stress that the earlier stages of development of the central nervous system, with a diffuse unspecialized structure, temporary connections are developed that are termed differently by different authors: as 'summation reflexes' by Pavlov, as 'Bahnung-reflexes' by Kreps, and as 'conditioned-reflex related phenomena' by Asratyan. The essence of these notions is that they are devoid of the main features of conditioned reflexes, as they are very labile and disappear with the same facility with which they are established. At the following stage of development, when the mesencephalo-cerebellar system of integration is formed, and the bulk of afferent pathways converges in it, temporary connections are developed after the pattern of the stronger positive and negative conditioned reflexes.

And finally, when this integration system reorganizes or disappears and the diencephalocortical system of integration is formed instead, a higher type of temporary connection — associative temporary connection — is developed.

Now an essential question arises: whether all types of temporary connection will disappear or completely corticalize as a result of the reorganization or extinction of the older integration systems. The old neurology and modern electrophysiology give convincing proofs of the preservation of the ability to establish temporary connections in the subcortical formations, and this ability is more distinctly traced when, for some reason or other, the influence of the highest level of integration — that of the cerebral cortex — is weakened or removed.

Thus, from the point of view of functional evolution, temporary connections may be set up at all levels of the central nervous system where conditions are present for switching from distant afferent systems to efferent ones, both somatic and vegetative in character. It may be debated how complex these reflexes are, and to what extent the structures participating in their formation are adapted to synthesizing the multiform external signals which are of primary importance in the current activity of the organism.

The new electrophysiological and conditioned-reflex data, obtained on Acrania, cyclostomes, plagiostomes, Osteichtyes, amphibians, reptiles and lower mammals, once again support our opinion stated earlier that the evolution of the vertebrate central nervous system proceeds by the principle of stepwise development, or changing functions, in the central nervous system, in the course of which a definite correlation is established between the nerve substrate and its function (Karamyan, 1956).

This point of view agrees with Filimonov's principles of multifunctionality and diencephalic formation, as well as with the general conclusions drawn by Voronin and Guselnikov (1963) on the structural principles of development of the analytico-synthetic activity of the brain in the phylogenic series of vertebrates. These views only indicate general trends in the development of the central nervous system, reflecting to some extent the idea of 'the law of changing functions' stated by Anton

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Dhorn in the second half of the last century. The question remains unsolved, however, of how the complex evolutionary process is effected, of progressive changes in some integration systems and regressive ones in others, of the reorganization and extinction of some functions and the beginning and development of others. A matter especially abstruse is the correlation between the phylogenically ancient and the new integration systems, normally and during a pathology of the central nervous system.

Such notions as 'dissolution', 'disinhibition', 'liberation', 'release' (Jackson, Pavlov, Orbeli, Fulton) remain uninterpreted to the present day, notwithstanding their reflection of an extremely important aspect of evolutionary neurophysiology and neurology.

It is hoped that further and more extensive studies of separate stages in the evolution of the brain, of the structural and functional bases of the change of functions in the central nervous system, and finally, a comparison of the results of these explorations with the disintegration phenomena observed under the conditions of experimental pathology of the central nervous system, will contribute to the development of this important direction in evolutionary neurophysiology.

SUMMARY

Summarizing the evolutionary ideas of Darwin and Spencer, Sechenov wrote in his work 'Elements of Thought' that the general pattern of evolution is the same at all the stages of development.

In representatives of the lowest stage of the animal world the sensibility is spread equally over the whole body. In the course of evolution separate systems emerge out of this diffuse sensibility. Finally, more highly organized species develop specialized functions to adjust the nervous activity and the organism as a whole to the environment.

Our task was to elucidate from this point of view the peculiarities of functional and structural organization of some parts of the central nervous system in a series of animals constituting the basic stages of the evolution, *i.e.* Acrania, Cyclostomata, Crosstomata, reptiles and lower mammals.

In *Amphyoxus lanceolatus* the responses to light or electrical stimulation of both the head and the tail of the nervous tube were of the same intensity. These reactions persisted even after surgical disconnection of the nervous tube. Combination of unconditioned and conditioned stimulation of the disconnected parts of the head and tail end of Amphyoxus resulted in the formation of temporary connections. This proves that the central nervous system of Amphyoxus is diffuse with no functional localization.

In Cyclostomata, despite differentiation and formation of various parts of the central nervous system, the elements of diffuseness are still inherent. For instance, illumination of the retina or electrical stimulation of the optic nerve produces surface-negative responses in the midbrain tectum with a latency of 6 msec, in the rhomboid fossa area with a latency of 13 msec, and in the spinal cord with a still longer latency. Transection of the midbrain tectum at the caudal level, or even its full extirpation,

has no effect on the reactions in the medulla oblongata and the spinal cord. Temporary connections in this group of animals possess no typical features of conditioned reflexes and are related, in their nature, to summation phenomena.

Considerable progress in further specialization of functions of the central nervous system is observed in Crosstomata. Our experiments carried out on *Raja clavata* and *Trigon pastinaca* showed that stimulation of the optic nerve evokes local responses in the midbrain tectum and cerebellum, but has no effect on other structures, such as the forebrain hemispheres.

The experiments performed on reptiles (*Varanus griseus*) demonstrated that stimulation of the dorsal thalamus evokes recruiting responses in the anterior and medial parts of the common and hippocampal cortex with a latency of 8–30 msec. Intravenous injection of nembutal (over 5 mg per kg) or application of a 0.1–1 % solution of GABA to the hemisphere surface depresses or reverses these responses. Thus between the dorsal thalamus and isocortex of reptiles certain functional connections are formed, which, by their functional characteristics, may be related to the diffuse polysynaptic type of nervous activity.

The experiments carried out on rats and cats showed that low-frequency stimulation (5–10 per sec) of non-specific thalamic nuclei evokes responses in different cortical areas different in their latency and the degree of recruiting. Two regions can be differentiated: the region of maximum recruiting with surface-negative potentials (latency 12–30 msec) and that of a mixed type, where negative-positive responses are recorded along with positive ones. The frontier of these regions is dynamic and fluctuates, depending on the depth of anaesthesia and the functional state of the animal. Nembutal, as well as cortical application of GABA, depresses all the reactions of the recruiting type.

From the comparison of our data and the data obtained on cats and primates by Jasper, Naquet, King, Starzl and Whitlock, it follows that in reptiles, *i.e.* on the stage of thalamo-cortical system formation, connections between thalamus and cortex are effected only through the non-specific system. In rodents the recruitment area is restricted to definite cortical regions. Finally, in more highly-organized mammals the tendency towards concentration of non-specific systems in certain cortical areas is still more pronounced.

Extirpation of the paleocerebellum brings about intensive preterminal degenerative changes localized mainly in non-specific structures of the caudal parts of the brain. Extirpation of the neocerebellum produces changes in rostral brain structures, including changes in cerebral cortex. Electrophysiological and conditioned reflex investigations carried out on mammals show that the paleocerebellum system exerts a generalized, adaptive influence on the cortical activity. Elimination or stimulation of the neocerebellum is accompanied by a more differentiated alteration in the cortical electrical and conditioned reflex activity.

The results of our investigations show that the classical neurophysiological concept of a general pattern of evolution from diffuse to more local forms of nervous activity is one of the main principles of the functional evolution of the brain as a whole as well as of its separate integration systems.

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Age and Seasonal Differences in the Effects of Some Neurotropic Substances

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At the present stage in the development of experimental and theoretical pharmacology and its applied branch, pharmacotherapy, the problem of revealing biochemical and physiological mechanisms that determine the difference in reactions of this or that individual to the effect of medicinal substances is of extreme importance. In the course of investigations having this aim (Petkov, 1962, 1963) we tried to elucidate, on the one hand the influence of pathological processes on the effect of different pharmacological substances, and on the other hand the influence of other medicinal substances used preliminarily or simultaneously. Further, we made some observations on the role of age and seasonal factors in the effect of medicinal substances. A part of these observations is the subject of the present paper.

In some of our experiments (Petkov, 1957) on old (two year) white rats we observed that caffeine (*Coffeinum purum*, 0.02 g/kg body weight injected subcutaneously), instead of improving cortical activity, almost regularly caused prolongation of the latent period of positive conditioned reflexes, increase in the time of a run, paradoxical phasic states up to complete suppression of conditioned reflex activity, and disinhibition of differentiations. (We worked with conditioned nutrient-motor reflexes using the method of Kotlyarevsky.) Among 9 old rats caffeine improved the positive conditioned reflex activity in only 2.

As a result of a pharmacological analysis (Petkov, 1956, 1957, 1958, 1960, 1961) of the Far-Eastern plant *Panax ginseng* we found that one of the most important peculiarities of this drug rich in pharmacodynes is its stimulating effect on the processes both of cortical excitation and active cortical inhibition. The experiments were done on white rats according to the method of Kotlyarevsky. A single injection of small doses of the drug studied, improving the excitability of cortical cells, enabled us to restore rapidly and clearly the apparently lost dynamic stereotype by using adequate stimuli. Our investigations on the effect of *Panax ginseng* on the bioelectrical activity of the brain (on cats in an acute experiment and with chronically sewn-in electrodes, Petkov *et al.*, 1961a, showed that the standardized *Panax ginseng* preparation caused a prolonged (30–60 min) moderate synchronization of the basic bioelectrical brain activity. The primary evoked potentials were facilitated though in a somewhat peculiar form; on the background of *Panax ginseng* effect the stimuli used

more easily caused EEG desynchronization. In conditions of desynchronized bioelectrical activity too appearance of primary evoked potentials was facilitated (Fig. 1).

Changes which developed under the influence of the same dose of *Panax ginseng* injected into old rats (aged about 2 years) were different. These animals often developed protective inhibition: manifestations of superinhibition, increase in latent period values and the time of conditioned stimuli run, irradiation of inhibition process, phasic states. The protective character of all these changes in cortical activity was shown by their complete reversibility.

Using the logokinetic method of Ivanov-Smolensky, and the method of associative experiments in healthy, relatively young (20–40 years old) persons we observed (Petkov and Shivacheva, 1957) that under the influence of a dose of *Panax ginseng* extract (with a placebo preparation as control) in the majority the power of cortical process of excitation increased: shortening of the latent period and increase of conditioned reaction value, objective registration of the restriction of generalization of cortical excitation process and weakening of the passive inhibition effect. Simultaneously an improvement in extinctive, conditioned and differential inhibition was observed. *Panax ginseng* also improved the mobility of cortical processes. In the majority of the persons examined a relief in the elective irradiation of excitation process in the second signal system was also observed. With the help of associative experiments we also observed a distinct stimulation of the second signal activity: shortening of the latent time of responses, improvement of mobility of excitation process, decrease of its exhaustibility.

In the majority of older persons (41–65 years) subjected to the examination after receiving the same dose of *Panax ginseng* the latent period increased while the value of a conditioned reaction decreased. In some of the persons in this group the generalization of excitation processes somewhat widened. This age group did not show elective irradiation to the second signal system. However, in some of the older persons administration of *Panax ginseng* resulted in the improvement of excitation process mobility in the second signal activity and sharp reduction of its exhaustibility.

Using the defensive conditioned reflex method (according to Cook and Weidley — conditioned reflex of running by clambering) we (Ovcharov *et al.*, 1964) found that reserpine, in a relatively high dose (1 mg/kg body weight) in young (1 month) rats, to a certain degree suppressed positive defensive conditioned reflex activity. It did not, however, exercise any influence over differential inhibition. In adult (one year and a half) rats reserpine gradually (the experiment lasted 6 days) caused not only full suppression of positive conditioned reflexes but also resulted in the disappearance of the unconditioned defensive reaction. The experimental animals developed a peculiar state of complete areflexia being awake with pronounced ptosis and dishevelled fur.

On another series of adult (older than one year) and young (one month) white rats we tested the effect of reserpine (1 mg/kg body weight), sodium bromide (100 mg/kg body weight) and *Panax ginseng* (100 mg of dry extract per kg body weight) by intraperitoneal injection with the purpose of altering conditioned reflexes. The same conditioned reflex defensive method was used. Reserpine facilitated the transformation of a positive stimulus into a differential one both in young and adult rats, but it

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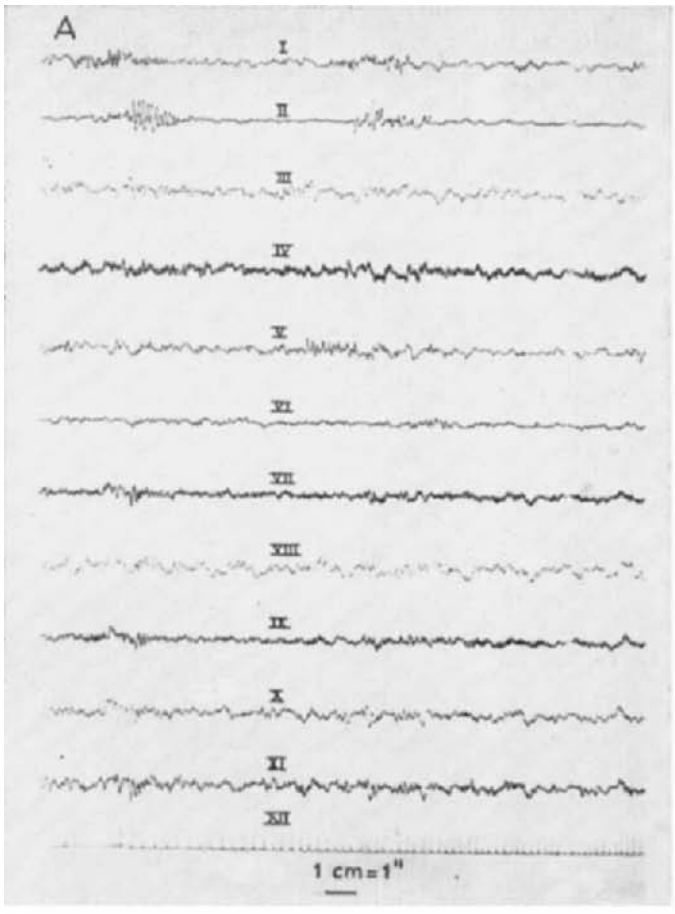
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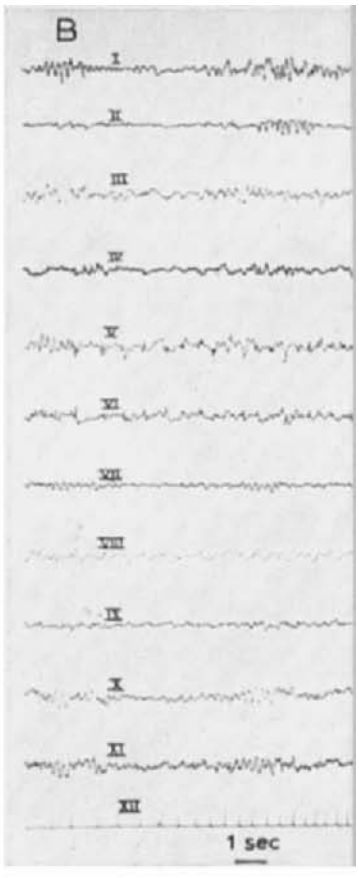
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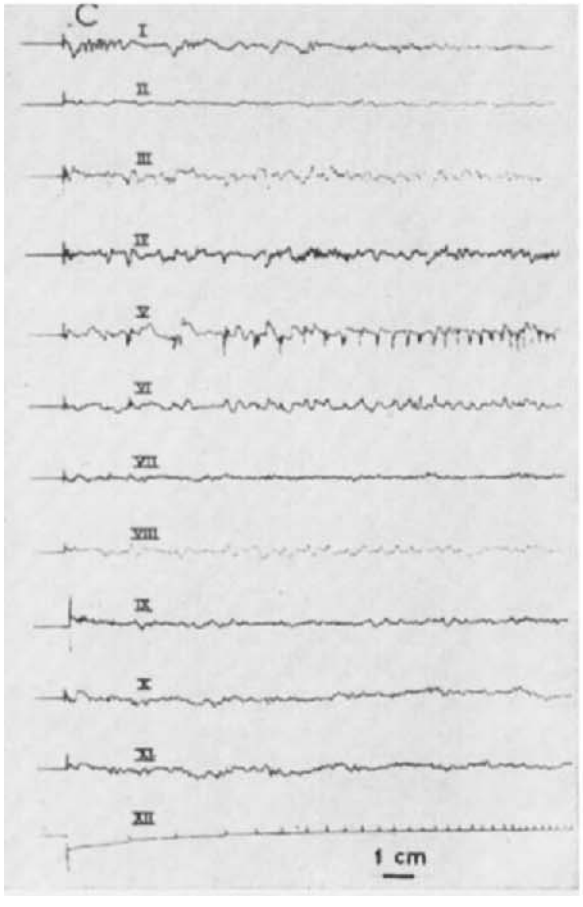
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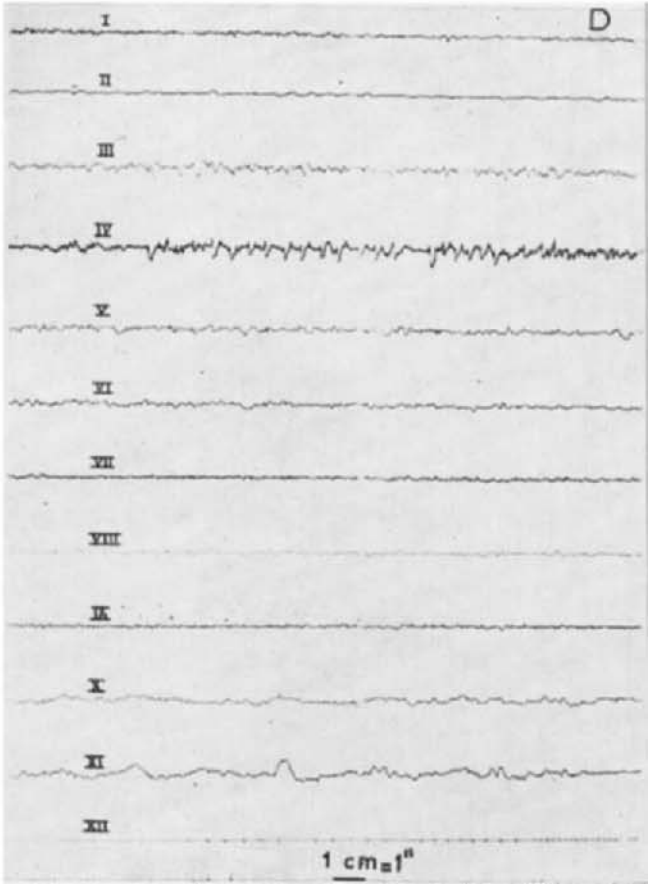
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hampered transformation of a differential stimulus into a conditioned signal of the beginning of painful irritation.

Panax ginseng produced a favourable influence on the transformation both of a positive conditioned stimulus into a negative one and a negative stimulus into a positive one in young rats. In adult rats the dose of *Panax ginseng* used did not cause any effect on the transformation of conditioned reflexes (Fig. 2).

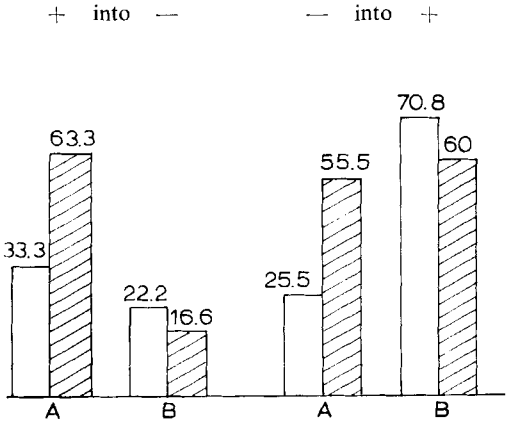

Fig. 2. Effect of *Panax ginseng* on the transformation of conditioned reflexes in young and adult rats. Administration during 5 days in isotonic solution. The columns show transformation of a bell (+) into a differential signal (—), and a buzzer (—) into a positive signal (+) in control young (A) and adult (B) animals (white columns) and in those which were given *Panax ginseng* (shaded columns).

Sodium bromide caused a moderately facilitating influence on the transformation of a differential conditioned stimulus into a positive one in young rats. In adult rats, however, the same dose slightly hampered this transformation.

The following observation is also worth mention. In the process of the transformation of a negative defensive reflex into a positive one (when a buzzer was combined with transmission of an electric current through the latticed floor of the chamber) about 40% of the young rats developed epileptic convulsions. Such con-

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Fig. 1. Facilitated appearance of primary evoked potentials under the influence of *Panax ginseng*. Experiment on a cat. Cortical leads; I, frontal, left; II, frontal, right; III, temporal, left; IV, temporal, right; V, occipital, left; VI, occipital, right. Subcortical leads: VII, nucleus reticularis sinister; VIII, nucleus reticularis dexter; IX, centrum medianum sinistrum; X, centrum medianum dextrum; XI, formatio reticularis mesencephalica sinistra; XII, registration of the stimulants used. (A) EEG before *Panax ginseng* drug administration. Light stimulants in conditions of the experiment do not provoke the appearance of primary evoked potentials, and do not suppress slow waves. (B) EEG 8 min after *Panax ginseng* drug administration (0.05 g/kg of the dry extract). Light stimulants in the same experimental conditions provoke the appearance of clearly pronounced potentials. (C) EEG 20 min after *Panax ginseng* drug administration (and after repeated use of different stimulants). Light stimulants provoke clearly pronounced primary caused potentials. Basic bioelectric activity passes over to desynchronization. (D) EEG during 1 h after *Panax ginseng* drug administration. On the background of clearly desynchronized bioelectrical activity of different cortical and subcortical structures acoustical stimulants provoked pronounced primary evoked potentials in the leads of the temporal cortical region.

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vulsions were not observed either in adult rats that developed the transformation or in young ones that were subjected to the current of the same power but did not develop the transformation. The inhibition in young rats, insufficiently developed and easily wounded, probably becomes affected in the difficult process of conditioned reflex transformation. This causes a strain in nervous processes and the appearance of epileptiform convulsions under the influence of painful irritation due to the electric current.

We also studied the convulsant effect of corazol in young and adult rats. Contrary to our expectation, corazol (50 mg/kg body weight) caused epileptiform convulsions in 100% of adult animals and in only 50% of young ones (statistically significant difference when P is less than 0.05 was checked by the method of χ^2).

As the last example of our investigation of age differences in neurotropic effects of some pharmacological substances we shall mention the results of an experiment with cardio-active glucosides.

Investigations conducted by a number of pharmacologists, mainly by Soviet ones, showed that different cardio-active glucosides exert a definite effect on higher regions of the central nervous system. As a result of a complete pharmacological analysis of glucosides in the species of *Helleborus odorus L.* widespread in Bulgaria (carried out by one of my co-workers Staneva, 1961) it was found that they cause an influence on higher nervous activity to a considerable degree similar to neuroleptics. A serotonin liberating action of these glucosides is also of special interest. This action of *Helleborus odorus L.* glucosides (also observed with strophanthin) was manifested, on the one hand in the depletion of the serotonin depot in Kulchitsky's enterochromaffin cells; another argument for the serotonin liberating effect of these glucosides is the

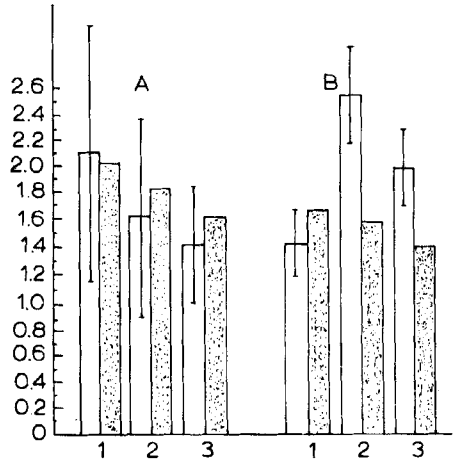

Fig. 3. Effect of acetyldigitoxin on the duration of the latent period of a defensive conditioned reaction in young (A) and adult (B) rats. White columns represent the latent period of positive conditioned reflexes within confident limits in rats which were given acetyldigitoxin. Shaded columns show the latent period of positive conditioned reflexes in rats which were given isotonic solution. (1) Preliminarily; (2) 30 min after administration; (3) 2 h after administration.

fact that their contracting effect on smooth muscles the intestine is prevented and eliminated by means of BOL 148, a typical serotonin antagonist.

These results being available it was interesting, from the theoretical and practical points of view, to elucidate whether there are differences, determined by age, in the effect of cardio-active glucosides on higher nervous activity. With this aim we set up experiments on adult (1 year) and young (3 months) white rats using crystalline acetyldigitoxin (Acylanid–Sandoz preparation) and desacetyllanatoside C (Cedilanid-Sandoz preparation). Both glucosides were administered intraperitoneally, acetyldigitoxin in doses of 0.4 and 0.2 mg/kg body weight, and desacetyllanatoside C at 0.4 mg/kg body weight. We used the defensive conditioned reflex method of clambering.

When positive conditioned reflexes were not yet sufficiently strong acetyldigitoxin in both doses caused a deterioration in excitation process in adult rats: 30 min after glucoside administration the percentage of successful defensive conditioned reactions decreased while their latent period increased (statistically significant difference when $P = 0.05$, Fig. 3), and 2 h after glucoside administration deceleration of the tempo of the defensive conditioned reflex consolidation was observed both as to the percentage of defensive conditioned reactions and diminution of their latent period. When defensive reflexes in adult animals were consolidated acetylcholine also suppressed defensive conditioned reflex activity, suppression being especially pronounced after a high dose.

In young rats neither dose of aceyldigitoxin exerted such an effect on the process of cortical excitation. Under its influence a certain tendency to excitation process intensification during the period of the development of conditioned defensive reflexes was observed, being mainly displayed in a faster tempo of conditioned reflex formation.

In the doses used by us desacetyllanatoside C showed effects on both age groups partly opposite to those of acetyldigitoxin. In adult rats its suppressing effect on defensive conditioned reflex activity was slight (moderate prolongation of the latent period) while in young rats this glucoside gave rise to more pronounced weakening of excitation processes: diminution of the percentage of conditioned defensive reactions, and more considerable increase in the latent period.

The above-mentioned examples from our laboratory show how difficult it is to define all the differences that are observed in the action of a pharmacological substance on higher zones of the central nervous system at different stages of an organism's development. The cited investigations in the field of age pharmacology have the basic character of a statement, and I shall not present a thorough explanation of the results. I shall only allow myself briefly to give two or three possible reasons for some observed differences in the effect of the neurotropic pharmacological substances that we have studied.

Experiment showed that reserpine used every day at 1 mg/kg body weight during 4 days prevented a convulsant effect of corazol. (Brain structures seem to become impoverished so far as biogenic amines are concerned.) Noradrenaline administered subsequently (50 μg per kg body weight) restored this effect of corazol, the same percentage differences being preserved both in young and adult animals. This observation gives us grounds for suggesting that insufficient development of central

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adrenoreactive structures in young rats can play a decisive role causing impeded manifestation of the corazol convulsant effect.

Data on the ontogenetic improvement of conditioned reflex activity, and in particular the process of active cortical inhibition, make it possible to elucidate mechanisms forming the foundation of different effects of a pharmacological substance at different stages of ontogenesis. Thus, one can understand why, for instance, *Panax ginseng*, stimulating simultaneously both cortical processes and thus improving an organism's adaptive possibilities, to a greater extent facilitates the transformation of conditioned reflexes in both directions, especially in young animals in which processes of cortical excitation and inhibition are still far from perfect. One could also explain some other differences observed in the effect of pharmacological substances on the transformation of conditioned reflexes in young and adult animals.

The weakness of cortical processes developing with age enables us to understand why *Panax ginseng* — at the dose that exerts an all-round stimulating effect in experimental animals and in people of middle age — is an excessive stimulus provoking phenomena of protective superinhibition.

Henceforth, no doubt, a leading role in a more profound insight into the essence of age differences in neurotropic effects of pharmacological substances will also be played by further enrichment of our knowledge about peculiarities of higher nervous activity at different stages of ontogenetic development. Further, we must emphasize the extreme importance of studying specific, biochemical processes of the central nervous system, at different stages of ontogenesis, combined with a more complete determination of biochemical mechanisms of action of pharmacological substances.

Another important problem of evolutional pharmacology that has not yet been undertaken is that of the reflection of biological rhythms of organisms on the effect of pharmacological substances. Rhythms are basic biological characteristics. Impoverishment and narrowing of biological rhythms reduces the possibility of adequate adaptation of organisms. The foundation of entire biological rhythms is formed by

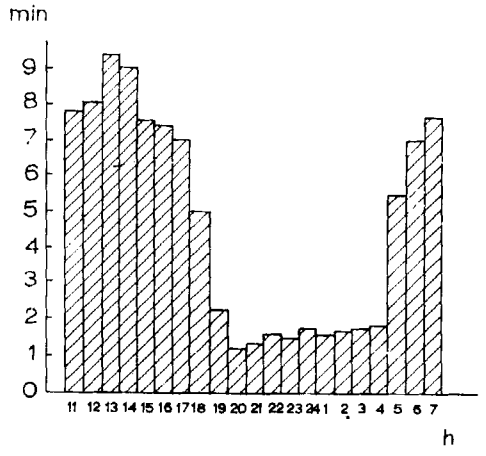

Fig. 4. Duration of the motionless state in each hour between 11 a.m. and 8 a.m. of the next day. Average results of 30 experiments on 10 rats.

the conditions of existence in which a given species develops. Peculiarities and changes of environment, and conditions of social environment, so far as man is concerned, are factors that determined and determine biological rhythms in phylo- and ontogenesis. However, it is the great adaptive significance of biological rhythms caused by external and internal factors that determine transformation of many of them, in the course of evolution, into basic hereditary features characteristic of every species. Thus, biological rhythms acquire considerable selfdependence and independence of the factors determining them.

As an example of the role played by an organism's biological rhythms in experimental pharmacology we shall mention one finding from our laboratory. Studying the effect of some medicinal substances of vegetable origin on motor activity of white rats we (Petkov *et al.*, 1961b) found, using the autographic method, that a sedative effect of Valeriana preparations on motor activity of rats was especially well pronounced at night. This sedative effect was more marked in summer than in winter.

In trying to discover the cause of these differences in effect we investigated natural oscillations of motor activity in white rats. Using our own calculation method we found that the period of time during which rats were motionless by day (11–17 h) was 500–600% longer compared with that at night (from 20 p.m. till 5 a.m.) (Fig. 4). On the other hand, at a given period during the 24 h, white rats were motionless far longer in winter than in summer. Thus, the period of rats' motionless state from 17 p.m. till midnight in December was 160% longer than in July (Fig. 5). (Experiments were carried out in a heated room.) It is evident that considerable motor activity of white rats at night and in summer resulted in better manifestation of a sedative effect of the preparations studied.

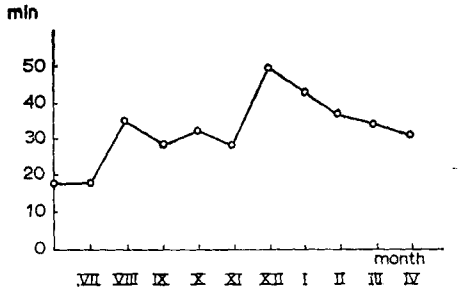

Fig. 5. Duration of the motionless state during separate months of a year from 17 p.m. till 1 a.m. Average results of 340 experiments on 39 rats.

This well-known diurnal and annual biological rhythm of spontaneous activity in white rats is evolutionally conditioned by the way of life of wild ancestors of this species. An important adaptive significance of this life rhythm of rats is so strong that even radical changes in living conditions of these animals during laboratory growth throughout hundreds of generations could not shatter this peculiarity which has become typical of their heredity.

I have tried to show by these examples from our laboratory that in studying pharmacodynamics of this or that medicinal substance one should seek to reveal the influences

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of age and seasonal biological and physiological peculiarities of experimental animals affecting its action. Hence, in pharmacotherapeutical use of a medicinal substance one should consider these factors in man. The exceedingly important theoretical and practical significance of these problems requires more profound research in the field of evolutional pharmacology.

SUMMARY

The investigation of differences in reactivity of an organism to pharmacological drugs, depending on age, time of administration (by daytime or at night, in summer or in winter) and other factors is of theoretical and practical importance. The higher parts of the central nervous system play an important role here.

Our experiments with the conditioned reflex method have shown that a definite dose of ginseng increased the general tone of cortical activity in young people (simultaneous stimulation of excitatory and inhibitory processes), but in elderly subjects it acted as a depressant (emergence of preventive cortical supramarginal inhibition). Similar results have been obtained in young and old rats. A certain dose of ginseng facilitated the reversal of conditioned reflexes only in young rats.

Reserpine (1 mg/kg) in young rats improved the differentiative inhibition, somewhat decreased the positive reflexes (Ovcharov *et al.*, 1964) and impeded the reversal of the differentiation stimulus into the conditioned signal of pain. In old rats the same dose of reserpine completely depressed both conditioned and unconditioned reflexes.

Corazol (50 mg/kg) evoked epileptiform convulsions in 100% of old rats, and in young ones only in 50%.

The extracts of different sorts of rad. valerianae (Petkov *et al.*, 1961b) produced a more pronounced sedative effect at night and in summer, *i.e.* in periods characterized by more significant locomotive activity of rats.

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Modification of Ontogenetic Patterns in Mammalian Brain

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INTRODUCTION

The postnatal development of feline cerebral and cerebellar cortex has been studied in sufficient detail in recent years to provide a satisfactory basis for preliminary examination of the effects of various pathological processes on the immature brain (Noback and Purpura, 1961; Purpura, 1961; Purpura *et al.*, 1960, 1963; Voeller *et al.*, 1963). Studies aimed at modifying ontogenetic patterns can be expected to contribute to the clarification of several problems. Of particular importance is the problem of specifying the manner in which different insults to the immature nervous system are expressed in different functional disturbances. Closely related to this problem is the question of the selective vulnerability of different neuronal systems at various developmental stages. Studies of altered ontogenetic patterns may also provide clues to the role of a particular population of neurons in the sequential development of different synaptic organizations. Here emphasis may be placed on attempts to correlate changes in electrophysiological activities with alterations in morphological features of elements presumably involved in the production of these activities. Such studies also permit greater definition of the morphological substrate of evoked activities in the immature brain, especially in those situations in which selective damage to specific types of neurons results in unequivocal changes in particular components of evoked responses.

In the present report examples are illustrated of the effects of two types of injuries to the immature brain which produce fundamentally different modifications of ontogenetic patterns. The first involves interruption of pyramidal neuron axons during isolation of regions of the cerebral neocortex in the immediate neonatal period. The second type of injury is that produced by x-irradiation. The alterations in developmental patterns produced by these injuries are particularly pertinent to the problems noted above.

* Supported by Public Health Service, Research Career Program Award, NB-K3-5280-03, National Institute of Neurological Diseases and Blindness, National Institutes of Health.
** Special Fellow, National Institute of Neurological Diseases and Blindness, National Institutes of Health, 2 F11 NB-999-02.

CONSEQUENCES OF THE RE-ORGANIZATION OF SYNAPTIC PATHWAYS IN THE IMMATURE NEOCORTEX

The changes in the morphological and physiological properties of chronically isolated immature neocortex are best appreciated by prior consideration of normal developmental patterns (Noback and Purpura, 1961; Voeller *et al.*, 1963). Pyramidal neurons in the neocortex of newborn kittens have well developed apical dendrites which extend into the molecular layer. Basilar dendrites and axon-collaterals are poorly developed or absent on most pyramidal neurons at this developmental stage. Electron microscope studies of the superficial neocortex in newborn kittens have revealed a relatively large number of axodendritic synapses (Voeller *et al.*, 1963) which have ultrastructural characteristics that are entirely similar to those observed in adult cat neocortex (Pappas and Purpura, 1961). Only towards the end of the first postnatal week are axosomatic synapses readily detectable. At this time also basilar dendrites undergo a rapid phase of proliferation which continues until the end of the third week. During this period of basilar dendritic growth spines appear on dendrites and many collateral branches of apical and basilar dendrites are detectable. Axon-collaterals of large pyramidal neurons increase in number and length during the 2nd–4th weeks. At the end of this period available evidence suggests that axon-collateral–interneuronal synaptic pathways are well established (Purpura *et al.*, 1963). The general features of pyramidal neuron development are summarized in Fig. 1. It should be noted that myelination of corticospinal axons and increase in axon diameter occur well after

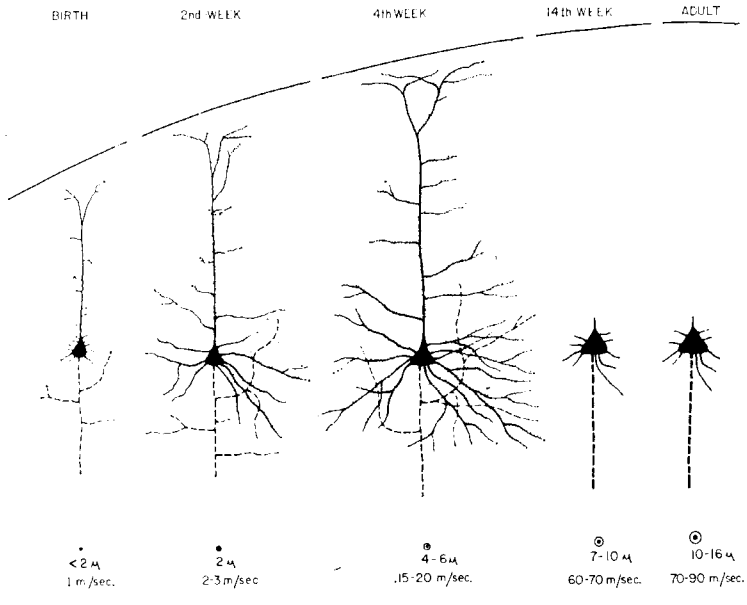

Fig. 1. General features of morphogenesis of large pyramidal neurons in the feline neocortex. Dendritic ramifications were not added in the 14th week and in adult animals since there is little change in dendrites after the first month. Below, representation of axon diameter of largest pyramidal fibers and their conduction velocities at various ages. (From Purpura *et al.*, 1963).

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the elaboration of apical and basilar dendritic systems of cortical pyramidal neurons.

Electrophysiological studies of evoked cortical activities have confirmed morphological observations indicating a differential development of superficial axodendritic synaptic pathways in the neocortex of newborn kittens (Purpura, 1961). Some of the observations in support of this view are summarized in Fig. 2 with respect to

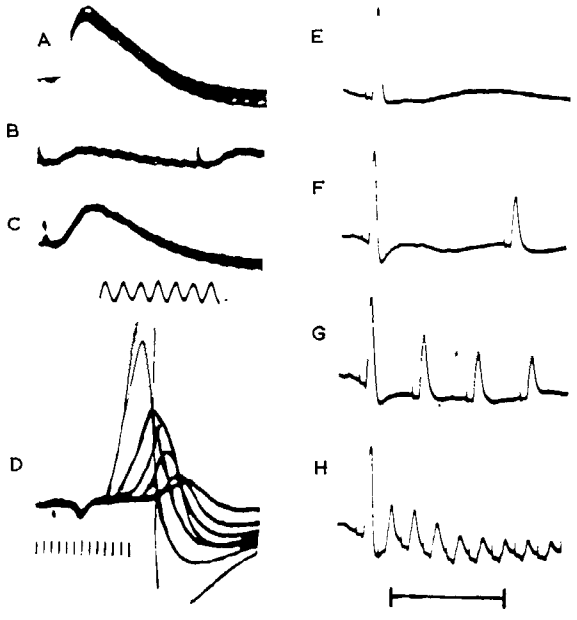

Fig. 2. Composite of different varieties of potentials evoked in the neocortex of neonatal kittens. Negativity upwards in this and all subsequent records. A = superficial cortical responses (SCR) to local surface stimulation consist in long-duration negativity. Stimulus frequency in A, 0.5/sec. B = increase in stimulus frequency (12/sec) produces marked depression of SCR which persists for many seconds after return to 0.5/sec stimulus frequency (C). Cal. 100 cycles/sec. D = superimposed responses recorded from the anterior suprasylvian gyrus during 0.5/sec stimulation in medial mesencephalic reticular regions in a 2-day-old kitten. Early low-amplitude surface-positive component is unaltered, whereas late prominent negativity rapidly attenuates at this stimulus frequency, indicating marked 'fatigability of reticulocortical evoked responses'. Cal. 100 cycles/sec. E–H = predominantly surface-negative specific responses evoked in posterior sigmoid gyrus following stimulation of ventrolateral thalamic nuclei and their projections in an 8-hour-old kitten. Stimulus frequency as follows: E = 0.5/sec; F = 2/sec; G = 5/sec; H = 10/sec. Depression of response is evident during 2/sec stimulation and profound during 10/sec stimulation. Time calibration, 0.5 sec.

the electrographic characteristics of several varieties of evoked cortical responses in the immediate neonatal period. Although surface-negative components are prominent in virtually all types of evoked cortical responses in newborn kittens, such activities exhibit a marked fatigability during repetitive stimulation. It is of interest that prior to the second or third week it has not been possible to elicit after-discharges by stimulation of the neocortical surface with stimuli maximal for local superficial negative responses. Similarly, paroxysmal activity has not been observed following strong stimulation of afferent pathways in young kittens.

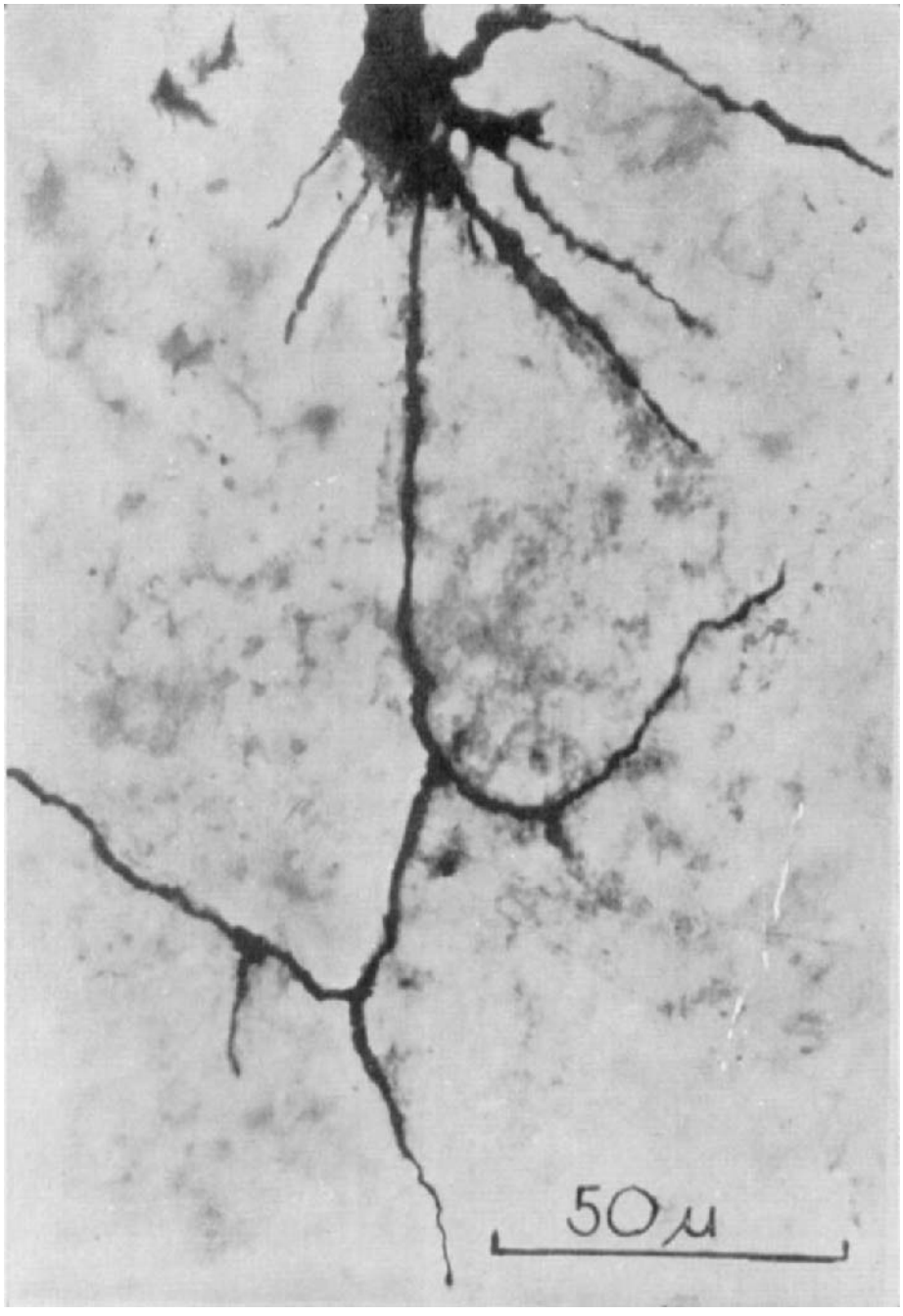

Fig. 3. Deep-lying arciform pyramidal neuron in a region of the isolated cortex from a 5-day-old kitten, 2 days after cortical isolation. This neuron was located in close proximity to the tip of the microelectrode with which the focal records shown in Fig. 5 were obtained. The photomicrograph was retouched to emphasize some portions of the basilar dendrites and the initial axonal segment that were out of the plane of focus. Note extensive axon-collateral development. Such collaterals have never been observed in the intact immature neocortex of a 5-day-old cat. (from Purpura and Housepian, 1961).

In attempts to modify the normal postnatal development of neurons in neocortex, studies were carried out on the effects of complete subpial isolation of a slab of the neocortex in the immediate neonatal period (Purpura and Housepian, 1961). This procedure produced little detectable change in the growth and elaboration of apical and basilar dendrites. Striking changes were observed, however, in the number and length of axon-collaterals of pyramidal neurons whose main stem axons had been severed in the isolation procedure. A pyramidal neuron in a region of the isolated neocortex from a 5-day-old kitten, 2 days after cortical isolation is shown in Fig. 3. Attention is directed to the remarkable proliferation of recurrent axon-collaterals which was observed in a large number of pyramidal neurons in different sites in the isolated slab. Several days after the preparation of neuronally isolated regions of the neocortex, weak surface stimulation elicited complex short-latency surface-negative responses which were succeeded by surface-positive repetitive discharges (Fig. 4). A

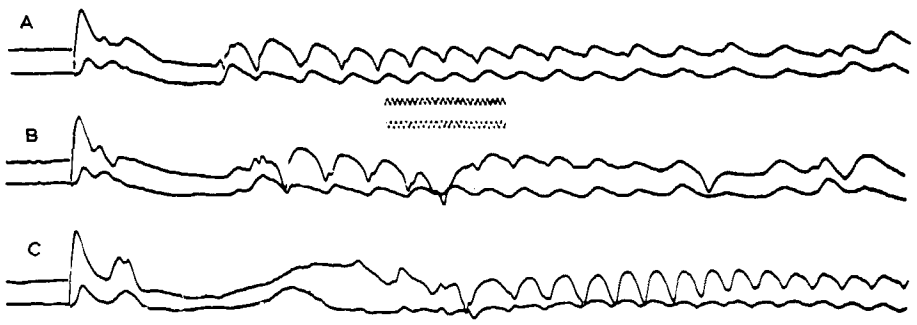

Fig. 4. The SCR's and repetitive discharges recorded 1.5 mm (upper channel) and 3.5 mm (lower channel) from the site of stimulation in a region of the chronically isolated neocortex in a 10-day-old kitten, 7 days after preparation of the isolated slab. Examples of three successive responses elicited at 5-sec intervals are shown in A to C. The SCR's evoked by weak stimulation and recorded at both sites exhibit multiple additional surface negativities on their late phases. These are succeeded by very long-latency predominantly surface-positive 10–14 per sec repetitive responses. Note in C that the repetitive responses are confirmed almost exclusively to the near electrode site. Time calibration, 100 cycles/sec; 0.1 mV.

prominent feature of the burst responses evoked in a chronically isolated immature neocortex was their remarkable regularity in contrast to the variability of burst responses in the chronically isolated cortex of adult animals (Burns, 1958). In attempts to define the loci in the isolated immature cortex giving rise to such repetitive bursts studies were undertaken in which microelectrode recordings were made at several intracortical depths during stimulation at surface and subsurface sites. The results of these studies are summarized in Fig. 5. Weak subsurface stimulation at a depth corresponding to the cell bodies of large pyramidal neurons elicited repetitive responses which were similar in overt configuration to burst responses elicited by strong surface stimulation. Surface-positive components of repetitive responses were associated with focal negativities in the cortical depths. These studies taken together with findings on the effects of topically applied aliphatic ω-amino acids (Purpura *et al.*, 1959b) and

strychnine have suggested that the repetitive bursts of the chronically isolated immature neocortex are attributable to synchronous activation of pyramidal neurons which effect synaptic contact with adjacent elements via recurrent axon-collaterals of the type illustrated in Fig. 3. The picture which emerges from studies of the chronically isolated immature cortex is consistent with the findings of Cajal (1959a) who first demonstrated proliferation of axon-collaterals of pyramidal neurons following

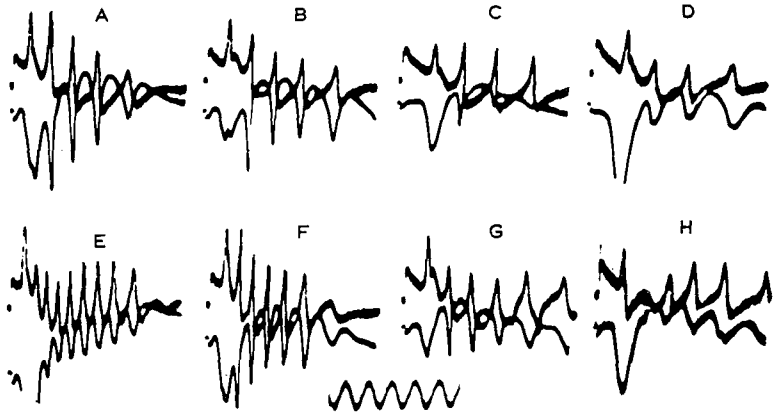

Fig. 5. Evoked repetitive responses in an isolated cortical slab. Stimulating wire electrodes located at a depth of 0.8 mm. Surface responses (lower channel) recorded 3 mm from the site of stimulation. Focally evoked responses (upper channel) simultaneously recorded with a microelectrode in the cortical depths (0.5–0.6 mm); stimulus frequency 0.5/sec. A = control repetitive sequence elicited by single intracortical stimulus. Surface-positive responses are associated with focal negativity. B = 3 min after topical application of ε-aminocaproic acid (C_6). Note depression of surface and focally recorded responses with greater depression of late components of surface activity. C and D = few seconds after addition of GABA during C_6 action. Surface responses augment but focally recorded activity is decreased. E = phase of post-GABA augmentation after removal of both ω-amino acids. F = control. G = maximum effect produced by topically applied strychnine sulfate (1 : 1000). H = few seconds after addition of GABA during strychnine action. Time calibration, 100 cycles/sec; 0.1 mV. (From Purpura and Housepian, 1961).

interruption of their main stem axons in the immediate neonatal period. It is of interest that Cajal also proposed that the conversion of type I neurons into type II arciform pyramidal cells could result in marked changes in the functional activity of such elements. The demonstration of increased hyperexcitability as seen in the appearance of repetitive bursts in regions of the isolated cortex containing arciform pyramidal neurons amply confirms Cajal's hypothesis. It should also be noted that since trauma to pyramidal neuron axons is an essential factor in initiating the proliferation of axon-collaterals and the development of hyperexcitability, it is not unlikely that these alterations may constitute an important mechanism involved in the delayed appearance of convulsant activity in traumatized mature as well as in immature cerebral cortex. If such is the case there would appear to be little necessity to invoke the phenomenon of 'denervation sensitivity' (Cannon, 1939; Echlin, 1959) to explain the alteration in excitability observed in regions of the neocortex that have sustained injury to their intracortical or corticofugal projections.

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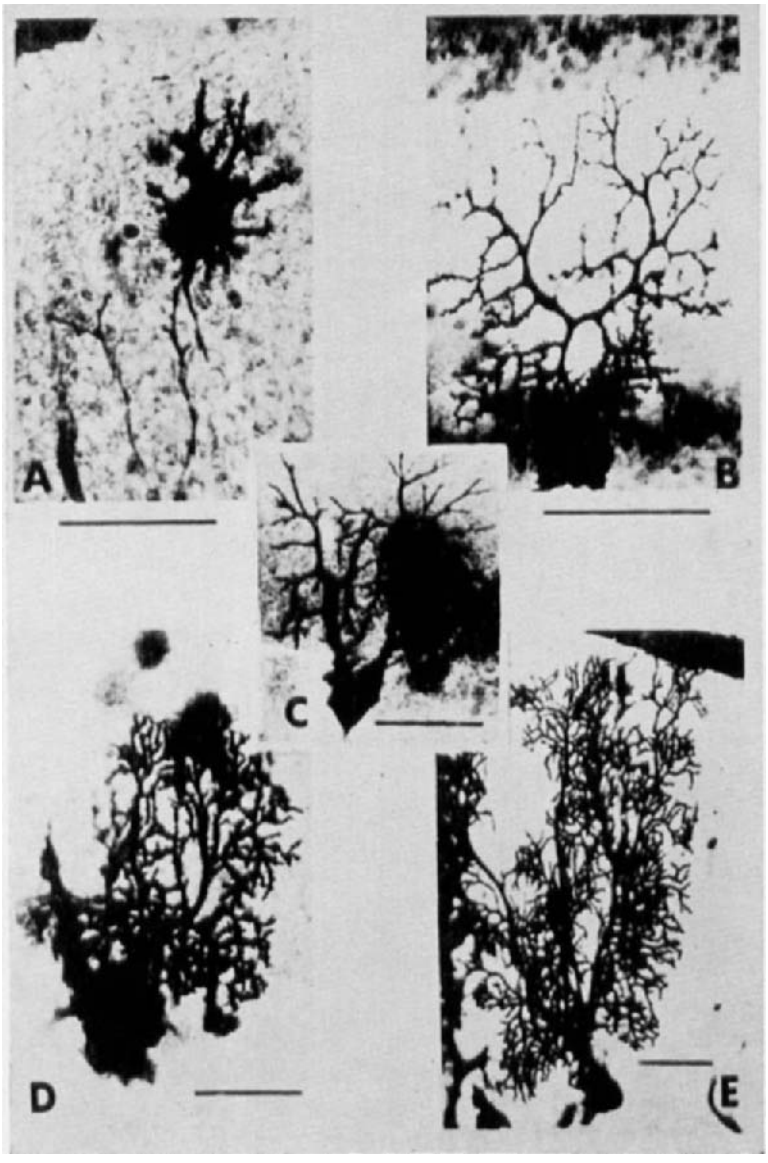

Fig. 6. Microphotographs of Purkinje cells revealed in 200-μ-thick Golgi–Cox sections of cerebellum in kittens of various ages. A = 2-day-old kitten. Main stem dendrites are short, occasionally branched and have several protuberances. Dendrite-like ramifications are seen emerging from the cell body. B and C = 2 different stages of Purkinje cell development seen in the same section but from different parts of the cerebellum in a 8-day-old kitten. Note in B terminal portions of dendrites do not penetrate into lower border of external granular layer. C = possible 'transitional' stage of Purkinje cell with smoothly contoured primary, secondary and tertiary rami. D = 20-day-old kitten; E = 42-day-old kitten. Note that during the second month a marked increase in length of dendrites occurs but not in density of tertiary rami and spiny branchlets. Horizontal bar below each microphotograph is 50 μ. (From Purpura *et al.*, 1963).

ALTERATIONS IN POSTNATAL DEVELOPMENT OF SYNAPTIC ORGANIZATIONS IN THE CEREBELLAR CORTEX

The changes in physiological properties of the isolated immature neocortex illustrate the manner in which stereotyped patterns of evoked electrocortical activity may result from relatively specific developmental changes, *i.e.*, an increase in excitatory synaptic drives involving pyramidal neurons with hypertrophied axon-collaterals. The effects produced by x-irradiation of the cerebellum in newborn kittens provide information on the interaction of several maturational alterations which result in more complex pathophysiological effects. For present purposes radiation-induced alterations may be considered with respect to two of the major postnatal developmental features of the cerebellar cortex: the differentiation of cells of the external granular layer and the development of Purkinje's cells. Although a variety of electrophysiological activities may be utilized as indices of cerebellar maturational changes and subsequent effects of radiation, it has been found convenient to utilize two types of responses in these studies, one evoked by local folial stimulation and the other elicited by stimulation of the contralateral motor cortex.

NORMAL DEVELOPMENTAL PATTERNS IN CEREBELLAR CORTEX

Purkinje's cells in neonatal kittens have well developed main stem axons which can be traced into the underlying white matter (Fig. 6A). Numerous small highly branched axon-collaterals are observed intermingled with axons of climbing fibers and mossy fibers. The cell body does not have a smooth contour such as is observed in Purkinje's cells of slightly older kittens. Numerous short, perisomatic protoplasmic projections are seen which are similar to those of pyramidal neurons of neonatal kitten neocortex (Noback and Purpura, 1961). These processes appear to be resorbed sometime shortly after birth. The main dendritic trunk is frequently not much more prominent than the perisomatic extensions. The tips of these dendrites terminate at the lower border of the external granular layer (EGL), which is approximately 10 to 12 cell layers thick in the immediate neonatal period (Chiarugi and Pompeiano, 1954). During the early part of the second postnatal week an expansion of the dendrites of Purkinje's cells is detectable (Figs. 6B and C). Most Purkinje's cells have well developed primary, secondary and early tertiary branches by the end of the second week. The delicate distal tertiary branches give rise to spiny branchlets. As in earlier stages, dendritic processes terminate at the lower border of the EGL. The EGL appears less densely packed than in neonatal animals, but is still 4 to 8 cell layers thick. Increased cellularity of the molecular layer seen during the second week is referable to inward migration and differentiation of elements from the EGL (Cajal, 1911; 1959b). From the third to sixth weeks there is further elaboration of Purkinje cell dendrites with considerable elongation of main stem dendritic trunks (Figs. 6 D and E). Progressive thinning of the EGL and continuation of the processes of inward migration of EGL cells also occurs but at a diminished rate. Towards the end of the second month the EGL has virtually disappeared although in some areas a single-layered remnant is

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detectable immediately below the pial surface. Purkinje cell dendrites appear to be fully developed in the 2-month-old kitten and for the most part, spiny branchlets of dendrites are contiguous with a single layer of granule cells or closely related to the pial membrane (Purpura *et al.*, 1963).

Local responses to surface folial stimulation in adult animals consist of 10–20 msec surface-negative waves generally preceded by variable short-latency inflections (Dow, 1942; Purpura *et al.*, 1959a). These responses, which are here referred to as superficial cerebellar responses (SCbR's) were not recorded in young kittens (Fig. 7). Typical responses elicited in neonatal preparations consisted in diphasic or triphasic 'spike-like' components. These were followed by broad surface-positive waves especially after the first week. Surface-positive responses to folial stimulation were more prominent during the 2nd week, whereas during the 3rd week diphasic spikes of considerable

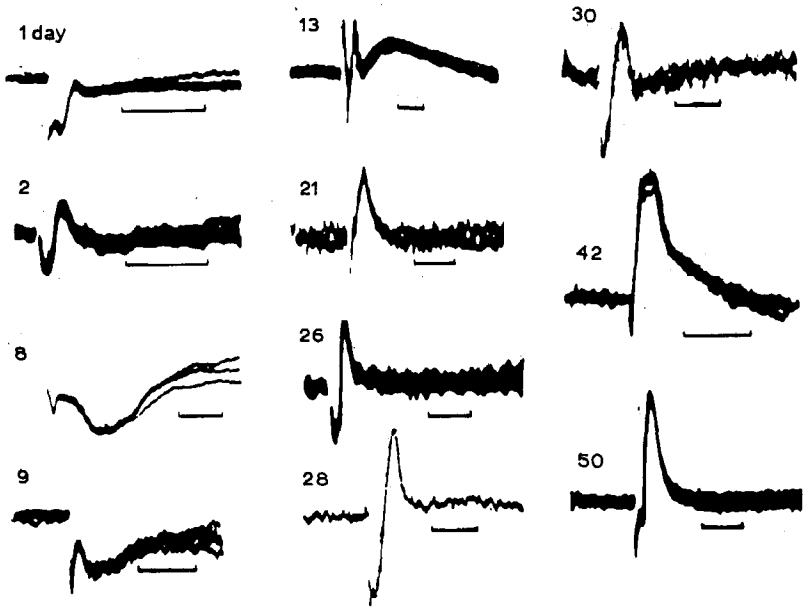

Fig. 7. Electrographic characteristics of local responses of cerebellar cortex to surface folial stimulation in normal kittens of various ages as shown. Each record is formed by 3–10 superimposed traces except that of the 28-day-old animal. First postnatal week (p.n.w.) responses consist of 'spike-like' components followed by slow positive waves. During the second p.n.w. slow positivity becomes well developed and early positive-negative spikes are occasionally seen. Early surface negativity of superficial cerebellar cortical responses (SCbR's) of adult animals are seen by the third week. Thereafter SCbR's with adult characteristics are noted until at 5–6 weeks responses are identical to those of mature animals. Time calibrations, 20 msec for all records. (From Shofer *et al.*, 1964).

amplitude were occasionally followed by slow, variable negativities. Diphasic positive–negative sequences were obtained with greater regularity during the 4th week. By the end of the 6th week, adult SCbR's were consistently elicited (Fig. 7).

Afferent responses recorded from the paramedian lobule (PML) (Dow, 1949;

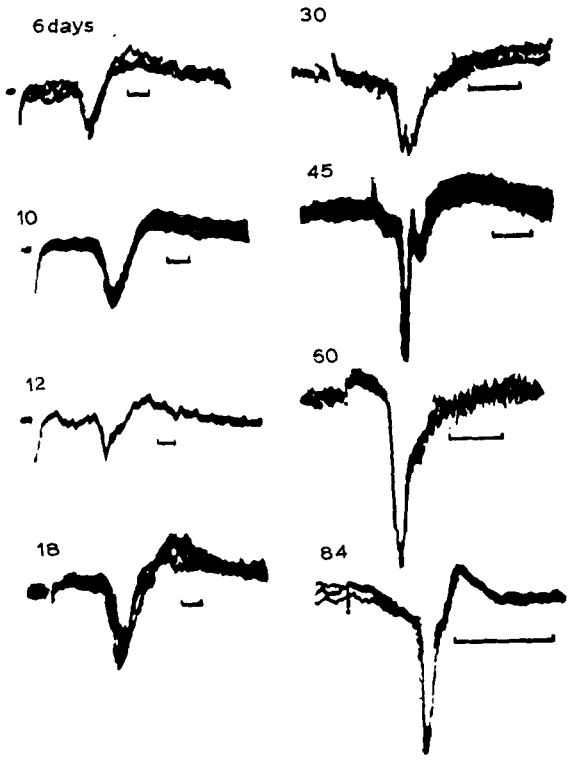

Fig. 8. Characteristics of responses evoked in cerebellar paramedian lobule (PML) by single-shock supramaximal stimulation of contralateral pericruciate (motor) cortex in normal kittens of various ages as shown. Each record consists of 3–5 superimposed traces except that of the 12-day-old kitten. Note long latency of responses in first 3 weeks and progressive shortening of latency and increased complexity of responses after the first month. Positivity downwards in this and all subsequent records. Time calibrations, horizontal bars, 20 msec for all records. (From Shofer *et al.*, 1964).

Purpura *et al.*, 1959a) following moderately strong stimulation of contralateral pericruciate cortex in kittens of different ages are shown in Fig. 8. Typical PML responses observed during the first 3 postnatal weeks consisted in long-latency (60 msec) surface-positive waves succeeded by low-amplitude, variable long-duration negativities. Diphasic positive–negative sequences of similar, relatively simple configuration were recorded from different folia in the paramedian lobule. The stereotyped wave form of PML responses in young kittens was not altered by changes in stimulus strength (Fig. 9), although some enhancement of the long-latency negative wave was observed. Towards the end of the third week additional early components preceding the peak of the positive wave were occasionally observed.

Further examination of PML responses in Fig. 8 indicates that significant developmental alterations occur towards the end of the 4th–6th week. During this time PML responses exhibited both latency and wave form changes. Particularly striking was the appearance of high-amplitude 15–25 msec sharp positive waves with or without additional smaller components. These are illustrated in records taken from 45-, 60- and

84-day-old kittens, whereas a third type of response characterized by a broad double positivity is shown in records from a 30-day-old kitten (Fig. 8).

PML responses after the first month begin to resemble in many respects those observed in adult animals (Jansen, Jr., 1957; Purpura *et al.*, 1959a). In most instances, however, additional response complexities were obtained with strong stimulation as illustrated in Fig. 9. Progressive increases in stimulus strength required to elicit PML responses resulted in the development of a short-latency component (Fig. 9D, 3–6).

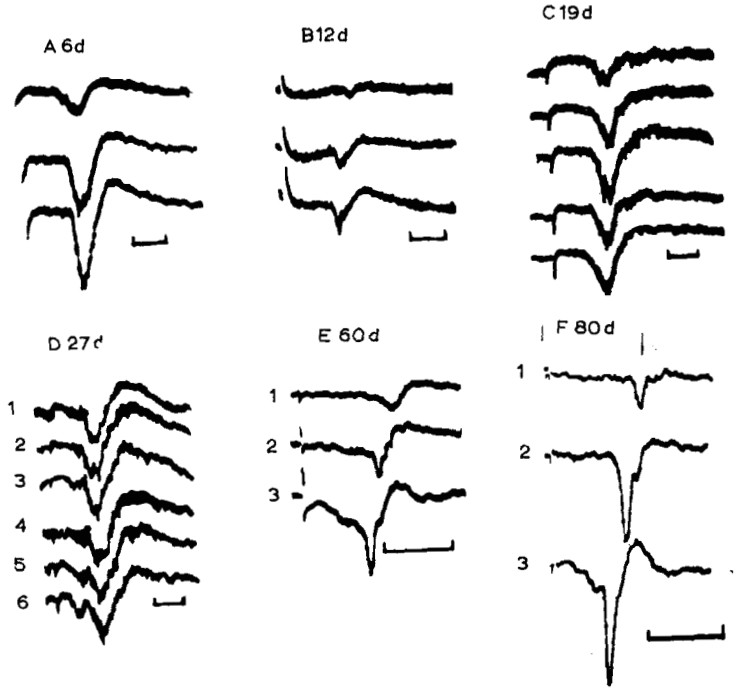

Fig. 9. PML afferent responses evoked by stimuli of progressively increasing strengths in normal kittens whose ages are indicated. A = in a 6-day-old kitten simple wave form is maintained and no latency shift is observed with strong stimulation. B and C = essentially similar to A. D = weak stimulation (1 and 2) elicits prominent positive–negative sequence, whereas stronger stimulation introduces an early positive component of short duration (3–6). E = weak stimulation (1) elicits long-latency response. Shift in peak latency of positive response is seen with stronger stimulation (2 and 3). F = similar to E, but note prominent slow positivity preceding the major deflection elicited by strong stimulation (3). Calibrations, A–D, 50 msec; E and F = 20 msec. (From Shofer *et al.*, 1964).

The full response evoked by supramaximal stimulation had characteristics similar to the mixed short- and long-latency responses of adult animals (Jansen, Jr., 1957). It is to be noted that the short-latency component observed in mixed responses of 4-week-old kittens was obtained with stronger stimulation than that required to elicit the long-latency positivity. This is in contrast to findings in adult animals in which both short- and long-latency components usually have identical thresholds and are obtained with equal facility. PML responses with characteristics similar to those of long-latency responses of adult animals are illustrated in Figs. 9E and 9F. Responses shown

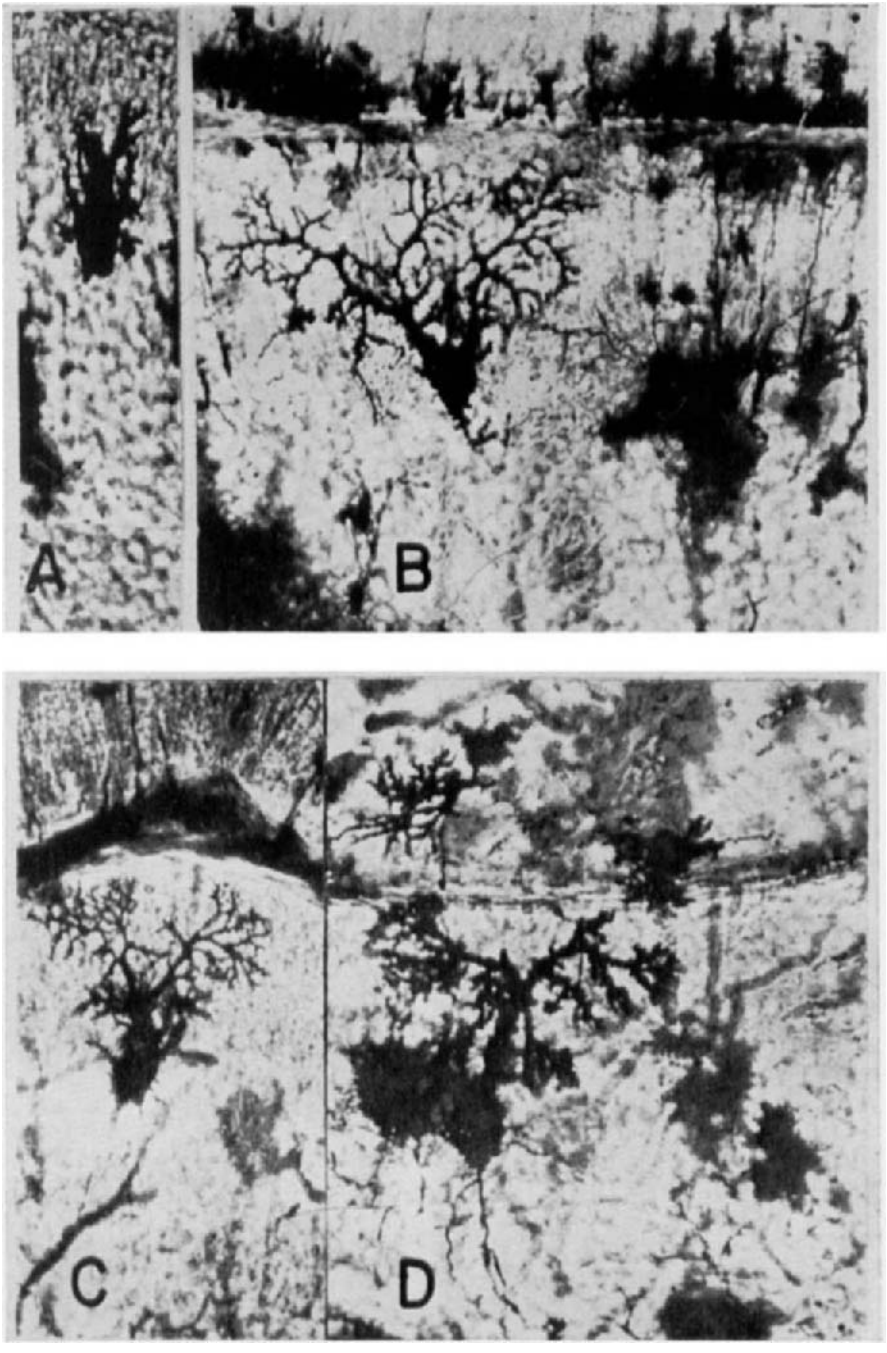

Fig. 10. Characteristics of Purkinje cells in irradiated immature cerebellar cortex. A and B = cells from different regions exhibiting markedly different maturational features in a 5-day-old kitten. Cell in A was in close proximity to a region of maximal damage. B = shows an unusual degree of dendritic proliferation. C and D = from 5-day-old kittens. Dendritic terminals are in close relation to the pial surface elements. Additional details in text. All cells × 320. (From Shofer *et al.*, 1964).

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in Fig. 9E differed from those of Fig. 9D with respect to the characteristics of the major deflection and their mode of evolution during progressive increases in stimulus strength. The large broad positive-negative deflection in Fig. 9D was virtually fully developed with relatively weak stimulation and was unaltered even with very strong stimulation. The initial very long-latency positivity elicited with weak stimulation in older kittens (Fig. 9E) not only exhibited shortening of latency with stronger stimulation, but underwent marked increases in amplitude and decreases in duration. The fully developed PML responses exhibited additional complexities consisting of early slow positive components, not unlike those seen in younger kittens (Fig. 9D) and the development of a late prominent negativity (Fig. 9E, 3).

RADIATION-INDUCED ONTOGENETIC CHANGES IN CEREBELLAR CORTEX

Several days after irradiation (2000 r) of the posterior occipital region in newborn kittens, general loss of the external granular layer was a characteristic finding. In addition to the loss of external granule layer cells hypercellularity of the molecular zone was observed along with some loss of internal granular elements. The general architectural alterations observed in the second and third postirradiation[4] weeks consisted in attenuation of granule cells and areas of diffuse and focal necrosis. Loss of the EGL, hypercellularity of the molecular layer, heterotopic displacements of Purkinje cells and unusual configuration and pyknosis of Purkinje cell bodies were also noted in the second and third postirradiation weeks (Shofer *et al.*, 1964).

Purkinje cells in the first postirradiation week exhibited a number of common features. Unusually rapid maturation of some Purkinje cells was seen in the remarkable development of tertiary dendritic branches (Figs. 10B and C). Marked differences were also noted in Purkinje cells in the first postirradiation week. Thus cells from the same midline section of a 3-day-old kitten exhibited considerable maturational differences (Figs. 10A and B) which were comparable to those ordinarily seen in normal newborn and approximately 10-day-old kittens, respectively. Particularly striking was the relationship between Purkinje cell dendrites and the pial surface. The cell in Fig. 10D displays widespread primary dendrites which appear to course obliquely toward the surface. Secondary and tertiary branches extend mostly downwards, with only a few ascending branches contacting the pial surface. Terminal branches of the Purkinje cell in Fig. 10D appear smaller and pleomorphic, ranging from globular to fusiform in shape. Occasional delicate spines are seen, but these are far less numerous than in the cells illustrated in Figs. 10B and C.

Progressive alterations are seen in Purkinje cells in the second and third postirradiation week. Little increase in dendritic branching is noted at 12 days and even less at 15 days (Fig. 11). Some indication of growth of the dendritic trunk is inferred from observations on primary branching of tangentially oriented dendrites in subpial locations. In these elements primary branches are directed in a lateral and inferior direction. A number of large spiny processes are seen on smaller branches, whereas more proximal branches have smooth contours. Spines are irregular and coarse and their development is minimal.

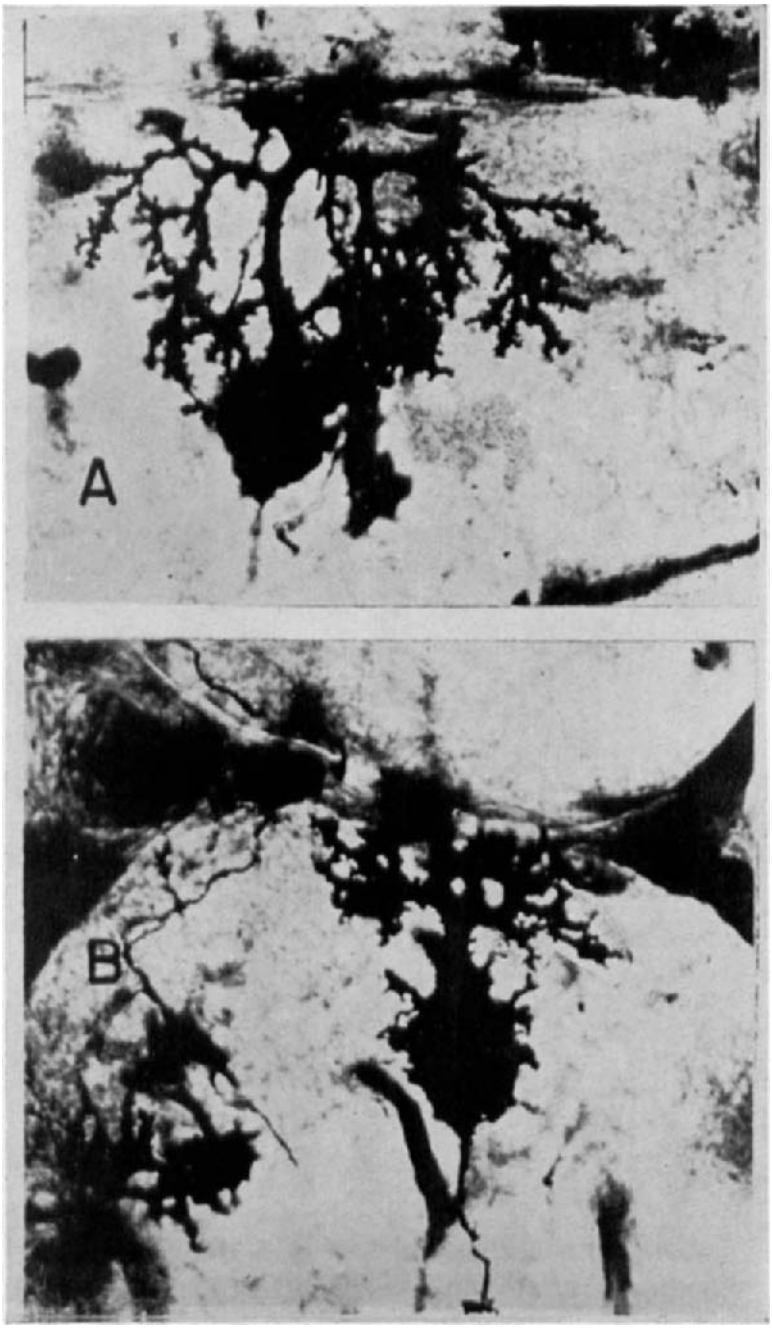

Fig. 11. Characteristics of Purkinje cells in irradiated cerebellum during the second postnatal week. A = 12 days. Large dendrites at surface are deflected laterally and downward during their attempted maturation. This unusual growth pattern is related, in part, to loss of the EGL. (× 660) B = 15 days. Marked retardation of dendritic growth is evident. Other features are similar to those noted in A. (× 660) (From Shofer *et al.*, 1964).

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Profound effects of irradiation were noted in responses to folial surface stimulation. Particularly impressive were findings of complex single and multiple spikes of relatively large amplitude superimposed on various multiphasic components of slow waves. In early postirradiation periods initially small spikes followed by slow positive waves were obscured in their development by slow positive and negative sequences (Fig. 12A). With weak surface stimulation (Fig. 12A, 1) responses were not unlike those observed

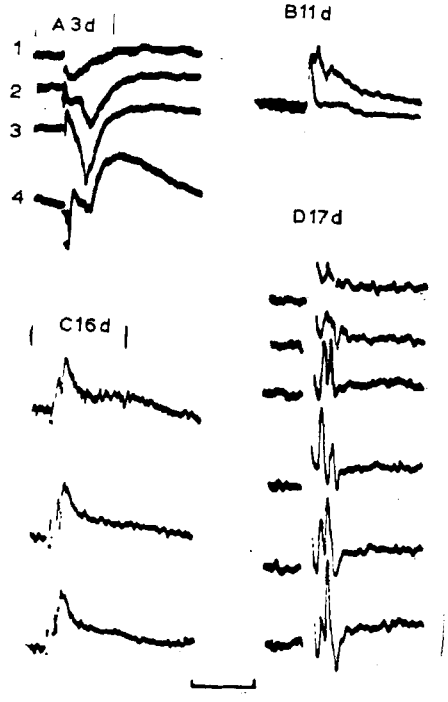

Fig. 12. Characteristics of local responses to surface folial stimulation in irradiated kittens; ages as indicated. A = weak stimulation elicits prominent positivity and barely detectable spike (1), whereas stronger stimulation (2–4) evokes additional complex positive–negative components and large amplitude early multiphasic spike. B and C = examples of spikes superimposed on slow negativities. D = stimulus increment series illustrating reciprocal changes in magnitude of early and late components of multiphasic spikes. Time calibration, 20 msec for all records. (From Shofer *et al.*, 1964).

in the second week in normal animals. However, stronger stimulation revealed progressively more complex activities including a prominent second positive wave (Fig. 12A, 2) which was enhanced with further increases in stimulus strength (Fig. 12A, 3). These changes were accompanied by increases in magnitude of the initial spike-like component. Finally with very strong stimulation apparent inversion of the positivity occurred with the appearance of a prominent prolonged surface negativity (Fig. 12A, 4).

Several variations of responses shown in Fig. 12A were recorded in other kittens in the immediate postirradiation period. In older irradiated animals multiple spikes superimposed on slow negative waves were especially prominent (Figs. 12B and C). Dramatic reciprocal changes in the magnitude of early and late spike components of

responses elicited in the third postirradiation week are shown in the stimulus increment series of Fig. 12D. These responses, as well as other complex electrographic activities, are indicative of an extraordinary change in the 'excitability' of elements responsive to surface stimulation. They also indicate that the relatively simple early surface negativity of SCbR's which appears during the third week in normal kittens, has no counterpart in records of irradiated kittens of comparable ages.

Afferent responses of paramedian lobule (PML) evoked by motor cortex stimulation in kittens several days after irradiation consisted in complex positive–negative sequences of extremely long latency (100–120 msec) (Fig. 13A). The major positivity of responses evoked by weak stimulation was frequently preceded by a shorter-latency positive deflection and followed by a low-amplitude negativity of variable duration (Fig. 13A, 1). Increases in stimulus strength resulted in broadening of the major

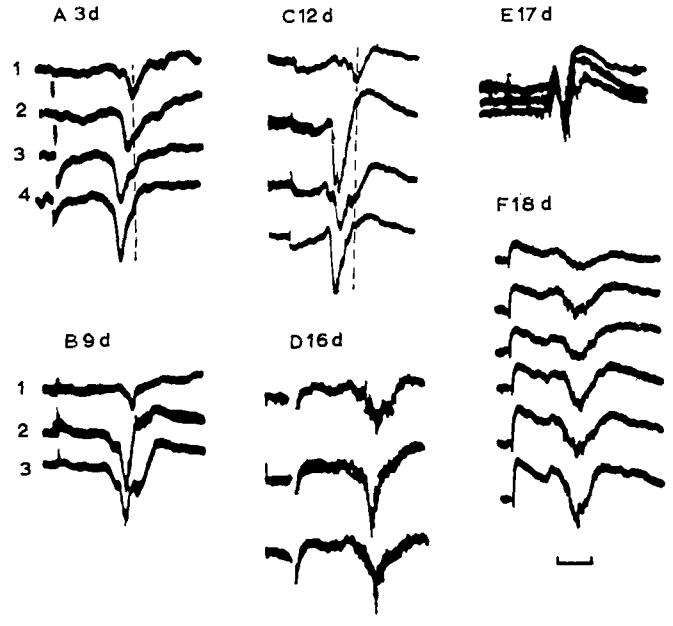

Fig. 13. Changes in characteristics of PML afferent responses evoked in irradiated kittens of various ages as indicated. A = weak motor cortex stimulation (1) elicits long-latency positivity, whereas stronger stimulation (2–4) evokes responses with major positive deflections of shorter latency. B and C = essentially similar changes in configuration and latency of evoked responses during stimulus increment series. Vertical dashed lines in A and C drawn through peak of positivity elicited by weak stimulation. Note peak-latency shift and inflection on responses to strong stimulation that are temporally related to long-latency positivity of responses to weak stimulation. D–F = lack of significant changes in latency and overt configuration of responses in stimulus increment series. Time calibrations, 50 msec for all records. (From Shofer, *et al.*, 1964).

component with decrease in latency of peak positivity. With supramaximal motor cortex stimulation PML responses were initiated by a slow positivity fused with the fast positive component of the major deflection.

Some idea of the overall complexity of PML responses elicited during the second

postirradiation week is gained from the stimulus increment series shown in Fig. 13C. It is of interest that PML responses in this preparation exhibited cyclic alterations with respect to latency shift and amplitudes of peak positivity, as well as relative changes in magnitudes of preceding and succeeding components. Not infrequently during the course of these variations PML responses were almost entirely blocked only to return explosively with the next stimulus of a train of low-frequency (0.5/sec) stimuli.

The analysis carried thus far permits several inferences concerning the effects of irradiation on the development of responses to surface stimulation and activation of afferent pathways. Ontogenetic data on the SCbR obtained in normal and irradiated kittens are perhaps reasonably explained by the assumption that the production of typical 10–20 msec graded negative waves to folial stimulation requires a number and a particular variety of axodendritic synapses related to fine spiny branchlets of tertiary rami of superficially located Purkinje cell dendrites. Such a substrate for the SCbR is present by the 2nd–3rd postnatal week in normal kittens. Due to the loss of the EGL after radiation Purkinje cell dendrites attain the pial surface within a few days but their spiny branchlets are meager and atypical. They appear to be deficient in the number of synapses apparently required for the SCbR. The loss of typical spiny branchlets in irradiated preparations is undoubtedly related to alterations in the differentiation of cells of the EGL since these elements eventually give rise to granule cells whose axons constitute the parallel fiber system (Fox and Barnard, 1957; Cajal, 1911, 1959b). However, other factors must be considered in attempts to explain the complexity of local responses to folial stimulation in irradiated preparations. It is to be recalled that many Purkinje cells acquire relatively mature morphological characteristics within a few days postirradiation. This finding taken together with observations on the bizarre and pleomorphic appearance of Purkinje cell dendrites in subpial locations could account for some of the complex responses elicited by surface stimulation. The development of membrane properties permitting conductile responses in dendritic systems of irradiated Purkinje cells may also be considered in attempts to explain excitability changes in elements responding to folial stimulation.

Several of the factors noted above in connection with ontogenetic changes in the characteristics of local responses to surface stimulation in normal and irradiated cerebellar cortex are undoubtedly applicable to postirradiation changes in afferent PML responses to contralateral motor cortex stimulation. Impressive differences have been observed in PML responses in young normal and irradiated kittens. Both long- and short-latency components were obtained with supramaximal motor cortex stimulation within a few days after irradiation in young kittens and frequently major components of PML responses exhibited marked latency shifts with different strengths of stimulation. Since these complexities have not been recorded in normal kittens of comparable ages, it is not unreasonable to suspect that initial postirradiation excitability increases are attributable, in part, to 'maturation acceleration' of synaptic pathways involved in the production of PML complexities. Another factor in the early development of excitability in irradiated kitten cerebellar cortex may be related to the loss of cells which may be inhibitory in nature. Clearly, both phenomena,

acceleration of development of some elements and destruction of others, must be recognized in attempts to explain the excitability changes in PML responses (Shofer *et al.*, 1964).

SUMMARY AND CONCLUSIONS

Normal morphological and physiological developmental patterns in feline cerebral and cerebellar cortex are summarized in order to provide a basis for analysis of the pathophysiological effects of two types of injuries to the immature brain. Surgical interruption of axons of pyramidal neurons markedly accelerates development of intracortical axon-collaterals which establish synaptic relations with adjacent arciform pyramidal neurons. One consequence of this re-organization of synaptic pathways is reflected in the development of powerful excitatory synaptic activities in traumatized regions of immature neocortex.

Effects of x-irradiation of cerebellar cortex in newborn kittens are defined primarily in terms of loss of the external granular layer and modification of Purkinje cell morphogenesis. Major alterations in electrophysiological properties of irradiated cerebellar cortex are seen in evoked responses to folial surface and motor cortex stimulation. These alterations are considered expressions of radiation-induced maturation acceleration and re-organization of synaptic pathways and possible changes in membrane properties of Purkinje cell dendrites. The data obtained in these ontogenetic studies of pathophysiological processes indicate that different types of insults to the immature brain may produce similar overt effects, *i.e.*, changes in excitability by entirely different mechanisms.

ACKNOWLEDGEMENT

This study was supported by the National Institute of Neurological Diseases and Blindness, National Institutes of Health, NB-01312-07, the United Cerebral Palsy Research and Educational Foundation, RO135-62 C, and the Epilepsy Foundation.

DISCUSSION

ROITBAK: In your records of cerebellar primary responses in newborn animals there is a negative potential after the initial positive one; at the same time direct stimulation of the cortex surface produces no dendritic potential. How do you explain it? Dow (*J. Neurophysiol.*, **12** (1949) 245) was the first to record a response near the stimulating electrode on the surface of the cerebellum, and he proposed the hypothesis according to which the negative potential recorded expresses the postsynaptic potential of the dendrites of Purkinje cells arising under the action of impulses from the stimulated fibers of the surface layer. You have brought weighty arguments in the favor of this hypothesis.

PURPURA: The low-amplitude negative waves observed in primary or afferent re-

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sponses of the cerebellar cortex in very young kittens probably does not arise in the dendrites of Purkinje cells as is the case of the superficial cerebellar response to local folial stimulation. However it is not unlikely that some synaptic activation of proximal dendritic regions, especially dendritic trunks could occur at this time to produce the low-amplitude negativity. We are convinced that the typical SCbR requires a suitable substrate of parallel fiber–Purkinje dendritic terminal synaptic complexes and these are not present in the very young kitten.

KREINDLER: I would only like to know if the x-irradiation of the cerebellum has an elective action upon the granular external layer of the cerebellum and not upon other structures of the cerebellum.

PURPURA: The major effects of x-irradiation of the cerebellum in the neonatal period are exerted on the external granule cells as has been shown by many other workers. However, I believe we have produced satisfactory data indicating that x-irradiation also produces dramatic effects on the growth and maturation of Purkinje cell dendrites. In addition we suspect that many cells may undergo maturation acceleration and there need not all be elements derived from external granule cells.

SZENTÁGOTHAI: First of all I want to express my admiration for this really magnificent piece of work. As at the time of irradiation there already is a considerable inner granular layer, I presume that some part of the parallel fiber system still must be present in your irradiated animals. What is then your explanation for the lack of the negative wave? Do you think that this is due to the fact that activity in a very considerable fraction of the parallel fibers, running through the dendritic arborization of a Purkinje cell, is necessary to bring about the activation of the dendrites? If so, this might be of very considerable importance for our understanding of the function of the cerebellar cortex.

PURPURA: There is no doubt that some part of the parallel fiber system is still present in our irradiated animals. However, it is our belief that the failure to observe a negative wave following local folial stimulation is a consequence of the relative paucity of typical axodendritic synaptic contacts on tertiary branchlets of Purkinje cells. In other words it would appear that a 'critical mass' of substrate (parallel fibers–Purkinje dendritic terminal synapses) is required for a prominent negativity or superficial cerebellar response. We are currently attempting a statistical analysis of the relationship of this 'critical mass' to detectable evoked potentials.

KARAMYAN: Prof. Purpura's presentation leads to the conclusion that the neocortex morphogenesis is accomplished in the process of ontogenetic development earlier than the morphogenesis of the cerebellar cortex. Possibly this is so for the neocerebellum. As to archi- and paleocerebellum, the whole history of their phylogenesis shows that their formation begins much earlier than the morphogenesis of the neocortex. Still more, the old cerebellar structures already form a fully developed function-

al and morphological system in those representatives of the animal classes who have no neocortex at all.

May I beg Prof. Purpura: (1) to precise whether his data pertain to neo- or to paleocerebellar structures; (2) to tell his opinion on the above mentioned contradictions relative to the cerebellum and cortex morphogenesis.

PURPURA: The results I have presented pertain both to neo- and paleocerebellar structures. Actually I do not believe there is any contradiction here but there is some confusion as regards the extension of phylogenetic development and ontogenesis. Thus, although the cerebellum appears earlier phylogenetically speaking, it has a longer period of ontogenesis in the course of mammalian postnatal development. This, of course, is quite evident from classical studies of the comparative development of the cerebral and cerebellar cortex by Cajal and others.

DSUGAJEVA: As a morphologist, I am much impressed by Prof. Purpura's presentation But I should like to see morphological control at cellular level showing the electrode insertion into the Purkinje cell and proving that the biopotentials are actually lead off from Purkinje cells and not from other structural elements of the cerebellum.

In our laboratory we have detected direct connections between peripheral links of the analyzers and the cerebellum which provide for a direct contact with the cerebellar cortex.

Such connections must effect first a reaction of rapid action in the cortex and only after that a delayed action sets in transmitted along relays. All these facts must be taken in account when recording the biopotentials.

PURPURA: Prof. Dsugajeva has asked about controls at the cellular level of the positions of insertion of microelectrodes into Purkinje cells. Actually we have not attempted intracellular recordings in the cerebellar cortex of kittens. The responses we have described following surface folial stimulation are inferred to be due to synaptic or direct activation of Purkinje cell dendrites, whereas responses from stimulation of afferent pathways to cerebellar cortex undoubtedly involve many elements besides Purkinje cells.

GRIGORIAN: Prof. Purpura's report dealing with the postnatal morphophysiological differentiation of the neocortex and the cerebellum cortex is a fairly thorough and very interesting research.

At the same time I have some questions on this report. First: all the oscillograms presented here including those made in grown-up animals failed to show the first positive deviation (the short-latent one) which, as many believe, represents an activation of the cells of the cerebellar granular layer. What is the reason for such a failure? Second: what is the connection existing between the electrogenesis of the cerebellum cortex and the motor activity of kittens (according to your opinion) provided the complete maturing of the granular layer is known to be somewhat behind the development of the motor activity of the kittens?

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PURPURA: We have observed a small amplitude positivity preceding the major positive component of the cerebellar response evoked by motor cortex stimulation. In our studies this early positivity is best seen following peripheral nerve stimulation. In the very young kittens the absence of this early positivity is probably attributable to the immaturity of the mossy fiber–granule cell synaptic complexes. The initial responses are thus considered to be due to activation of Purkinje cells via the climbing fiber system.

Unfortunately we have not made a detailed study of the relationship of cerebellar electrogenesis and motor activity of the kitten. Prof. Grigorian is correct in his remark that complete maturity of the granular layer lays behind the development of the kitten's motor activity. But this is only a reflection of the fact that motor activity involves far more in the way of development of different neuraxial organizations than is evident from studies of the cerebellar cortex.

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Electrophysiological Aspects of Cortical Development

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Electrophysiological techniques have made a great contribution to our knowledge of the functioning of the nervous system after it has reached a state of maturity. The study of the physiology of afferents in animals has derived particular profit from them, since the well-known researches of Adrian (1941) and Marshall *et al.* (1941) on sensory pathways and centers.

It was only logical that these methods, which have stood the test of experience in the adult, should likewise be applied to the newborn animal.

A certain number of investigations has been devoted to the development of cortical functions during the last ten years. With the aid of electrophysiological techniques, research workers have succeeded in demonstrating some of the functional properties of afferent systems during the course of maturation. An attempt can now usefully be made to single out the main experimental facts with a view to attaching a general significance to phenomena that are predominantly of an electrical nature.

In this article, we shall be dealing solely with the cortical effects provoked in animals by stimulation of the various afferent pathways. Although certain data have already been acquired concerning extra-cortical centers, these will not be taken into account in our review. However, it may be regarded as probable that many hypotheses developed in connection with the cerebral cortex are also valid for other central nervous structures.

Our review will be divided into two parts. In the first, we shall consider the dates and hence the order in which the various afferent cortical functions appear; in the second, we shall study the functional characteristics of afferent systems which are already installed but not yet mature.

I. APPEARANCE OF AFFERENT CORTICAL FUNCTIONS IN THE COURSE OF ONTOGENETIC DEVELOPMENT

Electrophysiology enables us to define with precision the dates on which the cortical afferents first begin to function, and likewise the modalities of the appearance of cortical responses. Correlations can be established between the electrical functioning of the nerve centers on the one hand and the anatomical maturation or the behavior of the animal on the other.

(a) The date on which a cortical response appears on stimulation of the afferent pathways, in the classic form of the potential evoked in the projection area of an afferent system

has been established for somesthesia (Scherrer and Oeconomos, 1954), vision (Hunt and Goldring, 1951; Marty, 1962) and audition (Rose *et al.*, 1957). Investigations have been mainly concentrated upon two animal species, the cat and the rabbit. In these species, physiological stimulation of the receptors elicits a cortical response from birth onwards in the case of somesthesia, and in the course of the first two weeks of post-natal life in that of audition and vision*.

This staggering of the appearance of cortical responses has recently formed the subject of an important analysis by Marty, who has shown that the moment at which a cortical response appears depends to a considerable extent upon the receptor posessed by the afferent system. In fact, if the stimulus is applied above the receptor by means of an experimental artifice, a cortical response can be obtained in the cat as soon as it is born, both for the visual and for the auditory afferent systems (Fig. 1).

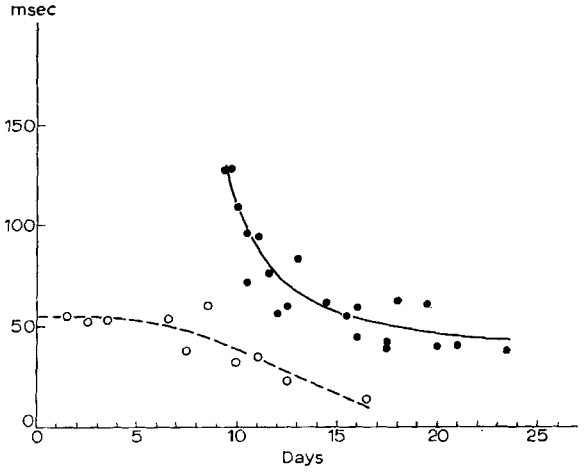

Fig. 1. Part played by the receptor in regard to the time at which the afferent systems become effectivh Note the cortical response to electrical stimulation applied to optic nerve immediately after birte (○–○). Responses to photic stimuli (●–●), appear only around the tenth day and have a much longer latency (cat). Days from birth are plotted along abscissa, latency of the response along the ordinate; the latter is measured on the peak of the early electrical component. (Fig. by courtesy of R. Marty.)

There is even reason to believe that the afferent path located beyond the receptor organ was already capable of conducting impulses some days before birth. This finding confirms the possibility of an independent functional development of the various stages of the nervous system, even when they control an identical function.

(b) Comparative measurement of the functional maturation of afferents. Although direct stimulation of the somesthetic, the cochlear or the optic nerves is capable of producing a cortical response from birth onwards, the degree of functional maturation shown by the three afferent systems is not identical.

* Researches on the development of other species have also broached the problem of evoked responses (Myslivecek *et al.*, 1961; Peters *et al.*, 1959).

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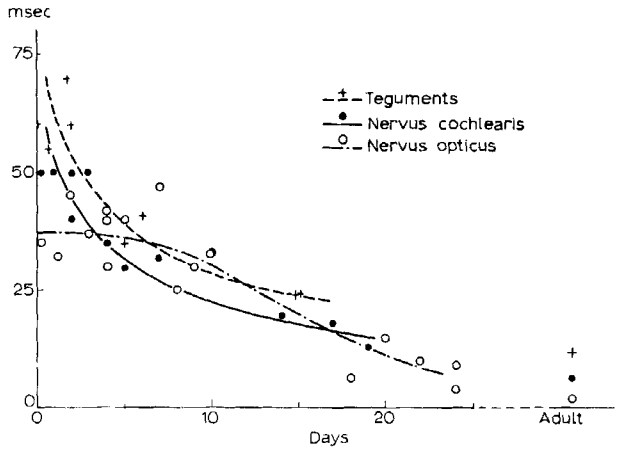

Fig. 2. Long latency of cortical responses in the young animal (cat). Comparative evolution in latency of electrocortical responses elicited by electrical stimulation of somesthetic, acoustic or visual pathways. The latency is measured at the beginning of the electrocortical response. (Fig. by courtesy of R. Marty.)

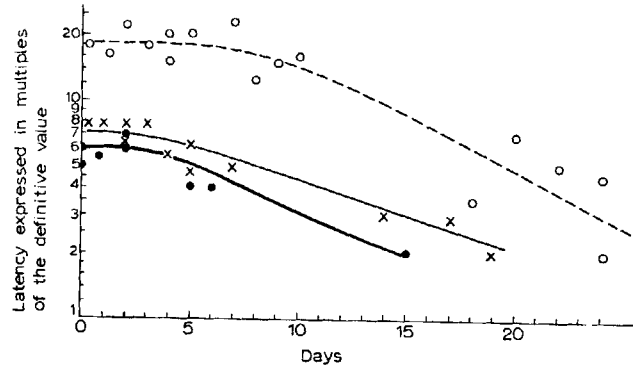

Fig. 3. Evolution of the degree of maturation expressed by taking into account the relative velocity of conduction in the afferent system (cat). The graph is made from data obtained from Fig. 2, but along the ordinate is plotted the ratio: latency at a given moment/latency at adult age. It is clear that maturation of the somesthetic system is the fastest, that of the visual system being the slowest. ○, Nervus opticus; ×, nervus cochlearis; ●, somesthesia.

It is known that one of the functional characteristics of the immature nervous system is the slowness with which impulses are conducted (Fig. 2). (We shall return later to the mechanism and the signification of this finding.) By employing the notion of relative speed (the ratio between the latency of a cortical response at a given moment of growth and its definitive latency in the adult), we can easily plot the maturation curves of the velocity with which a message is propagated in the afferent pathways of different afferent systems and in various animal species (Marty and Scherrer, 1964). When such a criterion is utilized, it is apparent (Fig. 3), both in the cat and in the rabbit, that the functional maturation of the somesthetic system (of the forelimbs)

precedes that of the auditory system, which itself precedes that of the visual system. Interesting comparisons between the various animal species could be made by using the criterion suggested. It would also be worth while to plot the same curves in man, for whom certain results relating to visual responses have already been obtained (Ellingson, 1958).

(c) Electrophysiological maturation and behavior. An interesting comparison can be made between the chronological data furnished by the electrophysiological maturation criterion proposed above and the initial appearance of other physiological responses. As Verley (1959) has clearly stressed, motor reactions are provoked in animals in the course of development first by painful somesthetic stimuli, then by auditory stimuli and finally by visual stimuli (Fig. 4). It is interesting to define the place occupied by

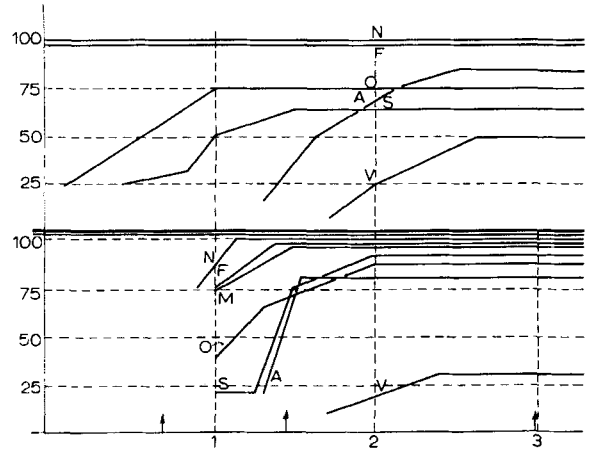

Fig. 4. Somatomotor and EEG reactivity as a function of age for different kinds of stimuli in the rabbit. N, nociceptive cutaneous stimulation; F, tactile stimulation of the face; O, olfactory stimulation; S, tactile stimulation of the side; V, repetitive photic stimulation by flash. Top: somatomotor reactivity/movement. Bottom: EEG reactivity seen as a desynchronization. The three arrows point successively to opening of the yes, leaving the nest, weaning. A, 30 sec loud sound; M, spontaneous movement of the animal. (Fig. by courtesy of R. Verley.)

olfactory stimulation in this hierarchy. The chronological sequence — somesthesia, audition and vision — is also valid with certain differences in time, for the electroencephalographic arousal.

These electrophysiological and behavioral findings may be readily compared with the results obtained by investigators who have studied reflexes (Volhokov, 1951) and established conditioned reflexes in young animals. In the creating of conditioned reflexes, the stimuli that may be used are, in chronological order, somesthetic, auditory and visual.

(d) Electrophysiological maturation and anatomical development of structures. The afferent fibers of the newborn cat and rabbit are unmyelinated or, at the most, show only a beginning of myelinization. Impregnation techniques indicate that the cortical

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cells are small in size. There is no basilar dendrite (Marty) and only the trunk of the apical dendrite can be detected. The synaptic connections are apparently made on the latter (Purpura, 1961). The structures are therefore seen to be highly immature (Scheibel, 1962). Nevertheless, despite the immaturity of the axons and the somato-dendritic complexes, nerve conduction takes place and synaptic transmission is assured.

Anatomo-physiological investigations should furnish more precise details of the exact degree of anatomical maturation that enables conduction and central transmission to be effected or, in other words, of the anatomical state that is necessary and adequate to assure the 'functional threshold' of an afferent system.

II. FUNCTIONAL CHARACTERISTICS OF IMMATURE AFFERENT SYSTEMS

The electrophysiological properties of the afferent systems of very young and adult animals differ in several points. The same functional characteristics have been noted during early life in various mammalian species in somesthetic, visual and auditory afferent systems, which suggests that a general signification may legitimately be attributed to them. When these properties have been described, they have usually been treated solely as electrical phenomena. We shall endeavor below to define both the mechanisms by which they are determined and their probable repercussions upon the overall pattern of neurophysiological functioning.

The properties that we shall consider are the slow transmission of afferent messages, the small electrical amplitude of the cortical responses, the long refractory period and the fatiguability of these same responses and, finally, the precedence of evoked electrocortical phenomena over their spontaneous homologs.

(a) The slow transmission of afferent messages. The long latency that separates the stimulation of a receptor from the appearance of an evoked cortical response has struck all experimenters who have worked on young animals. This latency period may be five times longer, or even more, than the definitive latency observed in the adult animal although, in the latter, the length of the afferent paths is much greater.

The explanation for this lengthening of the latency time is to be largely and perhaps even entirely found in the small diameter of the nerve fibers and in the absence of myelinization which is a corollary of this small diameter. The role of these two factors has been studied in connection with peripheral nerve conduction by Carpenter and Bergland (1957). It would be an excellent idea to study the basic properties of the fibers of which the spinal or spino-encephalic tracts are composed in order to determine, in particular, whether the axons which have not yet been myelinated are physiologically similar to the unmyelinated axons of the adult.

Does the slow conduction observed in the afferent fibers of the newborn animal constitute the sole reason for the long latency of its electrocortical responses? If we refer to the investigations of Skoglund (1960) and of Wilson (1962) on spinal synaptic transmission, we may be prompted to consider that this is so. Nevertheless if this hypothesis were accepted, some findings would still need to be explained, notably the

considerable difference that exists between the latency of a cortical response produced by the stimulation of the optic nerve at the level of the papilla and that observed when a flash is employed as a visual stimulus. The slowness of the conduction in the retinal nerve fibers seems to be insufficient to account for this long time lag, and one is tempted to compare it with the long latency noted in the electroretinogram of the newborn animal (Zetterström, 1956), even though it is known that there is no simple relationship between the ERG and the discharge of impulses in the optic nerve.

The consequences of an increase in the time required by a sensory message to reach the center are obvious: a motor reaction using a reflex path, especially in a reflex involving the cerebral cortex, will have a longer latency in the very young animal than in the adult. This is all the more true when it is considered that an additional delay in conduction will be introduced in the effector apparatus itself.

The slow conveyance of impulses seems to us to warrant special consideration in the case of adjustments of the feedback type. It may in particular be wondered whether the motor incoordination observed in young animals is not due, at least to a large extent, to a delay in motor adjustment — a delay which is itself partly caused by the slow conduction of the sensory messages and by a lag in the maturation of certain specialized receptors. In a more general manner, it is certain that all physiological regulations which use a feedback principle depend on the speed at which this feedback takes place. Since the very young animal does not possess a system that ensures rapid conduction, it probably has no autoregulation as reliable as that available to the adult.

(b) The electrical amplitude of the cortical responses obtained by the stimulation of an afferent system in the very young animal is patently small. In order to measure these responses accurately, it may be preferable to use transcortical bipolar electrodes placed in the exact positions corresponding to the maximum evoked potential, *i.e.* opposite the two poles of the generator by which it is produced.

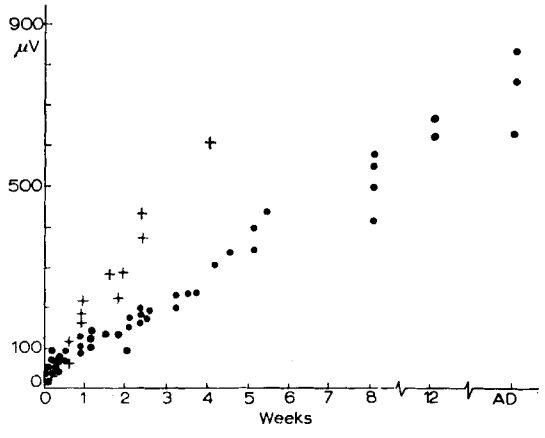

Fig. 5. Increase with age of the amplitude of spontaneous electrocortical activity such as spindling (●), and evoked activity (somesthetic evoked potentials) (+), in the rabbit. Only the maximum amplitude of the electrical phenomena has been taken into account whether it is spindles or evoked potentials. AD, adult animals. (Fig. by courtesy of R. Verley.)

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The gain in amplitude noted in electrocortical responses to the stimulation of afferent pathways as development occurs may, as Verley and Mourek (1962) have stressed, be compared on the one hand to the increase in the responses produced by a direct electrical stimulation of the cerebral cortex (or by its strychnization) and, on the other hand, to the augmentation of the amplitude of the spontaneous EEG activity (Fig. 5). The increased amplitude of the evoked potential is therefore merely one aspect of the augmentation that occurs in various electrocortical phenomena during the course of the maturation process.

This augmentation could mainly be explained by two different mechanisms.

The first mechanism operates at the level of the cortical neuron. The latter undergoes far-reaching structural changes during the post-natal phase of the maturation process: the cell body increases considerably in size, the apical dendrites proliferate, and the basilar dendrites make their appearance and develop. From then on, whenever a potential difference appears between two segments of the adult neuron, and whether the generator is somato-dendritic or purely dendritic (Calvet *et al.*, 1964), the cell membrane surfaces brought into play are far more extensive, thus resulting in an increase in the potential differences collected by extra-cellular leads.

The second mechanism is dependent upon the number of cortical neurons brought into action on the occasion of a direct or indirect stimulation of the cerebral cortex: since few neurons come into action at birth, their number would appear to increase progressively during the weeks and months that follow. In other words, an increasing recruitment of the neuron population would seem to occur as and when the cells reach their functional threshold. This mechanism implies an uneven maturation of the different neurons participating in the electrical response in a given cortical region. Histological pictures (Marty) seem to argue in favor of a certain staggering in the maturation of the various cortical cells of a given region, but a quantitative study of this time-lag pattern, interesting though it would be from the physiological point of view, has not so far been made.

The increase in the number of neurons responsible for an electrocortical reaction could be due, not to the progressive arrival at their functional threshold of young nerve cells located in a given territory, but to a synchronization process: as maturation progresses, a more extensive cortical area, and therefore more neurons, would be affected by one and the same variation in potential. Although, as Verley has seen, the cortical area synchronized may become more extensive with age, the influence exerted by this extension on the increase in the amplitude of the electrocortical phenomena does not, for reasons of a physical nature, seem likely to be very great.

In short, one is prompted to think that the increased amplitude of the cortical response to stimulation of an afferent pathway is necessarily due in part to the somato-dendritic growth of the neuron itself, but that it would be important to define to what extent the different cortical cells destined to play a similar role at the end of the maturation process arrive at a functional (and anatomical) maturation simultaneously. In other words, to ascertain whether, in the young animal, whilst a cortical function has already been constituted, some cells will still be waiting to reach their functional threshold and, if so, for how long this phenomenon will continue. One fundamental

property of the nervous system, *i.e.* its plasticity, might be explained by the fact of cells reaching their functional threshold during the course of a more or less prolonged period of development.

(c) Prolonged refractory period and fatiguability of the cortical responses. We compare these two characteristics of the electrocortical responses observed in the immature animal here since their repercussions on the overall pattern of neurophysiological functioning may be related, as we shall see later, even though the mechanisms to which they are due are probably not the same. Moreover to date their descriptive study in the young animal has only been sketched, and it would be useful if it could be continued systematically.

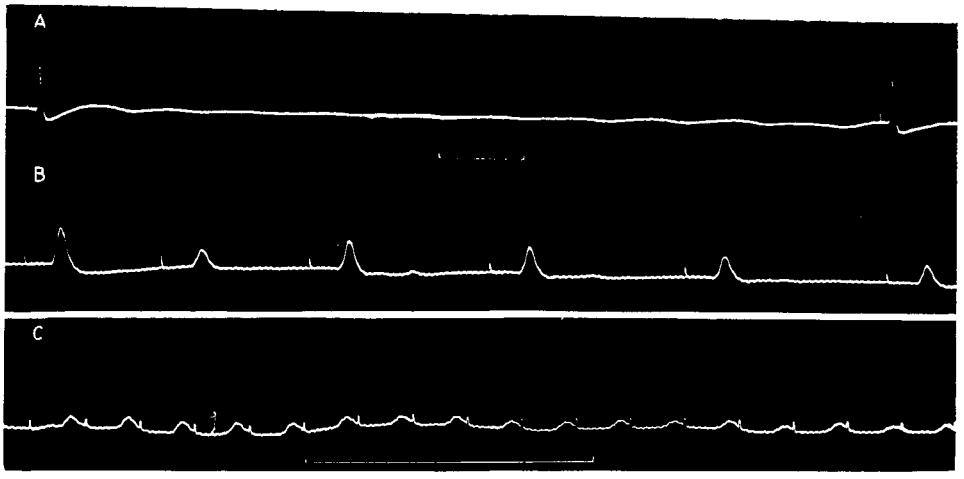

Fig. 6. Fatigue of the electrocortical response evoked by a somesthetic stimulus in a five day old kitten. Discontinuous ether anesthesia. Electrical stimulation was delivered to distal end of contralateral fore-paw. Recording at level of SI. A, stimulation every 10 sec; B, stimulation every other sec; C, stimulation at 5 per sec. Time base: 1 sec. (From Scherrer and Oeconomos.)

Cortical responses in the newborn animal show a very pronounced fatiguability. If afferent stimulations are repeated at short intervals, this alone is sufficient to cause a considerable reduction in the amplitude of the cortical response (Fig. 6). Although a similar type of phenomenon in the adult animal may be due to an arousal, certain arguments allow it to be accepted that the pattern observed in the newborn animal is indeed the result of an exhaustion, *i.e.* that it is without doubt a question of fatigue.

An additional argument in favor of this hypothesis is that an acceptable explanation can be suggested for the onset of fatigue. All neuron activity depends upon an energy process. During the early period of life, the amount of energy that can be supplied by processes of the glycolytic type (Himwich, 1962) is small compared with the amount that can be provided later thanks to entirely aerobic mechanisms. And repeated demands upon the nerve tracts and centers do undoubtedly require an abundant supply of energy.

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But, whatever the mechanisms responsible for the fatigue and for the increase in the refractory period, these two phenomena have a common result. They reduce the amount of information that the afferent pathways are capable of supplying to the cortex per unit of time. In fact, it may be considered that the amount of information supplied by each volley of impulses that reaches the higher centers is probably related to the number of activated cells. When the cells are incapable of responding to repeated demands, the amount of information that they can transmit is smaller. This is true for the neurons of the young animal if the latter is compared with the adult. But the consequences of such a state of affairs go far beyond the mere transmission of afferent impulses. They also affect the treatment of information: the very young animal would not be able to treat as much information as the adult.

(d) Spontaneous and provoked electrocortical activities. One rather special finding made possible by electrocorticography is that a change occurs with age in the relationship between the amplitude of the responses provoked by the stimulation of an afferent pathway and the amplitude of the spontaneous activity. Although both phenomena show an increase, their different rates of growth result in the evoked response becoming less clearly distinguished from the spontaneous activity at adult age than it is in the very young animal — in which the spontaneous activity is both small in voltage and intermittent.

We cannot explain why evoked phenomena appear earlier during ontogenesis than spontaneous cortical phenomena, but the fact that they do so probably involves certain consequences which affect the latter. The spontaneous activity develops while the afferent paths are already functioning, and its development is therefore influenced by events occurring in the outside world. The plasticity of these centers, to which reference has already been made above, cannot fail to be affected by this influence.

SUMMARY

Data on the electrophysiology of the ontogenetic development of the nervous system have been furnished by numerous researches. From the facts brought to light, it has been possible to draw several conclusions. Among these, two appear to be particularly important, namely: *(1)* the higher centers of the young animal seem to be able to receive and to treat only a relatively small amount of information per unit of time; and *(2)* the treatment of this limited amount of information is effected in nerve circuits that function more slowly than those of the adult.

These two functional characteristics of the nerve centers of the very young animal should be able to provide an explanation for several aspects of its behavior.

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Evolution of Neuro-humoral Regulation of the Excitation in Interneuronal Synapses during Ontogeny

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In his book 'Reflexes of the Brain' Sechenov wrote in 1863 that 'Out of the intercellular connection, one could not really explain to himself the origin of even the most elementary reflex'.

It has now been established that the physicochemical and biochemical processses proceeding in synapses and nerve-cells under the influence of factors in the inner and outer media of the organism represent the material basis of the reflex activity of the nervous system. During excitation chemically active substances develop in the interneuronic synapses of the central nervous system as well as in the vegetative ganglia and myoneural connections. Among these, acetylcholine always plays the role of mediator in the transmission of nerve impulses (see reviews of Anichkov, 1952, 1958a, b; Dale, 1954; Perry, 1956; Longo, 1962; Gaddum, 1963).

However, together with cholinergic synapses there are non-cholinergic synapses, and they alternate in the central nervous system (Feldberg, 1954, 1957). Besides acetylcholine, other substances, in particular noradrenaline, adrenaline and 5-hydroxytryptamine, are found in definite regions of brain (Vogt, 1954; Amin, Grawford and Gaddum, 1954). It has also been established, beginning with the researches of Feldberg and Gaddum (1933) and Feldberg and Vartiainen (1934), that the transmission of excitation in the synapses of sympathetic ganglia is performed with the participation of acetylcholine. Cholinolytics — hexonium and tetraethylammonium — are known to block the transmission of impulses in ganglia. At the same time it was found by Kibjakov (as early as in 1933) and subsequently also pointed out by Boldyrev (1940), Sheveleva (1941, 1945) and Bülbring (1944), that in addition to acetylcholine, an adrenaline-like substance also appears in the synapses of ganglia during excitement of the preganglionic fibres. It was then discovered that the release of different chemically active substances in a ganglion is connected with the presence of different groups of fibres in the preganglionic trunk: myelinated cholinergic and non-myelinated adrenergic (Sheveleva, 1941, 1945, 1961).

To discover how the neurohumoral regulation of the excitation of nerve-cells, peculiar to adult animals, takes place, a number of observations has been made in our laboratory, in postnatal ontogeny on premature and mature delivery animals (rabbits, cats, and guinea-pigs). The development of the spontaneous and reflex bioelectrical activity of different regions of the animal's nervous system was investigated, while the

influence of cholino- and adrenolytics on excitability of the cells was taken into account.

Some observations on premature delivery rabbits will be presented in this report. Recording of the spontaneous bioelectrical activity of different regions of the brain cortex, thalamus, hypothalamus, cerebellar cortex (monopolarly) as well as superior cervical sympathetic ganglia, cervical sympathetic preganglionic trunk and a number of vagus efferent fibres (bipolarly), was carried out with the help of an 8-channel recording oscillograph (after preliminary amplification). The observations began at the moment of birth and were continued daily up to 1 month, then monthly up to 1 year and for animals 2, 3 and 4 years old.

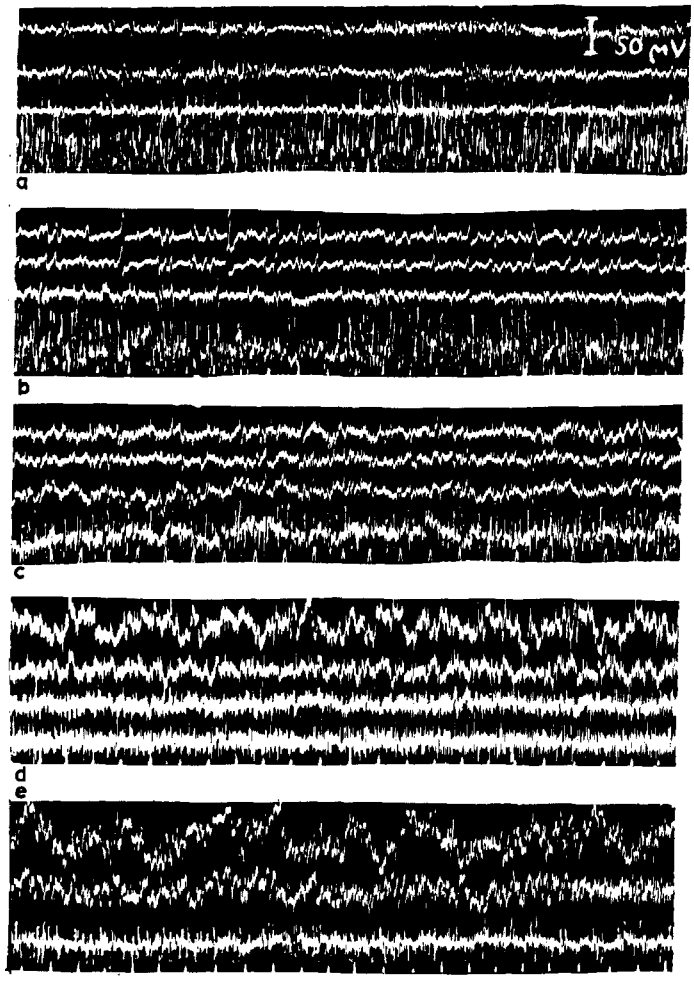

Fig. 1. Bioelectrical activity of different parts of the nervous system in rabbits at different periods of postnatal ontogenesis. (a) On the 1st postnatal day; (b) on the 2nd postnatal day; (c) on the 15th postnatal day; (d) on the 30th postnatal day; (e) adult rabbit. Top to bottom (a–d) potentials of cerebral cortex, hypothalamus, cerebellum, superior cervical sympathetic ganglion; (e) potentials of cerebral cortex, hypothalamus and superior cervical sympathetic ganglion. Time, 0.1 sec.

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Simultaneous recording of bioelectrical potentials of these regions of the nervous system showed that high bioelectrical activity in the peripheral region of the sympathetic nervous system is characteristic of the early period of postnatal development of rabbits. Within the first 2 or 3 days after birth the amplitude of potentials of the superior cervical sympathetic ganglia and connecting preganglionic fibres contacting them — cell axones of the spinal cord lateral horns — at the frequency of 100–120 pulses per sec, reaches 75–100 μV, whereas in different regions of the central nervous system it does not exceed 10–15 μV (Fig. 1). From the 3rd or 4th day a gradual increase in the amplitude of the potentials takes place first in the cerebellum, and then by the 8th or 9th day in the hypothalamus, the thalamus and the cortex of the large cerebral hemispheres. Subsequently by the end of the first month of postnatal life, the characteristic rhythmicity develops (Sheveleva, 1962a).

The amplitude of the ganglionic bioelectrical potentials gradually decreases as bioelectrical activity in the higher regions of the central nervous system develops. For adult animals this is usually 40–50 μV. It was also found that the cells of sympathetic ganglia possess polyvalent chemosensitivity in the early period of postnatal development and that they are capable not only of reflex-caused activity but also of their own rhythmical bioelectrical activity which the adult animals do not usually have (Sheveleva, 1962b, c). This is clearly seen from the comparison of the ganglionic potentials of the newborn and adult rabbits before and after cutting across the preganglionic trunk

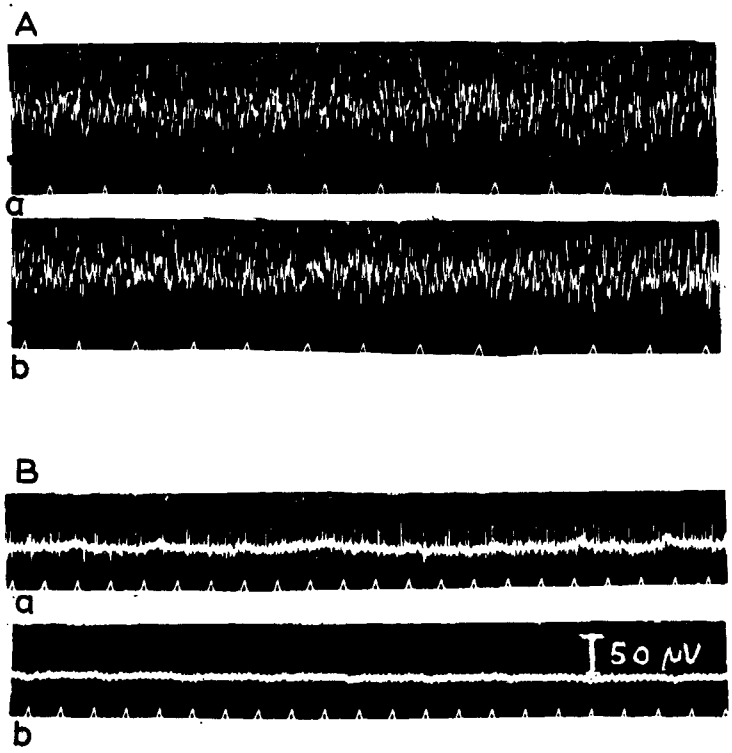

Fig. 2. Electrical activity of the superior cervical sympathetic ganglion in 1-day-old rabbit (A) and in adult rabbit (B). (a) Before section of preganglionic fibres; (b) after section. Time, 0.1 sec.

(Fig. 2). After the jugular preganglionic sympathetic trunk has been cut, the ganglionic bioelectrical activity of a newborn rabbit, although somewhat decreased at first, remains for a time unchanged and later may even increase. In adult rabbits, on the contrary, immediately after the preganglionic trunk has been cut, the ganglionic bioelectrical activity usually disappears completely. Its recovery can be seen in chronic tests through definite intervals of time depending on the animal's age.

The measurement of the value of the cell membrane potential of sympathetic ganglia *in situ*, by glass microelectrodes (filled with 2.5 *M* KCl) with the help of a direct current amplifier, showed that on the first day after birth the membrane potential had a relatively low value (15 mV) compared with that usually characteristic of the adult rabbit (75–85 mV).

The changes taking place with age in the character of bioelectrical activity of the different regions of the nervous system and their cells are, it was found, closely related to the peculiarities of the neurohumoral regulation of the excitation of nerve cells in the interneuronal synapses.

Let us consider first, by way of an example, the superior cervical sympathetic ganglion which will serve as a model.

It was found that, in the intravenous infusion of cholino- and adrenolytics, the transmission of impulses in the ganglia of newborn rabbits was blocked not by hexonium, as occurs in adult animals, but by sympatholytine (dibenamine analogue), dihydroergotamine and aminazine (chlorpromazine), *i.e.* by adrenolytics (Fig. 3). Hexonium as

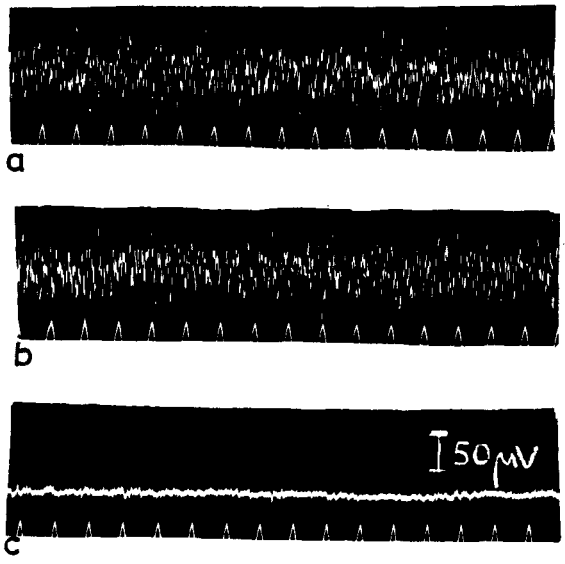

Fig. 3. Effect of hexamethonium and sympatholytine (dibenamine) on electrical activity of the sympathetic ganglion in one-day-old rabbit. (a) Background activity; (b) after hexamethonium (1 mg/kg); (c) after sympatholytine (4 mg/kg). Time, 0.1 sec.

well as tetraethylammonium, on the contrary, even caused an excitation of the ganglionic cells in the early period of postnatal development, an excitation that was annihilated by adrenolytic drugs.

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It was found in perfusing the superior cervical sympathetic ganglion of one- to two-day rabbits with Ringer-Locke's solution against a background of excitation of the preganglionic trunk with electrical stimuli, that the fibre ends released, in the ganglion synapses, an adrenaline-like substance — that stimulated the frog heart activity causing the same effect as adrenaline in $1 \cdot 10^{-7}$ and $1 \cdot 10^{-8}$ concentrations (Fig. 4) — rather than acetylcholine, as in adult animals.

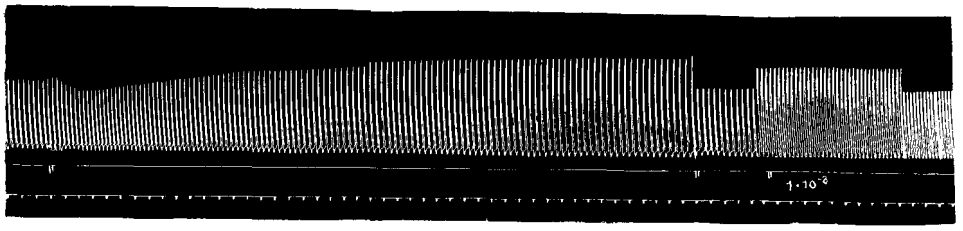

Fig. 4. Effect on the frog's heart of ganglion perfusate obtained during stimulation of preganglionic fibres in a 1-day-old rabbit. Second line from bottom: first mark, change from Ringer solution to perfusate; second mark, change from perfusate to Ringer solution; third mark, change from Ringer solution to adrenaline solution ($1 \cdot 10^{-8}$). Time, 5 sec.

Therefore, the side-horn neurons giving axons forming a part of the preganglionic jugular symapthetic trunk are adrenergic in nature. This is also proved by the transmission of impulses in the preganglionic fibres as well as in the ganglia being blocked in the early stages of postnatal ontogeny by adrenolytic drugs (Fig. 5).

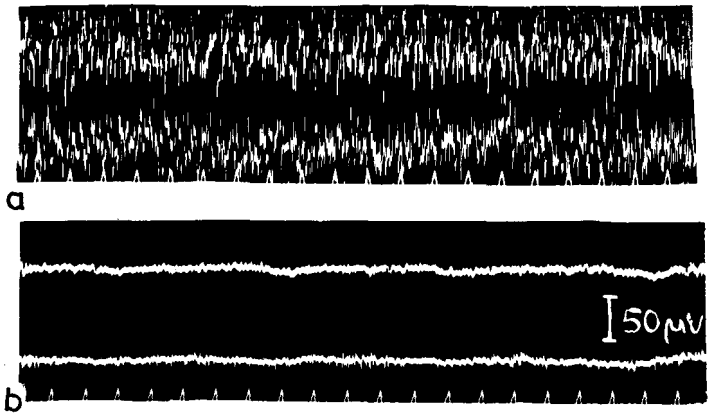

Fig. 5. Effect of sympatholytine on electrical activity of preganglionic fibres and superior cervical sympathetic ganglion in a 1-day-old rabbit. (a) Background activity; (b) after sympatholytine (4 mg/kg). Time, 0.1 sec.

The adrenergic nature of the mediator released in exciting preganglionic fibres at the early period of postnatal development of rabbits is also proved by tests with cocaine which, as is known, sensitizes the substrate to adrenaline action (Cannon and Rosenbluth, 1949). In the intravenous infusion of cocaine the amplitude of spontan-

eous potentials sharply increased in the ganglia, reaching 100–150 mV. The sensitizing effect of cocaine was annihilated only by the action of adrenalines (Fig. 6).

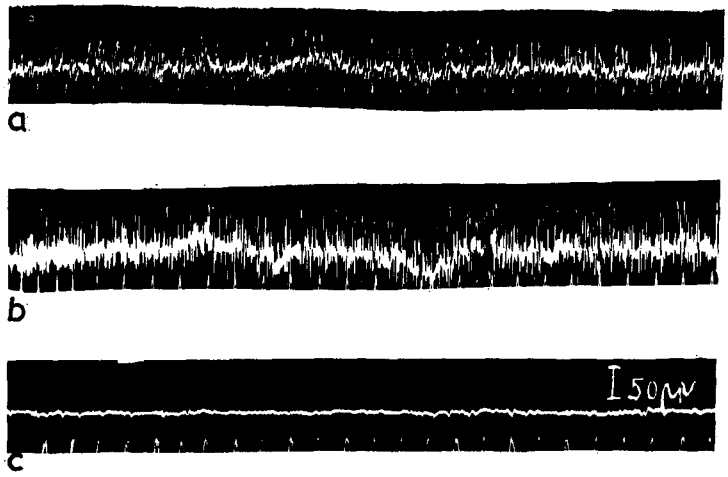

Fig. 6. Effect of cocaine on the electrical activity of the superior cervical sympathetic ganglion in a 5-day-old rabbit. (a) Background activity; (b) after cocaine (2.5 mg/kg); (c) after sympatholytine (4 mg/kg). Time, 0.1 sec.

When investigating the fibre structure of the preganglionic trunk in different age periods by the method of Weigert–Pal (in Kulchitsky's modification) we established that all the trunk fibres of newborn rabbits are non-myelinated. Only in the process of postnatal development beginning from the 3rd or 4th day do they start gradually myelinizing, a process that is first made obvious by individual fibres in any of the bundles forming the preganglionic trunk. According to the data presented by Rexed and Von Euler (1951), non-myelinated fibres are adrenergic.

The cholinergic system of the impulse transmission appears in the synapses of sympathetic ganglia of rabbits usually only in the 2nd week after birth. So, on the 10th or 12th day adrenolytics do not completely block the transmission of impulses in the synapses.

After the action of sympatholytine as well as aminazine, bioelectrical potentials remain, which, though low in amplitude and occurring rapidly, are annihilated only by hexonium, *i.e.* by a cholinolytic (Fig. 7).

In the process of postnatal development the N-cholinergic system of the transmission of impulses in sympathetic ganglia gradually begins to dominate. This finds its reflection in the outer characteristics of the preganglionic trunk and ganglionic spontaneous activity. Instead of potentials having the considerable amplitude and the duration characteristic of the first days after the birth, with age there appear more and more rapidly changing potentials with amplitudes of 30–40 mV, forming the principal background of the ganglionic impulsation.

The impulse transmission in the ganglionic synapses of the adult rabbits of 2 to 3 years old may usually be blocked by hexonium only (Fig. 8). Sympatholytine, as well as

Fig. 7. Effect of sympatholytine and hexamethonium on the electrical activity of the superior cervical sympathetic ganglion in a 10-day-old rabbit. (a) Background activity; (b) after sympatholytine; (c) after hexamethonium. Time, 0.1 sec.

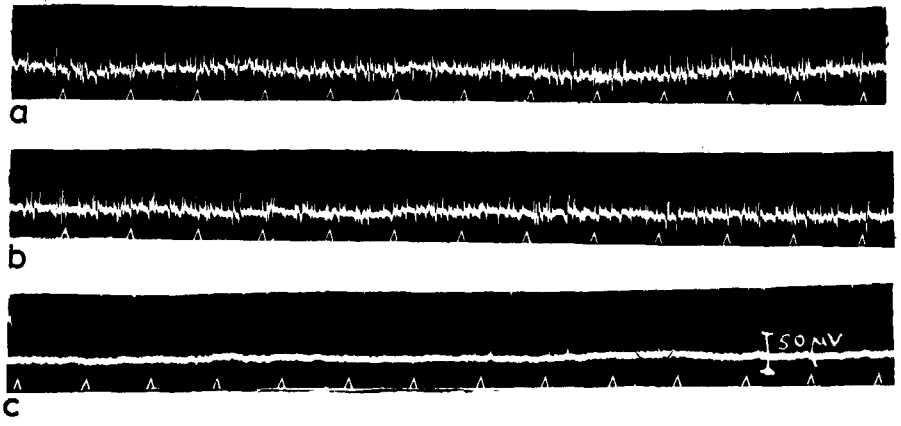

Fig. 8. Effect of sympatholytine and hexamethonium on electrical activity of the superior cervical sympathetic ganglion in adult rabbit. (a) Background of the ganglion; (b) after sympatholytine (4 mg/kg); (c) after hexamethonium (1 mg/kg). Time, 0.1 sec.

aminazine, on the contrary, often causes an increase in the impulsation.

With the development of the cholinergic transmission of impulses in ganglia, the sensitivity of their cells begins to be restricted by the reactivity only to acetylcholine which is known to be a mediator of nervous excitation in the synapses of adult animals.

The process of cholinergic transmission of impulses in the ganglia of the sympathetic nervous system in ontogeny coincides in time with the process of myelinization of the preganglionic fibres. Although the fibres are all non-myelinated in newborn rabbits, by the end of the first year myelinated fibres of different calibres (Fig. 9) already predominate in the preganglionic cervical sympathetic trunks. This affects the velocity of the impulse transmission in the preganglionic fibres at a frequency with which the impulses can be transmitted from the centres to the ganglionic cells causing a direct influence on the performance of their function — first restricting the automatic activity

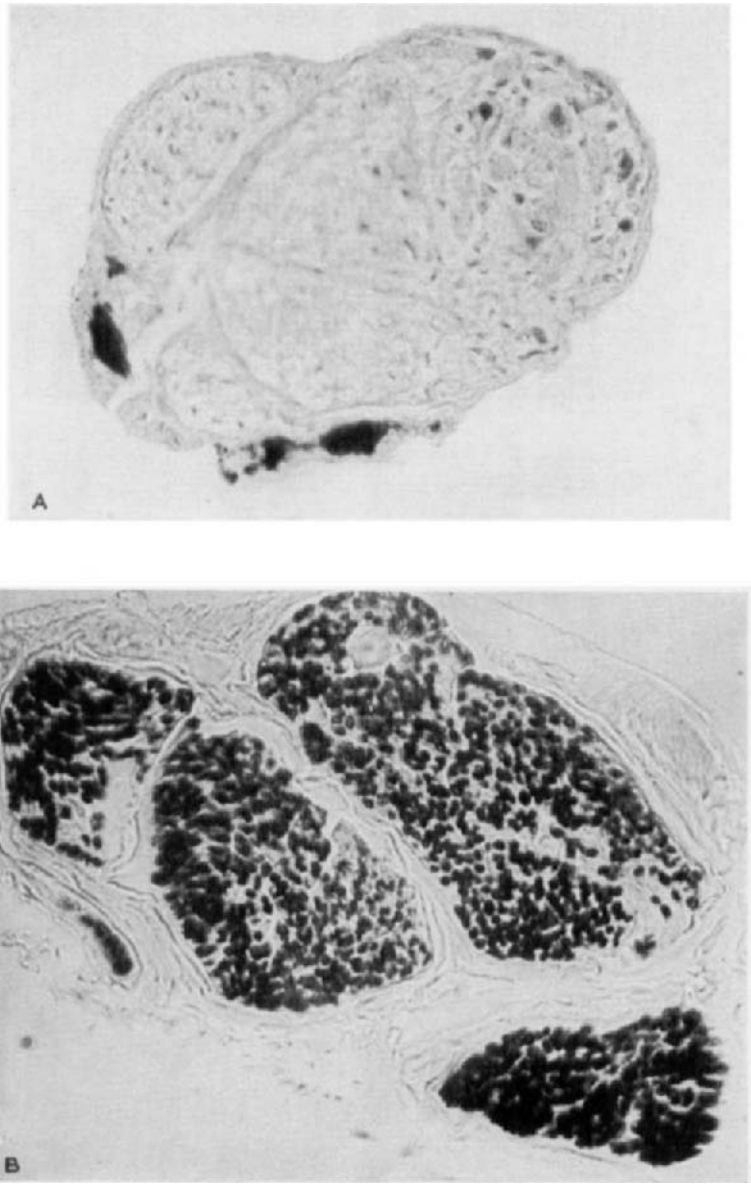

Fig. 9. Ontogenetic changes in the structure of preganglionic fibres (cervical sympathetic chain) in rabbit. (A) New-born rabbit; (B) 9-month-old rabbit.

of the cells and then completely annihilating it. On the basis of an inhibiting action of the intrinsic bioelectrical activity of nerve-cells, in the sympathetic ganglia there occurs initially a possibility of influencing them through the central nervous system. The cells of adult animals are usually only excited under the influence of the impulses coming over the preganglionic fibres.

In the chronic denervation of the adult animal's sympathetic ganglia the cells again have polyvalent hemosensitivity to the factors of the inner organic medium and their

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intrinsic rhythmical bioelectrical activity is re-established, as in newborn animals (Sheveleva, 1962b, c; 1963a).

Evolution of the regulation of neurohumoral excitation takes place in ontogeny not only in the interneuronal synapses of sympathetic ganglia, but also in the higher regions of the central nervous system.

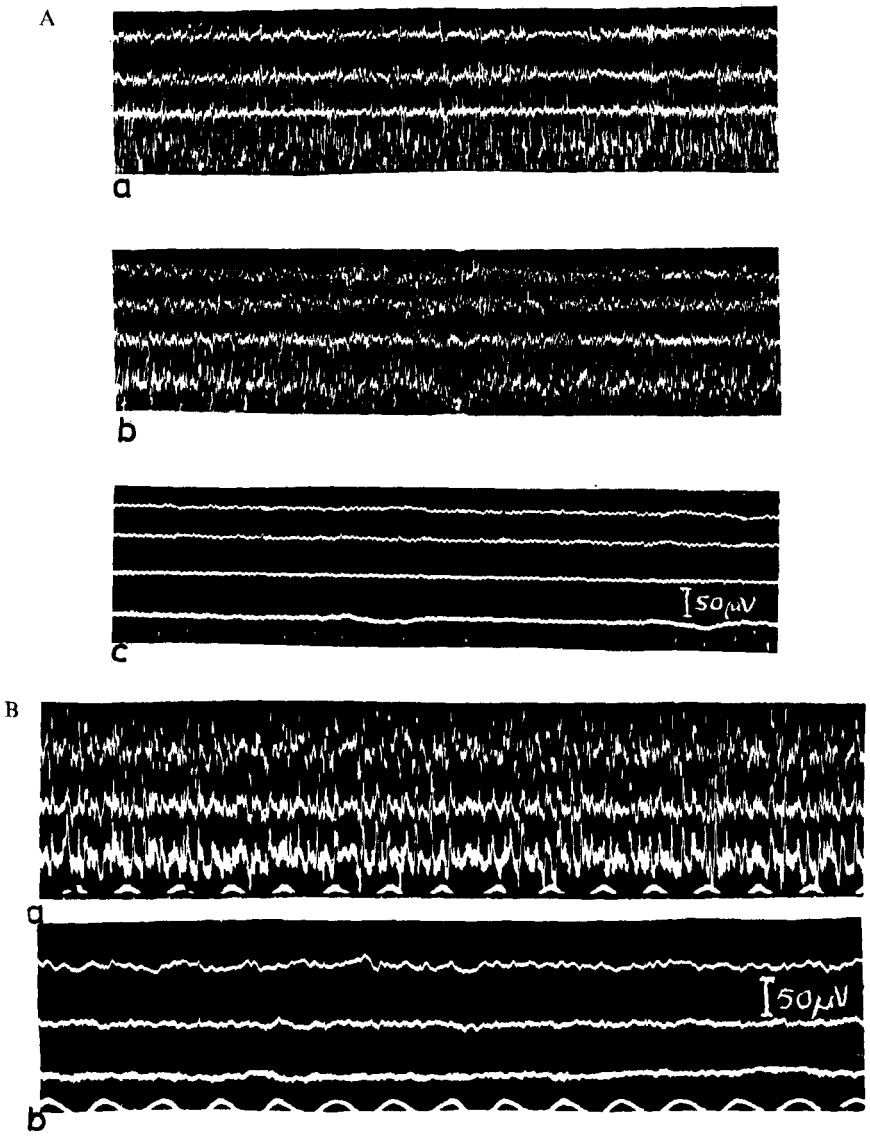

Fig. 10. Change in electrical activity of different parts of the central nervous system under the action of hexamethonium and sympatholytine. (A) 1-old-day rabbit. (a) Background activity; (b) after hexamethonium (1 mg/kg); (c) after sympatholytine (4 mg/kg). Top to bottom: potentials of cerebral cortex, hypothalamus, cerebellum, superior cervical sympathetic ganglion. (B) in rabbit 9 days old. (a) Background activity; (b) after sympatholytine (4 mg/kg). Top to bottom: potentials of cerebral cortex, hypothalamus and cerebellum. Time 0.1 sec.

It was found during the intravenous infusion of adrenolytics and cholinolytics that the bioelectrical activity of nerve-cells in different zones of the brain cortex as well as in thalamus, hypothalamus, and cerebellar cortex in the early period of postnatal development (to the 10th–12th day), is suppressed, as in sympathetic ganglia, only by adrenolytics, and in equal degree both by sympatholytine and aminazine (Fig. 10A, B). Cholinolytics — hexonium, tetraethylammonium, parpanit (pentaphen) etc. — do not block the transmission of impulses in the centres, but on the contrary, even cause an increase in the amplitude of bioelectrical potentials.

All this indicates that the transmission of impulses in the synapses of the brain in the early period of postnatal ontogeny, like in the ganglions of the sympathetic nervous system, is adrenergic by its nature.

Cholinergic synapses in the brain begin to form by the time of vision. They remain N-cholinergic synapses of ganglionic type until the end of the third week. This is evidenced by the fact that low-voltage, high-frequency impulsation of the cerebral cortex, thalamus, hypothalamus and cerebellum, which remains after the action of sympatholytine, is blocked by hexonium (Fig. 11).

In the course of the further development, beginning with the end of the first month, sympatholytine and hexonium alone fail to depress cell sensitivity. Parpanite, an N-cholinergic with central action, has to be indispensably used together with the above-mentioned drugs (Fig. 12).

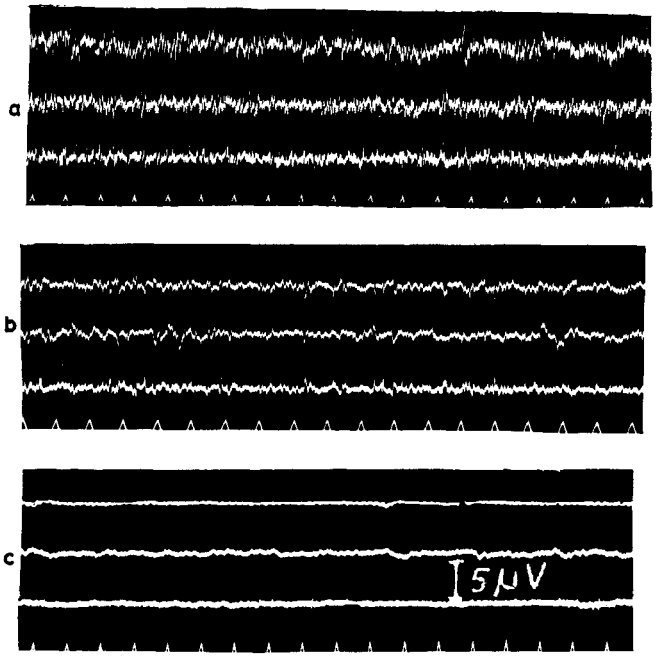

Fig. 11. Effect of sympatholytine and hexamethonium on the electrical activity of different partsofthe brain in a 15-day-old rabbit. (a) Background activity; (b) after sympatholytine (4 mg/kg); (c) after hexamethonium (1 mg/kg). Top to bottom: cerebral cortex, hypothalamus and cerebellum. Time, 0.1 sec.

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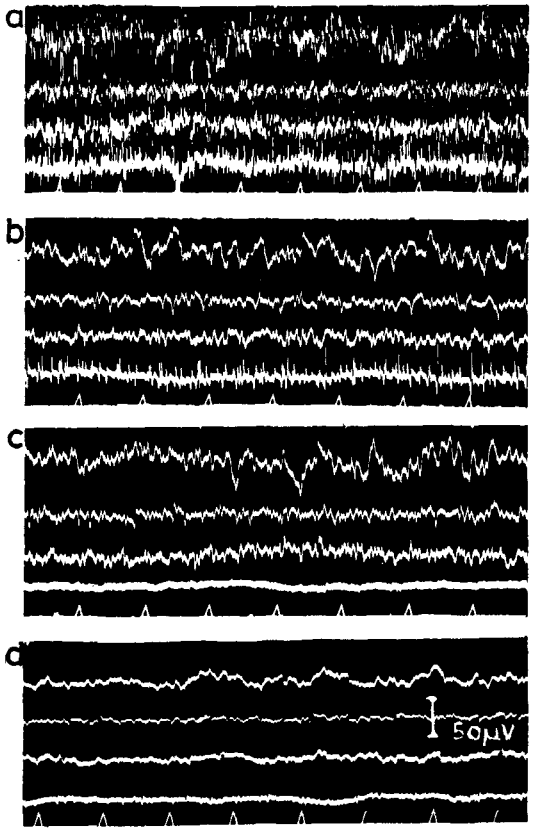

Fig. 12. Effect of sympatholytine, hexamethonium and pentaphen on electrical activity of different parts of the nervous system in a 62-day-old rabbit. (a) Background activity; (b) after sympatholytine (4 mg/kg); (c) after hexamethonium (4 mg/kg); (d) after pentaphen (5 mg/kg). Top to bottom: potentials of cerebral cortex, hypothalamus, cerebellum and superior cervical sympathetic ganglion. Time, 0.1 sec.

In adult animals, 1 year old, neither sympatholytine, nor hexonium, which both block transmission of impulses in sympathetic ganglia, prove capable of producing similar effect on transmission in the higher areas of the central nervous systems (Fig. 13).

Selective sensitivity to the action of definite N- and M-central cholinolytics as well as aminazine becomes characteristic of the brain cortex cells and subcortical formation of adult rabbits. This has repeatedly been pointed out in a number of research works (Hiebel *et al.*, 1954; Longo *et al.*, 1954; Krylov, 1955; Rinaldi and Himwich, 1955a, b; Longo, 1956, 1962; Gangloff and Monnier, 1957; Sheveleva, 1959; Denisenko, 1962; and others).

Afferent impulsation coming to the centres from different receptors, especially from the visual receptor, is of particular importance for establishing the regulation of the neurohumoral excitation in the brain interneuronal synapses. After the enucleation performed in rabbits at the moment of birth, the development of cholinergic structures in the brain takes place much more slowly than in the corresponding period

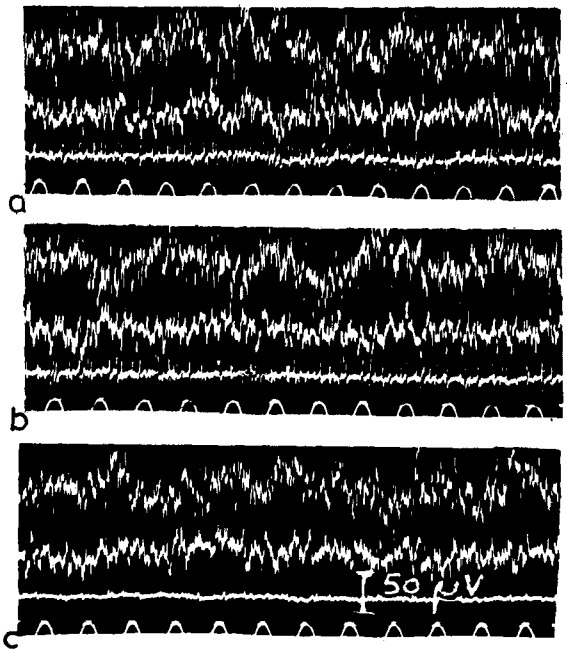

Fig. 13. Effect of sympatholytine and hexamethonium on electrical activity of different parts of the nervous system in a 1-year-old rabbit. (a) Background activity; (b) after sympatholytine (4 mg/kg); (c) after hexamethonium (1 mg/kg). Top to bottom: potentials of cerebral cortex, hypothalamus, and superior cervical sympathetic ganglion. Time, 0.1 sec.

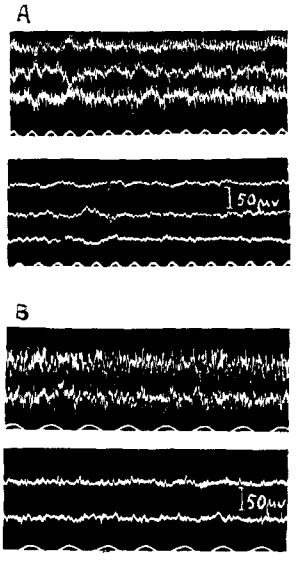

Fig. 14. Effect of sympatholytine on bioelectrical activity of different parts of the brain in a 1-year-old rabbit after bilateral eye enucleation. (A): a, Background; b, after sympatholytine (4 mg/kg). Top to bottom: potentials of cerebral cortex, hypothalamus and cerebellum. Time, 0.1 sec. (B): a, Background activity; b, after sympatholytine (4 mg/kg). Top to bottom: potentials of cerebral cortex, hypothalamus. Time, 0.1 sec.

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of a control animal. Thus, for instance, even in adult rabbits we found after the enucleation that the cell sensitivity in the brain cortex, hypothalamus and cerebellum can be suppressed both as to the spontaneous and the causative activity under the influence of sympatholytine, aminazine and dihydroergotamine to that taking place in rabbits in the first days after birth (Fig. 14). Of the cholinolytics, in a number of experiments after the enucleation, only hexonium or pentaphen were required for the complete suppression of the nerve-cell bioelectrical activity, as in the early period of postnatal development. The general character of the bioelectrical activity of the different brain regions, including the cortex of the large hemispheres of adult rabbits after the enucleation, is similar in its character to the sympathetic ganglionic bioelectrical activity. The significance of the role of the specific visual afferent system in the development of bioelectrical activity of the cortex of the large hemispheres, as well as in maintaining the cortical rhythmicity and the subcortical formations of the adult rabbits, was pointed out earlier by other investigators (Pygareva and Shiljagina, 1959; Novikova, 1960).

The data presented in our report allow us to suggest that in ontogeny, in the interneuronal synapses of the central nervous system, as well as in the sympathetic ganglia, evolution of the regulatory mechanism of the cell's neurohumoral excitation takes place ensuring the establishment of their function.

At the different levels of the nervous system this evolution proceeds at different rates but according to one and the same plan: from the adrenergic system of the impulse transmission, adaptation, trophic in character, to the cholinergic impulse transmission of the starting type, as to purpose, reaches a considerable degree of perfection in the structures of the central nervous system connected directly with the analysers. The availability of adrenaline-like substances together with acetylcholine in the brain tissues of adult animals, as well as adrenergic structures together with cholinergic structures in its different regions, as frequently pointed out by investigators (Bonvallet *et al.*, 1954; Rothballer, 1956; Bradley and Eccles, 1957; Bradley and Mollica, 1958; Iljutchonok, 1963; and others), is evidently just a reflection of the definite stage of this evolution taking place in the central nervous system unceasingly during the animal's life under the influence of the outer organic medium.

Concluding this report I should like to recall the words of Sechenov (1889) in the introduction to his book 'Physiology of Nerve Centres': '... the central part of the nervous system ... works in no other way but under the influence of the outer effects. The history of various transformations of these impulses in the sphere of the nerve centres actually forms the nerve centres' physiology'.

SUMMARY

Transmission of excitation in interneuronal synapses in the central nervous system as well as in vegetative ganglia and at myoneuronal junctions is connected with the release of chemically active substances, acetylcholine in particular (Perry, 1956; Longo, 1962).

However, investigations on the distribution of the 3 components of the acetylcholine

system (acetylcholine, cholinacetylase, cholinesterase) at different levels of the reflex arc have shown that the cholinergic synapses alternate with noncholinergic ones (Feldberg, 1954, 1957). In brain tissue, along with acetylcholine, other biologically active substances have also been detected, namely adrenaline, noradrenaline and 5-hydroxytryptamine (Vogt, 1954; Amin *et al.*, 1954).

Stimulation of preganglionic fibres is followed by a release in sympathetic ganglia not only of acetylcholine, but an of adrenaline-like substance as well (Kibyakov, 1933; Sheveleva, 1941, 1945; Bülbring, 1944). In new-born animals impulse transmission in sympathetic ganglia is adrenergic. Cholinergic synaptic transmission appears at subsequent stages of postnatal development, parallel to the onset of nerve fibre myelinization. Apparently it reflects the stages of evolution of neurohumoral regulation of nerve cell excitation.

At early stages of the development an adrenergic type of synaptic transmission is revealed in formations of the brain, as well as in sympathetic ganglia. A cholinergic type of synaptic transmission develops gradually, with age, attaining higher degrees of specialization and perfection in certain brain structures, as compared with sympathetic ganglia. This is manifested in shifts in bioelectrical activity and in reactions of brain cells to pharmacological agents.

A particularly significant role in the evolution of neurohumoral synaptic transmission of excitation in the brain is played by afferent impulses. Elimination of afferent impulsation (for instance, from the visual analyser) sharply reduces the development of cholinergic transmission in synapses.

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Unconditioned Reflex as Specific Character and Problem of Studying Instinct

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The reflex automatisms described and used by I. M. Sechenov for the interpretation of the physiological principles of behaviour and the notion about unconditioned reflex created by I. P. Pavlov do not cover the vast field of the phenomena known for centuries and usually called instincts.

According to I. P. Pavlov's notions there is no difference between the instinct as an innate and hereditarily conditioned act and the unconditioned reflex, if one does not take into account the complexity of the very structure of the reflex act and the reflex chains forming it. This notion about instinct has been recently extended to the idea of complex reflex activity where the instinct as a specific form of behaviour is considered as a combination of the innate and the acquired activities forming themselves at different stages of prenatal and — mainly — of postnatal development (Bykov and Slonim, 1949, 1960; Slonim, 1961, 1963). The complex reflex activity includes not only the elements developing at the different stages of ontogeny, but also those conditioned reflexes, obligatory for individuals of a given species or a given population. These elements of the so-called obligate training have essentially the same taxonomic character as the innate elements of behaviour and regulation of physiological functions. Thus, for instance, the origin of food-seeking reactions of a beast of prey (cat, bird of prey, lion, lion's whelp, tiger cub) is not related to 'training' and becomes apparent for a species at definite periods of time in postnatal development (Uzhdavini, 1955, 1958a, 1958b; Ugolev, 1950, 1953; Kossobutsky, 1951; Shepeleva, 1961; Thomas and Schaller, 1954). On the other hand, such foraging activity as pasturage of ungulated animals is only formed on the base of imitative conditioned reflexes (in the herd) (Rakhimov, 1958a, b).

Lambs and kids isolated from rough foodstuff for 6 months and reared on milk have no grazing habits at all. Consequently, even within the limits of the food-seeking activity the specific strength of the innate elements acquired in the individual experiment can be different and has a specific character. Natural conditioned reflexes and the generating on their base of complex stereotypes of the behaviour of the obligate training — in contrast to the artificial or facultative training — are part and parcel of the specific adaptive behaviour under different ecological conditions of existence.

Of the great number of instinctive (innate and inherited) acts taking place in nature

comparatively few can be used for experimental study in the laboratory. These are mainly those that possess the character of 'activities' established for a long time and that must be estimated quantitatively. Besides, these forms of activity must — in accordance with the desire of the experimenter — be reproduced in the laboratory, and lastly, it is highly desirable to be able to work on animals that can live in cages and thus observe the generation of these hereditary forms of the behaviour of animals in ontogeny.

Many complex forms of the instinctive behaviour of rodents, such as burrowing, gnawing, food storing and playing and some others that form at definite stages of development to a great extent meet these three requirements.

The following form of experiment was adopted. The animals, kept under the usual

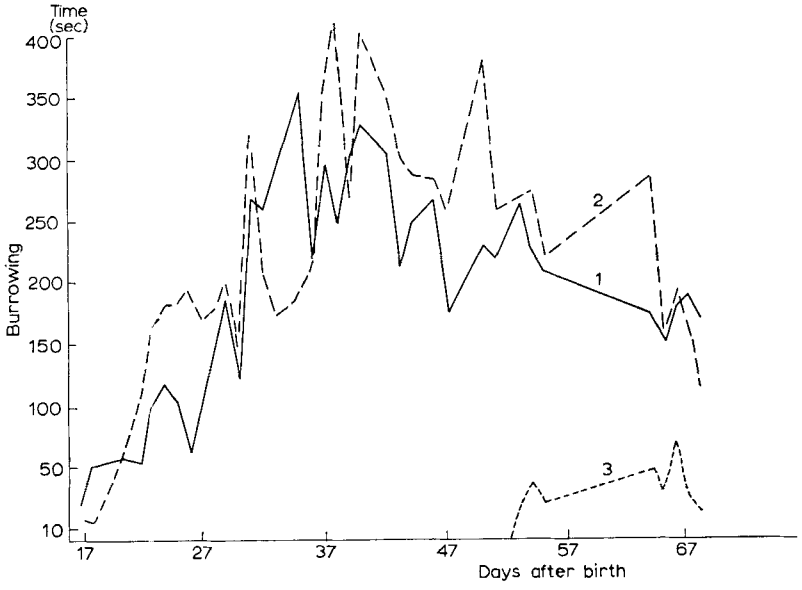

Fig. 1. Intensity of burrowing activity (time in sec) of large sand rodent cubs in breeding by mother sand rodent (1) and albino rat (2). (3), the same for young albino rats. (From Shcheglova, 1958a,b.)

conditions in cages (in nests, groups or separately depending on the task), are placed for a definite period of time in a separate room where there are objects required for revealing one or another form of activity: calcined clean sand for burrowing, wood for gnawing, seeds for food storing, partner for playing, etc.

The total time of performing the specific forms of activities and the work carried out by an animal (for instance, the weight of the food stored in g, the weight of the soil dug, etc.) are recorded. During the development of the instinct gaseous exchange, blood circulation, electromyogram and body temperature are studied.

This experimental design allows us to study the forming of the innate activity at different stages of postnatal ontogeny as well as the formation of a number of conditioned reflexes changing these innate forms of behaviour in different directions. Thus,

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for instance, we observed the formation of various natural and artificial conditioned reflexes changing considerably in intensity and the conditions of displaying such instinctive acts of an animal, as, for instance, gnawing dry and wet wood by large sand rodents (Shcheglova, 1956, 1958a,b), storing food by rodents (Ponougaeva, 1960), digging and foraging activity of rodents (Ponougaeva, 1954; Radko, 1957; Slonim, 1954), gregarious reactions of lambs (Rakhimov, 1958a,b). Hence, there exists an extreme changeability in the conditions under which the display of the enumerated forms of the instinctive activity and the differences in the role of individual afferent systems of even one and the same species takes place.

In the present report, I shall dwell on the two aspects which are of importance for understanding the problem of forming the instincts: on the problem of their constancy as the specific character and on the role of external and internal irritants (stimuli) in displaying the instinctive activity. Investigators often consider the instinct as a form of activity conditioned wholly or to a large degree by internal stimulation including also that generating spontaneously in the central nervous system of an animal.

Many complex forms of an animal behaviour generated in definite periods of

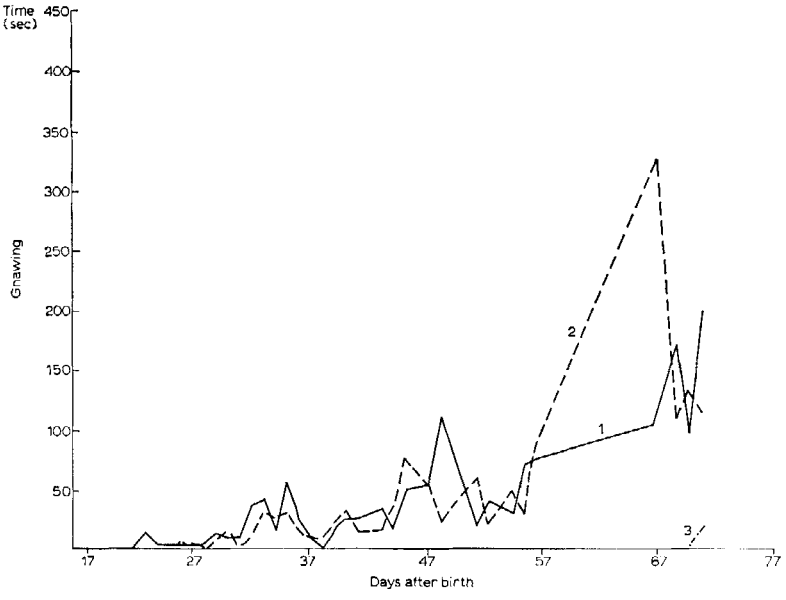

Fig. 2. Intensity of gnawing (time in sec) of large sand rodent cubs in breeding by mother sand rodent (1) and albino rat (2). (3), the same for the albino rat cubs. (From Shcheglova, 1958a,b.)

development in ontogeny are displayed independently of the action of any irritant from the external or the internal medium. The following forms of activity, which may be referred to such phenomena, were studied in our laboratory, thanks to their easy reproducibility and sufficiently definite qualitative characteristics: burrowing (Shcheglova, 1958a,b; Ponougaeva, 1960), food storing (Ponougaeva, 1960, 1963b), gnawing (Shcheglova, 1958a,b) and others.

If one breeds sand rodents such as *Rhombomis optimus* under conditions of complete separation from their mothers (nursing them artificially or under an albino rat) then the periods of generating the burrowing activity or the gnawing in no way differ from the periods of the control animals. This activity also does not differ as to the time taken to produce the gnawing or burrowing activity (Figs. 1 and 2).

Similar results were obtained with the golden hamster (*Hemicricetus auratus*) with food storing. Feeding animals with liquid food deprives them of the possibility of food storing. Young golden hamsters bred under such conditions start to store food at the same periods of time and with the same intensity as the animals kept together with their mother continually storing the food (Fig. 3).

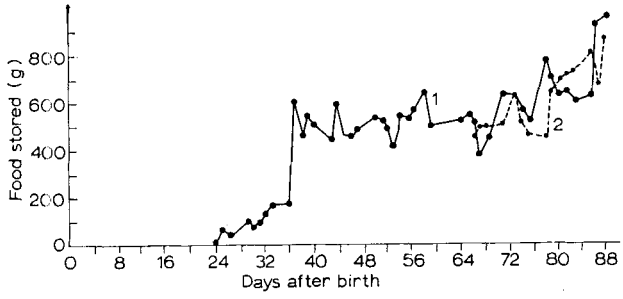

Fig. 3. Quantity of food stored by golden hamster cubs: 1, bred together with their mother; 2, separated from their mother and bred up to the 64th day on liquid food. (From Ponougaeva, 1960.)

Consequently, not only the simpler innate acts as for instance, foraging but also the complex ones comprising chains of individual motor acts can generate and repeat themselves stereotypically independently of the conditions of an animal's postnatal development.

One finds perfectly clear relationships between the periods of forming the instincts and the physiological state of animals. Thus, for instance, the 'gregarious' reaction of reducing the general metabolism of the rodent cubs in the presence of the female in the nest disappears at the time of the nest disintegration and, apparently, by the beginning of settling the young animals in new places (Ponougaeva, 1953). Consequently, the pronounced increase in metabolism here precedes the behaviour changes. A clear relationship was found between changes in the gaseous interchange and the blood circulation in the process of postnatal ontogeny on the one hand, and playing activity on the other one. As the investigations of A. G. Ponougaeva and D. A. Rashevskaya showed, a considerable parallelism is found between changes in oxygen consumption, pulse frequency and heart capacity per min, beginning from the 18th–22nd day after birth (of a golden hamster). Up to the 18th day an increased oxygen consumption from day to day (per g body weight) and quickened pulse are observed. The appearance of the motor activation is observed: playing activity is absent until the time (the 18th day) when a decrease in the pulse frequency and the general gaseous interchange begins. Ponougaeva's researches showed that the periods of the appearance and the intensity of the rodent cubs' playing activity represent specific charac-

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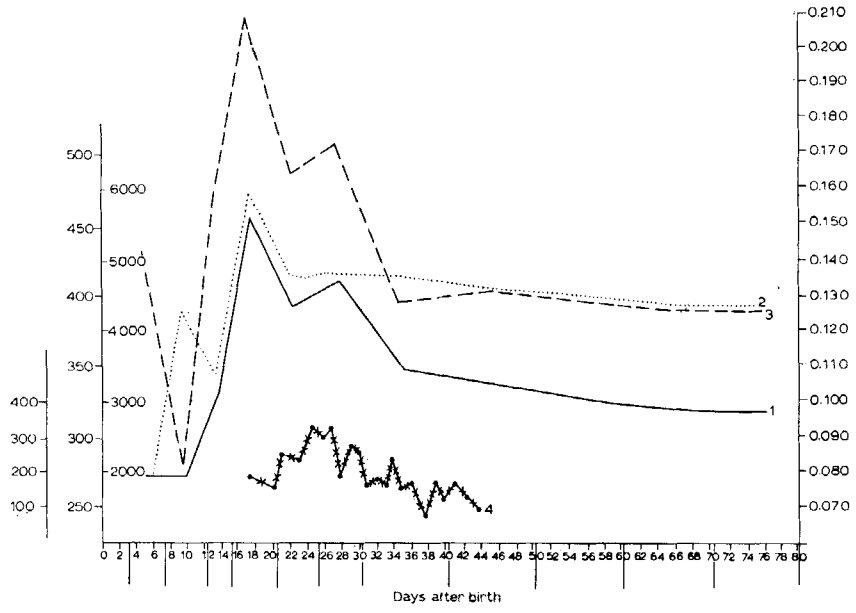

Fig. 4. Relation between the development of playing activity (4) and changes of pulse frequency (2), oxygen consumption (kg/h) (1) and oxygen pulse (O_2/min/pulse/min) (3) of golden hamster cubs in the postnatal development. X - axis, days after the birth. Y - axis, from left to right: pulse rate, oxygen consumption (kg/h), oxygen pulse (ml), playing activity (min). (From A. G. Ponougaeva and D. A. Rashevskaja.)

ters. This activity occurs against a background of the decrease, due to growth, in the general metabolism, and has its maximum on the 32nd–36th day for the golden hamster; it disappears on the 58th–60th day (Fig. 4). Previous observations (Slonim *et al.*, 1960) showed that, among the mature-delivery animals, lambs have elements of the playing activity almost immediately after birth and that the gregarious playing activity reaches its maximum by the 25th day against a background of continuous decrease in oxygen consumption (per g weight). This relationship between the intensity level of the tissue processes and playing activity led us to study the role of the metabolic intensity as this form of activity occurred 'spontaneously' and unrelated to stimulation of any exteroceptive or interoceptive zones. A close relation between playing activity and ambient temperature was found (Fig. 5). At one and the same temperature of the experiment (+22°) the playing activity of the animals bred at a temperature of 25° proceeds at a lower level than of those bred at a temperature of 10–12° (Ponougaeva, 1961). Similar results were observed with rats. The question naturally arose whether this relation is a reflection of the muscular state observed at different ambient temperatures and consisting in the appreciable functioning of the muscular thermo-regulation tonus at the lower ambient temperatures (Ivanov, 1962a,b; Ivanov and Alimoukhavedov, 1963). The muscular thermo-regulation tonus is known to cease functioning not only on ambient temperature increase and cessation of the functioning of the chemical thermo-regulation, but also on increased heat generation in muscle as a result of separation between breathing and oxidative phos-

phorylation (Veselkin, 1961; Nejfakh, 1961). This served as the basis for studying the influence on playing activity of some substances that change the state of thermo-regulation.

It was found that in the infusion of α-dinitrophenol at 0.045 mg/g body weight an appreciable increase in the body temperature (2°) occurs; a decrease in playing activity is found when the body temperature returns to normal. The dose of 0.015 mg/g body weight also causes a decrease in the playing duration at a preliminary moderate increase in the body temperature. In the first example the playing duration

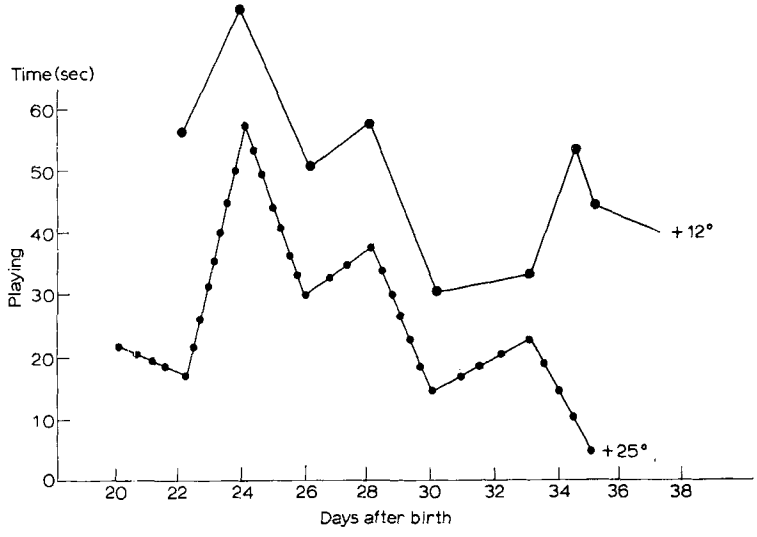

Fig. 5. Intensity of playing activity (time in sec) of golden hamster cubs, bred at different ambient temperatures. (From Ponougaeva, 1963.)

decreased on the average by 52%, in the second by 22%. Statistically both values are valid (P = 99 and 98%) (Fig. 6,a). At both doses the latent period of playing also increases somewhat, especially at the dose of 0.045 mg/g body weight.

The infusion of a pyrogen at 0.005 mg/g body weight also causes a decrease in the playing duration and a very small increase in the latent period. The body temperature remains practically unchanged. Thus, both α-dinitrophenol influencing the muscular energetics and a pyrogen mainly influencing the heat production markedly decrease the playing activity (Fig. 6,b).

As the data of Veselkin's laboratory showed, pyrogenal has a highly peculiar effect on the thermo-regulation centres markedly increasing their sensitivity. Both the step-wise change in the reaction, as if thermic irritants are summated, and the subsequent increase in the body temperature are also characteristic here. The pyrogen effect is mainly a central one, whereas the action of the α-dinitrophenol is peripheral, separating the processes of oxidation and oxidative phosphorylation. In the experiments of Ponougaeva the infusion of a low dose of aminasine, 0.02 mg/g body weight, caused, against a background of unchanged body temperature, a very marked increase in the

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latent period (almost 5 times — 483%). Besides a restriction in the playing activity a decrease in the general activity was also found here (Fig. 6,c).

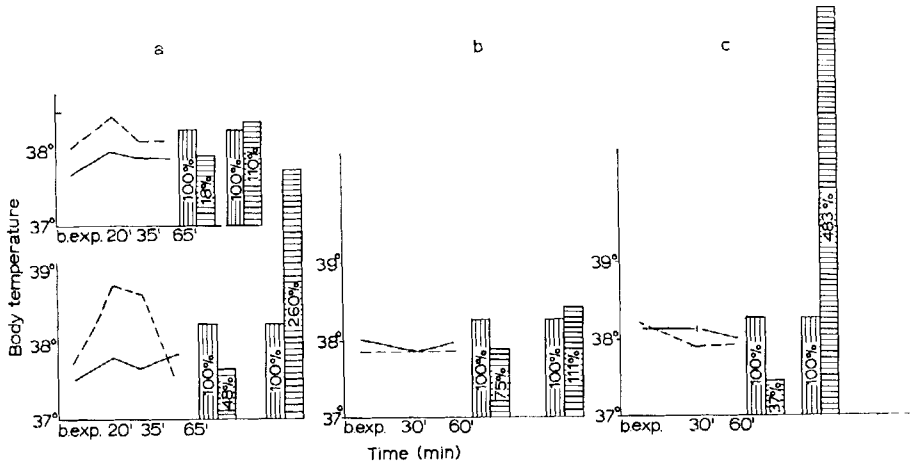

Fig. 6. Changes in playing activity of the golden hamster during the infusion of (a) dinitrophenol, (b) pyrogenal and (c) chlorpromazine (column 1, time of playing in %; column 2, the latent period of playing in %). Solid line, body temperature in the control test; dashed line, after the infusion of preparation. (From A. G. Ponougaeva.)

Aminasine acts centrally and in greater measure causes hypothermal effects resulting from the considerable decrease in muscular activity (both spontaneous and reflex). Consequently, the depression of the playing activity is possible both in the

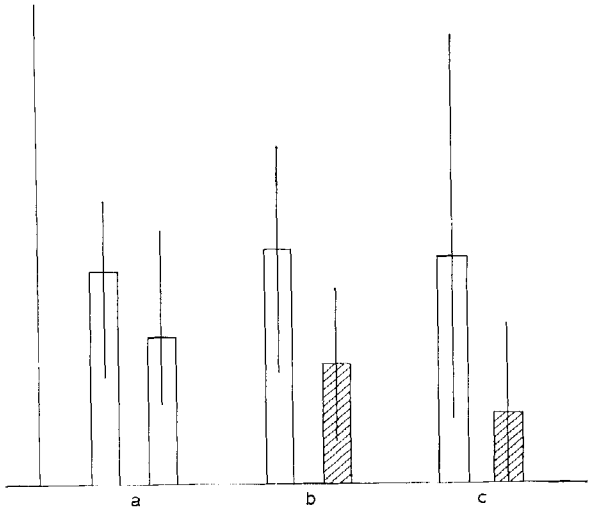

Fig. 7. Influence of muscular activity (running in the treadmill) on playing activity of golden hamster cubs in sec. a, repeated tests, control; b, running for 10 min; c, running for 60 min. White columns, before the run; shaded, after the run. (From E. L. Sklyarchik.)

effect on the central and on the peripheral links of the motor system and the system of thermo-regulation.

Thus, whenever the heat balance, heat generation or heat production are altered the playing activity is selectively disturbed. The use of aminasine also depresses this activity, but only against a background of the general suppression of the activity including the food behaviour. This also affects the marked increase in the latent period at the beginning of the playing.

Further work was aimed at studying the influence of the muscular activity and repetitions of the playing functioning on the playing activity. The purpose was to reveal the specific character of playing as a form of muscular activity, because emphasis is being placed on the storage of the energetic potential (specific energy) causing the appearance of the spontaneous (vacuum — Vakuumprocess) reaction. The method of compulsory muscular loads and the method of isolation were used to analyse these phenomena.

It was established by E. L. Sklyarchik that compulsory muscular work (running in the treadmill), depending on its duration, causes a marked depressing influence on the playing activity of the golden hamster. Other forms of muscular activity also markedly decrease after such loads, as was previously shown for a number of rodents by Maiselis (1953) in our laboratory.

The repeated placing of the animals in the playing cage for 30 min at 1 h intervals causes a stepwise decrease in the playing activity. This decrease is not the result of the daily dynamics of physiological functions; thus with the absence of playing for 6 h the decrease in the duration of the experimental playing is considerably reduced (Fig. 7).

The second version of excluding the playing activity from the general regimen consisted in isolating litter mates and breeding them in individual cages. The isolation of hamsters completely deprives them of playing activity. If the animals bred in this way are placed in the 'playing' cage every other day, then one finds a marked increase in the playing duration and a decrease of the latent period (Fig. 8). This occurs in spite of the fact that the more pronounced orientating reaction takes place after

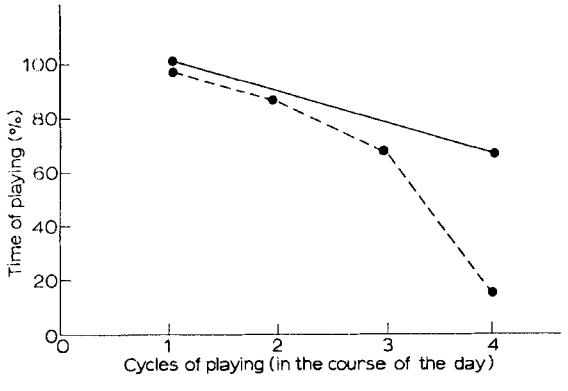

Fig. 8. Influence of repeated cycles of playing on playing activity (in sec). (From A. G. Ponougaeva.)

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replacing the animals into another cage. Consequently, the cessation of the specific form of activity — playing — leads to a marked increase in the 'potential'. The animals, as it were, compensate for the period during which they did not perform the playing activity. Thus, the repetition of both nonspecific and, especially, specific activity — playing — leads to the depression of this activity. On the contrary, the cessation of playing causes a marked increase in the 'playing potential', or 'exercise' of the corresponding nerve centres.

A peculiar 'centres exercise' to which attention is drawn by contemporary investigators of instinct (Lorenz, 1937, 1957; Tinbergen, 1955) occurs in the opinion of Sechenov as a result of the action of below-threshold irritants. Sechenov wrote: '... if the irritants, acting on the nerve centres, with the resulting impulses cannot find the natural way out (in our experiments in movements), then they are stored in the nerve centres and appear in an increased degree as soon as the cessation disappears' (Sechenov, 1935).

Exercise markedly increases the excitability of centres relating to the complex forms of the activity called instincts. This effect, whether we explain it by the appearance of the special dominant according to Ukhtomsky or by the state of the so-called appetency according to Craig (1918) discriminates many of the innate forms of behaviour from unconditioned reflexes. Many investigators (for instance, Thorpe, 1956) see in instinct a form of spontaneously-occurring activity. This distinguishes the notion of instinct from the notion of an unconditioned reflex. Every instinct may seem to be distinguished with such a peculiar ability of exercising the corresponding centres with the cessation of this activity.

This point of view may be considered with the statement of Craig (1918) concerning the appetent-directed character of instinctive behaviour. The possibility of typical instinctive acts being intensified by the formation of the corresponding conditioned reflexes or by the cessation of instinctive activity — an increase in the hypothetical 'potential' — is noteworthy.

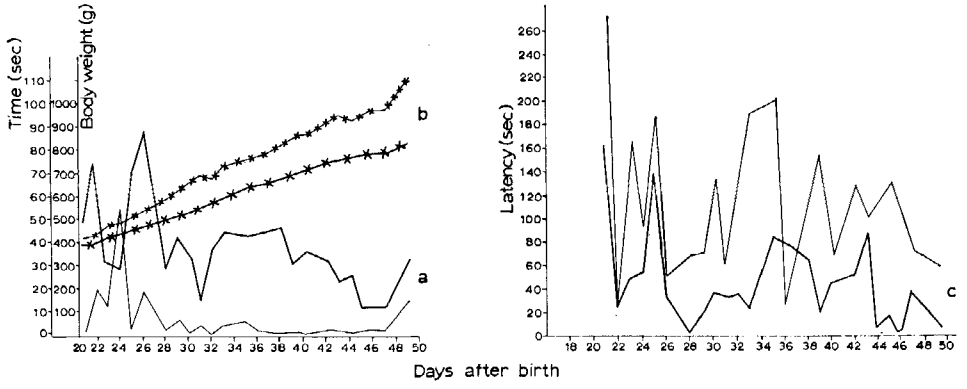

Fig. 9. Influence of periodic isolation of the golden hamsters from litter mates on playing activity, latent period and body weight in the process of development. Thin line, control animals; thick line, animals after the isolation. a, playing in sec; b, body weight in g; c, latent period of playing. (From A. G. Ponougaeva.)

As distinct from foraging activity related to the irritation of the synocarotid zone (Chernigovsky, 1960, 1962), or food storing by hamsters related to the irritation of cheek pouches (Ponougaeva, 1963b; Slonim, 1961), playing activity is most related to metabolism and also, perhaps, to the presence of tonic thermo-regulational excitation in individual muscular groups. Particular attention is given to this aspect as related to the experiments on the infusion of α-dinitrophenol.

Instinctive activity undoubtedly reflects the state of high excitability of definite nerve centres, excitability imitating, as it were, the spontaneous character of its display. This aspect of the problem was reflected in the statements of Ukhtomsky (1923, 1925).

Consideration of all the data at our disposal — I can only refer to few — allows us to conclude that instinctive activity depends, to a less degree than the simple reflex one, on well-known factors of the external medium, and is determined by the state of the nervous and hormonal systems developing in the course of onto- and phylogeny. There is a chain of transitions between instinct and unconditioned reflex as the innate acts of behaviour and regulation of physiological functions occur, depending on the reflex or automatic nature of an excitation.

SUMMARY

Certain methods, used in the author's laboratory for studying innate and acquired behaviour (unconditioned reflex and natural conditioned) as well as regulation of physiological functions are discussed.

The use of these methods, especially that of isolation of an animal from certain environmental factors, facilitates the separation of the elements of innate activity and their quantitative study during postnatal ontogenesis. The innate forms of mammalian activity, well displayed in rodents (burrowing, food storing, gnawing and playing), are conveniently studied in laboratory conditions. The regularities of complex behaviour formation in ungulates in the herd (herd reactions, grazing) are also studied as well as the onset of activity in the search of food (predatoriness) by carnivorous mammals.

Special investigations have shown complete independence of innate behaviour patterns of developmental character on breeding conditions, and, at the same time, great significance of conditional reflex formation for species behaviour.

'Charging' of centres and the onset of 'spontaneous' activity have been observed during playing activity. This form of activity may be strengthened or weakened under the influence of environmental temperature, by changes in heat production by the organism (with α-dinitrophenol treatment), or in heat losses (with pyrogenal treatment), and under the influence of chlorpromazine on the CNS. Chlorpromazine inhibits the general activity of an animal, playing activity included. With α-dinitrophenol treatment, which uncouples the oxidation and oxidative phosphorylation processes, only playing activity is decreased, body temperature remaining stable. Thus, playing activity may be weakened both by influencing the CNS and by changing the chemical processes in the effector apparatus in skeletal muscles. Temporary isolation of young

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animals from their mates caused the phenomena of 'charging' centres and energy accumulation, resulting in the strengthening of playing activity.

Consideration of the experimental observations permits the conclusion that between the instinct and the unconditional reflex (according to I. P. Pavlov) there is a chain of stages, dependent either on reflex or on the automatic nature of excitement.

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Some Regularities in Cellular and Subcellular Evolution of Structure, Chemism and Function of Sense Organs

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The statement made by Sechenov in 1863, that physiology is the physics and chemistry of the living being, becomes more and more evident. Indeed, nowadays nobody has any doubt of the fact that the specific response of sense organs — exteroreceptors — to particular phenomena of the outside world, that is, the adequate perception of energy in some form from the outside, takes place through cellular, subcellular and molecular changes in their organization. The act of reception of energy in some form from the outside always commences with or is accompanied by molecular changes in the specific and non-specific chemical and biologically active substances located in quite definite structures (organelles) of receptory cells. It appears that these changes stimulate the so-called state of receptor stimulation, this stimulation being transmitted as impulses through synapses to the central nervous system.

In the present report we proceed from the general statement that the principle of reception is universal, and the cell at first responds to all outside stimulations universally. It is only after a lengthy evolution that the cell can sense differentially definite forms of energy from the outside, the structure, function and chemical composition of the cell being specifically altered. Although the cells have been changing into primary sensing cells (sense organs of the vertebrates and invertebrates) or into secondary sensing cells (sense organs of the vertebrates), both types of cells retain a series of initial common structural, chemical and functional features. It is our task to attempt to show in what way this or that cellular, subcellular or molecular organization of receptors originates in the course of a long evolution, this organization determining the cell's ability of differential perception of a definite form of outside energy. Further, we shall attempt, on the basis of the ideas of Orbeli (1958), to clarify what features of this organization are specific as being connected with the particular functional evolution of this or that sense organ.

From the data of phylogenesis and ontogenesis it follows that the basis of the structure and function of sense organs is a cell form having mobile cilia or a flagellum, which in protozoa serve for both locomotory and receptory purposes. Thus, these two indissoluble functions of sense organs are combined in structure and function. Later, as the organization becomes higher and higher, these functions are combined

through close co-operation of nervous and muscular systems (Sechenov, 1863). As will be shown below, the receptory cells, of an animal's or man's sense organs, provided with a flagellum or cilia, is almost the same as similar receptory structures of some protozoa, *e.g.* of Diflagella (Fig. 1).

The substructural organization of mobile flagella or cilia, which are frequently termed hairs or kinocilia (flagellum of the unicellular organisms, sperm caudal filum of animals and plants, cilia of epithelium vibratorium and sensing hairs of receptory cells), are usually characterized by the presence of nine (often twin) peripheral and two central fibrils. The proximal ends of peripheral fibrils terminate in basal corpuscles located in the cytoplasm under the plasmatic membrane (Fauré-Fremier, 1961; Fawcett, 1961; Sjöstrand, 1953a, b, 1961, etc.). The motion of cilia or a flagellum is stimulated by the contraction of the fibrillation apparatus. Fibrils are macromolecular combinations polypeptides, type β, close in nature to myosin (Astbury *et al.*, 1955). As has been proved experimentally, fibrils contract under the action of ATP even after being damaged (Hoffmann-Berling, 1951; Bishop, 1956; Alexandrov and Arronet, 1956). Fibrils are characterized by ATP activity (Mann, 1955). They are synthesized by basal corpuscles, *i.e.* by gigantic ribosomes containing DNA and RNA (Seaman and Gottlieb, 1957). We have succeeded in proving experimentally that mobile hairs — kinocilia or their derivatives — may be found in practically all the receptory cells of sense organs. Being activated by ATP, their motion becomes more active or starts anew after damage, as was proved experimentally in 1964 by Bronstein (olfactory cells) and in 1962 by Vinnikov and Titova (labyrinth ciliary cells). The olfactory cells of higher vertebrates have, as a rule, 5–6 mobile cilia crowning the olfactory clava. Some fish and invertebrates have only one flagellum or cilium. Undoubtedly such a mobile hair providing for contraction or expansion of retinal rods and cones in the light and dark (retinomotory phenomena) is the basis of the substructural organization of the inner and outer segments. The outer segment essentially represents a ciliary form. In addition to 9 fibrils it comprises a series of lamellae growing from the external plasmatic membrane. The lamellae contain the semi-carotenoid retinene (aldehyde of vitamin A) combined with the albumin opsin. Receptory structures of photoreceptors of the invertebrates (rhabdomeres of molluscs, arthropodes, and echinoderms) are also based on a ciliary form and contain retinene.

The secondary sensing receptory cells of the labyrinth of vertebrates and of the lateral line organs of fish, as was shown by the experiments conducted in our and other laboratories, are characterized by the presence of a mobile kinocilium with a specific (9 + 2) fibrillated structure located in a certain way next to immovable cilia which are free of fibrils (stereocilia). The direction in which the kinocilia are arranged is determined by that of an adequate stimulus, *i.e.* by the direction of the flow of endolymph in the structures of the membranous labyrinth (including the birds' organ of Corti) and of the flow of fluid in the lateral line organs of the fish. The researches of electrophysiologists have shown that the flow of liquid in the direction of the kinocilia is always accompanied by a decrease in the microphone effect and an increase in action currents (Trinker, 1957; Löwenstein and Wersäll, 1959).

There is no kinocilium in ciliary cells of the mammalian organ of Corti. On the

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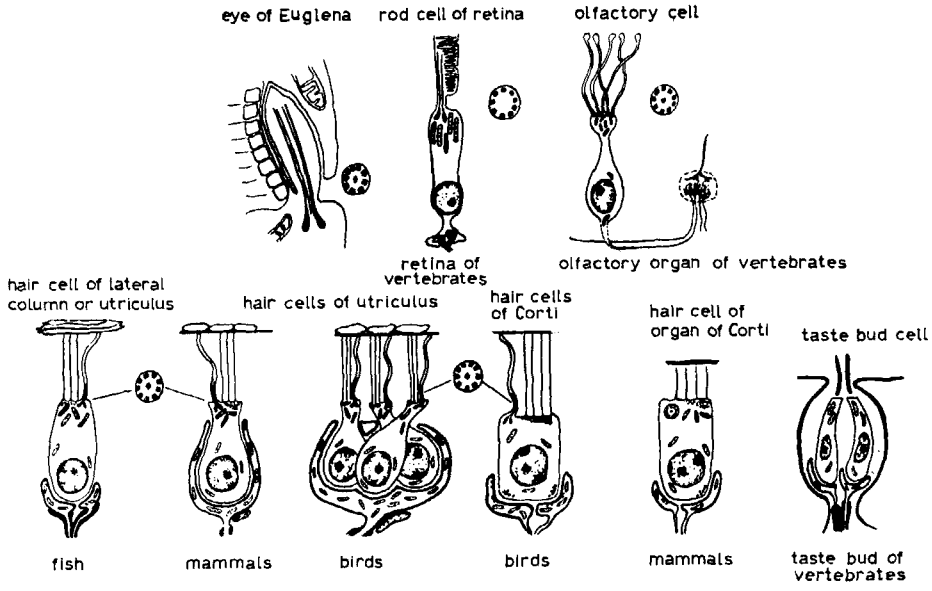

Fig. 1. The organization of the sensory cells and of their motion antennae. On the cross sections there can be seen 9 pairs of peripherical and 2 pairs of central fibrils. Schematic drawing.

other hand, according to the data obtained in our laboratory the kinocilium is found well developed in the bird's organ of Corti (Fig. 1). Also, a kinocilium of the typical fibrillated structure is found in the receptory cells of the hearing organ of insects (Gray, 1960). The kinocilium is absent as well in the gustatory cells of the vertebrates' taste organ (Fig. 1). However, as will be seen later, the loss of a kinocilium is associated with the formation of other similar structures possessing a new cytochemical organization.

Thus, the basic sensing element in all receptory cells of the vertebrates' and invertebrates' organs of sense is a specific form of a mobile kinocilium or its derivatives. We think that the kinocilia of receptory cells are to be looked upon as a kind of receiving antenna. These antennae are ancient instruments that have been modified in both structural and chemical respects in the process of development, and that serve for active interaction of the unicellular or multicellular organisms (animals and man) with the outside world. It is difficult to comprehend now the reason why kinocilia of the fibrillation apparatus had a constant number of fibrils (9/18/ + 2), this quantity remaining the same nowadays. In the process of evolution natural selection transmits kinocilia having the above number of fibrils from the protozoa to colonies and then to some cells of multicellular organisms, including the cells of the sense organs of animals and man. Perhaps the mystery of this structure and of the constant number of fibrils will be discovered through bionic modelling. No one will underestimate the fact that various forms of energy from the outside (photons, molecules of redolent and flavouring substances, gravitation, vibration, and sound) are sensed in this or that way by means of these antennae characterized with different and

variable amplitudes of motion but having a similar structure, chemism and function. Indeed, the reception principle is universal.

But along with the similar substructural organization and affinity of antennae, the receptory cells of sense organs have some divergent co-ordination distinctions. One can trace in what way the receptory cells of various sense organs, in the process of their development, have acquired new features undoubtedly associated with the influence of some form of energy from the outside and providing for perception and transformation of this energy. The formation of new structures and redistribution of biologically-active chemical substances may occur, as a rule, both within the antenna proper and in the cytoplasm of the cell body. This particularly relates to such primary sensing cells as photoreceptors of the vertebrate retina. A specific photosensitive substance, retinene, whose molecule is isomerized by the action of photons, is accumulated in lamellae of the kinocilium itself, that is, in the outer segments — retinal rods and cones (Wald, 1960; and others). The enzymic activities of succinoxidase and cytochrome oxidase might be detected in the rods and cones of cattle, the fraction being isolated in our laboratory by Etingoph and Shukolyukov in 1963. The effect of adenosinetriphosphatase is also found in the lamellae of outer segments (McConnel and Scarpelli, 1963). Consequently, even from these preliminary observations, we may suggest that there is a transport of electrons in the photoreceptor lamellae, which may be explained by their specific function. Accumulations of mitochondria (the so-called ellipsoids) are found arranged in a certain order in the photoreceptor inner segment. In 1962 and 1964 Luckashevich proved, when experimenting on vertebrates in our laboratory, that the rather high activity of oxidizing enzymes in mitochondria of ellipsoids varies in the light and dark. These results were soon underscored by the experiments of other researchers. Thus, we conclude that photoreceptors have one centre of ATP regeneration located in the lamellae proper, the said centre likely being power-connected with retinene and the other centre located in the ellipsoid. It may be supposed that during the excitation of the photoreceptor by photons feeble power processes in the outer segment lamellae are largely increased by a powerful source, ATP, located in the mitochondria of the inner segment ellipsoid whence they are transmitted *via* peculiar synapses to bipoles. Biopotentials are manifested in the retina starting only with bipoles. According to unpublished results obtained in our laboratory by Govardovsky and Charkeyevich, in the process of embryonal development of photoreceptors the mitochondria-containing ellipsoids having structural cilia with 9 fibrils are formed earlier than are the outer segment lamellae. Hence, the powerful ellipsoid system is employed both in embryonic development, differentiating the outer segment lamellae, and for the further functioning of the photoreceptory cell as a whole. Similar regularities in the development and differentiation of photoreceptors show themselves in tissue cultures, as proved by Govardovsky and Charkeyevich in 1965. The information on the chemical organization of the vertebrates is so far limited by the fact that we do not know much about the presence of retinene in the receptors (Wald, 1960).

The study of the cytochemical organization of primary sensing olfactory cells of the vertebrates (Bronstein, 1962a, b, c, 1964, 1965) has shown that the distribution

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of a number of biologically-active chemical substances is of mosaic character, which resembles photoreceptors. For instance, the enzymic activity manifesting in mitochondria of the olfactory clava is to be explained mainly by its olfactomotory ability. Mitochondria of the olfactory clava are disposed in the vicinity of basal corpuscles, praximal ends of fibrillo-antennae of olfactory cells whose specific motion was mentioned above. We have succeeded in observing that when the olfactory cells are stimulated by molecules of redolent substances, changes occur in the distribution and content of RNA, total protein and its functional groups, as well as of some enzymes located in the cytoplasm of the cell body. As for the role of carotenoids (vitamin A) in the reception of energy of fragrant substances*, it still requires many experiments to be carried out to clinch it, although we consider this role to have been proved. In whatever way the kinocilia of the olfactory cells come into contact with the molecules of fragrant substances (chemically or physically), they presumably take up the energy of these substances and convert it into a nerve impulse (biopotentials of an olfactory nerve) through power processes intensifying in the area of the olfactory clava where mitochondria are located. The further course of these processes is likely to be determined by biologically-active chemical substances whose orientation in space is rather specific in olfactory cells of the vertebrates, as shown by Bronstein in 1962. As to the cytochemical organization of olfactory cells of the invertebrates, no information is yet available.

Studies on secondary sensing cells of Corti's organ of mammals and birds, the gravitation organ (utriculus) connected with the labyrinth of the vertebrates, and the cells of the side line organ of the fish have shown that the structural and chemical organization of their receptors formed in the cells of the ancestors of the vertebrates Agnathi as early as Silur have a few distinctive features. Thus, there remains in ciliated cells of Corti's organ one kinocilium oriented with regard to the bundle of stereocilia (as proved by Vinnikov, Titova, Osipova, and Govardovsky in 1965). Mammals, however, have none; yet the sterocilia of mammals are characterized by high enzymic activity, acetylcholinesterase activity included, thereby becoming specific enzymic antennae (Vinnikov and Titova, 1961). These antennae are the first to receive mechanical stimuli sent from tectorial and basilar membranes and *via* moving endolymph, and they convert the stimuli to enzymic activity. Further, under the action of stimulation in the cytoplasm of ciliated cells certain changes occur to enzymes and substrates of anaerobic and aerobic metabolism, nucleic acids, proteins and functional groups, these changes resulting eventually in changes in the enzymic activity of acetylcholine esterase located in the synapses from which stimulation impulses are transmitted to further links in the auricular analyser (Vinnikov and Titova, 1961 and 1962a, b). Comparison of histochemical data with biochemical, pharmacological and electrophysiological data makes us think that in the external ear, not only in the organ of Corti but also in other receptory structures of the labyrinth — organs of gravitation

* This problem is being investigated by, for example, Duncan and Briggs, who say that the activity of carotenoids is based on the structural and chemical homology of olfactory and optic cells, which was noted by the author and Titova as far back as 1947.

(utriculus), of vibration (sacculus), of angular accelerations (crests of semicircular canals), including lateral line organs — the nervous impulse is transmitted through the cholinergic mechanism (Vinnikov and Titova, 1961 and 1963; Titova and Aronova, 1963). Thus, histochemical analyses confirm the onto- and phylogenic unity of the acoustic-lateral system.

Comparative studies of the cellular and subcellular organization of ciliated cells of the gravitation organ of fish, mammals and birds have shown impressive peculiarities which may undoubtedly be explained by the position of these species in the gravitational field. The orientation of animals of these classes in the gravitational field is determined by their mode of life formed in the process of ecological evolution; that is, the position of fish is determined by their life in the water, of mammals on the ground, and of birds in the air (as shown by Vinnikov, Osipova and Govardovsky in 1963). Evolutionary changes are not concerned with the oriented mobile kinocilium or a bundle of stereocilia supporting the otolithic membrane with the otolith, *i.e.* not with the nature of the still-obscure stimulation of the organ by gravitation. These changes mainly occur in the nature of synaptic transmission. The so-called first-type cells have been formed in the gravitation organ of the terrestrial vertebrates. These cells have cup-shaped synapses, largely developed with birds, 3–5 cells being disposed in one cup. One cannot help connecting this with the specific position of birds in the gravitational field during flight when the utriculus may serve also as a navigation instrument (Screber *et al.*, 1962).

The study of cytochemical changes in the organization of the gravitation organ of animals, *e.g.* guinea-pigs, during acceleration of 10 g for 3 min has shown that the RNA nucleus takes part in the functioning of the receptory cell, in the synthesis of protein consumed in excitation. It has been established with experiments on guinea-pigs that the RNA nuclei are arranged in the direction of the gravitational field vector. With birds the whole picture is more complex. Through an electronic microscope it has been found that the RNA-containing ribosomes running from the nucleus into the cytoplasm are surrounded by membranes of the endoplasmatic network; in other words, a local centre of protein synthesis was demonstrated morphologically (Vinnikov *et al.*, 1963a).

The studies of gustatory bulbs of all classes of vertebrates, conducted in our laboratory by Pevzner in 1963, showed that their substructural and cytochemical organization is similar, which may be related with the similar character of stimulation by the four main flavouring substances. It is noteworthy that, in the gustatory cells of the vertebrates, a kinocilium is replaced by a pin containing protein, mucopolysaccharides and phosphatase. We may assume that the pin serves as an ion-exchange apparatus interposed between flavouring substances and receptory cell. Impulse transmission in gustatory cells is effected by means of a cholinergic mechanism.

SUMMARY

Summarizing the foregoing, we may conclude that irrespective of the remarkable resemblance in reaction of mobile antennae that are irritated by a specific stimulus,

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the substructural and cytochemical organization of receptory cells of sense organs has some principal distinctions as a result of their different functions. The distinctions expressed in the photoreceptor are determined by the presence, in the lamellae of its antennae, of molecules of a specific photosensitive substance, a carotenoid that is common not only for animals, but also closely related to those in plants. On the other hand, these lamellae and the other structures of the photoreceptor also contain a series of non-specific biologically-active chemical substances providing for some processes employed in the functioning of the cell as a whole. But in contrast to other receptory cells, these substances are located in the photoreceptor in a particular structural way. As was noted above, they are located in the lamellae of the outer segment, in mitochondria of the inner segment ellipsoid, in synapses, etc. We may therefore conclude that these chemical substances and processes associated with them have functional significance, depending on the character of their substructural localization either in optic or in olfactory cells, in ciliated cells of the organ of Corti or gravitation, or of the lateral line organs, or in the receptor cells of gustatory bulbs. Thus, with some exceptions, a similar range of biologically-active chemical substances acquires in the process of development a varied structural localization depending on the specific features of the receptory cell. This ensures the specific functioning of the cell, *i.e.* perception of a certain form of energy from the outside at cellular, subcellular and molecular stages of its organization. The concept covered was developed by Creps *et al.* in 1963 experimenting with other objects.

The foregoing facts and ideas show that changes in structure and chemism caused by changes in the function of receptory cells may be discovered at cellular and subcellular stages of their organization.

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Comparative Studies of the Functional Development of Analyzer Systems in Animals in the Process of Ontogenesis

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According to Pavlov, the study of the most important function of the nervous system, *i.e.* the function of analysis of external stimuli, should be performed strictly objectively, with the help of the conditioned reflex method. In his opinion, every analysis of the external world starts with a special activity of peripheral receptor apparatuses which is detected in the form of simple reflex actions or in complex orienting reactions, and finishes in the cerebral cortex with participation of active inhibitory process, with elaboration of positive and negative conditioned reflexes.

The problem of analyzers has become the spotlight of attention for many scientists. Solution of many questions on the physiology of higher nervous activity, on dynamic localization of functions and pathological disorders in brain activity depends on further investigation of the analyzer functions. The literature, covering different aspects of this problem, has recently been enriched with fresh and important physiological and biophysical data. The activity of different analyzers has been studied with the intention of elucidating not only the mechanism of centripetal propagation of excitation, but also to estimate the significance of reverse influences, going from the centres to relay-links and receptor endings. In other words, perception of a stimulus has been considered from the standpoint of interaction of complex afferent and efferent systems. Here much attention has been paid to assess the role of the so-called 'specific' and 'non-specific' conduction systems in functional manifestations of analyzer apparatuses (Anokhin, 1949, 1958; Snyakin, 1948; Gersuni, 1957, 1962; Granit, 1957; Sokolov, 1958; Gastaut and Rogé, 1962; Hernández-Peón, 1962, and many others). However, despite a considerable progress, our knowledge on the physiological peculiarities of certain analyzers remains incomplete.

One procedure used in the study of functional properties and mechanisms of the analyzer activity is to investigate them in ontogenesis. It is interesting to ascertain the sequence of development of different analyzer systems in ontogenesis, their functions becoming more complex with the animal's aging. We have consulted a number of works in which some aspects of the analyzer activity were studied with the help of both the conditioned reflex method (Troshikhin, 1953, 1957; Obraztsova, 1955, 1961a; Wool, 1958; Airapetiantz, 1960, and others) and the electro-physiological method (Grossman, 1955; Tuge *et al.*, 1960; Schadé, 1959; Scherrer and Verley, 1960); Shilyagina, 1960; Farber, 1963, and others).

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During the last few years in collaboration with Nikitina, Novikova, Panchenkova, Krylov, Bykov, Osepyan and Shilyagina, we have also made investigations combining these two methods on rabbits, rats, guinea-pigs, cats, dogs and monkeys. Our task was to study the appearance and development of motor reflexes, orienting reaction and positive and negative conditioned reflexes from different analyzers in ontogenesis. To evaluate the functions of a certain analyzer we recorded somatic and vegetative components of unconditioned and conditioned reflexes, as well as the bioelectrical activity of the brain. Our primary concern was to find out at what age and in which form the activity of this or that analyzer system is displayed. It transpired that the functional activity of a number of analyzers (such as cutaneous, kinesthetic and vestibular) is detectable even at the embryonic stage. For example, in rabbit from 16 to 22 days of prenatal development (the whole period being 30–31 days) various defense motor reflexes (local, generalized and specialized) are detected in response to mechanical stimulations of the skin of the head and extremities (Fig. 1). On the

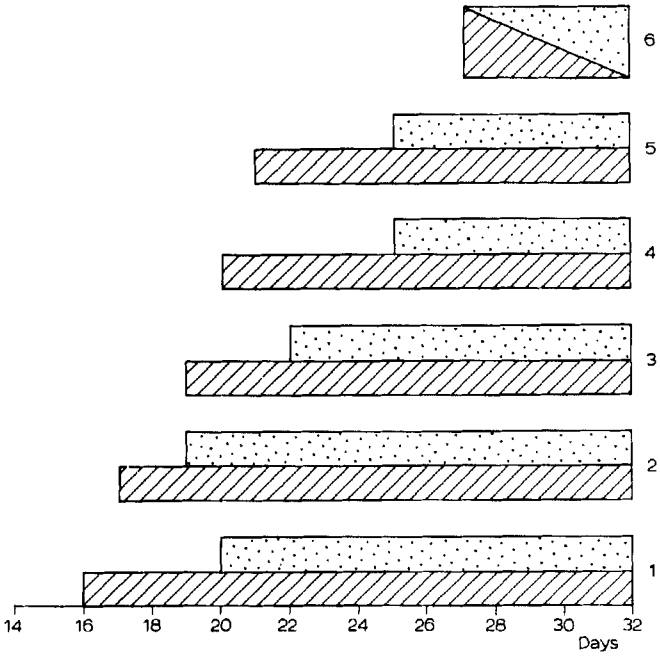

Fig. 1. Time of appearance of different motor reflexes evoked by stimulation of skin receptors of the extremity of the rabbit in embryogenesis. 1, Isolated flexion; 2, isolated extension; 3, simultaneous flexion or extension; 4, reciprocal relations in the reactions of the extremities; 5, alternating movements; 6, alternating movements of all four limbs. Hatched area: ant. limbs; dotted area: post. limbs.

22nd–24th day the rabbit embryo displays the function of the kinesthetic (proprioceptive) analyzer, which is revealed in reciprocal activity of muscle antagonists and in the appearance of the neck-tonic reflex caused by the stimulation of corresponding proprioceptive muscle apparatuses (Figs. 1, 2). During the last days of prenatal development (27–28th day) the function of the vestibular apparatus manifests itself

in the ability of the embryo to assume the normal position of the body, in the compensatory reaction of the head to turning, etc. (Fig. 2).

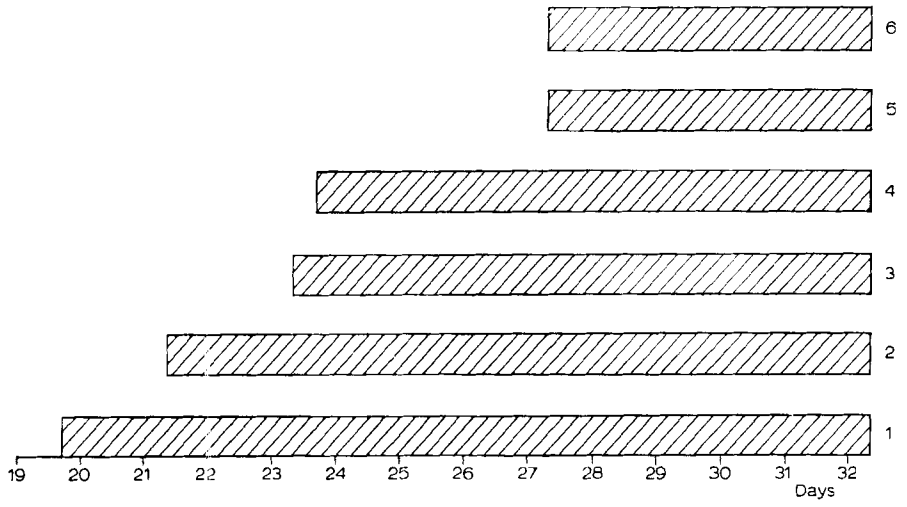

Fig. 2. Time of appearance of tonic and righting reflexes in rabbit in embryogenesis. 1, Tonic cervical reflex towards limbs; 2, tonic labyrinthine reflex towards limbs; 3, righting reflex of head from trunk surface; 4, cervical righting reflex; 5, righting reflex from trunk on same; 6, labyrinthine righting reflex (By G. A. Obraztsova, 1961b).

At the early postnatal stage in rabbits, dogs and other prematurely born animals these apparatuses begin to function more precisely, and at the same time other contact and distant analyzers (gustatory, olfactory, acoustic and optic) come into effect. This is proved by different reflex actions, particularly by the reaction of the orienting type, evoked by adequate stimulations of these analyzers. The time of appearance of the orienting reaction is the best indication of functional establishment of this or that analyzer. For example, in puppies it shows that in ontogenesis the olfactory and cutaneous analyzers come into action before the acoustic and optic ones (Table I).

TABLE I

THE TIME OF APPEARANCE AND EXTINCTION OF THE ORIENTING REACTION FROM DIFFERENT ANALYZERS IN DOG IN ONTOGENESIS (POST-NATAL DAYS)

Analyzers	*Appearance of:* *Primitive orienting reaction*	*Orienting-exploring reaction*	*Extinction*
Olfactory	1st day	1st day	38–48th day
Tactile	1st day	12–16th day	40–45th day
Acoustic	9–15th day	16–24th day	40–45th day
Optic	15–19th day	20–28th day	52nd–68th day

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During postnatal ontogenesis the orienting reaction undergoes certain changes which reveal functional maturation of analyzer systems. It has three consecutive stages (Table I): (1) primitive generalized motor reaction (startling, standing still) with preceding manifestation of vegetative (modification of respiration and of cardiac activity) and electrographic (EEG changes) components; (2) distinctly pronounced orienting-exploring motor reaction (turning of head, fixing of eyes, movements of ears etc.) also with preceding vegetative and electro-encephalographic components; and (3) unstable orienting reaction. It is a characteristic feature of the orienting reaction that it is preceded by vegetative components. In its primitive stage it is nearly always accompanied by a slowing of the respiration and of the heart-beat, while the orienting-exploring reaction is accompanied by quickened respiration and rapid heart-beat. The responding ability of the vegetative component is different for different species (Fig. 3) and depends on the modality of stimuli; for example, they are better pronounced to acoustic stimuli than to photic. As concerns the changes in the brain bioelectrical activity as the efferent component of the orienting reaction (Sokolov, 1958); in ontogenesis they also precede the motor manifestations of the reaction.

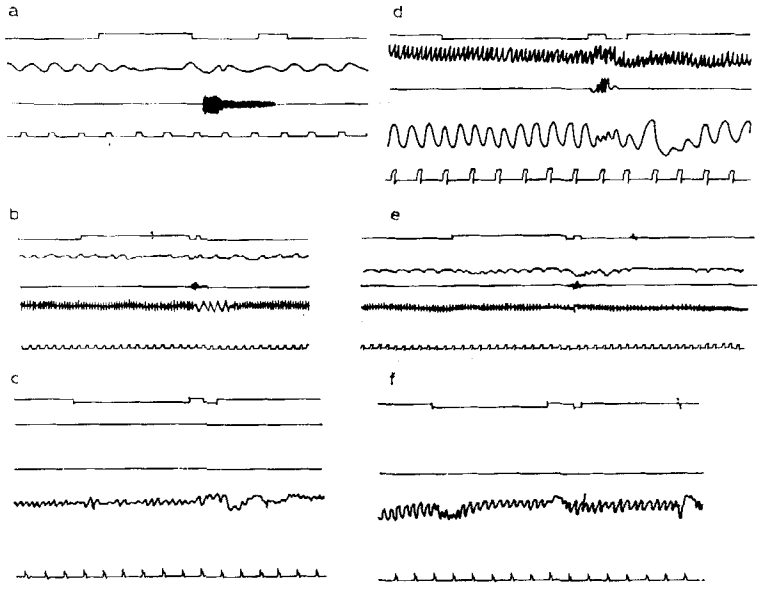

Fig. 3. Different types of modification of respiration as vegetative component of orienting reflex to sound. Marks from the top downwards: stimulation, respiration, motor reaction, time in sec, (on b, e, the fourth line: ECG; on c, f, the fourth line: respiration; on d, the second line: ECG; the fourth line: respiration). (a) Monkey, 17 days; (b) puppy, 11 days; (c) rut, 6 days; (d) rabbit, 16 days; (e) puppy, 21 days; (f) rut, 11 days.

In collaboration with Dr. Shilyagina, we tried to follow up ontogenetically changes in the electrical activity in different structures of the optic analyzer and in the 'non-specific' subcortical structures during the orienting reaction to photic stimuli in rabbits. The results of the experiments showed that in the early postnatal period (7–9th day), *i.e.* when the orienting reaction is still primitive, rhythmic photic stimulation

produces distinct changes in the bioelectrical activity in the form of depression of the background rhythmics (sometimes with an increase in the oscillation frequency) or in the form of feeble enhancement of the transformed rhythm, depending on the

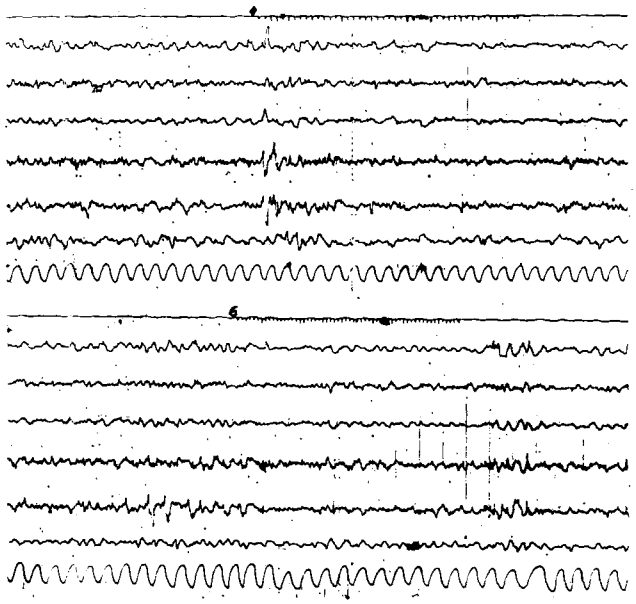

Fig. 4. Changes in bioelectrical activity of cortex and brain subcortical structures during orienting reaction to flickers in 7-day-old rabbit (chronic implantation of electrodes); upper portion of curves, to flashes 4/sec; lower portion, to flashes 6/sec. Marks from the top downwards: Photic stimuli, midbrain reticular formation, reticular nucleus of thalami, lateral geniculate body, visual cortex, auditory cortex, sensorimotor cortex, respiration.

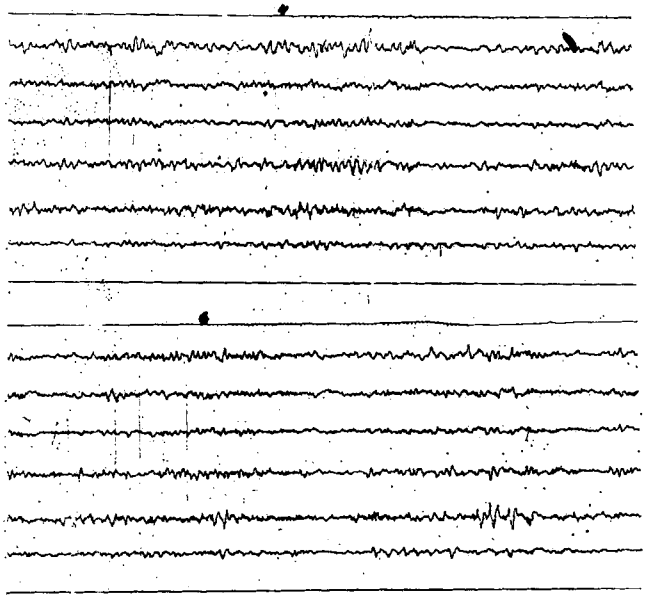

Fig. 5. The same as in Fig. 4 in 9-day-old rabbit.

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rhythm of stimulus presentation (Figs. 4, 5), In the later postnatal period (15–18th day), when the orienting-exploring reaction is already well pronounced, presentation of rhythmic photic stimuli provokes, as a rule, activation of the electric activity in the form of a more or less distinct driving of the rhythm of the stimuli applied (Figs 6, 7). With aging of the animal, the frequency range of rhythm driving increases

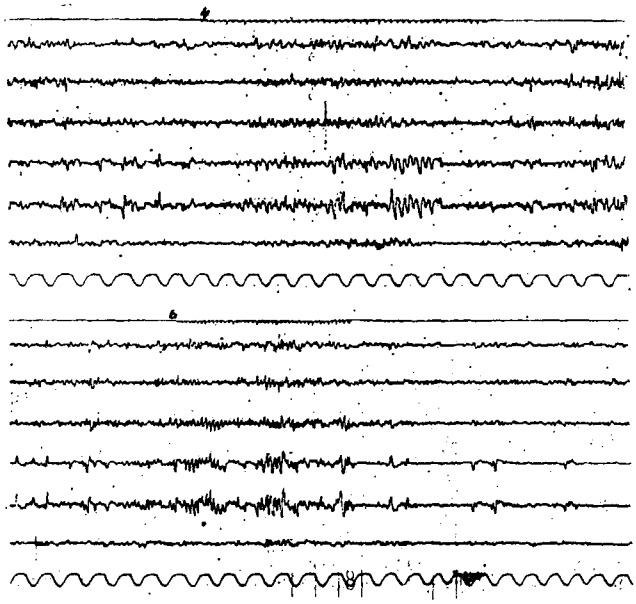

Fig. 6. Changes in bioelectrical activity of cortex and brain subcortical structures during orienting-exploring reaction to rhythmic photic stimulation in 15-day-old rabbit. Upper portion of curves, to flashes 4/sec; lower portion, to flashes 6/sec. Marks as in Fig. 4.

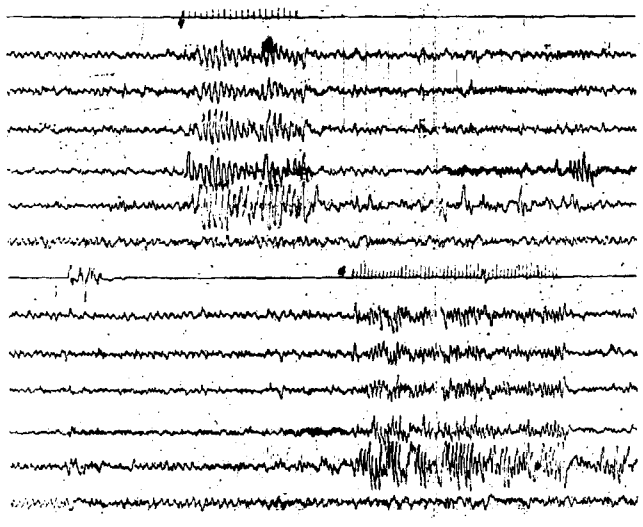

Fig. 7. The same as in Fig. 5 in 18-day-old rabbit.

Thereupon, the 'specific' structures of the optic analyzer often showed a tendency towards an early driving response. The phenomena observed call for their further and thorough study in ontogenesis not only within the system of the optic analyzer, but within other analyzers as well. This study may elucidate the mechanisms for transmission of excitation through the analyzer systems during perception of various external stimuli.

The process of appearance and development of the orienting reaction in ontogenesis, judging by the external manifestation of its components, takes in principle a similar course in all prematurely born animals; *i.e.* all of them at first have a primitive generalized motor reaction which later acquires the features of the orienting-exploring reaction (Table II).

TABLE II

THE TIME OF APPEARANCE OF THE ORIENTING REACTION TO AN ACOUSTIC STIMULUS IN DIFFERENT SPECIES IN ONTOGENESIS (POSTNATAL PERIOD)

Species	*Appearance of:*	
	Primitive orienting reflex	*Orienting-exploring reflex*
Guinea-pig		1st day
Rat	5–8th day	10–12th day
Rabbit	7–10th day	12–15th day
Cat	8–12th day	13–16th day
Dog	9–15th day	16–24th day
Monkey	14–16th day	40–45th day

But the time course necessary for appearance and development of this reaction into the orienting-exploring reaction varies with different species, depending on the period of postnatal maturation, on the place of the animal in the phylogenetic scale and its ecology.

It can best be proved on the example of prematurely born animals (guinea-pig, for one) whose analyzer system activity, judging by the orienting reaction, from the first day of birth corresponds in the main to that of adult animals.

Comparison of both the external manifestation and the time of appearance of the orienting reaction to sound, light, odour, mechanical stimulation of the skin or other external stimuli in all the experimental animals led us to the conclusion that in the neonatal period the contact of the animal with its environment and the elementary analysis of the latter on the basis of the orienting reaction is effected by means of phylogenetically old analyzers (cutaneous, kinesthetic, olfactory, vestibular) whereas in the more mature stages of postnatal life the main role in effecting this contact comes over to phylogenetically newer analyzers (optic, acoustic). It is important that in early ontogenesis, when the brain function of coupling and analyzing is still underdeveloped, the orienting reaction together with other inborn reflexes, provides the elementary analysis of external influences.

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Further, the orienting reaction plays an important role in formation and manifestation of the conditioned reflex. Investigations of recent years have shown that excitation of subcortical structures which takes place upon the appearance of the orienting reaction, exerts tonic influences on the brain cortex, thus providing in it the excitation necessary for coupling of the conditioned connection. However, it is known that the orienting reaction alone cannot ensure a complete and exact analysis of the changes in the environment. For this, participation of the conditioned reflex mechanism is indispensable. Only with the help of this mechanism does differentiation of stimuli attain a high degree of precision, and it is this mechanism which determines the analyzing ability of this or that analyzer as an integrative system.

The study of this problem necessitates studying the question of the time and nature of formation of conditioned reflexes from different analyzers in ontogenesis. The present paper gives a brief survey of this question on the example of formation of motor alimentary and defense conditioned reflexes in different species in ontogenesis.

The experimental results obtained in our laboratory (Volokhov, 1954, 1959; Nikitina, 1954; Panchenkova 1957; Bykov, 1958; Novikova, 1955; Volokhov and Nikitina, 1963, etc.) as well as the data of other authors, despite some discrepancies, give us grounds for believing that in growing animals elaboration of the alimentary motor conditioned reflex takes place earlier than elaboration of the defense conditioned reflex. In ontogenesis elaboration of alimentary motor conditioned reflexes from phylogenetically ancient analyzers (olfactory, skin, vestibular) precedes that from phylogenetically younger ones (acoustic, optic) (Table III).

TABLE III

THE TIME OF APPEARANCE AND STABILIZATION OF THE ALIMENTARY MOTOR AND DEFENSE SHAKING CONDITIONED REFLEXES FROM DIFFERENT ANALYZERS IN DOGS AND RABBITS IN ONTOGENESIS (POSTNATAL DAYS)

Analyzer	*Alimentary reflex appearance*	*Defensive*		
		Appearance of generalized motor reaction	*Appearance of shaking reaction*	*Stabilization of shaking reaction*
	Puppies			
Cutaneous	7–9th day	15th–21st day	26–34th day	42nd–45th day
Olfactory	1st–2nd day	15–19th day	24th–31st day	35–45th day
Vestibular	10–12th day	—	—	—
Acoustic	15–16th day	17–25th day	26–34th day	34–45th day
Optic	22nd–25th day	29th–33rd day	35–40th day	40–60th day
	Rabbits			
Olfactory	1st day	—	—	—
Cutaneous	10th day	11–14th day	12–19th day	12–19th day
Vestibular	—	—	12–15th day	17–19th day
Acoustic	10–11th day	11–12th day	12–13th day	12–13th day
Optic	13th day	11–12th day	11–12th day	15–24th day

As concerns defense conditioned reflexes from different analyzers (in our experiments, predominantly the conditioned reflex of shaking studied according to the method of Volokhov and Obraztsova — see Volokhov and Obraztsova, 1953), the time of their elaboration does not show any particular divergence despite the unequal morphophysiological maturation of the analyzers to the moment of birth. The only exception is a somewhat delayed elaboration of the conditioned reflex from the optic analyzer; from the rest of the analyzers, they are formed approximately at the same time (Table III). Such a proximity in the time of elaboration of defense conditioned reflexes may be due to some peculiarities of maturation of certain relay links of the reflex arcs from the afferent systems to efferent. These links evidently come into operation in each given animal at a particular time.

However, when we consider the time of elaboration of defense conditioned reflexes from different analyzers in different species, a great divergence leaps to the eye. For example, the defensive shaking conditioned reflex in the rabbit in postnatal ontogenesis develops much earlier than that in the dog (Fig. 8) (Table III). In guinea-pigs the defense conditioned reflexes from different analyzers are formed on the 2nd–3rd day of their life (Fig. 9). In general, the time of elaboration of defense conditioned reflexes to the stimulus of the same modality depends on the place of the given animal

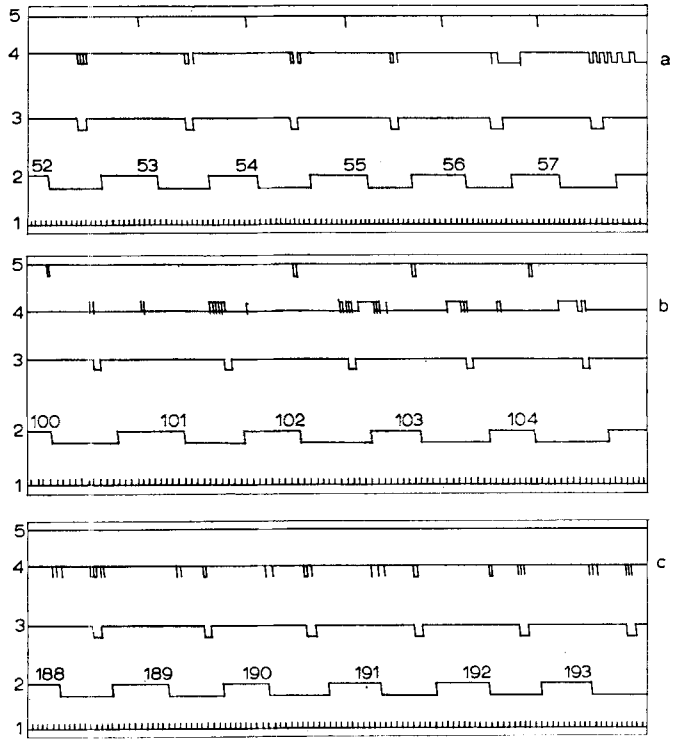

Fig. 8. Formation of defense shaking conditioned reflex to sound in puppy; beginning of elaboration on the 13th day: a, 19 days; b, 27 days; c, 39 days. Marks from the bottom upwards: 1, time in sec; 2, action of conditioned stimulus; 3, action of unconditioned stimulus; 4, shaking reaction; 5, orienting reaction.

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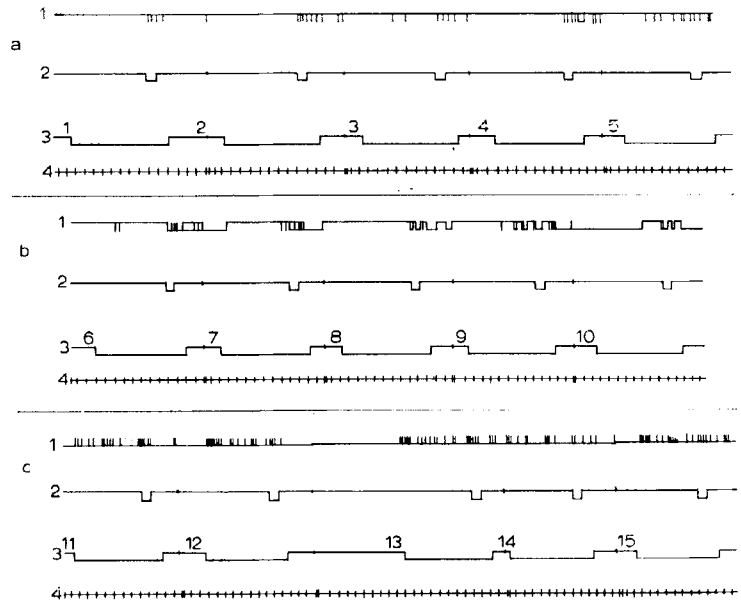

Fig. 9. Elaboration of defense shaking conditioned reflex to sound in new-born guinea-pig; a, period of appearance of conditioned reflex; b, period of unstable conditioned reflex; c, period of stable conditioned reflex. Marks from the top downwards: 1, shaking reaction; 2, action of unconditioned stimulus; 3, action of conditioned stimulus; 4, time in sec.

on the phylogenetic scale and on its ecological peculiarities. As to the nature of these reflexes, from all the analyzers studied in growing animals, the first to appear were always generalized motor reflexes; followed later by specialized conditioned reflexes, for example, the shaking conditioned reflex.

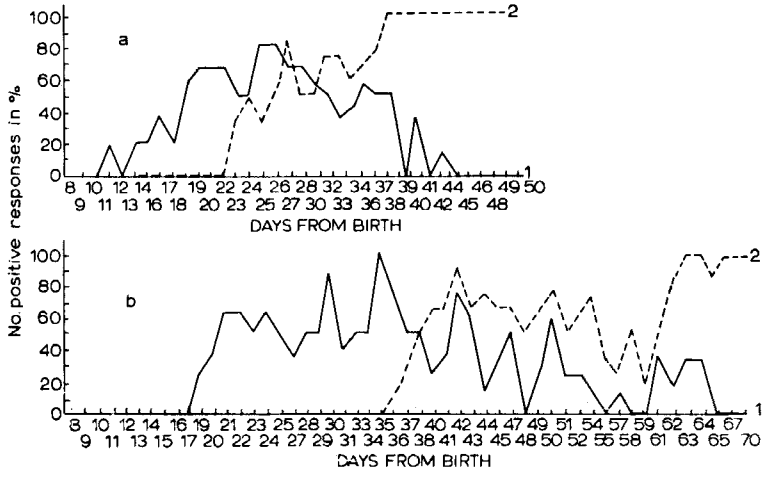

Fig. 10. Development of orienting (1) and conditioned shaking (2) reflexes to sound (a) and light (b) in puppies.

As mentioned above, the defense conditioned reflexes from different analyzers appear approximately at the same time. However, the periods necessary for their stabilization are divergent. For example, in rabbits and dogs a distinct stabilization of the shaking conditioned reflex takes place first from the cutaneous and acoustic analyzer, then from the optic one (Fig. 10).

Stabilization of the defense conditioned reflexes in ontogenesis coincides in time with a marked differentiation of subcortical and cortical structures of analyzers (Troitskaya, 1963; Kalinina, 1956), as well as with intensification of the cortical bioelectrical activity, in particular, with augmented ability of rhythm recruitment for photic and acoustic stimuli. The latter bespeaks an increased functional mobility and lability of the cortical structures. The sequence of stabilization of conditioned reflexes to a certain extent proves the regularity with which specialization and stabilization of conditioned reflexes from phylogenetically more ancient analyzers always come before that from phylogenetically newer analyzer systems.

Even though the formation and stabilization of the positive conditioned reflex undoubtedly testify to the functional activity of higher parts of the analyzer, its integrative activity as the apparatus of analysis and synthesis of the changes in external environment is fully displayed upon formation of positive and negative conditioned reflexes, *i.e.* in the process of differentiation of stimuli. In our laboratory, an investigation of the development of differentiative and other kinds of internal inhibition was carried out on puppies, baby rabbits and baby rats, according to the method of shaking conditioned reflexes. Similar studies according to the method of defense conditioned reflexes were performed by other authors (Chinka, 1953; Troshikhin, 1957, Myslivečck, 1957; Obraztsova, 1961a, etc.). However, in all these experiments the differentiation performed was rather crude, because the parameters of the stimuli applied were not taken into quantitatively precise account. Differentiations were usually elaborated to very different stimuli (metronome sound, tones of generator, light stimuli, mechanical stimulation by touching etc.). The data obtained are mostly concerned with the development of the process of stimulus differentiation for acoustic, visual and cutaneous analyzers; rarely for other analyzers.

As to the time of formation and stabilization of differentiative inhibition in animals of different stages of development, certain differences are marked between phylogenetically older and younger analyzer systems. Differentiations in the optic and acoustic analyzers are stabilized later in ontogenesis, than in other analyzers. However, this question cannot be considered as studied in detail.

Great differences are observed in elaboration of differentiation in animals occupying different places in the phylogenetic scale. For instance, in white rats differentiation of acoustic stimuli at an early age is elaborated with great difficulty and often breaks down. In growing guinea-pigs, differentiation of acoustic stimuli is also elaborated very slowly and is often somewhat relative. In contradistinction to this, differentiation of sounds in kittens when they reach a certain age (32–36 days) manifests itself with much regularity and precision as compared with that in rodents (Fig. 11), although it is elaborated with some delay. In puppies the capability to differentiate acoustic and other stimuli is still higher than in the animals mentioned. It has three distinct

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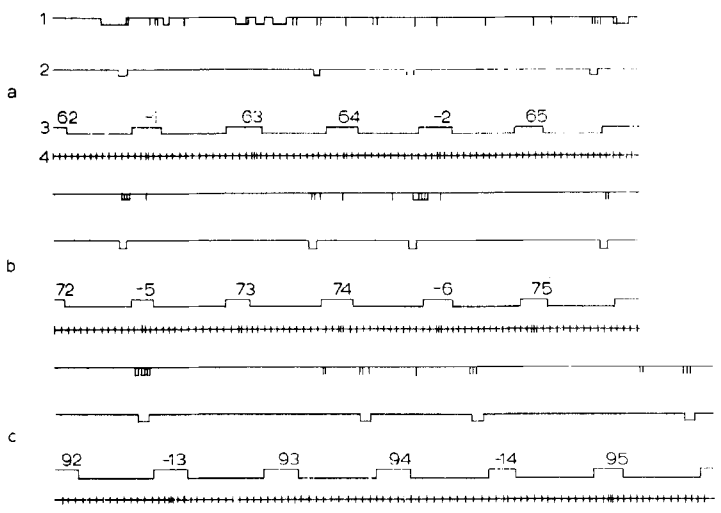

Fig. 11. Elaboration of differentiation to acoustic stimuli in 34-day-old kitten. a, Beginning of elaboration; b, disappearance of positive conditioned reflex after application of differentiative stimulus (figures with minus); c, appearance of differentiation. Marks have the same meanings as in Fig. 9.

stages: (1) narrow irradiation of the generalization process; (2) wide irradiation of the generalization process; and (3) unstable differentiative inhibition. On the second month of the puppies' postnatal life, elaboration of stable differentiation takes place, first in the cutaneous and acoustic analyzer, then in the optic. By the middle of the second month the stereotype of positive and negative conditioned reflexes becomes elaborated to cutaneous, vestibular, olfactory and optic stimuli (Obraztsova, 1961a), *i.e.* by this time the puppy can differentiate complex stimuli.

Comparison of the data on the development of differentiative inhibition to qualitatively the same stimuli (different tones or rhythms of metronome with the intensity 60–70 dB above the threshold of hearing in humans) in rabbits and puppies shows that in the former the ability to analyze stimuli appears at earlier stages, but it is not so regular and precise as in puppies. In monkeys development of differentiative inhibition is not subject to systematic studies. However, judging by the experiments performed in our laboratory (Krylov, 1959), in baboons 1.5–2.5 months old differentiation of acoustic and optic stimuli is very easy and fine.

Thus there are certain grounds for assuming that with the progress of phylogenetic development of the animal the analytico-synthetic activity becomes more perfect which is revealed at comparatively early stages of postnatal ontogenesis.

SUMMARY

Brief comparative characteristics of the analyzer systems of animals in ontogenesis show that development of analyzer functions has certain age stages. Morphophysiological development of some phylogenetically ancient analyzer systems begins at the embryonic stage and manifests itself in a number of defense and other simple reflexes evoked from these analyzers. Functional and structural development of

peripheral, stem and subcortical parts of the analyzer systems is revealed in early postnatal ontogenesis in the form of more complex defense and orienting reflexes. In the first postnatal weeks cortical ends of various analyzers mature and this results in the ability to elaborate multiform conditioned reflexes. Formation and development of orienting and conditioned reflexes from phylogenetically older analyzers (cutaneous, olfactory, vestibular, kinesthetic) takes place earlier than from younger ones (acoustic, optic). Further morphophysiological development of analyzer systems ensures the ability of precise and fine differentiation of conditioned stimuli, thus providing the complex analytico-synthetic activity of the animal's central nervous system.

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On the Problem of the Evolution of the Vertebrate Afferent Systems

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I. M. Sechenov, I. P. Pavlov, A. A. Ukhtomsky, A. A. Zavarzin, L. A. Orbeli, Ch. S. Koshtoyants, K. D. Herrick, and many other scientists have repeatedly stressed in their works that progressive development of the higher regions of the brain and integration of the central co-ordinating mechanisms in the course of evolution, can be explained as the perfection of some afferent systems.

It is well known that comparative physiological studies of afferent systems with various classes of vertebrates are promising for tracing their structural and functional evolution, exploring basically general and individual aspects in the organization of nerve centres, the ways of development of inter-central co-ordination and other nerve mechanisms underlying relations between organism and environment gradually becoming more complex in the process of vertebrate phylogenesis.

The present report is an attempt to demonstrate the ways of the evolution of afferent systems in view of some general problems of evolutionary physiology. This attempt is based on literary sources, and information obtained in our own comparative electrophysiological studies and experiments with conditioned reflexes on various vertebrates.

Our studies were aimed primarily at discovering the functional peculiarities and specific features of projection of various afferent systems on the structures of the endbrain, the tectum opticum of the midbrain and the cerebellum, in all classes of vertebrates. With this in view, we used the method of recording evoked potentials induced by afferent excitation.

First, our information shows that within the ascending line of vertebrates — fish, amphibia, reptiles— the common structure of the central links and the conductor part of analyzers undergo considerable transformation. This is particularly true of the endbrain and the cerebellum and, to a lesser extent, of the tectum opticum of the midbrain. The changes are mainly directed towards progressive enhancement of co-ordination of different analyzers and convergence of specific afferent system in the same regions of the brain.

Separate representation of analyzers is observed in fish in different parts of the brain: the olfactory one in the endbrain, the optic one in the tectum opticum of the midbrain and the analyzer of the lateral line in the cerebellum. We shall now illustrate the above statement.

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Evoked potentials caused by photic excitation are found in fish not only in the tectum opticum but also in the forebrain and the cerebellum (Buser, 1955; Buser and Dussardier, 1953; Karamyan, 1956; Enger, 1957; Schade and Weiler, 1956; Malyukina and Flerova, 1960; etc.). When potentials recorded in different areas are compared, it becomes clear that all of them have some features of similarity and are composed of the same number of fast oscillations superimposed on slower ones, while latencies and the durations of the respective oscillations coincide completely (Fig. 1). As for

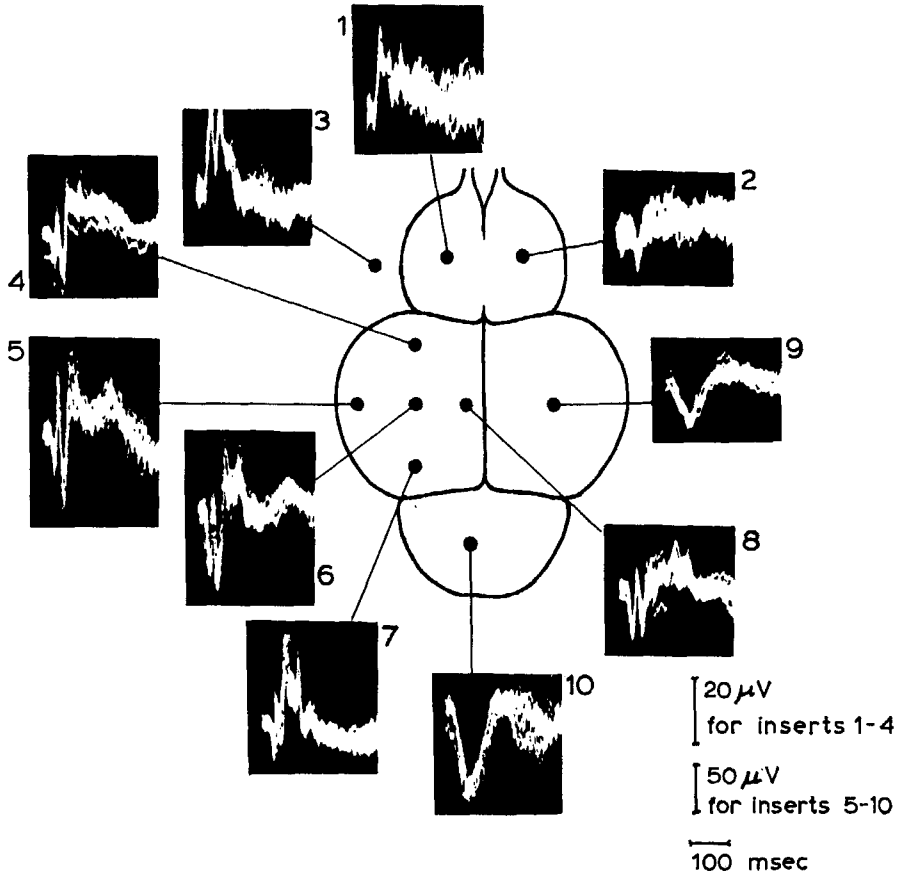

Fig. 1. Shape and topography of evoked potentials recorded on the fish brain with single excitation by light. Excitation by light is applied to the right eye. Leads are shown on the chart for each graph. 1, Lead from the counterlateral side to the side of excitation of the forebrain; 2, from the ipsilateral part of the forebrain; 4–8, from the counterlateral tectum opticum; 9, from the ipsilateral tectum opticum; 10, from the cerebellum; 3, from the adipose matter surrounding the fish brain. Here and afterwards: every shot is built up by superimposition of 10 runs of the oscillograph's ray starting at the moment of excitation. Monopolar lead, electrical negativity of the active electrode corresponds to an upward deviation of the ray.

the variations, they are determined entirely by the ratio of the amplitudes of these oscillations or their polarity. When these peculiarities are regarded in view of a much greater amplitude of response from the tectum opticum, compared with responses from

other regions of the brain, one comes to think that the potentials recorded in the endbrain and the cerebellum might be determined by entirely physical causes resulting from a widespread powerful electric field generated in the tectum opticum. This suggestion is supported by the fact that a similar potential is measured in the adipose matter which surrounds the brain of the fish (Figs. 1, 2). The variations in the ratios of amplitudes and polarity of the response components generated in different parts

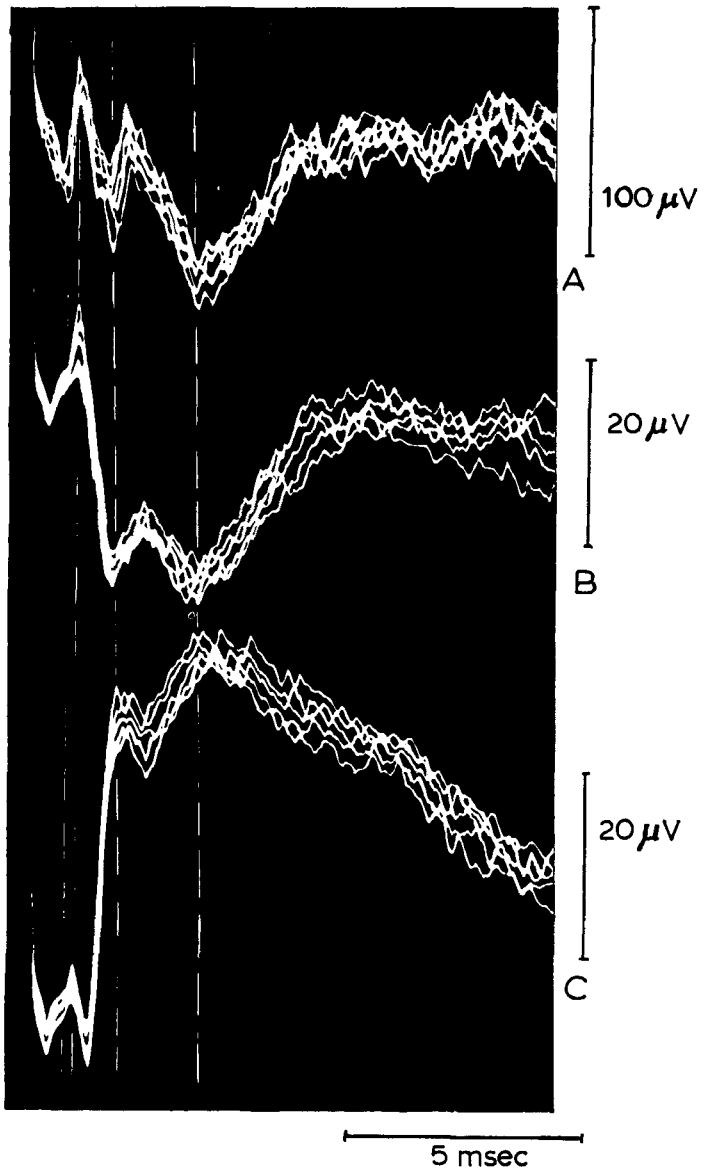

Fig. 2. Evoked potentials in the fish brain to electrical excitation of the optic nerve. A, recorded from the tectum opticum; B, from the body of the cerebellum; C, from the forebrain. Vertical dotted lines denote coinciding temporal constants of evoked potentials.

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of the brain may result from the differences in the structures of the optic lobe generating these components, which involves different spreading of their respective electric fields.

Several series of experiments were conducted to find an adequate solution to this problem. The first of them showed that with extirpated optic lobes (Fig. 3) the potentials of the forebrain and the cerebellum die out completely. True, it may be objected

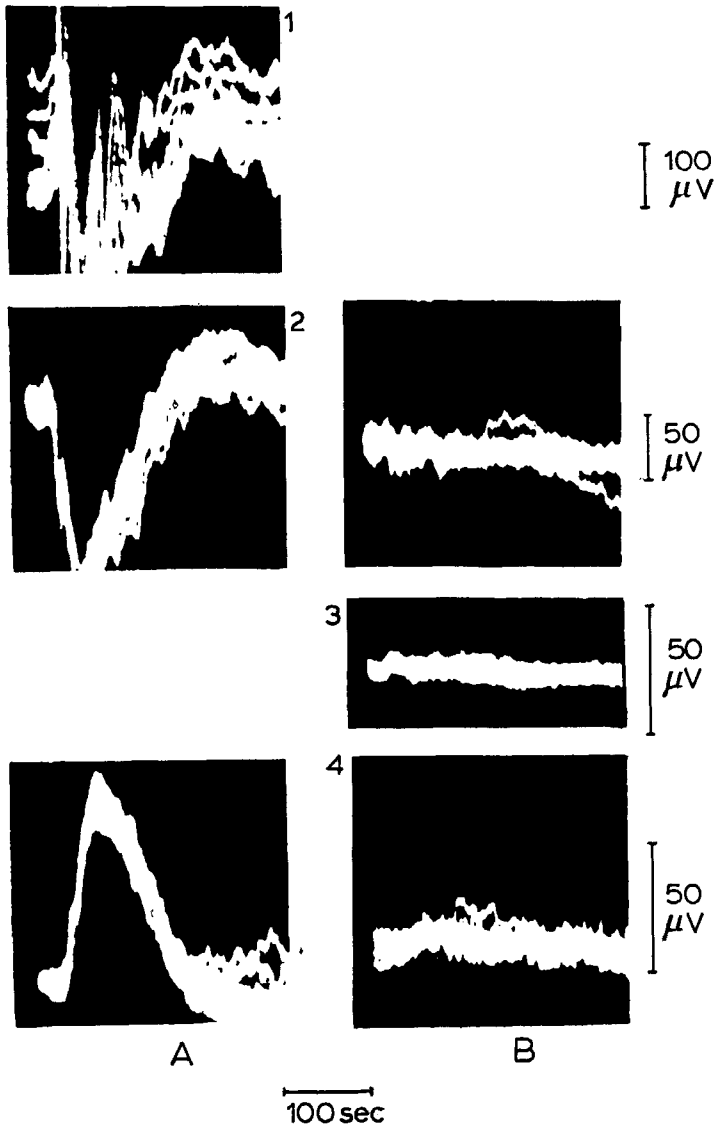

Fig. 3. Genesis of evoked potentials in the fish brain to excitation by light. 1, Recorded from the tectum opticum; 2, from the body of the cerebellum; 3, from the shutter of the cerebellum; 4, from the forebrain, A, normal; B, after removal of the tectum opticum. Single photic stimuli are applied to the eye counterlateral to the lead side.

that either the forebrain or the cerebellum generate their own responses not to direct impulses from the optic nerve but to impulses switched to the tectum opticum. Another objection is that the responses of the forebrain and the cerebellum might be suppressed because of the shock caused by the removal of the tectum opticum. The former objection can be disregarded because of the exact coincidence in response latencies (which can be measured with a tolerance of up to 0.1 msec) of different parts of the brain to electric excitation of the optic nerve (Fig. 2). The latter objection is opposed by the fact that, according to specific studies, removal of some parts of the brain of fish never exercises considerable influence on the response potentials in the other parts to stimulation of the afferent tracts with which they preserved immediate connection.

The artifact nature of the potential recorded in the forebrain under excitation is also confirmed by the observation that severance between the midbrain and the forebrain did not remove potentials in the latter. If the forebrain is removed and replaced with a cotton tampon saturated with physiological salt solution, an 'evoked potential' can be recorded on it.

A similar series of experiments was conducted to study the genesis of evoked potentials in various areas of the brain of the fish under excitation of the olfactory tract and the lateral-line nerve. The result of the experiments presented evidence that the lateral-line analyzer is immediately represented only in the cerebellum, and the olfactory analyzer only in the forebrain; that the evoked potentials recorded in other parts of the brain are artifacts similar to the above-mentioned artifact 'responses' to photic stimuli in the forebrain and the cerebellum.

Thus our results suggest that in fish different afferent tracts are projected on to different parts of the brain. No convergence of direct afferent impulses from different receptors was found in any part of the brain under study.

Nevertheless, a certain functional solidarity between different analyzers can be found in fish. Thus, if one region of the brain is drugged with strychnine and evoked potentials to corresponding afferent stimuli are recorded from another, longer latencies and smaller amplitudes of evoked potentials are generally observed (Fig. 4, A). A similar effect is achieved, by tetanization of the afferent paths in one part of the brain, in evoked potentials in another; for instance, when tetanization of the olfactory tract influences optical evoked potentials in the tectum opticum.

This co-ordination most probably takes place at the reticular brain stem level, as severance of the medulla oblongata from the parts lying above, or anaesthesia with barbiturate, remove it completely (Fig. 4, B). Thus with fish, functional solidarity of analyzers is, evidently, exercised only through the polysynaptic structures of the brain stem.

Much closer relations between analyzers appear in amphibia compared with fish: there emerge straighter patterns leading from different receptors to one region, namely to the endbrain or, more precisely, to the structures of the primordium hippocampi.

Studies of evoked potentials in the frog's endbrain showed that, with excitation of the eye by light or excitation of the sciatic nerve electrically (Fig. 5), similar evoked potentials are recorded from the dorsal surface of the forebrain counterlateral hemi-

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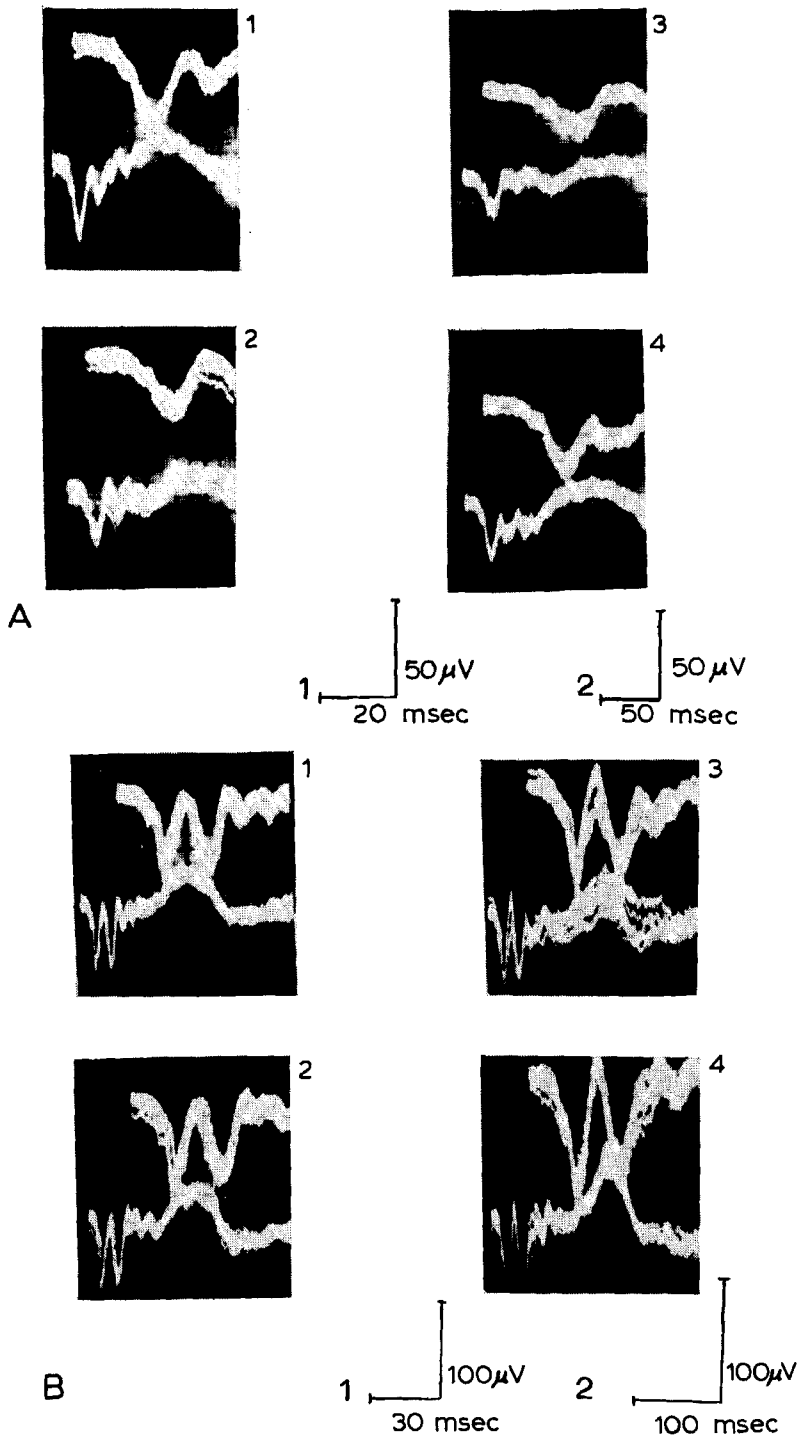

Fig. 4. Co-ordination of the forebrain and the tectum opticum of the midbrain in fish. A, response of the tectum opticum to a single photic stimulus, within normal brain (1), and against the background of strychninized forebrain (2, 10 min after strychnine was applied; 3, in 15 min; 4, in 20 min). The upper ray in each shot, an increased speed of reading; the lower ray, slow speed. Calibration: 1, for the upper ray; 2, for the lower ray. B, the same after transection of the brain stem between the medulla oblongata and the cerebellum. Designation the same as with (A).

sphere. These are surface-negative slow oscillations (150–250 msec) generally preceded by a fast component. Responses to single acoustic stimuli are similar but weaker.

Studies of the distribution of evoked potentials across the dorsal surface of the hemisphere revealed that a slow wave of response to excitation of any modality has the peak of its amplitude in the medial part of the hemisphere, which can easily be

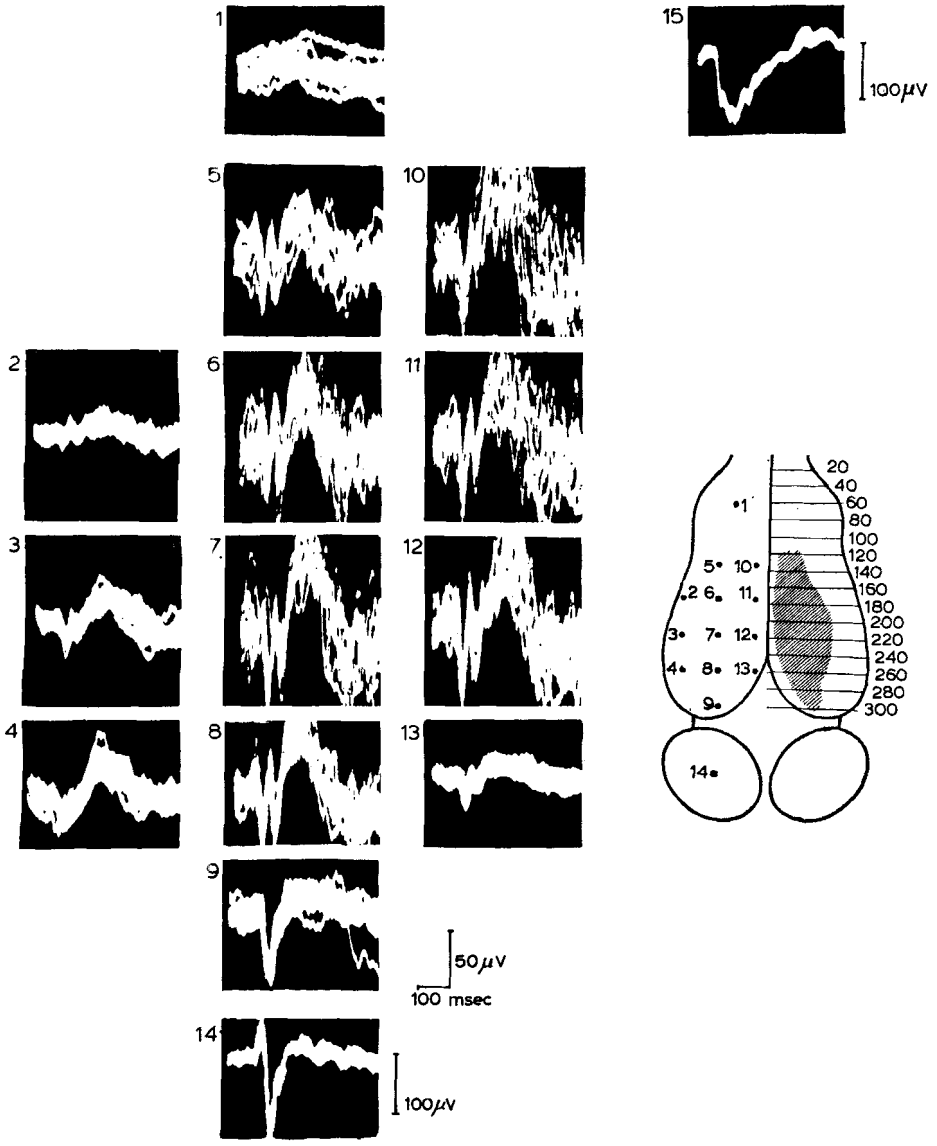

Fig. 5. Shape and topography of the response of the frog endbrain to single photic excitation of the counterlateral eye. Leads for each shot are designated with dots accompanied with their respective numbers on the supplied chart of the frog brain (1, olfactory bulb; 2–13, hemisphere of the forebrain; 14, tectum opticum of the midbrain; 15, electroretinogram). Shading on the right side of the chart denotes the projection of the primordium hippocampi on the dorsal surface of the hemisphere, reconstructed with a series of frontal sections.

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seen in Fig. 5. This area coincides well with the projection of the primordium hippocampi on the dorsal surface of the hemisphere.

Potentials were recorded with depth electrodes placed inside the hemisphere to investigate the genesis of the slow component of responses to various stimuli. One can see in Fig. 6 that a slow electrically-negative wave similar to the surface response is recorded only within a limited area in the dorsomedial edge of the hemisphere. Outside this area it is either not detected at all or has reverse polarity.

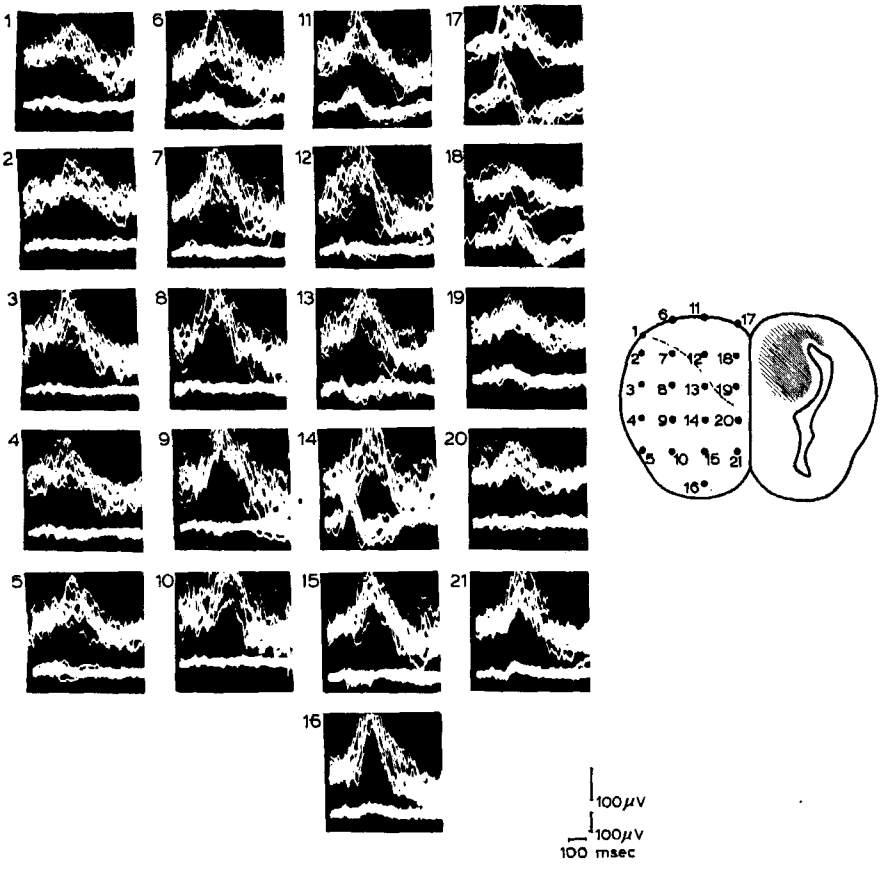

Fig. 6. Distribution of evoked potentials in the frog endbrain to photic stimuli across the volume of the hemisphere. Recorded from a number of points on the hemisphere located in one and the same frontal plane. In each shot: the upper curve is recorded from a surface electrode fixed during the whole experiment; the lower curve is recorded from a depth electrode. The position of the tip of the depth electrode is shown for each shot with a dot supplied with a number on the chart of the frontal section of the hemisphere. In the right side of the chart the area of the primordium hippocampi is shaded.

Consequently, the only area where this wave can be generated is the primordium hippocampi situated in the dorsomedial part of the hemisphere, as the side of the primordium hippocampi facing the surface of the hemisphere assumes a negative electric charge, while that facing the ventriculus becomes electrically positive.

The fact that impulses of different modality converge on one and the same structure — the primordium hippocampi — incites one to further studies directed towards finding out to what extent these impulses are interconnected. Experiments with evoked potentials to paired stimuli of different modality recorded in the primordium

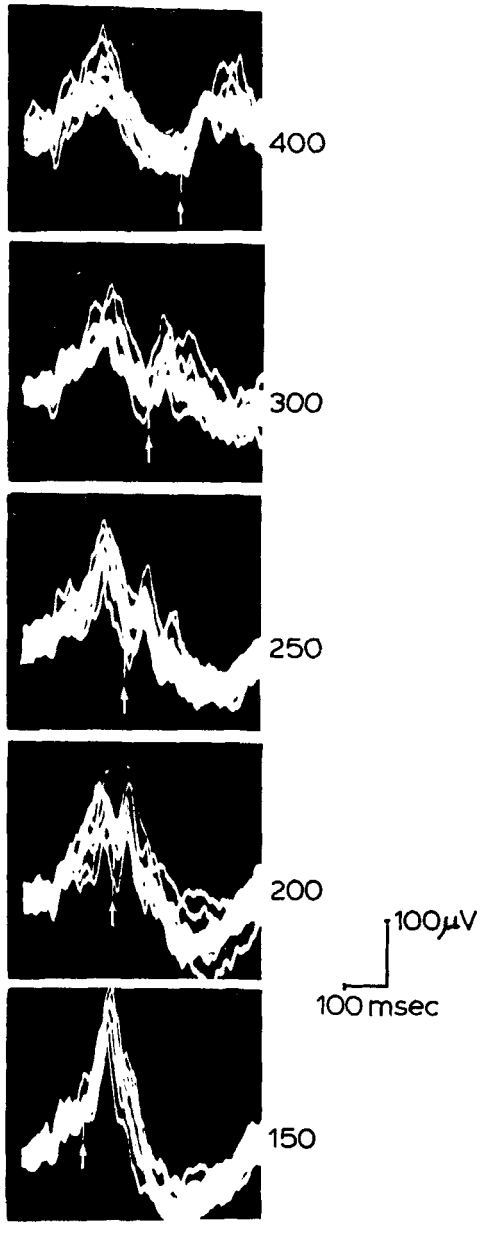

Fig. 7. Co-ordination of evoked potentials in the frog endbrain to stimuli of different modalities. Paired stimuli: light to the counterlateral eye and electrical excitation of the sciatic nerve. Paired excitation is applied when recording begins; the moment of the second excitation is shown by the arrow. The time interval between the stimuli (in msec) is given beside each shot.

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hippocampi (in Fig. 7 it is light-stimulation of the sciatic nerve), showed that induced potentials to the second (test) stimulus are not depressed, no matter how small the interval between the stimuli be, and may be superimposed on the first evoked potential. This information shows that, up to the elements generating evoked potentials, the paths of different kinds of sensitivity in the primordium hippocampi do not coincide, and, probably, the very elements are also separated.

Consequently, with the amphibia different analyzers, though converging to one and the same area of the endbrain, are but loosely interconnected.

In reptiles, further structural integration of afferent systems takes place, different analyzers being not merely represented in one and the same region, but also having a common link at the cortical or subcortical level. The cortical projection zones of different analyzers undergo further differentiation; here the region of projection already embraces not the whole of the hippocampal cortex, as in the amphibia, but is concentrated in its specific part. This may be illustrated with the following data obtained from recordings of evoked potentials in lizards.

With single excitation by light or sound and with somatic excitation, similar responses consisting of an initial surface positive potential, 40–50 msec long, and a slower surface-negative wave, 150–200 msec long, are recorded from a greater part of the dorsal surface of the counterlateral hemisphere in the forebrain. Fig. 8 illustrates the shape and topography of the response to light; responses to sound and excitation

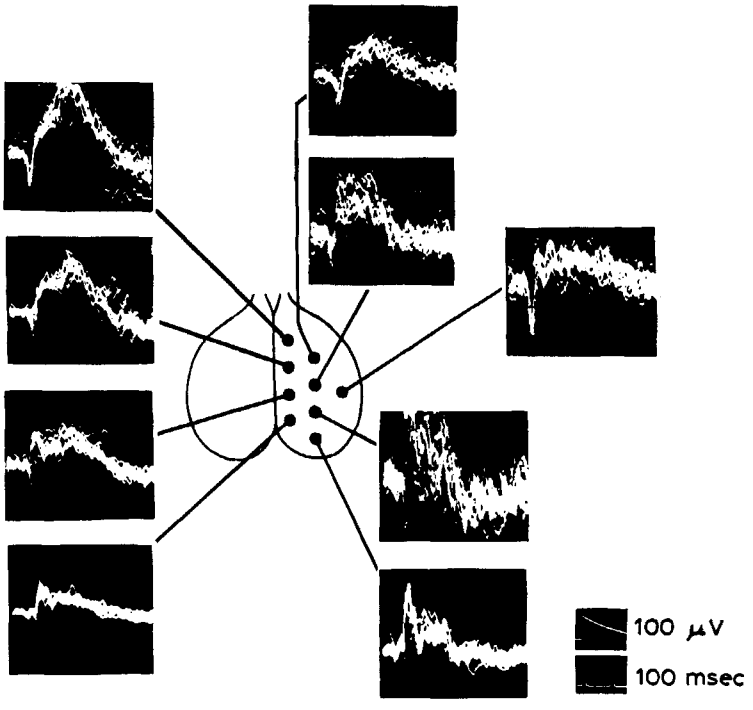

Fig. 8. Shape and topography of responses of the lizard's endbrain to single photic stimulation of the counterlateral eye. Leads for each shot are shown on the chart of the dorsal surface of the hemisphere.

of the somatic nerves are basically the same in their shape and topography and may be omitted from the demonstration.

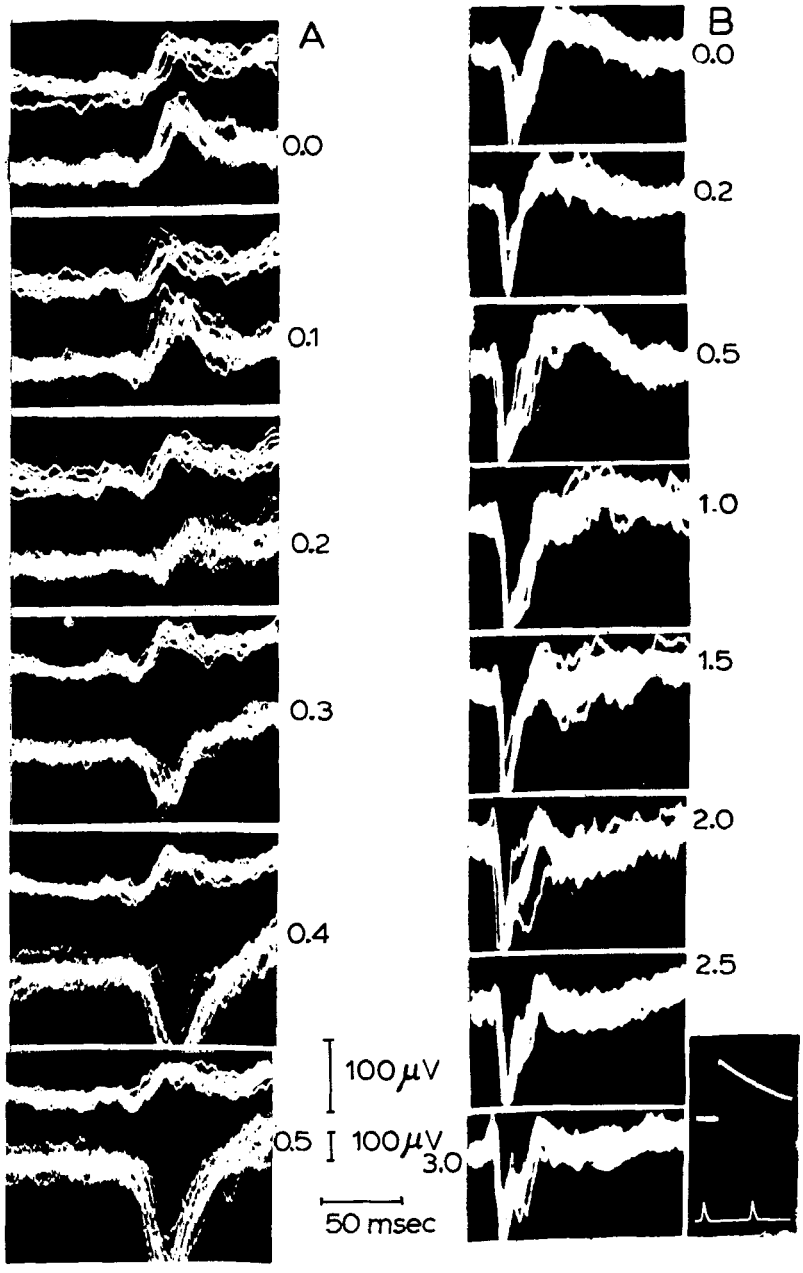

Fig. 9. Reversal of the evoked potential to photic stimuli when the recording electrode is sunk into the hemisphere of the lizard's endbrain. A, electrode inserted in the medialcaudal part of the hemisphere (the upper ray of each shot represents record with a surface electrode; the lower ray, recording with a depth electrode). B, sinking of the electrode in the central part of the hemisphere. The depth of sinking (in mm) is shown beside each shot.

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Within a limited area of the dorsal surface of the hemisphere adjoining its medial-caudal edge, and on the adjoining medial surface, responses are recorded to all kinds of excitation with the same temporal characteristics, but their initial oscillation is of reverse, *e.g.* negative, polarity (Fig. 8). Comparison with the architectonic structure of the lizard's forebrain shows that the area of initially negative responses coincides with the location of the fascia dentata.

In experiments with gradual advancement of the recording electrode at various points of the hemisphere, it was shown that when the electrode passes through the fascia dentatum, inversion of the initial (surface-negative in this region) component of the response is observed: at the depth of 150–200 μ it becomes positive and coincides in shape with the response recorded from the surface of the hemisphere outside the fascia dentata (Fig. 9). When the electrode is submerged at a point outside the fascia dentata, the initial (positive) component undergoes only slight changes in amplitude. These observations prove that the initial component of the response is not generated by the whole of the hippocampal cortex, but by the fascia dentata alone. The record of the response outside this region is probably the result of entirely physical spreading of electric lines of force from the inner surface of the fascia dentata facing the bulk of the hemisphere.

As far as the second (slow) component of the response is concerned, it is not reversed when the electrode is submerged deeper, but reveals changes not only in amplitude but in time as well. Such a slow wave is probably generated by different structures of the hemisphere both cortical and striatic, more or less concurrently.

This interpretation of the genesis of both the response components is also supported by the results of experiments on a lizard whose forebrain cortex had been removed. When the removal of the dorsal cortex does not involve the fascia dentata, the initial component of the response recorded from the exposed striatic structures is similar in shape to the check response recorded from the surface of the cortex (Fig. 10); when the fascia dentata is removed, this component disappears. The second component does not disappear when any region of the cortex is removed, but its shape is transformed considerably (Fig. 10).

To solve the problem of how closely different analyzers converging into the region of the fascia dentata co-operate, the method of coupled stimuli different in modality was used, as it was in amphibia. It was found that, in contrast with amphibia, evident blocking of the lizard's response to the second stimulus is observed, and it is the greater the smaller the interval between the stimuli (Fig. 11). Thus convergence of different afferent fibres in the region of the fascia dentata is not mere space matching of different projection systems, but actual convergence on the same elements, as the paths of various sensations leading to the cortex have a common link at some level, which is either cortical or subcortical.

The cortex of the turtle is differentiated to a still greater extent. Orrego and Lisenby (1962), as well as ourselves, noticed a definite differentiation in turtle cortex between the zones of the optical, olfactory and somatosensory analyzers, which is not so with the lizard. Evidently, after all analyzers were united at the forebrain level in the process of evolution some differentiation between their projection zones started anew within

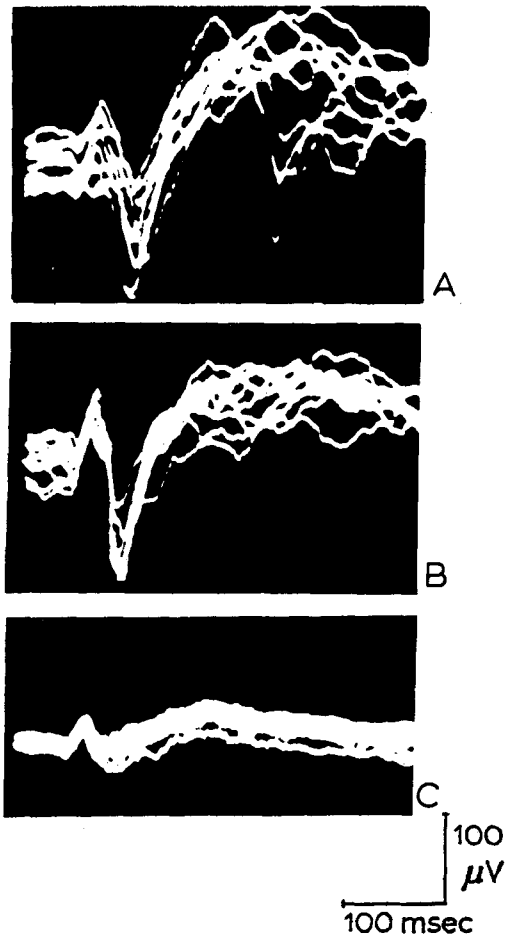

Fig. 10. Effect of decorticating on responses of the lizard's endbrain to photic stimuli. A, normal, recorded from the central part of the dorsal surface of the hemisphere. B, after extirpation of a small (about 1 mm^2) area of the cortex with underlying white matter; recorded from the surface of the exposed striatic structures. C, complete extirpation of the dorsal cortex including the fascia dentata; recorded from the surface of the striatic structures.

the cortex of the forebrain hemispheres, which found its highest expression in mammals. Alongside this, close interconnection of analyzers which had been established by that time was evidently preserved, but differentiation of stimuli became more precise.

In reptiles the cortex must be a highly specialized nerve structure, and it possesses properties of both projection and association zones of the mammalian cortex; this is shown by our observations on the properties of some neurones in the lizard's cortex.

On the one hand, with the cortex neurone we could observe convergence of impulse of different modality, which is most characteristic of the neurones in the associative zones of the mammalian cortex. On the other hand, we could also note the presence

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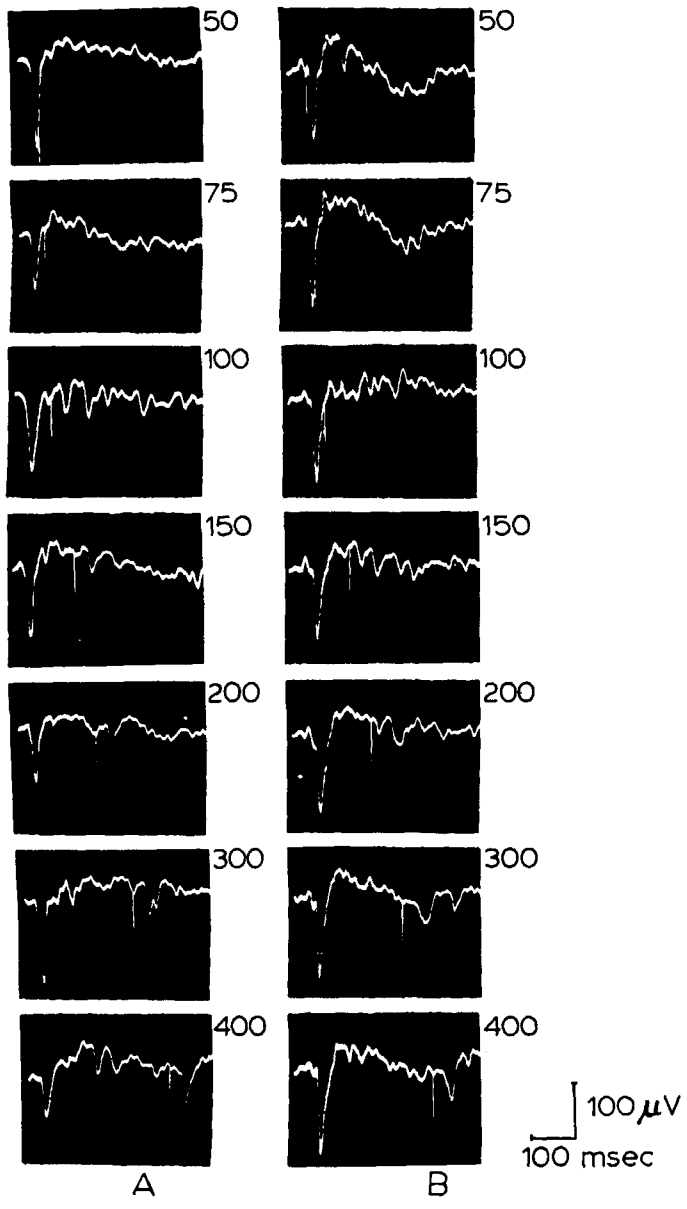

Fig. 11. Co-ordination of evoked potentials in the lizard's endbrain to stimuli of one and the same and different modalities. A, paired similar stimuli of the counterlateral sciatic nerve. B, paired stimuli: light to the counterlateral eye and excitation of the counterlateral sciatic nerve. The first stimulus is applied when recording begins; the time of the second stimulus is shown by the downward artifact. The interval between the stimuli (in msec) is shown beside each shot. Recorded from the central part of the dorsal surface of the hemisphere.

of specific neurones responding to stimuli of one and the same modality with short latencies (Fig. 12), even though polyvalent neurones constitute a great majority.

We can summarize the above statements as follows. There are two basic structural

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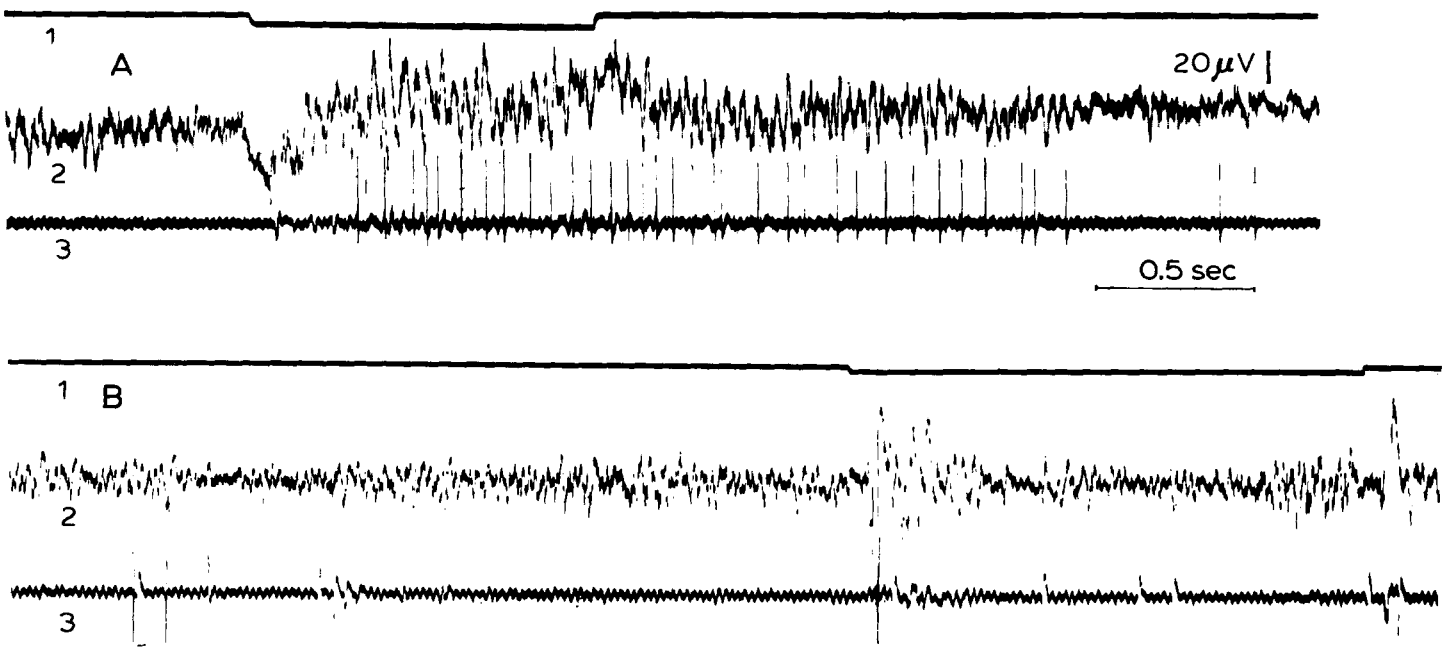

Fig. 12. Reaction of different neurone types in the lizard's cortex to photic stimuli. A, neurone responding with a long burst of activity (simultaneous with increase in summary bioelectrical activity) with a long latency. B, neurone responding with an impulse of short latency: 1, photic stimulus; 2, slow cortical bioelectrical activity; 3, activity of a single cortical element.

types of specific projection systems in the phylogenesis of the vertebrates. The extreme place in the first type is occupied by the fishes in which we observe differentiated representation of the main receptors in different regions of the brain with loose interconnections between the latter. The second type includes the mammals in whom all the main receptors are represented in one region of the brain, namely in the cortex of the cerebral hemispheres, and closest functional ties are established between analyzers. Amphibia and reptiles occupy an intermediate position: though in them receptors are mainly represented in different regions of the brain, they develop specific cortical associative structures in the endbrain which, most probably, plays an important role in the functional co-ordination of the analyzers.

Birds, in our opinion, occupy a peculiar position, and we do not think it appropriate to dwell on this problem in the present paper.

There are two levels of analysis–synthesis in the phylogenesis of the vertebrates. The first level, as shown by our experiments, is characterized by a poorly developed ability of animals (fish) to analyze and synthesize stimuli addressed to different analyzers. This feature is probably connected with the above-mentioned information on separate brain projection of analyzers.

The second type, or level, of analysis–synthesis performed by the vertebrate brain is characterized by better developed ability to individualize single and complex stimuli and unite them into systems, that is patterns. This level of analysis and synthesis is characteristic of birds, and it attains its highest development in higher mammals. This peculiarity of mammalian higher neuropsychic activities must be connected with that type of the analyzer's structure which is characterized by convergence of afferent impulses of different modality on one and the same region of the brain. The cortex of the cerebral hemispheres is known to be such a region in mammals with its association fields, which is probably organized after the pattern of the primordium hippocampi of the amphibia and the endbrain cortex of reptiles.

In the light of the above information, some developmental theories concerning the neopallium, problems of localizing functions in pre-mammal vertebrates, as well as some data on closure of their conditioned-reflex ties, and the role played by this or that part of the brain in performing the closure function, should, in our opinion, be reconsidered. Space limitations prevent us from dwelling on these problems in the present report.

The second part of our report deals with the development of non-specific afferentation in the phylogenesis of the vertebrates, and the proportion of non-specific structures of the brain stem in the overall integrative reactions of the nervous system.

The question of functional phylogenesis of the reticular formation, though raised by many scientists including Kuhlenbeck (1927), Poliakov (1959), Dzugaeva (1959) and Bishop (1962), has not as yet been answered experimentally.

Proceeding from our results, we may summarize that with representatives of all pre-mammalian classes of vertebrates the ascending activating effect of the reticular formation is well developed and expressed the better the higher is the place of the aninal on the ladder of evolution.

Experiments on fish, amphibia, reptiles and birds show that in practically all regions

of the brain, and with all kinds of animals, every external stimulus provokes generalized changes in the background bio-electrical activity expressed either in its enhancement or in depression.

Experiments on repeatedly operated animals (amphibia, reptiles and fish) demonstrated that as orientative reaction to some kind of excitation dies out, generalized reactions also disappear. Application of an external stimulus results in the recovery of the orientative reaction and generalized changes in background bioelectrical activity.

In experiments with applied barbiturates, direct electrical stimulation of the reticular systems of the brain stem and severance of the brain stem at various levels (Fig. 13),

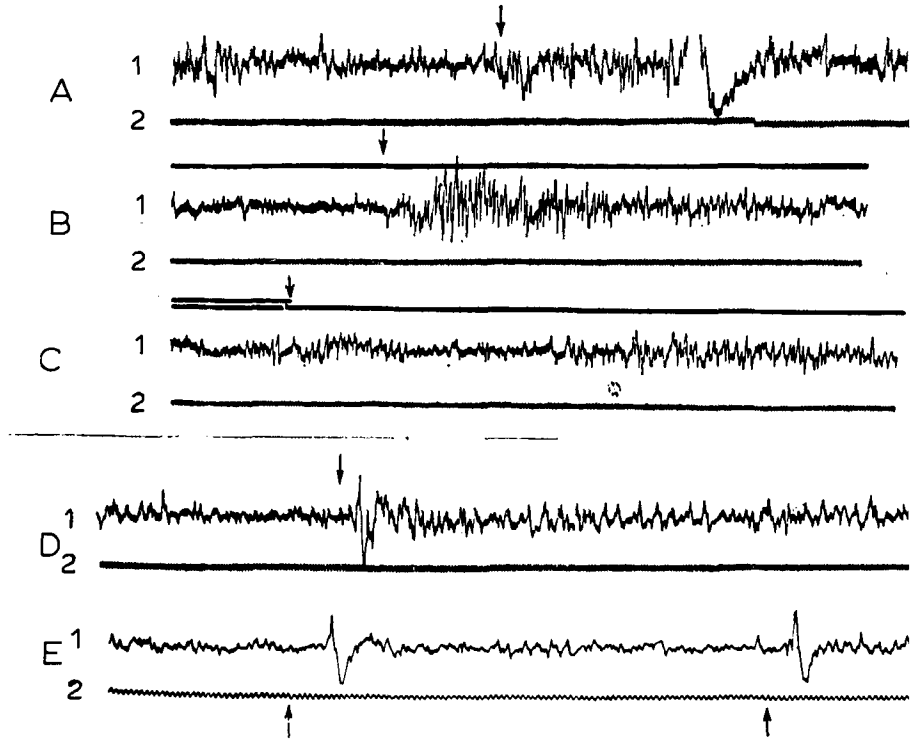

Fig. 13. Generalized reactions in the cortex of the lizard's forebrain. A–C, effect of stem extirpation on generalized reactions. A, normal; B, after a low severance of the brain stem; C, after a high severance. D, E, effect of hexenal anaesthesia on generalized reactions. D, normal; E, anaesthesia. Application of excitation (light) is shown by the arrow: 1, EG of the cortex of the forebrain; 2, time for 50 c/s.

we found that generalized changes in the background activity of the endbrain in fish, amphibia, reptiles and birds are determined by the ascending activating effect of the brain stem and, probably, its reticular formation first and foremost.

This enabled us to draw an analogy between the generalized reaction in EG hemispheres in the animals under study and the arousal reflex in EEG mammals.

The role played by non-specific afferentation in transforming background activity in fish is far less prominent than in amphibia, and it is the greatest with reptiles and

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fishes. With high severance of the brain stem and deep barbiturate anaesthesia, background bioelectrical activity in fish is never suppressed altogether, and photic excitation goes on causing generalized reactions. This is especially true of the tectum opticum where specific optical afferentation must play an important role in maintaining and enhancing background bioelectrical activity.

But in amphibia and, especially, in reptiles and birds, barbiturate anaesthesia and high severance of the brain stem result in almost complete suppression of background bioelectrical activity (Fig. 13).

Our experiments on reptiles (lizard) revealed the closest ties between the structures of the endbrain and the non-specific afferent system. The presence of such ties is proved by the above-mentioned slow electrical negative oscillation of the evoked potential in the endbrain of the reptiles which is probably similar to the secondary response of the mammalian cortex. It is depressed completely under the effect of barbiturates and when the thalamus is destroyed, and is generated concurrently by the cortical and subcortical structures of the endbrain. Similar results were obtained also with birds.

Further, as we have already mentioned, most neurones of the lizard hippocampal cortex are polysensory. The latencies of their reactions range between 100 and 400 msec, *e.g.* their reactions start simultaneously with generalized transformation of summary background bioelectrical activity.

Thus, a greater part of the neurones of the lizard's cortex hippocampi receive their afferent impulses through non-specific polysynaptic systems*.

Alongside this, simultaneous studies of summary slow (both background and evoked) activity and the impulsation of some neurones showed that a summary slow activity is also formed, mainly, due to non-specific impulsation.

Moreover, we think that the slow activity of the lizard's cortex hippocampi is built up primarily by postsynaptic potentials of the neurone bodies, induced by non-specific impulses. This suggestion may be supported with the following observations (Fig. 14): (1) close parallelism between the value of summary slow activity and the frequency of the discharge with most neurones; (2) simultaneous — in most experiments — disappearance of slow and impulse activities under the influence of barbiturates; and (3) a considerable increase in slow activity as the electrode is submerged deeper into the cortex to the level of cell bodies.

The reversal in the permanent potential of the cortex towards surface negativity under the influence of various stimuli reflects the role played by surface dendrites in shaping the electrical activity of the cortex. Thus non-specific afferentation in pre-mammalian vertebrates exercises a considerable influence on the activities of the brain, including the cortical structures of the endbrain.

If we regard the function of the reticular formation in its evolutionary aspect, we must clearly distinguish between its role in co-ordinating the functions of different regions of the brain and its activating function. In the course of evolution the reticular

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* Similar results were obtained by Moore and Tschirgi (1962) in their studies on the dorsal cortex of the alligator.

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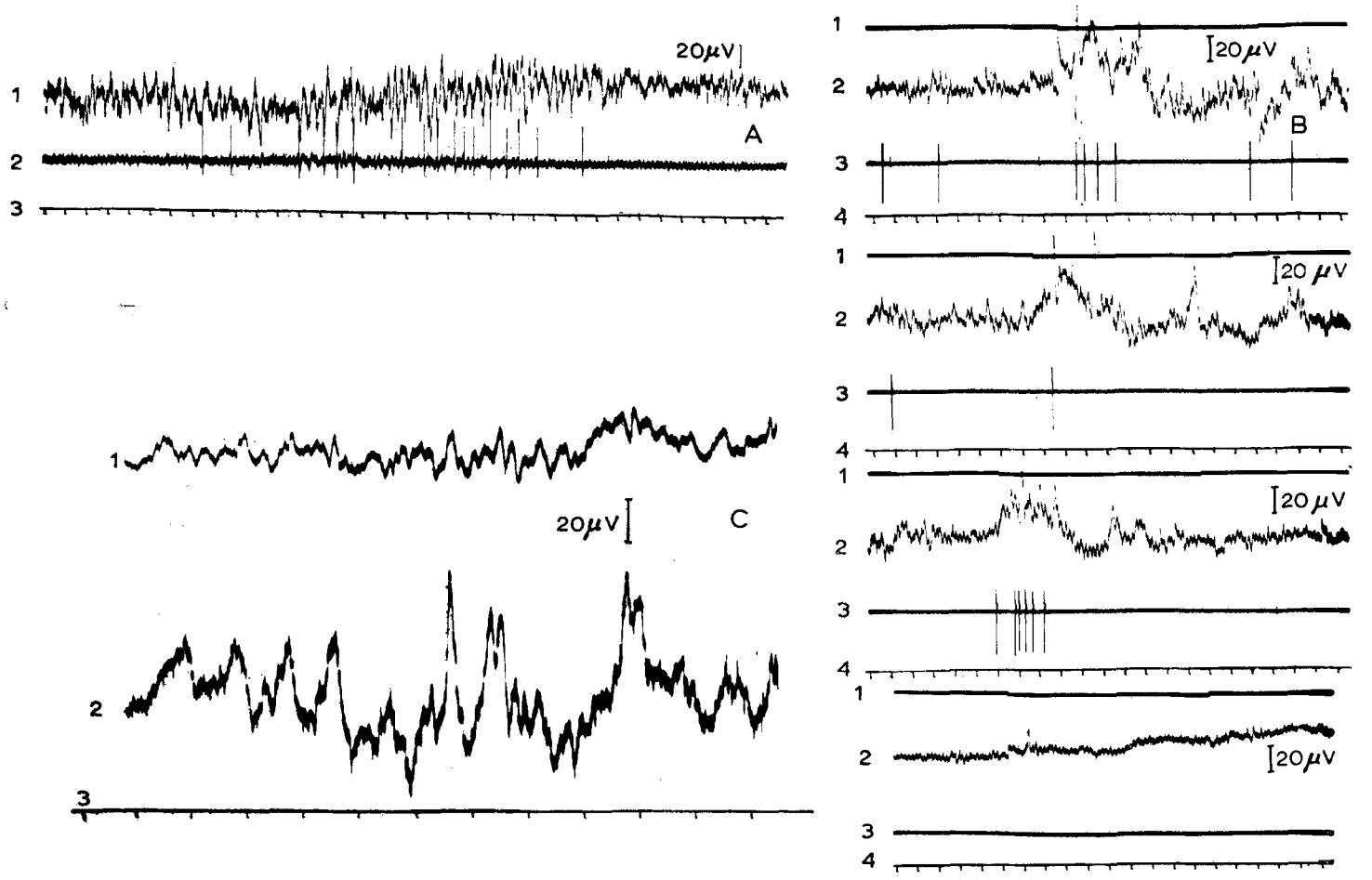

Fig. 14. Connection between slow and impulse activity in the cortex of the lizard's forebrain. A, concurrent appearance of slow activity and burst of activity of a single neurone in spontaneous flash (1, slow summary activity; 2, activity of a single neurone; 3, time, 10 msec). B, concurrent transformation in slow activity and activity of a single neurone under the effect of nembutal anaesthesia. Downwards; normal, 3 min, 5 min and 7 min after introduction of nembutal into the peritoneum. 1, Photic stimulus; 2, slow summary activity; 3, activity of a single neurone; 4, time, 100 msec. C, summary bioelectrical activity recorded from various cortical levels (1, lead from the surface of the cortex; 2, lead from the level of cell bodies; 3, time, 100 msec).

formation of the vertebrates loses its significance as the main correlation and associative centre and begins to play an ever greater role in overall regulation mechanisms, including activation of all the above-lying brain structures and, primarily, the cortex of the hemispheres of the forebrain. With all the above in mind, it is difficult to agree with those scientists who allot the reticular formation universality in co-ordinating functions and associative processes in pre-mammalian vertebrates, to say nothing of mammals.

Thus Bishop (1962) ascribes the main role in integrative activity of analyzers in amphibia and reptiles to the reticular formation alone. Proceeding from morphological information, he thinks that all the basic connections in the amphibian endbrain are exercised through the reticular formation. Our information on separate transmission of impulses of different modality to the primordium hippocampi disagrees with his opinion. Bishop also rejects specific projection of the sensory systems into the reptilian cortex and thus neglects its role in associative functions, referring them to trunk formation. This is why in his evolution scheme he has to postulate a hypothetical 'pre-mammal' animal in which the cortex already had differentiated areas connected with the main sensory systems but devoid of specific sensory projections. But data provided by Orrego and Lisenby (1962) and our information, make it reasonable to suppose that with modern reptiles — primarily the turtle — the cortex satisfies the requirements of Bishop's theory, and, hence, its functional role in this connection must be of extreme importance.

Considerable variations are observed in different classes of vertebrates when their regular 'synchronized' bioelectrical activity is investigated. As evolution progresses, there emerge new kinds of synchronized activity which are not found with older forms.

The only kind of regular (synchronized) activity found with pre-mammalian vertebrates is rhythmical oscillation revealed in specific structures of the olfactory analyzer — the olfactory nerve, the olfactory bulb and the cortex piriformis. Bursts of these oscillations (spindles) generally appear with every respiratory cycle and may be caused by artificially forcing the air through the nasal cavities (Adrian, 1942). Activity of this type is observed in almost every class of vertebrates: amphibia, reptiles, birds and mammals.

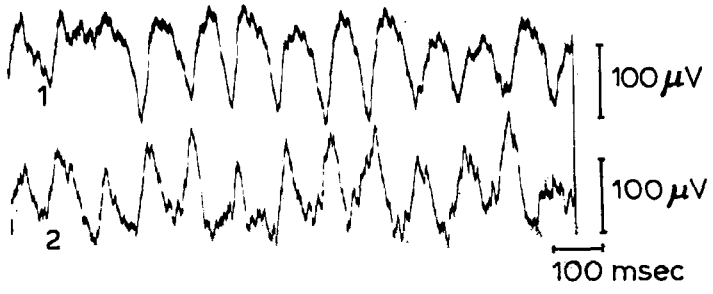

Fig. 15. Regular rhythmic oscillations in the olfactory bulb and the turtle cortex piriformis. 1, Olfactory bulb; 2, cortex piriformis. Oscillations are caused by forcing air through the animal's nostrils.

Oscillations of this kind recorded simultaneously from various part of the olfactory analyzer always preserve a permanent phase ratio. (For example, see Fig. 15; the oscillations recorded simultaneously from the olfactory bulb and the cortex piriformis of the turtle with artificial forcing of air through the nose.) This fact points to the common origin of this kind of activity; evidently activity spreads along the specific olfactory epithelium *via* the olfactory bulb and to the cortex piriformis.

As a rule, regular synchronized activity is best expressed in the olfactory bulb, but it is also distinctly observed in the cortex piriformis. Proceeding from the above information on the probability of broad physical spreading of electrical fields, one might suggest that the synchronized activity of the cortex piriformis also reflects entirely physical or electrotonic spreading of the potential away from the olfactory bulb.

However, this suggestion must be rejected because of the great distance between the olfactory bulb and the cortex piriformis in many animals, and also in view of direct experimental results, namely, comparison of synchronized oscillations recorded simultaneously from these regions shows that these are not exact replicas, or each other's mirror reflections, which they have been with physical or electrotonic mechanisms of spreading, but some of them are shifted compared with the others to a significant time interval (in Fig. 15 (turtle) it is about 10 msec.)

Further, an analysis of the distribution of activity throughout the depth of the olfactory bulb and the cortex piriformis shows that in both formations inversion of recorded potentials is observed when the electrode crosses the diameter of the structure under study (Fig. 16). Consequently, both the olfactory bulb and the cortex piriformis are separate generators of this kind of activity. While one of them (the cortex piriformis) is dependent on the other (the olfactory bulb), the latter serves as a 'pacemaker' for the former. The leading role of the olfactory bulb may be demonstrated by experiments with severance of the olfactory tracts as well as by the direction of the above-mentioned temporal shift of the synchronized oscillations recorded simultaneously from the olfactory bulb and the cortex piriformis (Fig. 15): the bulb potentials forestall those of the cortex by approximately 10 msec.

Synchronized oscillations of the type described here can be recorded not only from the cortex piriformis but from other areas of the cortex as well. In their shape and temporal characteristics they are generally exact mirror reflections of the potentials appearing in the cortex piriformis. This phenomenon makes one suggest that appearance of these potentials outside the cortex piriformis is of entirely physical origin. In fact, when the electrode passed through the bulb of the hemisphere outside the cortex piriformis, the recorded synchronized oscillations were not reverted but only increased as the electrode approached the inner (facing the bulb of the hemisphere) surface of the cortex piriformis.

Consequently, among the cortical structures it is the cortex piriformis alone which directly participates in generating regular synchronized oscillations.

Compared with the other classes of the vertebrates, mammals reveal a much wider diversification of synchronized activities. In addition to the above-mentioned 'olfactory spindles', new kinds of rhythmic activity appear in them, including: rhythmic activity in the septum–hippocampus–neopallium system, in which the septum is the basic

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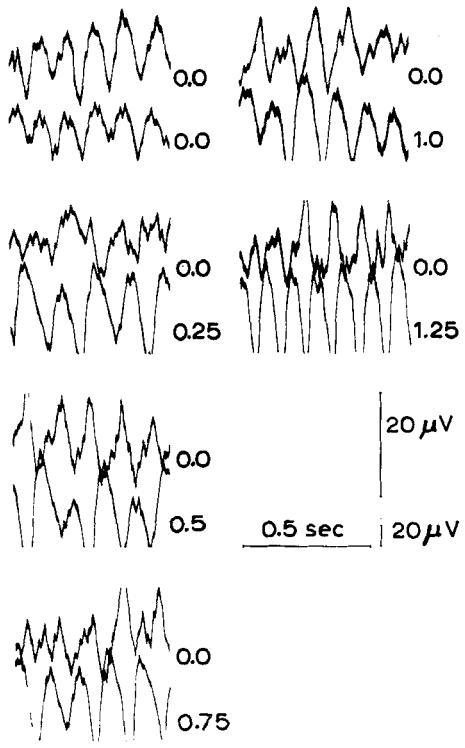

Fig. 16. Transformation in regular rhythmic oscillations of the cortex piriformis with deepening of the recording electrode. In each shot: the upper ray represents recording with a stable surface electrode, the lower line-recording with a depth electrode (figures denote the depth in mm). Oscillations caused by forcing air through the animal's nostrils.

leader; rhythmic activity of the opiate or barbiturate type with its leader in the thalamic nuclei; and, last, activity of the human α-rhythm type the mechanism of which has been least studied. (Many authors draw analogies between α-activity and opiate spindles, but, in our opinion, without sufficient ground.)

Nothing definite can as yet be said about the causes of the new kinds of rhythmic synchronized activity in the vertebrate phylogenesis. In fact, this problem is interconnected with one of the basic problems of modern electrophysiology — that of the functional role of rhythmic electrical activity of the brain. Emergence of new kinds of rhythmic synchronized activity in the process of evolutionary development may be regarded as indirect evidence of the fact that this activity plays some important role in the functions of the brain. It may play a certain role in the functions of closure and processing information received by the brain from the receptors. (Suggestions of this kind have already been found in the literature.) Hence the probable significance of rhythmic activity for processing information is indicated by data obtained both from electrophysiology and from studies of human reflex reactions.

Thus, evolution of afferent systems in vertebrates may be outlined in the following way. At earlier evolutionary levels of the vertebrates (fish), convergence of the major

streams of afferent signals most probably took place in the stem structures of the brain and, primarily, the reticular formation of the brain stem which was connected with the other regions with bilateral communication. Neither the endbrain, the midbrain, nor the cerebellum is the centre where afferent systems converge in fish. These regions of the brain are central links of the respective analyzers, while their interconnections are exercised through the structures of the brain stem.

In the evolution of the vertebrates when the aqueous way of life was abandoned for land, distance reception and, primarily, olfactory reception assume considerable significance. In this connection development of more direct paths between the olfactory and other analyzers is stimulated; paths of the other kinds of sensitivities find their way to the olfactory structures of the endbrain.

In earlier vertebrates these tracts probably have a merely corrective function (Ukhtomski, 1945), that is, they do not as yet serve as conductors of the main streams of information. At this developmental level of the vertebrates the brain and the reticular formation of the brain stem may act as the basic co-ordination and association inter-analyzer centre; the reticular formation's activating influence is still poorly expressed.

In the course of further evolution, the endbrain develops a special apparatus for more exact transmission and selection of incoming information, there appear special lummelar cortical structures which assume an important co-ordinative role. The main regions of the brain analyzers (nuclei) have not yet been shifted into the cortex of the endbrain, but the reticular formations of the brain stem already lose their role as higher correlation centres, and their ties with the central regions of the analyzers become looser and gradually transform their functional designation. One may suggest that at this phase of vertebrate evolution the thalamic structures still exercise diffuse projection of the sensory systems on the cortex of the endbrain. It is only in mammals that, alongside diffuse projection, specific projection areas develop as well.

In this connection note should be made of the changes in the microstructure of the cortical zones in the analyzers.

Morphological and electrophysiological observations show that in pre-mammal vertebrates the synaptic connections of specific projection fibres with the neurones of the tectum opticum of the midbrain and the cortex of the endbrain are, generally, of axo-dendritic nature, while in the mammalian neopallium it is primarily non-specific fibres that are characterized by axo-dendritic ties (Chang, 1956; Akimoto and Creutzfeldt, 1958; and many others).

The significance of such a shift in synaptic terminations is difficult to appreciate. One may suggest that this change predetermined various properties of dendrites (at least optic ones) of the pyramidal cells in the mammalian and pre-mammalian cortex. In the latter, dendrites can probably be stimulated by electric current (Orrego and Lisenby, 1962) and are capable of orthodromic conduction of stimulation (Buser, 1955; and others). As for the major role of cortical neurone dendrites in mammals, it evidently consists in electrotonic regulating of the excitability of the neurones. Moreover, according to Purpura and Grundfest (1957), electrical non-excitability of dendrites in the mammalian cortex is their evolutionary gain, as here accidental interference of alien electrical fields with the regulating function of dendrites is excluded.

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In the course of vertebrate evolution, in connection with progressively complicated relations of the organism with the environment, there emerged a necessity for still more exact and expedient transmission of information throughout the nervous system, which probably resulted in replacement of axo-dendritic ties of specific afferents with axo-somatic ones, and in the development of special regulating systems as non-specific axo-dendritic terminations.

Based on data from the literature and our own observations, we suggest the following theory of this transformation in afferentation. At earlier phylogenetic phases both specific and non-specific afferents form axo-somatic as well as axo-dendritic synapses with cortical neurones. In fish, amphibia and reptiles, in a number of nerve structures (the tectum opticum and fascia dentata of the lizard, projection zones in turtle cortex) axo-dendritic ties of specific afferents dominate over axo-somatic ones, as shown by electrical reactions of the said formations.

In the course of further evolution of cortical formations a greater role in transmitting specific afferent signals is already played by axo-somatic connections. The presence of axo-somatic synapses of specific afferents in the cortex hippocampi and tectum opticum in reptiles and birds is confirmed by Buser (1955), Smirnov and Manteifel (1962), and by some of our observations mentioned above.

Proceeding from the theory presented here, we may suggest that as the number of specific axo-somatic ties increases, the former mechanism of axo-dendritic transmission of specific impulses is gradually reduced, which results in a transformation of the properties of dendrites themselves, and the whole dendrite apparatus consisting of a number of highly specialized cortical neurones is transformed into the apparatus for regulating processes at the same level.

As a result, the role of non-specific afferentation as the overall regulating mechanism becomes still more important.

SUMMARY

Specific features of the different sensory projections in the phylogenetic series of vertebrates were investigated by a method of recording of evoked potentials in the forebrain, tectum opticum and cerebellum. The separate representation of different sensory systems in different regions of the brain (vision in the tectum opticum, lateral line sensitivity in the cerebellum) is characteristic for fish. Sensory interaction in the fish can be accomplished only through the polysynaptic structures of the brain stem. In amphibians the convergence of impulses of different modalities is observed in the forebrain. Yet because of the absence of interaction of evoked responses to paired stimuli the pathways for different sensory systems should still be separate. In reptilia different sensory systems have common relays at cortical and subcortical levels; here the occlusion of the second response with paired stimuli is observed, which indicates the convergence of different sensory pathways upon the same neural elements.

Thus, the two main types of organization of specific afferent systems are shown. Representatives of the first type, with separate representation of sensory systems in different structures of brain are fish. The second type is characteristic for mammals

in which all receptors are represented in the forebrain. Amphibians and reptiles represent the intermediate groups. The role of ascending activating influences of the reticular formation in the integrative processes increases progressively in the phylogenetic series of vertebrates.

Presumably the reticular formation gradually loses its role as the main associative centre, and participates more and more in general regulatory processes. Co-ordination at higher levels is performed by developing structures of the forebrain, where in mammals, together with diffuse sensory projections, the specific projection systems develop. Evolution of microstructures of cortical projection sensory areas is characterized by an increase in the number of axo-somatic synapses. The system of specific axo-dendritic connections becomes the mechanism of regulation of processes, organized at the level of soma.

On a whole the data show that progressive development of co-ordinatory mechanisms of the highest levels of the brain is the result of the development of organization of afferent systems.

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Differentiation of Metabolism in Muscles of Different Function during Ontogenesis

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Neurons are characterized by a high degree of specifity of structure and metabolism (Abood, 1960; Hydén, 1960). Axons establish connections with non-nervous tissues and control their metabolism apparently by two different mechanisms: (a) synaptic contact connected with nerve impulse transmission, rapid liberation of a specific transmitter substance and excitation of the postsynaptic membrane; and (b) long-term regulations, related to 'trophic functions' of the nerve cell which maintain and restore structure and functional capacity of the cells. Generally, we may assume that the synaptic function of the nerve cell is relatively uniform, *i.e.* the neuron operates in a uniform manner inasmuch as its function is connected with characteristic changes in permeability, depolarization or hyperpolarization of the post-synaptic membrane of neuron or non-neuron structures. Compared with this relatively uniform mechanism the 'trophic' long-term regulation of metabolism of muscle, which we assume is also under nerve cell control, shows a much greater variety of mechanisms. These two mechanisms of nerve cell function which establish intercellular metabolic communications are of course interconnected but apparently not identical (Gutmann, 1962). The existence of 'trophic' long-term regulations is probably best shown in neuro-embryological studies (*e.g.* Hamburger, 1954) which demonstrate, as well as regeneration studies, the double dependence (Young, 1946, 1951) between nervous and non-nervous tissues mediated apparently by transmission of nerve impulse and non-impulse (trophic) activity.

However, some of the basic problems of these nerve cell influences discussed by Sechenov in his paper on 'The influence of nerve on tissue metabolism' (1866) have remained unsolved. Sechenov stressed the points that (1) the substrate of the 'hypothetical nerve-influences, *i.e.* basic metabolic tissue processes, cannot so far be tested by direct studies', and (2) 'the solution of the problems will be possible only if their border lines become exactly defined'. Sechenov pointed out that the first task is to differentiate vasomotor functions from direct metabolic 'trophic' nerve functions. A critical analysis of denervation experiments on the vagus and trigeminal nerve did not convince Sechenov of the existence of these hypothetical trophic influences. No doubt vasomotor influences have an important influence on the metabolism of muscle by control of substrate supply from the blood, but they are only one of the nervous

mechanisms establishing metabolic communications between nervous and non-nervous tissues. Sechenov's postulate of differentiation of vasomotor and direct nerve influences on muscle metabolism, however, remains valid.

The experiments reported in the present paper concern the metabolic differentiation of muscles during ontogenesis, and are thought to afford evidence for the existence of direct metabolic 'long-term' connections between nerve and muscle cells, *i.e.* for the 'direct substrate of the hypothetical nerve influences' according to Sechenov.

Comparative muscle physiology has shown far-reaching differentiation of metabolism in muscles of different function, apparently related to adaptation to different functional demands (Needham, 1926; Yakovlev and Yakovleva, 1953). Functional differentiation is clear-cut between the fast or twitch and the slow or tonic muscle fibres of frogs and toads, the latter required for posture and maintenance of tension for long periods of time (Peachey, 1961). A similar differentiation of structure ('Felder and Fibrillenstruktur'), pattern of innervation (multiple and focal end-plates), physiological responses (lack of conduction or conduction of action potentials) and pharmacological reactions (*e.g.* contractures or lack of contractures produced by acetylcholine), (Krüger, 1952; Kuffler and Vaughan Williams, 1953a, b; Sommerkamp, 1928; and others) have also been described in some avian muscles, such as the latissimus dorsalis anterior (LDA) and posterior (LDP) *e.g.* of the chicken (Hess, 1961; Ginsborg, 1960; and others). No exact equivalent of the slow muscle fibres of frogs or birds has been found in mammalian muscles, and the definition of fast and slow fibres in mammalian muscles does not rest on comparable differentiation. However, some structural and metabolic differences are apparently related to 'fast' and 'slow' muscle fibres in mammals, and the terms 'fast' and 'slow' fibres of mamma-

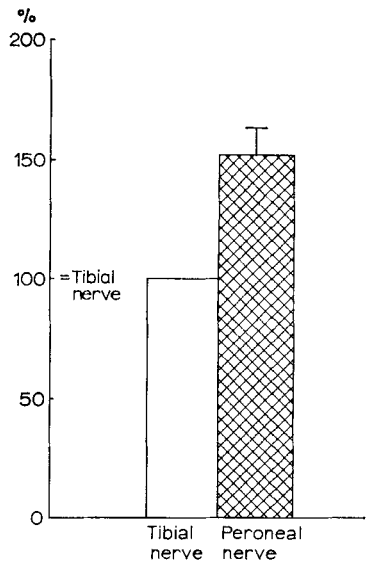

Fig. 1. Incorporation of [^{35}S] methionine into the proteins of the peroneal nerve. Number of impulses/mg protein expressed as % of incorporation into tibial nerve (100%) after 1 h incubation in 5 ml Krebs-Ringer solution containing 1 μC [^{35}S] methionine.

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lian muscles will be used, though this differentiation is probably related to other mechanisms than those found in muscles of frogs or birds. With this specification in mind, fast and slow fibres in mammals can be differentiated especially in relation to speed of contraction.

Ontogenetic studies have shown that all the muscles are slow at birth, and differentiate into fast and slow types during the first weeks of postnatal life (Banu, 1922; Denny-Brown, 1929; Koshtoyants and Rabinovskaya, 1935; Buller *et al.*, 1960a). In our studies we have followed the metabolic differentiation especially of the 'fast' m. extensor digitorum longus and the 'slow' m. soleus of the rat. The extensor muscle has higher levels of glycogen and potassium (on mg protein nitrogen) than m. soleus. This difference is apparently the result of adaptation of the neuro-muscular unit to more frequent conditions of anaerobic metabolism related to fast contractions. It appears that this metabolic difference is related to a differential behaviour of the nerve cells. 'Slow' motor nerve fibres have smaller axons (Eccles and Sherrington, 1930), a slower rate of conduction and a higher threshold of excitation (Eccles *et al.*, 1958; Eccles and Sherrington, 1930). Apparently there are also differences in metabolism of different motor nerve fibres (Fig. 1), *e.g.* there is a higher rate of proteosynthesis and proteolytic activity in the peroneal than in the tibial nerve (Gutmann *et al.*, 1963). This may be related to differences in number or metabolic activity of Schwann cells. The differentiation of metabolism in the two muscles is established progressively (Fig. 2).

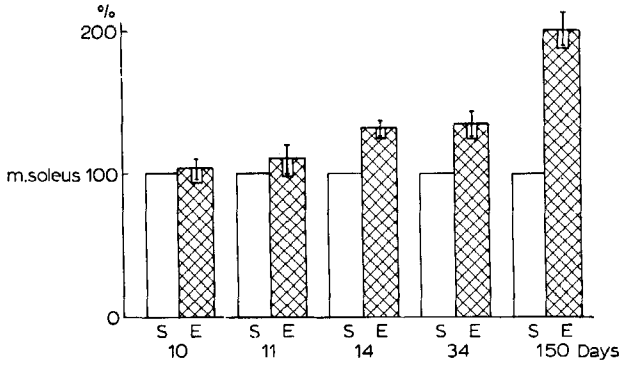

Fig. 2. Glycogen content in m. extensor digitorum longus (in % of the glycogen content of m. soleus) of rats 10, 11, 14, 34 and 150 days after birth. (Drahota and Gutmann, 1963.)

Differences in levels of glycogen are established between the 11th and 14th days of postnatal life (Drahota and Gutmann, 1963). M. extensor digitorum longus also has a higher proteolytic activity than m. soleus, this difference however being established at a later date (Fig. 3) (Gutmann *et al.*, 1964). Denervation and re-innervation experiments demonstrate the neural control of this differentiation of metabolism (Fig. 4). After denervation the differences are lost, but they are re-established with re-innervation of the muscles (Drahota and Gutmann, 1963; Hájek *et al.*, 1964).

A similar loss of differentiation to that in denervation is also observed in muscles in old age (Fig. 5). Many analogous metabolic changes were found in both denervated

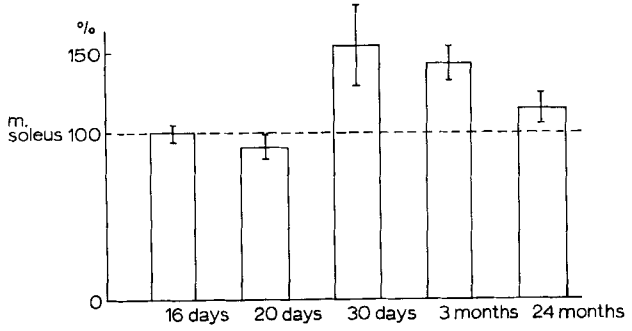

Fig. 3. Proteolytic activity in m. extensor digitorum longus expressed as % of proteolytic activity in m. soleus of rats 16, 20 and 30 days, and 3 and 24 months after birth.

muscles and muscles of old animals, and a progressive decline in trophic functions of the nerve cell was assumed (Drahota and Gutmann, 1961, 1963; Gutmann, 1962). Thus proteolytic activity increases in both denervated muscles and muscles of old animals, the increase being especially related to the sarcoplasmic fractions of the

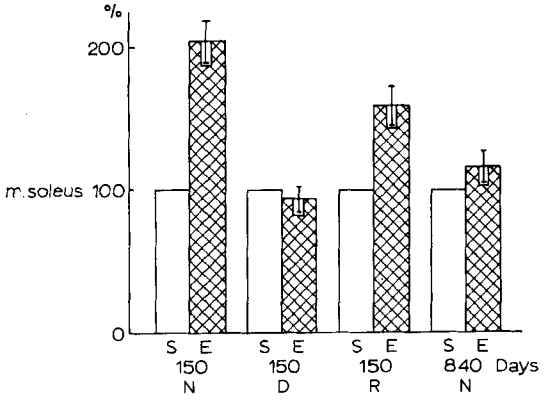

Fig. 4. Glycogen content (expressed as % of glycogen content of m. soleus in normal 150-days-old animals) of denervated (D_{30}, 30 days of denervation) and re-innervated m. extensor digitorum longus (R_{30}, 30 days after crushing the sciatic nerve) and of normal muscles of 840 -days-old rats. (Drahota and Gutmann, 1963.)

proteins (Hájek *et al.*, 1965). With re-innervation the metabolic differentiation is renewed, apparently owing to the progressive re-establishment of the modulating influence of nerve on muscle cell metabolism (Fig. 4). This is best demonstrated by cross-union experiments in which the 'slow' soleus muscle is re-innervated from the peroneal nerve, *i.e.* from a nerve cell which originally supplied the 'fast' extensor muscle. With re-innervation the 'foreign' nerve supply changes the metabolism of the soleus muscle which gains the characteristics of the 'fast' extensor muscle.

After long-lasting re-innervation from a foreign nerve source the soleus muscle was found to have a higher level of glycogen and potassium and a higher proteolytic

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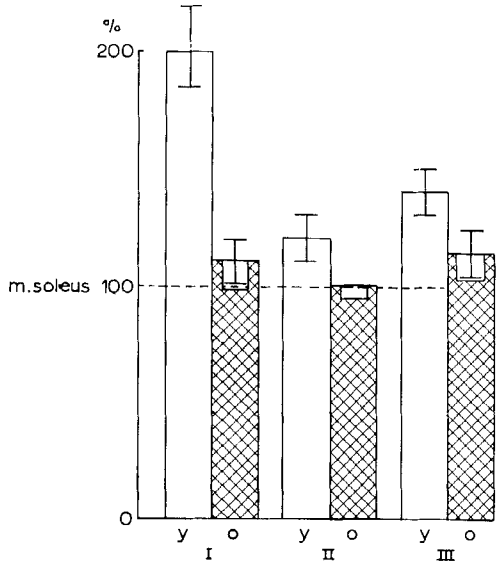

Fig. 5. Glycogen content (I), potassium content/mg PN (II) and proteolytic activity (III) of m. extensor digitorum longus, in % of these values in m. soleus (100%) in young (Y) and old (O) animals.

activity than the control muscle (Figs. 6 and 7). It was also possible to change the speed of contraction of the muscles by cross-uniting nerves innervating muscles of different function, the slow muscle gaining the speed of contraction of the fast one (Buller *et al.*, 1960b).

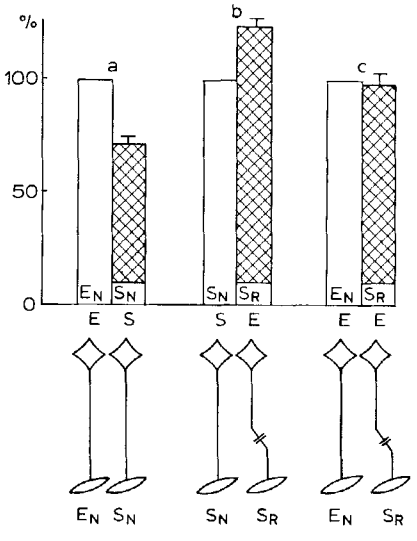

Fig. 6. Content of glycogen in a, normal soleus muscle (S_N) expressed as % of glycogen content of normal extensor muscle (E_N); b, cross-re-innervated soleus muscle (S_R) expressed as % of normal soleus muscle (S_N); c, in cross-re-innervated soleus (S_R) expressed as % of normal extensor muscle (E_N). (Drahota and Gutmann, 1963.)

The mechanism by which long-term regulations of metabolism are mediated by neurons is still not clear. Cross-union of nerves to fast and slow muscles results in transformations of fast to slow and of slow to fast even in adult animals; thus the neural differentiating influence must be continuously operating throughout life (see Eccles, 1963). The differentiation of metabolism could be due to different frequency modulation through fast and slow nerve fibres and the related motor neurons. However, in the cross-union experiments there is very little or practically no recovery of function. Moreover, in the experiments of Buller *et al.* (1960b) a change of speed of contraction was achieved even in muscles innervated from an isolated 'acquiescent' spinal cord segment, *i.e.* after cross-unions, myelotomy above and below the nerve cells and deafferentiation.

A further factor could be a different reaction of the muscles to chemical agents and mediators (Orbeli, 1945; Paton and Zaimis, 1951). The different stages of evolution of the neuro-muscular connections are characterized especially by restriction of chemosensitivity of muscle fibres to the junctional region (Orbeli, 1945). In

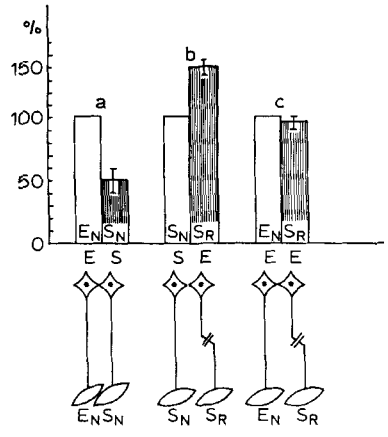

Fig. 7. Proteolytic activity of a, normal m. soleus (S_N) expressed as % of proteolytic activity of normal m. extensor digitorum longus (E_N); b. cross-re-innervated soleus muscle (S_R) expressed as % of normal soleus muscle (S_N); and c, in cross-re-innervated soleus (S_R) expressed as % of normal extensor muscle (E_N).

respect to acetylcholine these relations and their changes during denervation were first shown by Ginetzinsky and Shamarina (1942) and later studied in more detail by Thesleff (1960), Miledi (1960) and Diamond and Miledi (1960). However, there seems to be little differentiation in the reaction to acetylcholine in the fast and slow muscles studied by us, and it is difficult to conceive different types of specific mediators for the muscles concerned.

The denervation and re-innervation experiments suggest that chemical systems mediating long-term regulations operate, and that they inhibit degradation of proteins.

In denervated muscles an increase in proteolytic activity occurs, as also shown by Sheves (1955), and a return to normal levels is observed after re-innervation of the muscles. The increase in proteolytic activity can be correlated with the beginning of

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degeneration of the end-plates. Loss of metabolic connections between nerve fibre and muscle cell sets in about 24 h after nerve section, and coincides with the increase in proteolytic activity (Fig. 8). After a nerve is cut distant from the muscle on one side, and near to the muscle on the other side, proteolytic activity increases first in the muscle whose nerve was cut near to its entry. This finding suggests that the chemical systems in the nerve control the rate of degradation of proteins, and it appears that

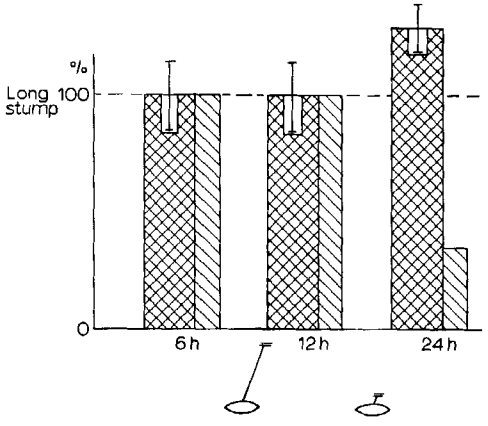

Fig. 8. Shaded columns: proteolytic activity in m. extensor digitorum longus after cutting the nerve near to the entry into muscle as compared with proteolytic activity of contralateral muscle after cutting the nerve high up in thigh (100%). Black columns: percentage of intact end-plates in muscle with 'short nerve stump'. Abscissa: hours after nerve section. (Hájek *et al.*, 1965.)

the increase in proteolytic activity is related to lack or loss of 'inhibitory chemical systems' which after nerve section are apparently exhausted earlier in a muscle with a short peripheral nerve stump. It is clear that the difference between the muscles with short or long peripheral nerve stumps cannot be explained by vasomotor influences or mechanisms connected with nerve impulse activity. It is difficult to believe that long-term nervous regulations mediating 'maintenance' functions in the muscle are due to the action of only one 'key substance' or mediator, though sub-threshold release of acetylcholine apparently plays an important role.

It is however possible, and this is suggested by the experiments reported here, that inhibitory nervous mechanisms operate in neuro-muscular metabolic connections. It will be interesting to find whether and in what way these inhibitory nervous mechanisms might be connected with mechanisms controlling chemo-sensitivity of the muscle membrane to acetylcholine, which apparently are not similar to acetylcholine (Miledi, 1963). Study of these inhibitory mechanisms appears to be an important task in neurophysiological studies. The studies of nervous mechanisms, especially of the inhibiting spinal reflexes, by Sechenov represent a forerunner of the exact analysis of inhibitory influences on nerve cells (see Eccles, 1963). Studies of neural inhibitory mechanisms affecting muscle metabolism may very well be another aspect of neurophysiological and neurochemical investigations, and it appears that

these studies should progressively lead to the discovery of the 'substrate of the hypothetical nerve influences' postulated by Sechenov.

SUMMARY

Metabolic differences in 'fast' (m. extensor digitorium longus) and 'slow' (m. soleus) muscles of rats were studied during ontogenetic development. The extensor muscle has higher levels of glycogen, potassium (on nitrogen basis), and proteolytic activity than the soleus muscle. These differences are established progressively during ontogenetic development, and are lost in muscles of very old animals. On the basis of the analogy of these changes with those observed in denervated muscles some age changes in muscles are interpreted as a result of a decline in the trophic function of the nerve cell during old age.

During denervation some metabolic differences between the muscles are lost, but they are re-established with re-innervation. Cross-union of the nerves changes the metabolic pattern of the muscles, the 'slow' muscle (re-innervated from a nerve cell previously innervating a 'fast' muscle) acquiring the metabolic characteristics of the 'fast' muscle. Some indications of the nature of these 'long-term regulatory influences' of the nervous systems are given by experiments concerning nervous influences on degradation of proteins in muscle.

Proteolytic activity increases during denervation, and returns to normal during re-innervation of the muscle. Increase in proteolytic activity in a muscle after denervation can be correlated with the onset of degeneration of the end-plates, and occurs later in a muscle with a 'long' peripheral nerve stump.

Hence it is improbable that vasomotor or nerve-impulse activity is involved. It is therefore suggested that long-term regulatory ('trophic') functions of the nerve cell are also mediated by inhibitory mechanisms regulating the degree of degradation of proteins in the muscle.

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Some Problems of Elaboration of a Temporary Connection in the Prenatal Period

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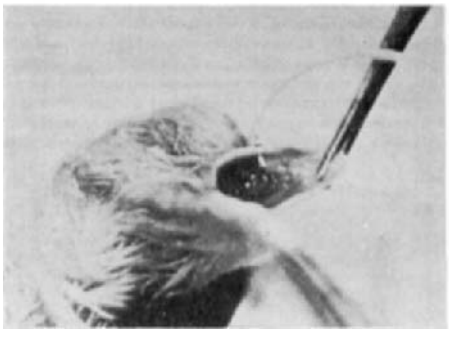
One of the most important problems that always attracts the attention of physiologists and evolutionists in the field of higher nervous activity consists in the question when did the very first conditioned reflex appear in the ontogenetic development of the organism. The solution of this problem has not only descriptive but also theoretical importance for understanding the evolution of one of the main mechanisms in the integrative activity of the central nervous system.

Without going into detail we may say that improvement in methods of approach and careful observation of ecological conditions of a new-born organism have shown that some animals and even man have the first conditioned reflex from the moment of birth.

This led us to start investigating the problem whether the first temporary connections may arise in the prenatal development of the organism (Sedláček, 1962a, b, c, 1963). To study this problem, we experimented on chick and guinea-pig embryos.

Experimenting on chick embryos we elaborated temporary connections either on the basis of a general motor reaction to electrical irritation or on the basis of an unconditioned deglutitive reflex. In both cases the unconditioned stimulus was accompanied by a 3000 c/s tone which did not cause any motor reaction of the embryo per se. Fig. 1 is a view of the chick embryo head with a needle through which a liquid is delivered into the beak. This liquid initiates deglutitive motions recorded by means of an electromyograph. Fig. 2 shows the general position of the embryo in the in-



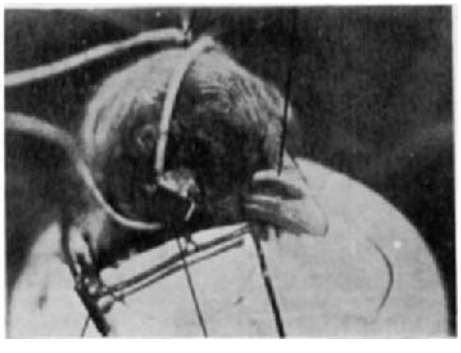


Fig. 1. Chick embryo head with needle for delivering liquid into beak.
Fig. 2. Chick embryo head with EMG- and EEG-electrodes.

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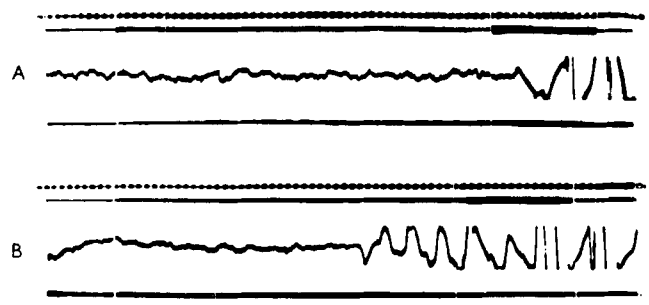
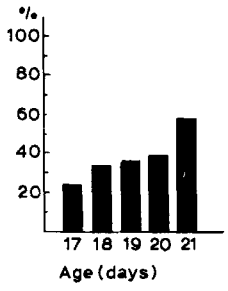


Fig. 3. Electromyogram of deglutitive motions of 18-day chick embryo prior to (A) and after (B) elaboration of temporary connection. Curves up to down: time, 0.1 sec, signal (first thick section of the line) and unconditioned (second thick section of the line) stimuli; electromyogram (sensitivity, 15 μV/mm), zero line.

cubator provided with EMG- and EEG-electrodes. Fig. 3 illustrates one of the EMG-recordings of deglutitive motions prior to and after elaboration of a temporary connection.

Through the new methods of approach we have shown that the first indications of a temporary connection in the chick embryo may be observed at the end of the 16th day of incubation (*cf.* Gos, 1933a, b; Hunt, 1949; Blinkova, 1960, 1961).

Fig. 4 shows the development of efficiency of elaboration of a temporary connection on the basis of a general motor reaction, and Fig. 5 on the basis of a deglutitive reflex. The index is the percentage of positive response signals to a sound stimulus. As may be seen, the efficiency of a temporary connection between the 17th and 21st days of incubation increases, but — and this is of great importance — not in a straight line.



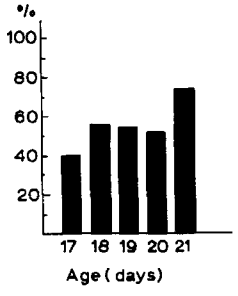


Fig. 4. Effectiveness of defensive temporary connection in chick embryos: combination of tone with electric irritation. The X-axis, age of embryos in days of incubation; the Y-axis, percentage of positive response signals.

Fig. 5. Effectiveness of deglutitive temporary connection in chick embryos — combination of tone with deglutitive reflex. Notations as in Fig. 4.

The efficiency sharply rises in the period between the 17th and 18th day and then between the 20th and 21st day; between the 18th and 20th day the efficiency remains unchanged.

These results raise the question whether this irregular increase in the temporary connection efficiency is just a quantitative indication of formation of a temporary

connection or if it shows some qualitative changes in the mechanism of the switching function of the central nervous system.

Fig. 6 illustrates a spectrogram of positive response signals during one experiment: 20 combinations with embryos of different ages. It is evident that in a 17-day embryo these signal reactions arise in a small number (1–2 per experiment) and separately.

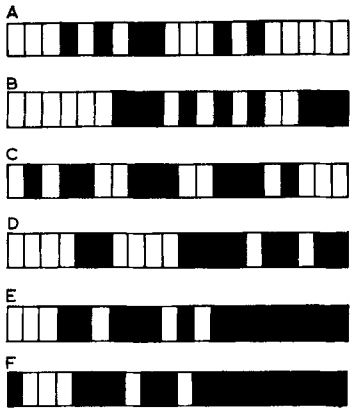


Fig. 6. Appearance of positive responses to 20 signal stimuli in chick embryos and in chicks. White columns, signal stimulus without response; black columns, positive response. A, 17-day embryo; B, 18-day embryo; C, 19-day embryo; D, 20-day embryo; E, 21-day embryo; F, 5-day chick.

The older the embryo the more are the reactions combined into groups so that we may say that the 21-day embryo has an actual temporary connection which manifests itself in continuous groups of positive response signals in the second half of the experiment.

Thus we have analyzed various parameters of the temporary connection elaboration process in chick embryos. Table I schematically shows the results of some groups of experiments: the analysis of strychnine action (Fig. 7), the action of signal and unconditioned stimuli, the effect of lag period duration, influence of stimulus coincidence

TABLE I

RESULTS OF EXPERIMENTS (FROM POSITIVE RESPONSES)

Experimental conditions	*Embryonic age*		
	17th day	*18th–20th day*	*21st day*
Increase in signal stimulus effect	Up	Up	Up
Increase in unconditioned stimulus effect	No effect	Down	Down
Effect of constant phonal stimulation	No effect	Up	Down
Increase in time for coincidence of signal and unconditioned stimuli	No effect	Up	Down
Increase in lag time	Down	Indefinite effect	Up

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duration, the analysis of phonal continuous stimulation value, of differential appearance, of generalization range and other parameters.

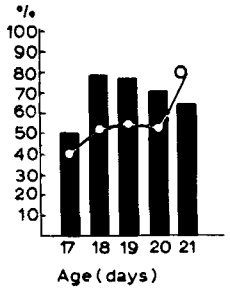


Fig. 7. Effect of strychnine on effectiveness of temporary connection in chick embryos. The X-axis, age of embryos in days of incubation; the Y-axis, percentage of positive responses. Line, reference embryos; columns, after application of strychnine.

The experiments have shown that our concept of the presence of nodal points in the development of a temporary connection mechanism is in agreement with the observations and that these nodal points are the result of quantitative evolutionary changes in the entire mechanism of a temporary connection. Based on the data obtained up to now we advance the following working hypothesis.

The first temporary connections on the 16th and 17th day of incubation are effected through the mechanism of simple summation reflex as a volatile adventitious sign of the combination of two stimulated points probably located in the lower part of the central nervous system and in no way affecting the activity of the central nervous apparatus. In the period between the 18th and 20th day the temporary connection is made on the basis of a dominant principle, on the basis of a dominant centre of excitation caused by unconditioned stimulation, which is capable of maintaining previous stimuli and thus facilitating the switching process. Only from the 21st day does the temporary connection begin to be based on the conditioned reflex mechanism whose effect depends not only on the chronic state of excitation centres but also on that of the manner of combination.

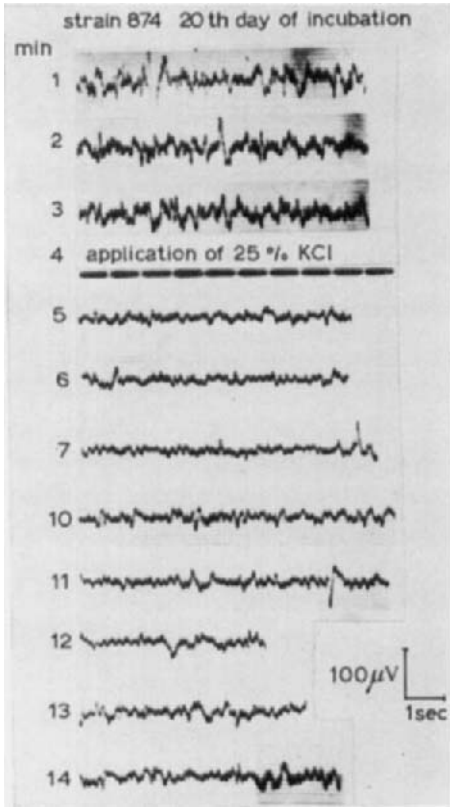
This is one of the cardinal problems of our investigation to be detailed next to prove the correctness of our supposition.

The other problem that confronts us is whether there are also distinctions in the structural apparatus of the switching process apart from supposed functional differences among the separate forms of a temporary connection. We have just started to investigate this problem. Although we cannot elucidate the problem completely we want to present the results of our experiments which show that this problem can be solved.

We have used the test procedure of spreading EEG-depression caused by application of a 25% potassium chloride solution to the surface of one cerebral hemisphere of chick embryos after 20–21 days of incubation. Even a 20-day chick embryo allows the initiation of a spreading EEG-depression. And this is another problem we are working

on. Without entering into detail we want to note the influence of this phenomenon upon the effectiveness of a temporary connection.

Figs. 8, 9 and 10 show how the electrical activity of surface structures of cerebral hemispheres changes under the suppressing action of potassium chloride. As may be seen, the amplitude of cerebral potentials sharply decrease within 60–120 sec. With the cerebrum being in such a state, we have experimented in producing temporary



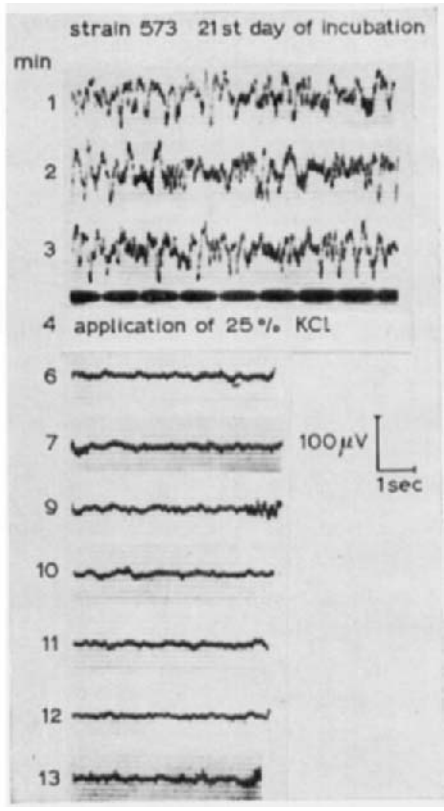


Fig. 8. Development of EEG-spreading depression in 20-day chick embryos.
Fig. 9. Establishment of EEG-spreading depression in 21-day chick embryos.

connections on the basis of a deglutitive reflex. From Fig. 11 we may see that whilst a depression had no effect upon the number of positive signal reactions on the 20th day of incubation, it reduced the effectiveness of a temporary connection by approximately 50% of the initial value on the 21st day of incubation.

From these results and those obtained by Bureš *et al.* in 1958 and 1960 we may assume that only from the 21st day of incubation do the higher parts of the central nervous system start to participate in elaborating temporary connections.

Proceeding from the results obtained in experiments on chick embryos we have also experimented on embryos of guinea-pigs, from the 60th day of pregnancy till

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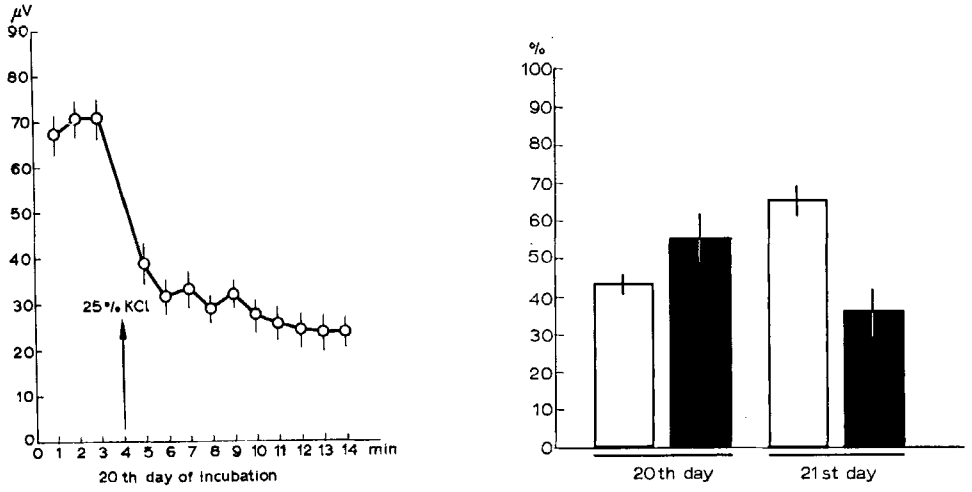

Fig. 10. Averaged data on change of EEG-amplitude during spreading depression in 20-day chick embryos.

Fig. 11. Influence of EEG-spreading depression on effectiveness of temporary connection in chick embryos. The X-axis, age of embryos in days of incubation, the Y-axis, percentage of positive responses. White columns, reference embryos; black columns, embryos with depression.

birth. Experiments were performed by combination of sound signal stimulus with electrical excitation, which brings about a general motor reaction.

As a result we have been faced with an unexpected situation. When a temporary connection had been created without disturbing the fetoplacental blood circulation in the fetus (Fig. 12), we were not successful in elaborating this connection. But it was sufficient to tie down the umbilical cord so as to obtain 50% of positive signals in response to a signal stimulus after 15 to 20 min of pulmonary functioning (Fig. 13).

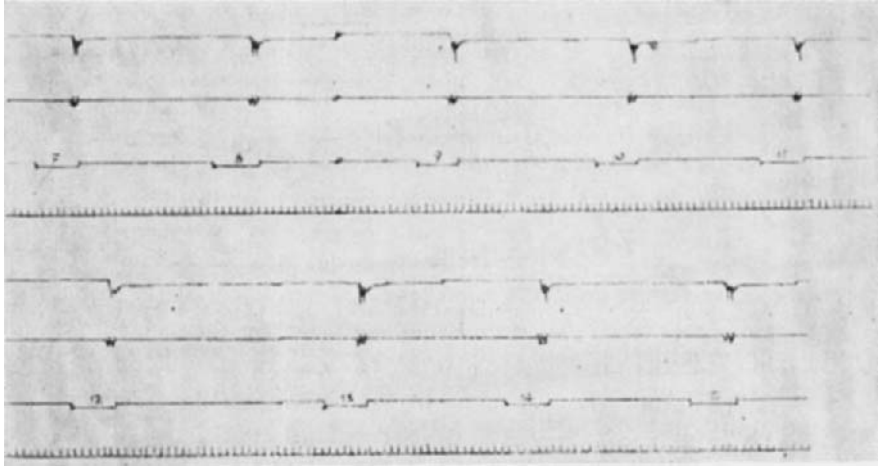

Fig. 12. Elaboration of temporary connection in 66-day guinea-pigs prior to tying of the cord. Curves up to down: embryo motory response, mark of electric stimulus, mark of conditioned stimulus, time in sec.

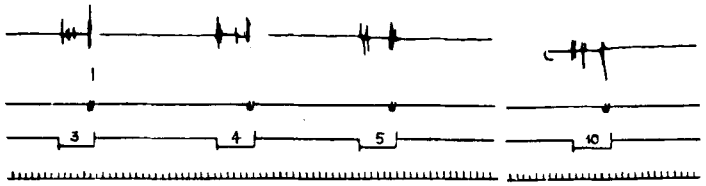

Fig. 13. Temporary connection in 60-day isolated guinea-pig embryos after 60 min of independent pulmonary breathing. Curves are presented as in Fig. 12.

The above percentage of positive signals is equal to the percentage in normally born guinea-pigs.

We next attempted to determine the cause of this effect. When we examine the correlation with chick embryos, we see that the greater the number of positive response signals the higher the oxygen consumption, and conversely (Fig. 14). This correlation

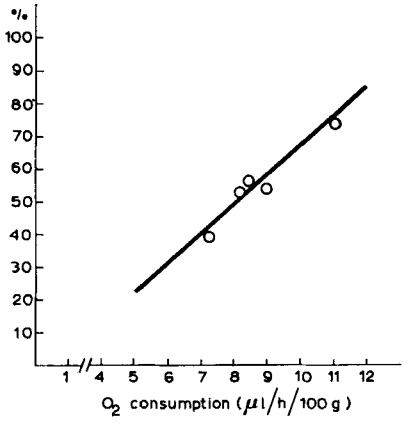

Fig. 14. Interrelation of O_2 consumption and effectiveness of temporary connection in chick embryos. The X-axis, O_2 consumption in μl/h/100 g; the Y-axis, percentage of positive responses.

may be taken into account to explain the phenomenon in guinea-pigs. Removal of fetal hypoxia, namely hypoxia of the cerebrum (65% blood saturation in the carotid) through pulmonary breathing makes it possible to ascertain the already-formed functional abilities of the central nervous system for combination activities.

The above mentioned phenomenon might also be explained as follows. If both vagi of the guinea-pig embryo are cut before the umbilical cord is tied, the temporary connection cannot be generated even after pulmonary breathing (Fig. 15).

With normally-born guinea-pigs after the fetal hypoxia period (95–97% blood saturation in the carotid artery), vagus section only results in a decrease in efficiency of a temporary connection (Fig. 16). Further experiments proved that the increase in excitability on using strychnine removes the effect of vagotomy which happens to new-born guinea-pigs (Fig. 17). From this it follows that vagotomy introduces a functional capacity decrease in the central nervous system.

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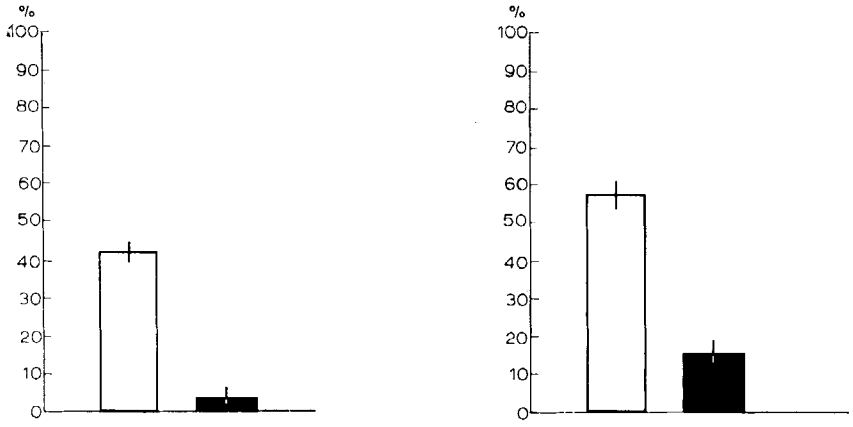

Fig. 15. Effectiveness of temporary connection in guinea-pig embryos. The Y-axis, percentage of positive responses. White column, reference embryos; black column, embryos after vagotomy.
Fig. 16. Effectiveness of temporary connection on new-born guinea-pigs. The Y-axis, percentage of positive responses. White column, reference guinea-pigs; black column, animals after vagotomy.

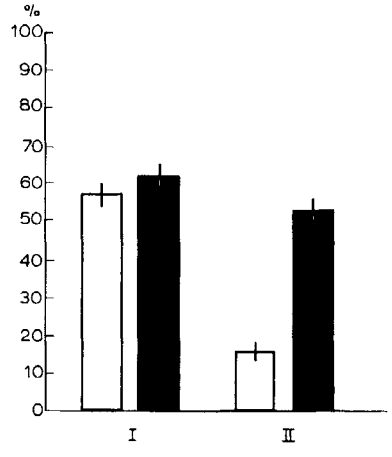

Fig. 17. Effect of strychnine on effectiveness of temporary connection in new-born guinea-pigs. The Y-axis, percentage of responses. I, Reference animals; II, guinea-pigs after vagotomy. White columns, animals without strychnine; black columns, animals affected by strychnine.

Taking into account all these results we came to the conclusion that the formation of the first temporary connections in a new-born guinea-pig is based on the important changes in the general functional state of the central nervous system, these changes being caused by the two factors: (1) increased oxygen consumption by the cerebrum, and (2) intensive supply of afferent impulses from vagus receptors excited during drastic changes in the respiratory system at the beginning of pulmonary breathing. Since none of these factors exists in the period of fetal development, the central nervous system is not yet capable of switching the temporary connection. To analyze this problem, we are now developing the method in which experiments will be conducted on an isolated embryo suspended from an artificial placenta.

These are some basic problems in our investigations that are not completed; or, it would be better to say, we are only beginning our research into the problem of prenatal elaboration of temporary connections.

SUMMARY

(1) We studied the development of the temporary connection in chick and guinea-pig fetuses and the development of electrical characteristics of the CNS.

(2) The temporary connection in chick embryos appears first on the 17th day of incubation. The effectivity of the temporary connection increases in the following days of incubation, especially between the 17th and the 18th day and between the 20th and the 21st day. The number of positive signal responses does not change in the period from the 18th to the 20th day of incubation.

(3) This quantitative characteristic of development of the temporary connection is accompanied by many qualitative differences. There are different effects of strychnine, of the intensity of conditioned and unconditioned impulses, different effects of the interval between both impulses, of periods of coincidence of both impulses, the appearance of differentiation and the development of the extent of generalization.

(4) On the base of these results we conclude that the mechanism of temporary connection in chick embryos develops in following three stages: 17th day, summation reflex; 18th–20th day, Ukhtomski's dominant; 21st day, conditioned reflex.

(5) We used the EEG depression by 25% KCl to eliminate the highest structures of hemispheres in chick embryos. This EEG depression had no effect on the temporary connection on the 20th day, but on the 21st day had an extensive inhibitory effect. From this finding we conclude that only on the 21st day do the highest levels of the CNS take part in elaboration of the temporary connection. It corresponds to the development of the steady potential of the hemispheres and to the development of high-frequency impedance (10–40 Mc/s) of the brain tissue of chick embryos.

(6) In the experiments on guinea-pig fetuses in term we found that the temporary connection occurs only after umbilical circulatory arrest and after the beginning of pulmonary ventilation. In this switching ability of the CNS an important role is played by oxygen supply and vagal afferentation. This is confirmed in the experiments with the effect of vagal deafferentation on the temporary connection in chick embryos.

These prenatal changes in brain functional activity are accompanied by an increase in the steady potential of the hemispheres (from 6.5 mV to 11.0 mV) and with the changes in high-frequency impedance of brain tissue.

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* Italics indicate the pages on which the author's contribution appears in this volume.

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