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THE BRAIN OF HIBERNATING ANIMALS

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by M. B. Shtark

"Nauka" Press, Siberian Branch
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By M. B. Shtark

Translation of "Mozg Zimnespyashchikh."
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THE BRAIN OF HIBERNATING ANIMALS

M. B. Shtark

Hibernation in mammals is accompanied by a prolonged decrease in brain temperature down to approximately 0°C. The return to normal temperature during natural arousal is characterized by a restoration of brain temperature, the neurohumoral regulation of physiological functions and the conditioned reflex mechanisms of the brain, as well as by restoration of memory and complex patterns of behavior. The special nature of this cerebral activity is one of the most fascinating mysteries of hibernation.

Based on an analysis of the bioelectrical, histochemical and electron microscopic changes in the hibernating brain, the author of this monograph formulates a neurophysiological concept of hibernation and attempts to establish the basic laws of functional organization of the "cooled" hibernant brain. For the first time, a detailed description is given of the electrical changes in the brain of hibernating animals during all stages of natural adaptation: from active wakefulness to incipient hibernation, torpor, and first tentative and then definitive arousal.

The data obtained by the author show that the conservation of cerebral activity at extremely low temperatures is not only a consequence of the genetically determined resistance of the tissues of the hibernant brain, but also of the characteristic organization of the interneuronal and systemic interaction between the cortical and subcortical structures.

To my parents, who inculcated
in me an honest relationship to
science and to people.

The author

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FOREWORD /5*

One of the characteristic features of the brain of hibernating mammals is its high resistance to low temperatures. This is reflected both in the low values of the thermal and "biological" zero-point of the cellular elements of the brain, and in their prolonged viability at a "biologically zero" level. One is led to think that this ability of the brain of hibernating animals is not so much the consequence of an insufficient development of the physiological mechanisms guaranteeing thermal homeostasis, as the result of special adaptation to specific conditions of life.

The study of these adaptive mechanisms is of considerable interest from both a theoretical and a practical point of view. There can be no doubt but that the discovery of the existence of these physiological and biochemical processes guaranteeing the natural and genetically conditioned resistance of the brain of hibernating mammals to a broad range of temperature fluctuations may lead to a significant expansion of the possibilities for the use of low temperatures in clinical practice.

It is precisely to these questions that the monograph by M. B. Shtark is devoted.

This book is the result of a great deal of experimental work, carried out by the author and his associates, into the bioelectrical changes taking place in various parts of the brain of hibernating animals under various conditions of temperature. In it one can find considerable new data on the changes in certain forms of bioelectrical activity ("burst" activity, evoked and dendritic potentials, impulse activity in individual cells) in various segments of the brain at low temperatures, as well as on the resistance of various segments of the animal brain to hypothermia. The book also contains a number of original ideas concerning the physiological mechanisms underlying the high degree of adaptation of the brain of hibernating mammals to changes in external temperature.

It seems likely that this book by M. B. Shtark will be received with satisfaction by a large circle of specialists.

Professor F. N. Serkov,
Corresponding member of the
Academy of Sciences of the
Ukrainian SSR.

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*Numbers in the margin indicate pagination in foreign text.

INTRODUCTION /6

The problem of the "cooled brain" is presently still at the level of intuitive deduction. There is no information about the most general laws of cerebral activity at temperatures approaching the "electrical zero-point", and few studies have been done on the physiological and chemical mechanisms underlying the resistance of the neurons of the central nervous system to low temperatures or the possibility of increasing this resistance in man and animals.

It seems to us that an interest in these questions leads directly to the necessity of a neurophysiological analysis of certain "natural experiments" — the phenomenon of natural adaptation to low temperatures; the most unique and yet the least studied of these "experiments" is probably mammalian hibernation.

By way of example to illustrate the importance of these questions, it may be recalled that electrical processes in the brain of hibernating mammals can be recorded at a brain tissue temperature of about 6-7°C (137, 231, 236, 244, 267, 292, 318, 320, 417, 437, 441, 470, 490, 493, 553, 563, 589-591). The transition to normothermia in hibernating animals is inevitably accompanied by restoration of the function of the central neurons.

At the same time, the "electrical zero-point" for the brains of homoiothermic animals and man does not fall below 16-17°C, and a return to normothermia in these animals (after more severe cooling of the brain) does not guarantee complete restoration of the activity of the central nervous system. It is obvious that this capacity of the homoiothermic brain severely limits the possibilities of cerebral hypothermia. There is thus a definite connection between the questions posed above and the phenomena of natural adaptation of hibernating mammals.

However, despite the obvious importance of the problem and the serious interest in the questions of the neurohumoral regulation of mammalian hibernation, the essential concepts concerning the cerebral activity of hibernating animals are still lacking.

In the non-Russian literature there are scattered publications dealing with the electroencephalographic phenomena of hibernation and providing some information as to the laws of the activity of the central neurons in hibernating animals. However, we have no definite information whatever about the interrelationship between the various cerebral systems guaranteeing, under conditions of natural

adaptation, the desired coordination of physiological functions during the torpor and arousal of hibernating animals. There are no experimental studies devoted to an analysis of the histo- and cytochemical changes in specific nervous structures and their relation to the functional state of the cerebral systems in hibernating mammals. Finally, there seem to be no reports in the contemporary literature of attempts to utilize data on the activity of the central neurons in hibernating animals for an analysis of the general characteristics of the waking and sleeping state in the brain of nonhibernating animals. /7

This discrepancy between the importance of the problem and the level of the contemporary ideas concerning the cerebral mechanisms in hibernating mammals has had a definite effect on the advances in our understanding of this unique natural phenomenon.

Before formulating the principal tasks of the present investigation, it seems appropriate to make one further comment concerning the present state of the problem. In the electrophysiological work reported in the literature on hibernation, the principal object of study has always been the animal itself: these have been subjected to physical cooling and artificial arousal and have generally been kept under conditions far removed from the natural situation (with the exception of the three papers by F. Strumwasser (589, 590, 591)). It seems to us that the electrophysiological results obtained under such conditions cannot adequately reflect the state of the neurophysiological mechanisms during hibernation. A neurophysiological analysis of the actual problem - mammalian hibernation - has remained outside the scope of these studies; this would seem to be the principal shortcoming of the previous work. The above thoughts serve to underline the theoretical and practical importance of a study of the laws governing the activity of the brain of animals falling into a torpor; on this basis, the specific content of the present volume can be formulated as follows.

It seemed essential to:

1) describe the electroencephalographic phenomena of mammalian hibernation. The most important in this connection would seem to be an electrophysiological analysis of the interrelationships of the various parts of the brain taking part in the regulation of sleep. A study will therefore be made of the role of the excitatory and inhibitory systems in the maintenance of cerebral homeostasis in hibernating animals;

2) characterize the specific electrophysiological characteristics of the functionally connected neuronal networks in the brains of hibernating mammals under conditions of natural adaptation on the basis of an analysis of the evoked potentials in the cerebral cortex. In this connection it seemed important to evaluate the characteristics of the transmission of excitation along specific and nonspecific pathways at various stages of hibernation;

3) evaluate the statistical structure of the pulsate activity of the central neurons in hibernating animals, especially in those parts of the brain where the most pronounced "hypothermoresistant" elements have been detected. Preliminary studies showed that most of these were associated with the hippocampus;

4) study the excitability of the cerebral cortex and clarify the synaptic organization of its upper layers;

5) study the sensitivity of the central nervous system of hibernating mammals to stimulatory neurotropic agents and attempt to analyze the neurochemical nature of arousal in these animals.

The answers to these new questions should make it possible, to a limited extent, to analyze the laws governing the cerebral activity of hibernating mammals, the functional characteristics of the "cooled" brain, and various borderline /8 problems of neurophysiology which may acquire a new interpretation on the basis of the data obtained.

By way of conclusion to the introduction of this book, it seems appropriate to point out that much of what it contains does not lead to a logical endpoint. This is true of the hypotheses and to a certain extent even of the factual material: the more one studies the problem of the neurophysiology of sleep, the less seems known and established. Factual data - the most essential material for an experimenter - seemed the most important, so that the recording of such data was looked upon as the primary task. It is possible that for this reason the relationships between the facts, deductions and hypotheses have not been brought out in perfect form.

All of what has just been said could not fail to be reflected in the style of this monograph, in a certain sketchiness. In the present state of knowledge, however, any other exposition of the problem of the cerebral activity of hibernating animals would seem extremely difficult, and possibly even unnecessary.

* * *

There can be no doubt but that the experiments which we undertook could never have been completed and the results would never have permitted the necessary generalizations were it not for the constant help received from colleagues and associates. Particular thanks are due to V. P. Danilyuk, N. G. Yurgelaitis and Ye. A. Zhosan, whose long hard labor contributed greatly to the completion of the work.

Constant interest in and full support for this work were also received from our scientific consultant: Corresponding member of the Academy of Sciences of the Ukrainian SSR Professor Filipp Nikolayevich Serkov. Thanks are also due to my newer colleagues N. N. Glushkov, H. I. Dubrovina, D. F. Kuznetsova, I. V. Popov and L. G. Chikovinskiy. Particular thanks are due to my constant helpmate and friend L. V. Voskresenskaya.

Part of the data on impulse activity in the neurons (Chapter VI) was processed in the Mathematics Institute of the Siberian Division of the USSR Academy of Sciences using programs developed by N. N. Glushkov (in the laboratory of Candidate in the Technical Sciences V.A. L'vov).

CHAPTER I.
NEUROHUMORAL REGULATION OF HIBERNATION /9

Statement of the Problem; Review and Analysis of the Literature; General Comments.

The range of questions which we have attempted to examine from the viewpoint of experimental neurophysiology and which we formulated in the brief introduction to the present work has also determined the character and structure of the traditional review of the literature. Schematically, its presentation and analysis will involve the evaluation of two types of questions: the first related to the physiological (and in most cases neurophysiological) mechanisms for the regulation of hibernation in mammals, the second dealing with data pertaining to the activity of the central nervous system in animals under the conditions of natural adaptation.

In the first part we will examine publications dealing with the analysis of the activity of the principal physiological systems in hibernating animals under conditions of natural adaptation. This part of the problem has been isolated primarily because the neurophysiological mechanisms of sleep can be adequately evaluated only in close relationship to the functions of the cardiovascular, respiratory and endocrine systems of the animal body. No analysis of the activity of the regulatory apparatus is possible without an evaluation of the principles of operation of the objects of this regulation. Besides, the contemporary biological literature lacks a systematic presentation of the problems of the physiological mechanisms for the regulation of mammalian hibernation, based on the latest experimental data. Practically the only review of studies of this type was made by Kayser in 1961-63, and this naturally does not contain the findings of the last few years.

An integral part of this first section is a critical presentation of the special studies devoted to the activity of the brain of hibernating mammals, the coordination of the physiological functions in these animals, and the neurochemistry of arousal and torpor. Questions of this type are the most closely connected to the goals of our own studies. The number of publications in this field is extremely limited, and most of them are purely descriptive in character. Nevertheless, it seemed essential to present an analysis of the experimental data on the activity of the hibernant central nervous system in a separate part of the present chapter. /10

The problems which are to be analyzed here may also demonstrate the connection between the general biological problem of mammalian hibernation and some problems of contemporary neurophysiology.

1. Activity of the Principal Physiological Systems of Hibernating Mammals

The latest reviews and analyses of studies devoted to the physiological processes in hibernating mammals were published relatively long ago in both the Russian and non-Russian literature (94, 214, 236, 256, 346, 437, 474). A few other papers of a review type, appearing more recently in the professional literature, deal with special aspects of the problem (95, 96, 441, 469).

In connection with the fact that the interest in the problems of the physiological mechanisms of sleep has not only not dried up but has recently acquired new aspects, in connection with the problem of artificial deep hypothermia and more particularly with the use of hibernating animals as promising objects for research in the field of space physiology (15, 190), a number of interesting and, to a significant degree, original findings have accumulated in the literature which require systematization. In this section we will evaluate material obtained principally between 1950 and 1968 and dealing with the regulation of mammalian hibernation. It is precisely on this aspect of the problem that the interests of research workers are now converging. The principal reports at the first, second and third international symposia on mammalian sleep, held in Boston, Helsinki and Toronto, respectively, were also devoted to the problems of the physiological mechanisms for the regulation of mammalian hibernation (98, 270, 363, 394, /11
415, 416, 434, 442, 446, 477, 538, 592-595).

In addition to the special interest in the problems of physiological regulation in animals falling into a torpor, the last few years have seen a broad penetration into practical ecological work of the contemporary research methods based on the advances in radioelectronics, telemetry and biological modelling. No evaluation of these methods as applied to the problems with which we are concerned here has yet appeared in the literature.

One final fact which confirms the necessity of this part of the book is the broad possibility of extrapolating data obtained in experiments on hibernating mammals to the plane of the general physiology of homoiothermic animals.

Contemporary Methods for the Study of Physiological Processes in Hibernating Mammals

The complexity of the registration of physiological functions in hibernating mammals arises from the requirements of a natural experiment, the design of which must never disrupt the natural activity of the animal organism, since this may significantly affect the results of the study (231, 232, 233, 234, 368, 369, 370, 371, 416, 437, 473, 580, 581, 589, 595, 596). This results in some rather complex problems, the solution of which has only become possible by the use of modern electronics.

The experiments are normally carried out in chambers or cages which simulate the natural living conditions of the animals (231-235, 251, 252, 368-371, 416, 437, 473, 580, 581). This is particularly important when studying the physiological rhythms of hibernants, particularly the circadian (24-hour) variations in activity (272, 496, 594).

The most important element in achieving a zone of comfort for the objects of these experiments is a smooth regulation of the environmental temperature. Such a chamber, proposed by Strumwasser (589), guarantees not only the necessary changes in environmental temperature but all the components of the habitual life of, for example, the Digger ground squirrel (burrowing, reproduction, natural methods for gathering food, the noise of the forest as reproduced by a reversible /12 motor, etc.). This can usually be accomplished merely by a refrigerator in which the temperature regulation is guaranteed by a freon device with a very broad range (231, 232, 233, 234, 291, 292, 600-605).

In order to carry out longer experiments lasting 4-6 months, one uses nonpolarizing electrodes which are oriented in the cerebral formations of the hibernating animals by means of systems of coordinates, stereotaxic atlases, or orienting bones selected in advance for any particular experimental animal (232, 291, 292, 417, 470, 471, 579). The special nature and duration of these experiments necessitates the use of specially designed electrodes which are fastened to the skull with the aid of a quick-drying plastic (231-234, 588-592).

Since the direct connection of the recording apparatus to the experimental animal naturally limits the possibilities of the experiment, this has served as a stimulus for the development of telemetry systems for the recording of physiological functions in hibernating mammals. Thus, Bonnin-Lafargue and Canivenc (298) have suggested transistorized radio transmitters implanted subcutaneously along the animal's spine. The transmitter was enclosed in a biologically inactive plastic sheath, and the animals were kept in burrows, the construction of which had previously been studied. The antenna received signals when the animals emerged from the burrow or when they moved about within it in connection with the changes in motor activity of the animals during the various periods of their daily life. The activity was recorded by means of a recording kymograph. For the same purpose, Miles (501) and Marshall et al. (488) used miniature radio transmitters working on the principle of a blocking generator. A radiolocator with a directional antenna guaranteed prolonged and stable translation over the course of several days.

The most interesting, technically advanced and promising in this connection are the experiments of Folk Jr. et al. (367-371); they suggested a highly promising system of electrodes for recording the ECG (367) as well as an armature for a transmitter which guarantees high reliability of reception. They implanted radiotelemetric sensors weighing 13-40 g in the abdominal cavities of golden hamsters, by means of which they succeeded in recording the temperature and electrocardiogram of these animals over prolonged periods.

Folk and Besler studied the arctic ground squirrel under conditions of deep hibernation and superficial sleep, in or out of the nest, as well as during hyperactivity and during constant or periodic illumination. They showed conclusively /13 that the daily activity of hibernating animals is uniformly accompained by periods of increased respiration, cardiac activity and body temperature.

Quite recently, Folk published new data obtained during telemetric studies of hibernation (370, 371). Analogous studies have been carried out by Strumwasser et at. (594, 595) on ground squirrels in the neurophysiology laboratory of the Walter Reed Institute in Washington.

Using the telemetry system of Folk and Besler, these workers determined the duration of hibernation and arousal in three species of ground squirrels observed over the course of a year. Thus, for the Digger ground squirrel (C. beecheyi), for example, the duration of uninterrupted hibernation was estimated at 47.7 days and that of arousal was 27.2 days, while for C. laterlis the corresponding figures were 174 and 22 days (594). These results are in agreement with the earlier conclusions of K. Fisher (363) .

Highly promising possibilities have been opened up by the electronic modelling of some periodic functions in hibernating animals. The first results obtained along these lines (566) showed that an electronic model of the diurnal rhythm consisting of a circuit for the endogenous rhythm, a control circuit for the conditioned reflexes and, finally, a consecutively operating computer able to reproduce all possible experimental situations produces a high degree of correlation with natural biological data.

An effective model which simulates the seasonal periodicity in connection with the changes in environmental conditions (temperature, light, etc.) has also been suggested (534). This model considers biological rhythms as self-organizing periodic fluctuations in the physiological functions, in agreement with the environmental temperature and other natural factors and represented by the authors in the form of an oscillator incorporating two systems (A and B) which are in feedback relation to each other. External changes in air temperature, humidity, etc. which bring about functional periodicity are recorded by system A and produce an appropriate response in system B, which controls the thermoperiodicity and follows the rhythm of photoperiodicity.

The development of an electronic model of the periodic functions of some parts of the brain of hibernating mammals also seems possible. The wiring diagram of this model will include a circuit for the endogenous rhythm which can reproduce the given periodic changes in electrical activity in connection with certain external factors. This will be realized in the form of a contour device which stabilizes the output effects by means of a negative feedback loop via an "intermediate" system of computer circuits. The model will also contain a permanent memory utilizing the accumulative principle and providing supervision over the work of a collating circuit which controls the level of agreement between the output and "internal" signals for the given model system. /14

There is every reason to believe that the progress in the physiological studies of the functional activity of hibernating mammals is directly related to the development of long-range (telemetric) recording techniques and methods of biological simulation.

Circulation and the Activity of the Heart. Regulation of Cardiac Activity and Vascular Tone. Regional Characteristics of the Circulation and the Nature of the Redistribution of the Velocity of the Blood Stream.

The hearts of hibernating animals continue to contract rhythmically at a body temperature of 0°C (416). This unique ability is also preserved in the isolated organ (212, 262, 264, 405, 475, 489) and in individual auricular myofibrills (489). A heart which has been removed from the body of a hibernating animal dies only at 0°C. These unique properties of the myocardium of hibernating animals could not but attract the attention of many investigators to the need for an analysis of the work of the circulatory system in hibernating mammals.

The methods for studying the circulation include electro- and phonocardiography (232, 264, 293, 310, 350, 351, 354, 356, 412, 414, 416, 432, 460, 472-474, 514, 567), the isotope dilution method, electrotonosphygmometry (415, 416), cannulation techniques, contrast roentgenography, and the application of prostheses to the large vessels (297, 298, 474, 476, 540, 546). Methods for studying the isolated hearts of hibernating animals have been widely described (212, 405, 476, 489).

In 1937, Andrew et al. became the first to record the electrocardiogram of the woodchuck (M. monax). These authors noted a progressively increasing bradycardia as the animals fell into a torpor. The heart rate decreased to 1-2 per minute. The table on the following page (Table I), which shows the relationship between heart rate and body temperature for hibernating animals during various stages of activity on the basis of experimental data obtained by means of electrocardiography, was compiled by Johansson (416). /15

Relatively recently (414, 415, 463), by the use of more advanced techniques, it was found that the progressive bradycardia is not the only change in cardiac activity, as had previously been reported. J. A. Lipp showed that a persistent tachycardia (up to 470 beats/min.), which seems to be connected with the activation of antiregulatory mechanisms, may also be recorded in the golden hamster during certain stages of hibernation. The force of cardiac contraction (minute volume) falls by a factor of 10 as the animal falls into a torpor, but the systolic volume remains relatively constant. While the peripheral resistance increases the velocity of the bloodstream drops (416).

Bradycardia is not the main feature in the evolution of cardiac activity in hibernating animals. If one analyzes the electrocardiograms obtained by Andrew et al., one finds definite changes in the structure of the cardiac complex, the interrelationships between the waves of the electrocardiogram. These workers were the first to show that atrioventricular dissociation develops in the ECG during a certain stage of torpor; this phenomenon was analyzed in more detail later (474, 582) and was also described by a group of Italian investigators working with the marmot (310). /16

TABLE I: HEART RATE (BEATS/MIN.) OF SOME ANIMALS UNDER CONDITIONS OF WAKEFULNESS AND TORPOR (DATA TAKEN FROM (416))

Animal	Wakefulness	Torpor	Author and Year of Publication
Bats	540-720	102	Eliassen, 1955 (350)
Birchmice	550-600	30	Johansen & Krog, 1959 (413)
Dormice	450	35	Pohe, 1959 (535); Kayser, 1961 (437)
Ground squirrels	300	4-7	Hiebel & Kayser, 1950 (416)
	120-456	7-8	Landau & Dawe, 1958, 1960 (337, 460)
		2-4	Dawe & Morrison, 1960 (338)
		2-7	Dawe & Morrison, 1960 (338)
	100-260	17	Strumwasser, 1959 (591)
Hamsters	500-600	6	Lyman & Chatfield, 1955 (474)
	260	6-10	Kayser, 1961 (437)
Hedgehogs	128-210	2-12	Shtark, 1963 (232); Kristoffersson, 1966 (453)
Marmots	80-110	8-10	Lyman, 1958 (416); Benedict & Lee, 1938 (416)
	73-200	5-40	
Pygmy possum	300-650	28-80	Bartholomew & Hudson, 1962 (286)
Hummingbirds	480-1200	48	Laziyevskiy, 1964 (416)

A definite relationship between the heart rate and body temperature, which persisted during artificial pharmacological arousal produced by veratrazine, was found during torpor and the onset of hibernation. An analysis of the electrocardiographic changes demonstrated conclusively that ischemic changes can also be observed during certain stages of torpor, along with significant changes in cardiac rhythm (473, 582).

The principal changes in the ECG, besides the progressive decrease in heart rate and amplitude, are related to the development of the atrioventricular blockade noted above. This develops at a body temperature of 18-19°C and is related to inhibition of impulse transmission in the AV node. As already shown by the studies of Lyman and Leduc (473), this part of the conducting system of the heart is the most sensitive to low temperatures. In addition to A-V dissociation, Swank and Johansen et al. (415, 607) reported prolongation of the QT and RT intervals and shortening of the ST segment in the ECG of hibernating hamsters. Arousal was characterized by the opposite changes in the ECG: prolongation of the ST segment and shortening of the QT and RT intervals (582, 612).

Disruptions of cardiac rhythm were first described by Landau and Dawe (337) in hibernating marmots; the deficit due to arrhythmia reached 3-15 beats/min. during torpor and (even more interestingly) during arousal or incomplete hibernation (581, 582). In the European hedgehog at a body temperature of

10-16°C, Aleksandrovich and Perkovskaya (264) observed consistent prolongation of the QRS complex accompanied by arrhythmia against a background of bradycardia.

One result of these changes in cardiac activity and circulation is the development of a significant temperature gradient between the anterior and posterior parts of the body which can reach 12-20°C under conditions of arousal: the head /17 then becomes significantly warmer than the tail (69, 303, 415, 416, 473, 582). As was conclusively demonstrated by Lyman (468) and then later by Chatfield and Lyman (474), this gradient is produced by deep vasoconstriction in the posterior part of the body. During thorotrast angiography in hamsters under these conditions, only the vessels of the anterior end of the body could be visualized. These findings are also true for other representatives of the hibernants, thus confirming the earlier findings of Marès (487) and Dubois (343).

The restoration of cardiac activity during arousal takes place very rapidly in hibernants: within 2-4 hours after a uniform increase in the heart rate the ECG already shows the usual parameters observed in the waking animal (232, 236, 412, 416, 582).

The circulation to the head and thorax is restored first of all, followed by that to the limbs and abdominal cavity (69, 262, 303, 494). The dynamics of the restoration of cardiovascular function were studied in detail in the work of Lyman and Leduc (473), who worked with the golden hamster, and then later in the great bat by Jansky and Hajek (420) and rather recently in the ground squirrel by Bullard (303) and in the hedgehog by Sivio et al. (581, 582). They all demonstrated an acceleration of the heart rate, which was slowed at the beginning of arousal and markedly so at a body temperature of 22-24°C. During the beginning of arousal there was a significant increase in blood pressure (353), 416), vasodilatation in the anterior end of the body, and a more rapid propagation of the pulse wave (467). At the beginning of arousal, at a body temperature of 10-16°C, the heart rate in the great bat was only 10-16/min., compared to 340-360/min. at 30°C.

During arousal of the hibernating animal, stabilization of the principal parameters of cardiovascular activity (blood pressure, rate of transmission of excitation in the myocardium and the cardiac nerves regulating its rate of contraction, nature and form of the principal peaks of the ECG, vascular tone) is already achieved at a body temperature of 20°C (351); in some animals these changes may be delayed until a temperature of 23-26°C. This process is accelerated by pharmacological arousal (580-582).

Valuable data on the dynamics of the blood pressure in hibernating mam- /18 mals have been obtained comparatively recently (416, 475, 540): cannulation of the carotid artery in hibernating hamsters and ground squirrels and measurement of the pressure by means of a volumetric manometer showed that this fluctuates between 40 and 72 mm Hg in the "sleeping" animal, averaging 55 mm Hg. At the moment of restoration of cardiac activity the initial blood pressure values were reached again, to be followed by a new decrease despite the progressive tachycardia accompanying arousal (416, 474, 476, 540, 542).

The changes in blood pressure have been studied in great detail by Johansen and his constant coworkers J. Krog and O. Reite (413, 415).

1. In the normothermic state (rectal temperature of 37°C), first of all, they described spontaneous fluctuations in blood pressure on the order of 5-10 mm Hg, due to respiratory excursions.

2. They then showed that electrical stimulation of the vagus nerve in the waking European hedgehog normally produces a drop in blood pressure by 25-30 mm Hg. This effect is completely preserved even at a rectal temperature of 18°C, but disappears in the range 5-6°C, despite an increase in the voltage of the stimulating current, to reappear again at 27°C.

3. The hypotensive effect of acetylcholine almost disappears at 5°C but reappears again in the range 15-16°C, while the hypertensive effect of noradrenalin at a temperature of 36°C remain unchanged during hypothermia (3°C). Adrenomimetic drugs potentiate the effect of acetylcholine and stimulation of the vagus nerve somewhat at a temperature of 4.5-6.5°C.

On the basis of these experiments it can be concluded that complex and characteristic interactions take place between the effects of cholinergic and adrenergic stimuli on the cardiovascular system during the process of hibernation. The preservation of vagal effects is indicative of a leading role for cholinergic tone during hibernation. The changes described are almost abolished only at temperatures approaching 0°C. Quite similar results were presented by Lyman and O'Brien (477) at the Second International Symposium in Helsinki.

On the basis of physiological data obtained on the perfused hearts of hibernating animals, it can be concluded that a predominant role in the processes of torpor and arousal is played by the following factors: a) a change in the rate of /19
transmission of excitation along the vagus and sympathetic nerves, b) the appearance of A-V dissociation, and c) the temperature optimum in the effectiveness of the mediators (212, 414).

In addition, it is possible that the changes in cardiac activity described above are based on a number of other factors: the increasing delay in the transmission of excitation along the cardiac nerves, which may be related to the observed changes in the axoplasm of the nerve fibers in hibernants (610), hyperpolarization of the presynaptic terminals, and desynchronization of the effects of acetylcholine which leads to a delay in the activation of the vesicles liberated by the mediator (426).

Respiration.
Respiratory Function of the Blood. Tissue Respiration.
Nervous and Humoral Regulation of Respiration.
Hypoxia in Hibernating Mammals.

During the onset of hibernation, bradypnea develops in parallel with the slowing of the heart rate (119, 425, 427, 460). In a state of torpor, rhythmic

respiration generally alternates with periods of dyspnea-apnea, which seem to be the most characteristic change in respiratory activity in hibernants during torpor and a marked decrease in body temperature (453, 474, 583). On repeating the older experiments of Tate (cited by 474), V. I. Telegin (208) showed that the "diaphragm-diaphragmatic nerve" preparation in the golden hamster still functions with a few changes at 5°C, while in physically cooled rats the transmission of excitation in this nerve is blocked at 10°C. Consequently, under low temperature conditions, the stimulus is transmitted in the same manner along the diaphragmatic nerve as along the tibial nerve (317).

This bradypnea, which can reach a rate of 2-4 excursions per minute during torpor (compared to an original respiratory rate of 80-120/min.), still supplies sufficient oxygen to the tissues due to the sharp drop in oxygen requirements in the "sleeping" animal: Alpine marmots consume 258 ml of oxygen per hour per kg of body weight at a body temperature of 27°C, compared to only 72 ml at 13°C (539). In experiments on respiration the oxygen requirements do not normally show cyclic variations. They remain high during the normal sleep of the hibernant, dropping only during a period of torpor (443). These findings may be in agreement with the results of the experiments of Bidet (294), who showed that the oxygen requirements in micrograms per gram of tissue remain relatively /20 high in the brain during torpor, decreasing in the waking state. On the other hand, the oxygen tension in the myocardium drops during hibernation, increasing only when the animals are aroused, which correlates to some extent with the ischemic changes in the ECG described above. In the brain (as confirmed also by electrophysiological studies), similar changes associated with oxygen deficiency fail to occur. The fact that the oxygen supply to the brain under conditions of torpor is generally sufficient to meet the needs of the animal is also shown indirectly by the data of L. A. Isaakyan and the results of the experiments of N. M. Shumitskaya (88, 248, 249). They are in agreement with the interesting data obtained quite recently (635) in the golden hamster. Here it was shown that the onset of hibernation is accompanied by a sharp drop in the oxygen requirements of the brain — from 2.179 ml/g/hr. to 0.071; the output of CO_2 fell from 2.295 ml/g/hr. to 0.043. Arousal was accompanied by a sudden increase in the oxygen consumption: this reached 0.435 ml/g in the course of an hour.

The act of respiration in hibernating animals is controlled by the respiratory center, which is capable of autorhythmic activity at very low temperatures. Prolonged survival under hypoxic conditions is observed in the suslik after evisceration and ligation of the aorta: in a state of torpor, the coordinating activity of the respiratory musculature is maintained for 13-27 minutes after interruption of the oxygen supply to the brain thanks to the "respiratory periodicity" which is characteristic of this stage of hibernation (119). The data of N. B. Lauer have been confirmed by the experiments of Andjus et al. (270). Occlusion of the trachea does not produce respiratory disturbances in marmots which have been cooled to 4-8°C. In rats, however, gross changes in respiratory periodicity are already noted at rectal temperatures of 12-16°C (270).

During this period, the efferent impulses in the diaphragmatic nerve acquire an unusual paroxysmal character. Analogous states of cerebral oxygen deficiency

are incompatible with the survival of homoiothermic mammals (270, 389, 460): in such animals, apnea develops 1.5-2 minutes after evisceration. These facts are particularly interesting in connection with the results of experiments by Lyman and Hastings (cited by (474)) which indicated that the respiratory center of hamsters remains sensitive to CO_2 during torpor. An increase in the concentration of the respiratory stimulator CO_2 in the surrounding air was still followed by an increased respiratory frequency. In addition, these experiments showed /21 that ground squirrels and marmots respond in a particular way (which is quite similar to that of the waking animal) to changes in blood oxygen tension during a state of deep torpor (270, 439).

The ability of the blood hemoglobin to bind and transport oxygen is the same in hibernants as in homoiothermic mammals. Rasmussen and MacBurney et al. have shown that, in connection with the sharp reduction in oxygen consumption during hibernation, the gradient of oxygen tension between the blood and tissues must be much less than in the active animal (see (474)). The displacement of the oxygen dissociation curve to the left (28°) under conditions of reduced body temperature is related to the fact that a lower oxygen tension in the blood is required for the dissociation of hemoglobin during torpor. However, since the percent saturation of hemoglobin in the venous blood of hibernating animals does not differ from that in homoiothermic animals, it can be concluded that the decreased blood-tissue oxygen gradient in hibernating animals is in complete agreement with the needs of the organism. A displacement of the hemoglobin dissociation curve to the right is also possible, as shown by MacBurney et al. (see (474)), due to the reduced pH or elevated pCO_2 which are usually found in a state of deep hibernation*. The blood CO_2 tension is quite clearly controlled in hibernating animals (445).

Hypoxia does not develop in hibernating ground squirrels, not even after prolonged occlusion of the trachea (270). Post-occlusion dyspnea and tachypnea are observed only at rectal temperatures on the order of 12-15°C. A deepening in the level of hibernation is not accompanied by any response of the respiratory center (270).

The restoration of respiratory activity during arousal proceeds in parallel with the restoration of cardiovascular activity (433, 437, 580-582). However, at the beginning of the rapid arousal period in hibernating animals (ground squirrels, marmots and hedgehogs), the effect of the respiratory center becomes generalized. The constellation of the respiratory neurons lacks the so-called "functional isolation" (206) which is characteristic of the waking state of these animals; this leads to generalization of the respiratory rhythms. This effect is reinforced by the convergence of the afferent impulses towards the neurons of the medullary reticular formation and from there along the descending pathways to the skeletal muscles, whether or not the latter are involved in active respiration (231, 376, 480, 481, 563).

Arousal is characterized by normalization of the respiratory rhythm and by /22 disappearance of the episodes of apnea and dyspnea. During the first 10-20 minutes of arousal the respiratory frequency already increases from 1-2 to 10-12 excursions per minute, while between the 40th and 100th minute it rises to 35-50 and

*This decrease in pH was not confirmed in the recent experiments of Kent and Peirce (445), performed on 13-lined ground squirrels.

after 2-3 hours it reaches 100-120 excursions per minute, with strict maintenance of the respiratory rhythm (441, 460, 473).

The results which we have obtained in this area (230, 231) lead us to suggest that the periodicity in the automatic activity of the respiratory center in hibernating mammals during torpor is based on a definite fixation of the refractoriness of the reticular neurons, based in turn on the changes in their sensitivity to CO_2. It is well known that the latter is the most specific stimulus of the respiratory neurons of the medullary reticular formation (340).

Thus, the regulation of active respiration in hibernating mammals is guaranteed by a respiratory center which is resistant to low cerebral temperatures. The periodicity and automaticity of the operation of this center is a specific adaptation permitting effective work under conditions of low intracerebral temperatures. The ability to stimulate respiration via the diaphragmatic nerve at low temperatures, along with the generalization of the respiratory rhythm to the skeletal musculature, guarantee a high level of tissue respiration under low-temperature conditions.

Thermoregulation.
Ontogenetic Determinants of Heat Production.
Heterothermia and "Antihibernation"
Synchronization between Thermal Periodicity and Factors in the External Environment.
Thermoregulatory Tone.
Contractile and Noncontractile Heat Production.
"Free" Oxidation and the Increase in Body Temperature during Arousal.

The capacity for reactive changes in heat production develops in hibernating mammals during the 2nd or 3rd week of postnatal development (14). In fieldmice studied over a temperature range of 10-40°C, the response to cold appears 10-11 days after birth and is accompanied by a 26% increase in oxygen consumption; i.e., the response is the opposite (in the generally accepted physiological sense) of that seen in adult animals, in which the oxygen consumption is known to be reduced by external cooling (433, 437). A turning point in the curve representing the effectiveness of the response to cold is noted around the 15th day, with a critical point on the 19th day. The maintenance of a constant body temperature in the waking state appears in the fieldmouse towards the 16-17th day of postnatal development. /23

As was shown quite clearly by Eisentraut (348), who studied the relationship between body temperature and environmental temperature in hedgehogs of various continents, the capacity for thermoregulatory responses is also conditioned by ecological factors. Eisentraut found that the periods of torpor in the Moroccan hedgehog lasted 5-6 days, began at an air temperature of 16°C, and were accompanied by a drop in body temperature. These changes were significantly less pronounced in dormice from Israel and were completely lacking in animals brought in from Africa, which did not normally experience hibernation.

It is well known that the body temperature of hibernating mammals follows, within limits, the temperature of the environment (69, 94, 431, 567). However, both persistent heterothermia and a tendency to hyperthermia are possible as a response to a drop in external temperature (195, 506). There is every reason to connect this fact with the activation of "antihibernating" contraregulatory mechanisms (which were already mentioned at the beginning of this section) serving to prevent the extreme cooling of the body in hibernating mammals.

Recent studies have not confirmed the point of view of Lyman and Chatfield, who suggested that natural hibernation, arising in connection with definite exogenous and endogenous factors, is identical with the torpor appearing in hibernating mammals as a result of physical cooling (404, 474, 613). Experiments have shown that physical cooling which is not "synchronized" to a significant degree with natural factors favoring hibernation results in the activation of antiregulatory mechanisms and prevents torpor (327, 335, 404, 508, 613). This fact is unfortunately not taken sufficiently into consideration in the design of experiments in electrophysiology.

The body temperature of an animal in a state of deep torpor still retains its diurnal periodicity (208, 368, 371, 529, 531, 595). As was pointed out earlier, this periodicity is also a property of other autonomic functions (respiration, cardiac activity), even during rather deep hypothermia. It is apparently an expression of an adaptive phenomenon guaranteeing the functional effectiveness of a biological system even under conditions in which the energy expenditures of /24
the system have been reduced to a minimum. Periodicity is genetically fixed as a characteristic form of temporal memory (38, 243, 246, 272, 594, 595, 608).

The periodicity of the body temperature is under the control of several endogenous and exogenous factors and, assuming a completed development of the endogenous thermoregulatory mechanisms (about which more will be said a little later), is clearly synchronized with environmental cycles. The controlling factor in the periodicity of the body temperature lies outside of the body (272). This postulate and the foregoing ideas have been arrived at on the basis of studies in which the thermal periodicity of mammals has been simulated. It should be kept in mind, however, that stabilized thermal periodicity is less subject to the influences of the external environment during the initial stages of hibernation and torpor (38).

The physiological possibilities created by the thermoregulatory mechanisms of hibernating mammals are most clearly demonstrated when animals which are in a state of torpor are being aroused. Arousal is accompanied by a very rapid rise in body temperature, the chemical nature of which is unclear. An important role in this warming process is played by the "thermoregulatory tone" of the skeletal muscles, which has been carefully studied by K. P. Ivanov in the laboratory of A. D. Slonin (79, 80, 81, 83). These studies showed that the increase in body temperature during arousal of bats from hibernation is accompanied by increased electrical activity, especially in various groups of muscles, and by an increase in muscular oxygen consumption (100-fold in comparison with normothermic conditions); when a body temperature of 32°C was reached, the thermoregulatory tone disappeared. Similar results have been obtained in susliks (66).

The analogous studies which Galletti et al. (376, 377) recently carried o t on marmots revealed dynamic changes in contractile thermogenesis in these hibernants. Thus, the temperature of the respiratory muscles reached maximal values during arousal (after 45-60 minutes), after which tachypnea developed. Subsequently, the temperature of the esophagus, the brown fatty tissue (in a somewhat different manner than observed in the experiments of Tucker (613)), and finally the rectum also returned to normal.

Similar results were also recently published by G. M. Daudova and N. V. Ipat'eva (69). In studies on the thermotopographic gradient, these workers showed that arousal is accompanied by increases in the temperature of the heart, brain, thoracic muscles and liver, followed by the remaining body parts. This gradient /25 can attain 12-14°C. Intensive studies of the nature of thermogenesis during arousal which are now in progress at the Department of General Pathology of the Institute of Experimental Medicine may serve to clarify the initial role of the muscles of the heart and forelimbs as well as of the brain in arousal.

From the earlier studies of Chatfield and Lyman (cited in (474)), it appears that muscle potentials can be recorded in hibernating hamsters at the very beginning of arousal, in the absence of any outward sign of motor activity.

Further increases in heat production during arousal are accomplished mainly at the expense of an increased activity of the central thermoregulatory elements in the brain (376, 481). Since the section of the brain just anterior to the pons does not prevent a rapid increase in body temperature after hibernation, these central mechanisms in hibernants must be localized in front of the midbrain. They may function primarily via activation of the hypothalamus, the pituitary gland, and then the adrenal system (581, 671).

The latest experiments carried out in Kayser's laboratory made possible a more precise localization of the central coordinating mechanism responding to torpor and a decrease in body temperature in hibernants (480, 481). Bilateral electrolytic injury to the lateral and dorsomedial groups of hypothalamic nuclei was found to prevent hypothermia and the onset of hibernation in animals kept for 8-25 days at an environmental temperature of 7 ± 1°C.

The work of Satinoff (571), which was carried out simultaneously with the experiments reported in (481), showed, on the other hand, that electrolytic disruption of the anterior preoptic zone of the hypothalamus interferes with the restoration of the body temperature in artificially cooled animals.

In any review of the problems of the regulation and chemical mechanisms of thermogenesis, particular attention should be devoted to the latest papers dealing with the so-called "rapid mobilization of animal heat" (144) during natural arousal in hibernating animals.

This refers to the role of uncoupled "free oxidation" which is accompanied by release of heat and which may be an important source of noncontractile heat production, the so-called "animal heat" (65, 67, 68, 144-146, 195, 575). It is

generally believed that this noncontractile heat production, which depends on the uncoupling of respiration and phosphorylation in the liver, skeletal muscle and brown fatty tissue, may provide all of the energy required for arousal (21, 65, 385, 386, 578, 583). /26

In one of the papers cited above (69) it was pointed out that a study of the dynamics of heat production during arousal necessitates an analysis of the site and role of the phenomenon of uncoupling, not only in the liver where it was first demonstrated but also in the skeletal muscles and brain. This is particularly connected with the fact that arousal is characterized by a rapid warming of the brain and the muscles of the anterior parts of the body. However, there is still no convincing data indicating that thermoregulatory dissociation also takes place in cerebral tissue (57, 68, 69).

Endocrine System.
Hormonal Factors in the Regulation of Hibernation.
The Hypothalamus — Pituitary — Adrenal Cortex System and the Mechanisms of Hibernation.
Neurosecretion in Hibernating Animals.

The adrenal glands do not undergo hyperplasia in hamsters subjected to physical cooling (297, 339, 397, 442). This distinguishes the hibernating mammals in principle from the homoiothermic animals, in which adrenal hyperplasia and hypertrophy are characteristic in adaptation to cold (181).

In recent studies, a definite seasonal rhythm has been noted in the activity of the adrenal glands in all species of hibernants: during hibernation the adrenalin content in the glands increases and the noradrenalin content falls. Several authors have come to the conclusion that reabsorption and inhibition of the sympathetic mediating hormone is the principal factor in the seasonal nature and endogenous rhythmicity of hibernation; this also plays a role in the regulation of the circulation and body temperature (266, 297, 398, 538, 574, 586, 587, 601).

After adrenalectomy, the European ground squirrel no longer hibernates (538, 541). In fact, the adrenalectomized animal very clearly manifests its "antihibernating" properties: it rapidly loses weight, neurosecretion in the posterior lobe of the pituitary disappears when the environmental temperature drops, and the ability /27
to develop torpor is sharply inhibited. This is apparently the result of a lack of adrenocortical hormones, since injection of desoxycorticosterone or cortisone brings about torpor. Transplantation of adrenocortical material into the ocular chamber of an adrenalectomized ground squirrel also makes possible the usual development of hibernation (538). This all leads to the conclusion that adrenal hypofunction is one factor in cold resistance which is quite clearly manifested in both hibernating mammals and animals which do not undergo torpor.

Hypofunction of the principal endocrine organs (adrenals, thyroid and sex glands (437), pituitary) usually precedes the onset of torpor. One exception, however, is the parathyroid gland (442). This indicated that there is still insufficient basis for listing adrenal hypofunction among the factors which must inevitably be

present to produce hibernation, as was formerly done by Kayser (428). In the context of the "endocrine nature" of hibernation, particular attention should be directed at the interaction between the thyroid and adrenal glands, and it is this interaction which should be looked upon as one of the links in the regulation of hibernation. Along with the above, consideration should be given to the results obtained in other experiments (512, 532) which indicate that catecholamines, which play a major role in the arousal of hibernating animals, are accumulated in the adrenals during hibernation.

Nevertheless, the study of the role of the adrenals in the regulation of hibernation can by no means be considered complete. The correctness of this conclusion is supported by the results of the latest experiments by Raths (558). The changes in catecholamine levels during hibernation are apparently more complex, for example, since their content varies even during torpor, i.e., when the temperatures of the brain and body reach very low levels. The activity of the mediating hormone decreases with the onset of hibernation and then increases, but remains below the levels in the waking hamster. The vago-insular tone increases while the sympathico-adrenalin tone decreases. As arousal approaches these proportions are altered somewhat but not essentially. These conclusions may be reinforced by the electron-microscopic studies recently carried out in Kayser's laboratory (586, 587).

The tendency to look upon hypofunction of the thyroid gland as one of the principal links in the endocrine regulation of hibernation has recently become stronger. Even in the older experiments of Adler (261) one can find an indication of the relative excitatory effect of thyroid extract. In the opinion of some investigators (203), hypofunction of the thyroid gland leads to torpor. This position is clearly illustrated by the experiments of Popovic (538) who fed one group of /28
European ground squirrels powdered thyroid gland (at a dose of 1 g per week) while another group was given a preparation of methylthiouracil which is known to suppress thyroid function. While the first group of animals did not "fall asleep" and their oxygen consumption increased, the second group of ground squirrels began to hibernate earlier than normal. Despite the skepticism of Popovic with regard to Adler's experiments, the results of his own studies differ little from those obtained in the 1920's (537, 539).

Under natural conditions, the activity of the thyroid gland in hibernants fluctuates periodically, but these fluctuations are not strictly synchronous with the seasonal fluctuations in adrenal function: the latter increases in the spring and decreases in the fall. In the experiments of Alia (265) on hedgehogs, artificial physical cooling produced an opposite effect if the hypothermia did not bring about the onset of natural torpor. However, experiments carried out recently with radioisotopes have shown that ^{131}I absorption by the thyroid gland of hamsters is maximal in March and December, i.e., precisely at those times when the animals are experiencing the onset of either arousal or hibernation (349). Studies carried out in dormice also showed that there is a rapid excretion of thyroid hormone labeled with ^{131}I during arousal; when the animals were placed in cages with a temperature of 5°C the activity of the thyroid gland decreased. In dormice hibernating at a body temperature of 8°C, thyroidectomy did not alter the nature of thermogenesis or the course of torpor (431, 458, 459). Thus, arousal is accompanied by a sharp increase in the

hormonal activity of the thyroid gland, while its function is depressed during torpor (440, 458). Analogous conclusions can be found in (605).

Rather interesting data have also been obtained on the neurosecretion of the hypothalamic nuclei in hibernating mammals (519); Gomori-positive granules of neurosecretion accumulated in the paraventricular and supraoptic nuclei of the hypothalami of hibernating hedgehogs and dormice. These data agree in principle with the results obtained more recently (282, 291, 448, 449, 603).

Kalachkovskiy and Vender succeeded in demonstrating that the neurosecretion of the hypothalamic nuclei in the suslik (studied by the chromohematoxylin-phloxine method of Gomori) is subject to rigidly predetermined fluctuations: in the summer the neurosecretory granules accumulate in the supraoptic nucleus /29
and lateral nuclei of the hypothalamus, while in a state of deep torpor the rate of accumulation of neurosecretion is significantly lower although, as confirmed in (603), the level of neurosecretion remains quite high during hibernation in the hedgehog. These changes are particularly marked in the area of the infundibulum. Significant amounts of neurosecretion accumulate in the peripheral parts of the neurohypophysis. In susliks, an anatomical connection has been noted between the paraventricular nuclei and the lumen of the third ventricle. It is thus possible that this is the pathway for the transport of neurosecretion which passes the hematoencephalic barrier.

Returning to the work of Barry (282), it should be pointed out that the neurosecretory structures of the paraventricular and preoptic zones of the hypothalamus are anatomically connected with the group of amygdaloid nuclei and the mesencephalic portion of the brainstem. This means that the factual date of Barry can be interpreted as evidence for a role of the hypothalamic region and the structures of the limbic system in the arousal of hibernating animals.

The role of the hypothalamo-pituitary system in the regulation of hibernation has been clearly examined in the work of Benetato et al. (291). They showed that the intraperitoneal injection of the pituitary hormone vasopressin at a dose of 250-500 IU in the course of 2-4 hours produced arousal in chipmunks hibernating at a temperature of 10°C. The control animals did not respond to injections of physiological solution. Reserpine either abolished or significantly weakened the excitatory effect of vasopressin. ACTH produced an effect analogous to that of vasopressin, which was of somewhat shorter duration. Both administration of vasopressin and that of ACTH consistently stimulated neurosecretion.

Despite the considerable amount of experimental work devoted to the problems of the endocrinology of hibernation, their level remains low; no work has been done with labeled hormones, and electron microscopic or cytochemical studies are rare. All this obviously has an effect on the experimental results and on the response to the problem of the "endocrine mirror" of hibernation. The hypothesis of an endocrine etiology for hibernation, which was so popular more than 50 years ago (380), is currently attracting fewer and fewer adherent

2. The Brain and Cyclic Functions in Hibernating Mammals /30

"Spontaneous" Bioelectric Activity of the Cerebral Cortex.
Cortical Electrogenesis, Hibernation, and
Artificial Cooling of Hibernants.
Characteristics of the Induced Electrical Responses.
Biochemical Changes in the Brain of Hibernating Animals.
Initial Hypotheses as to the Possible Neurochemical Nature
of Arousal in Hibernating Mammals.
Tracking Processes (Memory) in Hibernating Animals.
Hypothermic States and Learning.

The first studies on the electrical activity of the brain in hibernating mammals were carried out simultaneously at the Cambridge Medical College (USA) by Chatfield et al. on golden hamsters (318, 320, 470, 471) and at the Institute of Physiology of the University of Strasbourg (France) by Kayser et al. on marmots and ground squirrels (428, 441, 563). Their work was devoted mainly to the study of the "spontaneous" bioelectric activity of the cerebral cortex of animals under various conditions of temperature. Unfortunately, in the first as well as the succeeding publications on this question, the principal goal of the experiments was limited to a comparison of the "electrical zero-point" of the cerebral cortex and the temperature range over which the first signs of cerebral electrogenesis either appeared or disappeared in hibernants. These papers have only an indirect bearing on the analysis of the mechanisms of cerebral activity in mammals during the initial stages of hibernation.

The principal deficiency in all of the electroencephalographic work carried out to date is that the hibernants (ground squirrels, marmots, hedgehogs, birch-mice) have been subjected to anesthesia followed by physical cooling. This objection does not only apply to the work of Strumwasser (589-591, 594). As shown by our own studies (231, 232, 246) and by experiments carried out later by Massopust (492, 493), results obtained under such conditions cannot be considered satisfactory or valid. Under conditions of anesthesia and physical cooling (by contact), the object of the study becomes the specific hibernating animal rather than hibernation. /31

In the first experiments of Kayser et al. it was shown that cooling and rewarming in ground squirrels and marmots are characterized by definite changes in the electrical activity of the cerebral cortex (428, 441, 563). Signs of regular bioelectric activity disappear when the temperature of the cerebral cortex is decreased to 18°C and first reappear during rewarming when the brain temperature reaches 19-21°C, increasing as the temperature rises to 26-29°C.

Chatfield, Lyman and Purpura (318) studied the electrocorticogram of golden hamsters during physical cooling and found that the "spontaneous" electrogenesis in the cerebral cortex of these animals disappears below 18-19°C, i.e., within the same limits as reported for the suppression of electrical activity by the French physiologists. Irregular damped waves were recorded at cerebrocortical temperatures of 19-20°C, while at 21°C elements of synchronized rhythm of the "spindle burst" type could already be detected in the electrocorticograms.

Desynchronization of cerebral bioelectric activity could be recorded above 27-29°C.

In the experiments carried out by the American physiologists, attention is also called to the fact that the changes in bioelectric activity in the cerebral cortex of golden hamsters under conditions of natural torpor are quite similar to the changes in cortical electrogenesis observed in the same animals during application of physical cooling. However, a study of the illustrations in this paper shows that all of the experiments were carried out in the summer, which cannot fail to affect the results. We have called particular attention to these circumstances since in the following chapters (in evaluating our own results and analyzing those of foreign investigators) we will return repeatedly to these natural and experimental "analogies" in connection with the study of the bioelectric activity and the reactive changes in electrogenesis in hibernating animals.

The results of the experiments of Chatfield et al. and Kayser et al. in which they attempted to analyze the bioelectric activity in the cerebral cortex of hibernants showed that these changes are species-related and differ in definite ways in the various representatives of the hibernating mammals. Thus, in ground /32 squirrels and marmots in a state of deep torpor, as well as in those which have been physically cooled by the experimenter, cerebral electrical activity could still be recorded at 6-11°C, while in the golden hamster changes in the cerebrocortical potential differences were noted at higher temperatures.

Thus, the basic studies carried out in the laboratories of Chatfield and Kayser during the course of the last decade have been limited to an analysis of cortical electrogenesis in hibernants under conditions of unsuccessful simulation of natural conditions or "experimentally" induced hibernation. It is easy to see that these authors have concentrated their attention on two questions: the characteristics of electroencephalographic phenomenology and the establishment of a relationship between the temperature of the cerebral cortex and its ability to generate biopotentials. Only the complexity of design of experiments to be carried out on hibernating mammals under conditions of natural adaptation and the necessity of maintaining the natural course of all stages of their vital activity can explain the rather limited number of experiments carried out by these non-Russian investigators. However, the significance of the initial results which they obtained for the further development of the problem is difficult to overestimate.

Relatively recently, a number of experimental studies have appeared in the literature devoted to an analysis of the electrical activity of the brain of hibernating mammals during various stages of their vital activity. Attention should be called first of all to the work of Strumwasser (588-591, 594, 595), carried out while at the University of California. Despite the rather limited amount of material used in these experiments (the author studied the physiological indices in 9 Digger ground squirrels, he succeeded, thanks to the high technological level of the experiment, in obtaining new essential data pertaining to the neurophysiological mechanisms of hibernation in mammals.

Strumwasser used electrodes which were implanted into the brains of ground squirrels according to a special pattern (taking into consideration the orienting bones)

and a system of technically advanced receivers which guaranteed the prolonged and consistent recording of a number of bioelectric indices of the activity of physiological systems such as the cerebral cortex and the subcortical formations. This was essentially the first non-Russian study in which serious attention was paid to the question of the cortical-subcortical interactions under conditions of mammalian hibernation (the first similar studies in the USSR were /33 carried out by B. A. Saakov et al. in Rostov-on-Don (137) and later by us). Strumwasser partially confirmed the results of Chatfield and Kayser regarding the temperature range for cortical electrogenesis (590). The lower limits of this range were found to be 17-18°C. During deeper hypothermia, only episodic damped waves could be recorded in the electrocorticograms; the EEG of these animals showed no regular rhythmic activity.

The experimental results of Strumwasser make it possible to isolate several important aspects in the electrophysiology of mammalian hibernation, aspects which have to a significant degree affected the direction of our own work.

This pertains first of all to the changes which he detected in the "spontaneous" and induced activity in the mesencephalic portion of the reticular formation. He found that the "spontaneous" activity in this formation of the brainstem disappears somewhat later than the suppression of the bioelectric activity in the cerebral cortex. It should be noted, however, that even an examination of the illustrations provided by the author reveals simultaneous changes in the electrogenesis in these parts of the brain, which in turn enabled the author to draw certain conclusions regarding the space-time correlation between the electrical activity of the cerebral cortex and that of the reticular formation in the Digger ground squirrel.

The data obtained by Strumwasser are also of interest in connection with the problem of the cortico-subcortical relationships in the brain of homoiothermic mammals under experimental conditions. However, it was not actually these results that attracted our attention. From our point of view, the most important findings were those which indicated the possibility of reactive changes in electrogenesis in the brainstem reticular formation under conditions of very low brain temperatures. It is precisely in these structures that external stimuli produce the initial responses. The fact that these changes in the reticular formation can be suppressed by administration of pentobarbital also seems highly significant. Even on the basis of this alone it could be suggested that the neurons of the brain- /34 stem reticular formation may "eliminate" the external stimuli under conditions of low intracerebral temperatures, and that under certain conditions they may affect the subsequent activity of the cerebral cortex.

It was also shown clearly that the excitation of the neurons of the mesencephalic reticular formation under conditions of low intracerebral temperatures is incapable of generalization as a result of external stimuli, so that this is essentially a unique form of local excitation of the reticular neurons as a result of an external stimulus. These findings have been confirmed rather recently by Strumwasser using a microelectrode technique (592). The data obtained have also been confirmed in our studies on hedgehogs and in the work of Massopust and of Johnson et al. on European ground squirrels and prairie dogs (232, 417, 490).

During the following years, Strumwasser continued his studies on the cerebral activity of hibernants, using a telemetric technique to record the electrical activity (594, 595); these studies demonstrated a relationship between electrogenesis and the circadian rhythm or level of daily activity of the animals, this being the first example of a relationship between endogenous cellular rhythms and the state of the genetic apparatus of the central neurons (681). This work is also very important in connection with the special questions of the neurophysiological mechanisms of hibernation as a biological phenomenon. It is particularly interesting to us, however, because it enables us to evaluate some of the general laws of cortical-subcortical interactions from a new point of view — in the light of the characteristic way of life of the hibernating mammals.

We shall return repeatedly to the problem of the legitimacy of extrapolating data obtained on hibernating animals to the level of general physiological laws. In this connection, the "natural" experiment appreciably facilitates the solution of a number of experimental problems.

The persistent electrogenesis in the olfactory bulbs which was detected in the experiments of Strumwasser seems to us to be highly significant. This indicates that the structures of the rhinencephalon have a definite resistance to low temperatures, a resistance which is more pronounced here than in other formations of the brain. In his experiments, however, Strumwasser went beyond a simple detection of this fact and tried to establish some of the principles governing the interaction of the olfactory bulbs in animals, their relationship with the preoptic field of the hypothalamus and the sensorimotor region of the cerebral /35
cortex. Related data were first obtained in hedgehogs (234) and were later confirmed (553).

In their experiments on European ground squirrels and birchmice, K.M. Mokhin et al. (137) and Johansen, Andersen and Krog (267) determined the characteristics of the "spontaneous" activity of the cerebral cortex and hypothalamic region. These workers demonstrated the sequence in the disappearance of the "spontaneous" electrical activity during torpor and arousal of hibernating animals, pointing out that the cortical potentials disappear from the electrocorticogram earlier than the electrical activity in the subcortical areas. Johnson (417) , who studied the changes in electrical activity in the hypothalamic region in ground squirrels , came to analogous conclusions. The amplitude of the EEG was found to be decreased by 50% at a body temperature of 20°C. Below 15-16°C only electrical activity of an irregular arrhythmic type could be recorded from the hypothalamic region.

These studies also provided indirect evidence as to the temperature range over which it is possible to observe the reactive changes in electrogenesis produced by auditory stimuli or the administration of a stream of air into the nasal passages of the animals.

The reactive changes in the EEG of hibernating mammals at various stages of natural adaptation can only be evaluated very approximately on the basis of a review of the literature. The independent problems of studying the reactivity of the EEG and the changes in pulsate activity of the individual neurons in hibernating animals were not solved in a single one of the electrophysiological studies carried out up to 1963-64.

The data which were obtained in this direction were incidental and cannot be used to evaluate the reactive properties of the central neurons in these animals. The first studies of the induced bioelectric potentials in hibernant brain were carried out in our laboratory on hedgehogs and susliks (236, 244), after which the results were confirmed in the laboratory of Massopust (493).

It is obvious that the described changes in electrical activity are intimately connected with the specific biochemical characteristics of cerebral metabolism in hibernants.

When one speaks of biochemical studies on the characteristics of the /36
cerebral metabolism in hibernating mammals, attention should be called first of all to the results obtained in the laboratories of A. V. Palladin (17, 52, 151-153, 192, 193, 217), the Andjus group (3, 268-270) and Burlington (305-307). As will be shown below, these studies were devoted to the entire spectrum of biochemical transformations; they can be grouped somewhat artificially around the changes in protein and amino acid metabolism, carbohydrate and phosphate metabolism, and the oxidative metabolism of the brain.

A. Protein and Amino Acid Metabolism in Cerebral Tissue

The first studies on the cerebral protein metabolism in hibernants were carried out in the laboratory of A. V. Palladin (151-153). These showed that the incorporation of radioactive phosphorus into the phosphoproteins of the brain is significantly inhibited in susliks during hibernation and increases significantly during arousal. These experiments were expanded upon considerably by Ya. V. Belik (17) who studied the rate of incorporation of ^{35}S-methionine into proteins. Methionine-^{35}S was administered to the animals subcutaneously 18 hours before sacrifice in a state of either torpor, active wakefulness or arousal. The results showed that the rate of incorporation of ^{35}S was negligibly low during torpor, that it increased 10-20 fold in a state of active wakefulness, and that arousal was characterized by maximal radioactivity in the cerebral proteins. The author then studied the specific radioactivity of the acid-soluble fraction of the cerebral tissue of susliks in various physiological states. The highest radioactivity was found in the fraction from the hibernating susliks, followed by that from the waking susliks and finally by that from susliks which had been artificially aroused. This demonstrates clearly that the changes in radioactivity described above were not due to changes in the permeability of the hematoencephalic barrier but to the unequal rates of the processes of protein synthesis and turnover in the brains of hibernating animals.

In more recent work (570), the papers cited above have been indirectly confirmed and it has been shown that the RNA metabolism is significantly depressed in hibernating animals.

One of the well-known distinguishing features of the brain is the predominance in its amino acid composition of glutamic acid; this, together with its amide glutamine and the tripeptide glutathione, accounts for more than 50% of the α-amino nitrogen of the brain. Furthermore, in distinction to other tissues, the nonprotein fraction of the brain contains specific substances of the type of γ-aminobutyric acid (GABA), acetylaspartic acid, cystathionine /37
and homocarnosine. Several biochemical studies have recently been devoted to

the metabolism of these substances and their dynamics in the brain of hibernants. One of the first of these was the work of Robinson and Bradley (561), carried out in the Neurochemical Laboratory of the National Institute of Neurological Diseases in Bethesda on golden and European hamsters. The glutamine decarboxylase activity was determined in the homogenized cerebral tissue from two groups of animals, decapitated at 5°C and at 21°C (control). The results showed that the activity of this enzyme is significantly higher in hibernating animals in a state of torpor than in waking animals. This led the authors to stress the possibility of a high rate of GABA metabolism in the brain, since this is known to be connected with inhibitory processes in the central nervous system (355, 364).

The experiments which were recently carried out by E. S. Emirbekov (250-253), which showed that the glutamic acid content is increased by 7.6% in the brains of hibernating animals during the onset of hibernation while the GABA level is increased 2-4 fold, should also be described in this connection; analogous data were obtained by the same author by the study of ammonia levels. He concludes that GABA, glutamic acid and aspartic acid are used as source of energy in the brains of hibernating animals, although he does not categorically exclude a direct role for these substances in the inhibition and excitation of the central neurons. The changes in ammonia and amide levels noted by E. S. Emirbekov are related by him to a role for these substances in the adaptive mechanisms of hibernation. It will be obvious, however, that both conclusions are vague and preliminary in nature.

Very interesting results were obtained in experiments recently completed by N. I. Kalabukhov et al. (24, 25, 97-101) on the changes in tocopherol (vitamin E) levels in the brains of hibernating animals. These showed that the content of this antioxidant is significantly increased in the cerebral tissue of hibernating animals compared to the waking animal, in which the tocopherol content remained relatively constant. In view of this finding, as well as of the known role of vitamin E in the synthesis of acetylcholine, N. I. Kalabukhov may well be correct in suggesting that this substance plays an important role in the cyclic changes in cerebral activity seen in hibernating animals.

B. Oxidative Metabolism /38

This is the most interesting branch of the neurobiochemistry of the hibernants and has therefore been studied somewhat more intensively than the remaining metabolic pathways. However, even the theoretical questions touching on the high tolerance of the brain to hypoxia in these animals are still very far from being solved. Many of these questions have not even been formulated by the experimenters. As was mentioned at the beginning of this chapter, the resistance of these animals to hypoxia and anoxia was already described by the Russian investigators Val'ter and Khorvat at the end of the last and beginning of the current century (39, 220), after which it was confirmed in the West (see (441)). Respiration in hibernating animals does not cease at a body temperature of 5-6°C, while independent respiration in homoiotherms ceases at 17-18°C (39). In the experiments of Kayser on rats which had been acclimated to low temperatures, cessation of respiration was observed when the temperature at the base of the brain reached 15°C; hibernants, on the other hand, continued to

respire activity for long periods at even lower temperatures (270, 437, 441). When the barometric pressure was lowered from 760 to 10 mm Hg, ground squirrels and golden hamsters were found to be significantly more resistant than sparrows, pigeons and rats, tolerating a pressure of 8 mm Hg for 54 seconds while bats were able to tolerate this pressure for more than an hour.

The respiration of the brain in vitro was studied relatively recently by N. M. Shumitskaya in the laboratory of N. N. Sirotinin (249), and even more recently in (565) and (635). These studies showed that the highest level of respiration is attained in the cerebral hemispheres, and that a decrease in temperature leads to a decrease in respiration. The oxygen consumption by brain slices at temperatures of 10-36°C was highest in waking homoiotherms and lowest in hibernating hedgehogs. Intermediate levels were shown by incubated brain tissue from waking heterotherms. In the work of Finnish investigators (565), the following values were obtained for the energy of activation (in kcal. per mol of oxygen consumed): 14.3 in rats and 13.2 and 13.1 in waking and hibernating hedgehogs, respectively; electrical stimulation of brain slices increased the oxygen consumption at 25-36°C for all species of animals, but this effect diminished when the temperature was reduced to 10-15°C. The initial stages of hibernation were accompanied by a sharp drop in oxygen consumption, from 2.179 ml/g brain tissue per hour in waking animals to 0.071 in animals in a state of deep torpor. The output of CO_2 also fell from 2.295 to 0.043, and the respiratory quotient fell from 0.84 to 0.63 which indicates an increased catabolism of fat (400). Arousal produced a radical change in this picture: the oxygen consumption increased to 6.436 ml/g and the CO_2 output increased to 5.733 in the course of an hour. Arousal was also accompanied by an increase in the respiratory quotient to 0.8-1.0; this was connected with the onset of gluconeogenesis and protein catabolism (425, 583), which leads to the formation of metabolic intermediates capable of fixing CO_2. As was shown by the experiments of Popovich (537), the oxygen consumption increases significantly more rapidly than the body temperature during arousal. /39

These characteristics of the oxidative metabolism of the brain of hibernating animals, studied in vitro, may to a certain extent explain the high tolerance of the brain to hypoxia. Thus, the hedgehog can tolerate an oxygen deficit for several hours (295), regardless of whether this is produced merely by a low oxygen tension in the tissues or by interruption of the cerebral circulation at 5-10°C. These experiments were later confirmed, in broad lines, by the work of Kayser (441).

C. Carbohydrate and Phosphorus Metabolism

It is well known that a certain amount of lactic acid is formed in the brain when the oxygen supply is sufficient (aerobic glycolysis). About 10% of the glucose transported to the brain by the bloodstream is converted to lactic acid (50).

Even after only 21-25 hours of torpor, when the body temperature falls to 7.9-8.2°C (compared to a control of 33.8), the blood glucose level in hedgehogs falls from 135 to 60-70 mg % (569). It is also well known that the brain of hibernants consumes more glucose and produces more lactic acid than the brain of homoiotherms.

Glycogen catabolism decreases during hibernation as indicated by the decreased phosphorylase activity and the increased glycogen content in the myocardium (305-308) and possibly in the brain (218,235).

During hibernation blood glucose is formed in small amounts due to gluconeogenesis in the liver, where an active glucose-6-phosphatase can be detected (583). At both 37 and 6°C, the radioactivity of the glucose was higher in hibernating or artificially aroused ground squirrels than in waking ground squirrels (425). The glucose level in the brain decreased by 34% but at a body temperature of 10°C it rose gradually and reached values in excess of those in active animals. The /40 utilization of glucose by the brain is minimal during torpor. The lactic acid content of the brain normally decreases by 56.4% but during prolonged torpor (lasting more than 2 months) at a body temperature of 17°C it can drop by as much as 75.5% (122, 214, 217, 218, 585).

The study of the metabolism of high-energy phosphate compounds in the hibernant brain which was carried out by E. B. Skvirskaya and G. P. Silich (192, 193) showed that the rate of phosphorus metabolism decreases 20-30 fold during torpor; in "hibernating" susliks, there was a decrease in the content of acid-soluble phosphorus and phosphatides and a marked inhibition of the incorporation of ^{32}P into nucleotides and phosphatides. These findings were confirmed by the results of Zimny and Giegory (636) who demonstrated a decrease in ATP levels during hibernation and its rapid expenditure during artificial arousal. The level of creatine phosphate, which catalyzes the transformation of glycogen into ATP, also decreases significantly. Similar results have been obtained recently (3, 269, 270, 440).

Arousal in hibernating animals takes place mainly at the expense of the high-energy compounds which are formed as a result of glycolysis. The concentration of these compounds is maintained at high levels during hibernation (in contrast to the situation in rats which have been acclimated to the cold, in which the results are precisely the opposite). Completed arousal also produces no significant drop in the content of high-energy compounds (122, 192, 585, 636, 637).

* * *

As can be seen from an analysis of the papers reviewed, most of the experimental material has been devoted to definite stages in natural adaptation, particularly the incipient stages of hibernation and torpor. Significantly less work has been done on arousal, either the natural emergence from hibernation or arousal produced by administration of stimulants. Nevertheless, interest in the neurochemistry of arousal was already apparent in the 1920's, when Adler (260, 261) demonstrated the excitatory effect of adrenalin in hedgehogs. Extensive material on the adrenergic nature of arousal in hibernating animals was reviewed in the monograph by S. Ya. Arbuzov (5-8), which is the only major work devoted to this question. It was shown, for example, that the onset of hibernation is considerably more rapid and occurs at higher environmental temperatures in hedgehogs and susliks which have been subjected to prior cervical sympathectomy /41 than in control animals. Hibernating animals which have been subjected to bilateral

cervical sympathectomy succumb to extreme decreases in body temperature (down to 2-3°C). These facts are in harmony, to a certain extent, with the results of experiments which have been described earlier in this section (291, 292, 589).

The data of S. Ya. Arbuzov on the adrenergic nature of arousal in hibernants can also be brought into line with the results of experiments devoted to the effects of sympathicomimetic stimulants on the course of hibernation and the seasonal changes in catecholamine levels in these animals (618-620). Thus, A. N. Kratinov et al. showed that the sensitivity to adrenalin decreases sharply at the beginning of hibernation and under conditions of deep torpor (113). However, this effect, which was observed on an isolated suslik intestine preparation, was not confirmed in the experiments of T. M. Turpaev (212) who perfused the isolated suslik intestine with somewhat higher concentrations of adrenalin. Both the seasonal periodicity in the catecholamine content which was described by Suomalainen and Uuspää (601, 618) and the work described in (513), (611) and (633) indicates that catecholamines are accumulated during hibernation in ground squirrels and hedgehogs and are mobilized when these animals are aroused. Thus, when the body temperature decreased to 6-7°C, the catecholamine content in the liver of ground squirrels increased by 100% and that in the kidneys increased by 68%. The results of these experiments may be correlated with the data already described above (273, 276, 282, 449, 519) to establish a connection between the seasonal periodicity of the catecholamines and the level of hypothalamic secretion in hibernating animals: a neurosecretion which is known to contain catecholamines accumulates in the paraventricular and supraoptic nuclei of the hypothalamic region of hedgehogs and dormice. The experimental data thus confirm the accumulation of catecholamines in the internal organs and hypothalamic region of the brain of hibernants during torpor and their mobilization under conditions of natural arousal.

The hypothesis of the adrenergic nature of arousal in hibernating mammals is also partly confirmed by experiments (395, 397) demonstrating a marked inhibitory effect of the sympathicolytic drug chlorpromazine accompanied by a sharp decrease in body temperature (to 9-12°C) and a deepening of hibernation in ground squirrels.

In addition to the hypothalamic structures of the brain as areas which are /42
sensitive to adrenalin, attention should be directed at the area postrema; this area is located in the wall of the fourth ventricle, is excellently supplied with blood vessels in the hamster, hedgehog and common dormouse, and consists predominantly of glial cellular elements (450, 505). It is connected with the paraventricular nuclei of the hypothalamus and, like this part of the brain, contains maximal amounts of the catecholamines (618, 626). This indicates that it can be included with the hypothalamic nuclei in a single adrenergic system which is directly or indirectly involved in the arousal of hibernating animals.

All of these findings confirm the definite role of cerebral adrenergic mechanisms in the regulation of hibernation. It seems to us, however, that these findings are insufficient to prove that cerebral adrenergic structures play the sole role in arousal of hibernating mammals.

The excitatory effect of atropine, which was first described by Bruman (see (7)), is a well-known fact. Administration of 0.2-0.4 ml of 1-2% atropine solution facilitated artificial arousal in hibernating marmots and produced an increase in heat production, indicating that these animals are quite sensitive to this preparation. Essentially similar results were obtained by Oros, Pora and Chircoiasiu (517) as well as by Lyman and O'Brien (475, 477).

Data on the nature of the biochemical topography of acetylcholinesterase (AChE) in the brains of hibernating mammals are of considerable interest in connection with this problem (561). Experiments have shown that in brain slices suspended in an incubation medium at varying temperatures (10-40°C), the AChE concentration is depressed in hibernating animals compared to controls. The authors relate the decrease in activity of this enzyme to the suppression of "spontaneous" electrogenesis in the cerebral cortex.

Thus, experimental data obtained by various investigators on a variety of hibernating animals have elucidated the basic outlines of the neurochemical mechanisms of hibernant arousal and made possible the formulation of certain hypotheses as to the specific nature of this process in hibernating animals.

Torpor is accompanied by an accumulation of catecholamines, which are bound and fixed both in the neurosecretion granules of the hypothalamic nuclei and in various internal organs of these animals (538, 618-620). In this connection, the effectiveness of the sympathetic mediator hormone shows a definite /43 temperature optimum between 18 and 29°C. Below 18°C, the effectiveness of catecholamines in producing arousal is rather limited. Physiological doses of adrenalin and noradrenalin do not produce arousal until the body temperature has risen above the "critical level".

Nevertheless, cholinolytic substances such as atropine also have an excitatory effect at low temperatures, indicating that this should be looked upon as a possible independent mechanism for the arousal of hibernating mammals which may be significant under natural conditions. A hypothesis of this type envisions a "dual" mechanism for hibernant arousal which has a low-temperature and a high-temperature phase: the first phase is associated with the effects of cholinergic substances and the second with an adrenergic process.

* * *

In any analysis of data pertaining to the mechanisms of cerebral activity in hibernating animals, it is appropriate to include some papers dealing with memory in hibernants.

Interest in the problem of memory has increased markedly during the last decade. The reasons for this are discussed in a number of special monographs and reviews (246, 418, 644, 647, 654, 660, 681). Rather than attempting to review the whole problem again, I would like to refer the interested reader to the special literature. At this point it does seem suitable, however, first of all to examine some of the most recent papers dealing with the effect of low temperatures on the process of learning and habit fixation, and secondly to analyze some of the peripheral data pertaining to the problem of memory in hibernating mammals.

Most of the studies on memory have been carried out on homoiotherms. There are a few publications, however, in which the dynamics of conditioned reflexes with food reinforcement and without a subsequent effect of hypothermia on the established system of temporal connections have been studied in hamsters and susliks (170, 171) .

Thus, the hypothermic factor in the learning process can be studied only in nonhibernants. Data which have been obtained along these lines can be of value only for purposes of comparison. The limits of extrapolation are at the moment very difficult to determine, since the dynamics of short-term and long-term learning processes in hibernants have not been studied at all. /44

Within the framework of the question under discussion, it is possible to indicate the following principal trends of current research:

1) the retroactive effect of hypothermia on a learning process which has just been completed;

2) the effect of hypothermia on animal learning during various stages of rewarming;

3) learning during the cooling process;

4) the effect of prior hypothermia on learning in animals in a post-hypothermic state.

1. Retroactive effects of hypothermia. Conditioned reflexes are maintained and can even be developed anew in animals which have been cooled to 28-30°C. Rats can still orient themselves in the alternative situation of a labyrinth at a rectal temperature of 30°C. Analogous results have been reported by Caine (see (513)) and by Slonim (197, 575). These papers deal with temperatures in the neighborhood of 24-25°C. It is obvious that the brain temperature in such a situation is 4-6°C higher. Consequently, recall of an acquired habit from the memory is possible even when the body temperature has reached relatively low levels.

What can be the reasons for a disruption of the recall mechanism in cooled animals? Are these connected with a change in the afferent or the efferent part of the central reflex arcs? Is perception disrupted in the hypothermic animal or is the realization of the habitual act made difficult by the development of respiratory disorders? The data in the literature, which we have taken from the publications of Foy and Smith, Popovic and Panuska (520-527, 544), would seem to favor the second possibility.

2. Recall and hypothermia. Disruption of recall is observed inconsistently after rewarming (359, 509-512). This may be related either to the difficulty in administering uniform amounts of cold or to an actual irreproducibility of the results. Essman and Sudak (359-361, 596-598) managed to overcome this significant obstacle by cooling the animals during the first 10 minutes after learning. The results showed that even short periods of hypothermia produce severe disruptions in memory.

3. Learning during cooling. These experiments were set up in the following way: the cooled animal, following prior training, was given the possibility of /45 pressing on a lever regulating the admission of warm water or air into the experimental chamber, i.e., actually to regulate the increase in its own body temperature (358, 521). However, despite the original design of the experiment, the evaluation of the results was extremely difficult: the activity of the rats increased with an increase in body temperature, and this was accompanied by an increase in the percentage of accidental bar pressings. In order to alleviate this situation, the authors now plan to introduce a second alternative lever.

4. Effect of prior hypothermia on learning. A significant disruption of the learning process was found in rats which had been cooled to 0-1°C (268, 359-361). In the cooled animal, learning is inhibited more than the ability to perform a previously learned task. This was noted on a model of the passive avoidance conditioned reflex at a body temperature of 35°C (512). Not only was a retroactive inhibitory effect of hypothermia detected in these studies, but testing of the same animals for a definite period after cooling revealed a significant increase in the percentage errors.

Such are the extremely limited and totally insufficient data on the interrelationship between the effects of hypothermia on homoiotherms and short-term memory.

What effect does cooling have on consolidation?

It is well known that consolidation is based on the transformation of a short-term memory into a persistent memory with the production of plastic structural changes in the central neurons which cannot be wiped out by the application of electroshock or hypothermia. There is reason to believe that consolidation may be determined by the following factors:

a) the rate and stabilization of the distribution of electrical impulses entering the "mnemon", the rate of the so-called circulating processes of the "reverberating" type. The more rapidly these take place, the more obvious is the consolidation of the memory;

b) the time of onset of the post-tetanic potentiation which facilitates synaptic transfer during the first stage of short-term memory and thus makes possible the onset of the following stage — the accumulation of vesicles containing mediator in the working synapses. We have called this stage early consolidation;

c) the intensity of activation of the genetic apparatus of the neurons entering the "mnemon" in response to the increased activity of the electrogenic apparatus of the nerve cell. The rate of axoplasmatic flow of protein and RNA /46 along the nerve fiber, which is a prerequisite for the structuration of the memory, is a particularly important factor.

Reviewing these known theories in the light of studies on the effect of hypothermia on habit formation and learning in homoiotherms, it may be hypothesized that cooling of learning animals or those which have just completed a learning process may inhibit consolidation due to changes in the time course of

the various stages of short-term memory. Sufficient indications for this hypothesis can be found in classical neurophysiology. Thus, it has been shown that electroshock, which is known to have a marked retroactive effect, is significantly potentiated by prior cooling (382). Hypothermia thus makes the period of short-term memory more vulnerable to electroshock. This hypothesis is reinforced by the results of Agranov's experiments on goldfish (665). Their maintenance at a water temperature of 9°C during a learning process made the animals significantly less resistant to subsequent electroshock .

Thus, the available data, which are taken principally from the experiments which Agranov, Girard and Essman et al. carried out on nonhibernants, indicate that hypothermia has both a retroactive and a future inhibitory effect on learning.

Can cooling also have a position effect on the development of a habit? This is certainly not excluded in the very early stages of hypothermia when cooling blocks the ascending pathways to the brain, interfering with the learning stimuli (512). This mechanism may also affect the rate of consolidation in cooled homoiotherms.

Unfortunately, these thoughts concerning the specific characteristics of learning in hibernants lack almost any real experimental basis. No work has yet been done relating directly to the analysis of habit formation and habit recall in hibernating animals. One can find a few studies of higher nervous activity, carried out without any consideration for the periods of natural adaptation. Indirect evidence makes it possible to come to some very general conclusions regarding the physiological prerequisites for memory in hibernating mammals.

Ground squirrels are able to control their body temperature at 6 °C and to change their position in the nest at 2-3°C (589, 590). Birchmice begin to squeak at a body temperature of 4-5°C; in the EEG of deeply hibernating Digger ground /47 squirrels and European hedgehogs, external auditory stimulation can still produce activation shifts and induced potentials (236, 237, 318). These experimental results justify the conclusion that some forms of integrative activity are retained in hibernating animals at body temperatures which would be unusually low for homoiotherms.

There can be no doubt that the selection of the facts enumerated in this review has been rather tendentious, since it is known that it is quite difficult to arouse hibernants under experimental conditions: intracardiac injections and transverse acceleration are actually unable to disrupt the natural course of hibernation (15, 363, 460).

It is also interesting that hibernating animals may become habituated to repeated stimulation but that this habit, like all the others, also depends on the level of seasonal activity, the stage of natural torpor, and the biological rhythms.

Thus, neither the studies of the effect of hypothermia on learning in homoiotherms, which have been reviewed above, nor the indirect studies of some of the characteristics of learning in hibernants have been able to provide any concrete experimental evidence supporting an analysis of memory in hibernating

animals and of those physiological mechanisms of cerebral activity which may serve as the basis for learning phenomena in these unique experimental subjects.

* * *

Since the principal problems which we would attempt to cover in this paper have been formulated in the brief introduction to this chapter, there is no need to repeat them here. It is quite clear that the mechanisms of cerebral activity in hibernating animals, in view of the uniqueness and extreme originality of this natural phenomenon, deserve the most intense scientific attention and up-to-date experimental investigation. The data obtained will undoubtedly prove useful, directly and indirectly, to both theoretical neurophysiologists and practical workers interested in the problem of the "cooled" brain and increasing the resistance of man and animals to extreme environmental conditions.

CHAPTER II. /48
CHANGES IN THE BACKGROUND BIOELECTRIC ACTIVITY OF VARIOUS SEGMENTS OF THE BRAIN IN HIBERNATING MAMMALS

General Comments.
Design of the Experiments.
Choice of the Subjects and
Methods of Investigation.
Stereotaxic Atlas of the Brain.

Temperature changes in various parts of the brain in hibernants.

Background electrical activity in the cerebral cortex, the mesencephalic and diencephalic segments of the brainstem, and the structures of the limbic complex during various stages of natural adaptation. The temperature range of systemic changes in electrical activity: desynchronization, synchronization and depression of activity as indicators of cortico-subcortical interaction. Differences in electrogenesis among some representatives of the hibernating mammals. Possible role of the activating and inhibiting structures of the brain in the maintenance of homeostatic equilibrium in hibernants.

The European hedgehog (Erinaceus europaeus L.), in which the neural mechanisms for the regulation of hibernation are predominant, was selected as the principal subject of the present studies. It is these neural mechanisms which determine the regular periodicity in the activity of the physiological systems which is so characteristic of hibernating animals (196, 452). Some of the supplementary experiments and control studies were done on susliks (C. suslica P., C. erythrogenus Brandt).

The goals of this study necessitated the establishment of certain general /49
methodological conditions designed to guarantee an adequate solution to the questions presented. The first of these prerequisites was accurate localization of the electrodes in the desired cerebral formations. Fulfillment of this condition necessitated calculation of the stereotaxic coordinates of the gabaritic points of the principal cerebral formations (which are not to be found in the

literature), the functional characteristics of which were studied in the succeeding experiments, and the compilation of a stereotaxic atlas of the hibernant brain.

The coordinates of the gabaritic points were calculated in 15 European hedgehogs and 6 susliks, the heads of which were positioned in the headholder of an EMIB-1 or SEZh-1 stereotaxic instrument in such a way that the line connects the centers of the ear-holders and the lower edge of the orbits was perfectly parallel to the frame of the instrument; this guaranteed a stable reading of the gabaratic points taken in relation to the inion. In all cases we measured the distance from the tip of the inion to the center of the subcortical nucleus in the sagittal direction of movement of the electrode (fixed for this purpose in an electrode holder) and in the frontal plane with respect to the inion and the bregma. The position of the center and of the inner and outer edges of the subcortical nucleus was determined with respect to the immovable orientation points. The recording electrode was a steel needle, fixed in a holder and "attached" to a system of rectangular coordinates with the aid of an R.M. Meshcherskiy control column (131).

The brain of each animal was lightly fixed by injecting at most 50-60 ml of 10% formalin via the carotid arteries under a pressure not exceeding 100 mm Hg. The animals were then decapitated and 10-15 holes with a diameter not exceeding 4 mm were drilled into the base of the skull (after exarticulation of the lower jaw) and the fornix in order to facilitate subsquent fixation of the brain.

Calculations of the coordinates of the gabaritic points were carried out both on fresh brain and 15-20 days after fixation. The error in the calculations caused by switching from fixed to fresh brain did not exceed 8-10%. The numerical values of the points were read off after frontal bisection of the olfactory bulb at a distance of 1 mm from the anterior poles.

Tables 2, 3 and 4 show the statistically verified stereotaxic coordinates of the gabaritic points of 12 subcortical formations in 15 European hedgehogs, /51
while tables 5 and 6 show analogous data for 15 nuclear structures in the brain of the ground squirrel (worked out for 6 animals).

TABLE 2. BONE DISTANCES IN THE EUROPEAN HEDGEHOG (CALCULATED IN 15 ANIMALS) /50

Measured distance	(Mm ± mm)
Width of the skull (maximal distance between the most widely separated perpendicular lines drawn tangent to the frame of the instrument and the ear-holders)	20.3 ± 6
Distance between the centers of the acoustic ducts	13 ± 0.3
Distance from the tip of the inion to the coronal suture	17.6 ± 0.4
Height of the skull (distance from the highest point on the fornix to the os basilare)	14.7 ± 0.1
Distance from the tip of the inion to the frontal zero plane	2.6 ± 0.3
Distance from the frontal zero plane to the coronal suture	14.7 ± 0.3

TABLE 3. STEREOTAXIC COORDINATES OF THE GABARITIC POINTS OF THE SUBCORTICAL FORMATIONS IN THE EUROPEAN HEDGEHOG

Structure	F	H	L
Olfactory bulb	18.8±0.4	7.5±0.3	2.8± 0.2
Septum	17.0±0.2	9.6±0.1	1.4± 0.2
Amygdaloid nucleus	12.0±0.3	12.2±0.1	2.7± 0.1
Corpus callosum	11.0±0.8	4.1±0.2	2.8± 0.1
Medial nuclei of the optic thalamus	10.5±0.4	8.7±0.2	1.2± 0.1
Lateral nuclei of the optic thalamus	10.8±0.1	8.9±0.2	3.5± 0.2
Hippocampus	8.1±0.3	6.5±0.2	3.5± 0.2
Hypothalamus	10.5±0.4	12.2±0.2	0.3± 0.1
Central gray matter	10.5±0.3	13.0±0.2	0.8± 0.1
Subtegmental part of the mesencephalon	6.0±0.4	10.1±0.2	1.0± 0.1
Anterior prominence of the lamina quadrigemina	6.5±0.2	-	0.5± 0.1
Posterior prominence of the lamina quadrigemina	4.1±0.2	0.5±0.1	1.2± 0.1

TABLE 4. STEREOTAXIC COORDINATES OF THE GABARITIC POINTS OF THE SUBCORTICAL FORMATIONS IN THE EUROPEAN HEDGEHOG

Structure	F	H	L
Olfactory bulb	18.8 ±0.4	7.5±0.2	2.8± 0.2
Septum	17.0±0.2	9.6±0.1	1.4± 0.2
Amygdaloid nucleus	12.0±0.3	12.2±0.1	2.7± 0.1
Corpus callosum	11.0±0.8	4.1±0.2	2.8± 0.2
Medial nuclei of the optic thalamus	10.5±0.4	8.7±0.2	1.2± 0.2
Lateral nuclei of the optic thalamus	10.8 ±0.1	8.9±0.2	3.5± 0.2
Hippocampus	8.1 ±0.3	6.5±0.2	3.5± 0.2
Hypothalamus	10.5±0.4	12.2±0.2	0.3± 0.1
Central gray matter	10.5±0.3	13.0±0.2	0.8± 0.1
Subtegmental part of the mesencephalon	6.0±0.4	10.1±0.2	1.0± 0.1
Anterior prominence of the lamina quadrigemina	6.5±0.2	-	0.5± 0.1
Posterior prominence of the lamina quadrigemina	4.1±0.2	0.5±0.1	1.2± 0.1

TABLE 5. BONE DISTANCES IN THE SUSLIK (CALCULATED IN 6 ANIMALS)

Measured distance	(Mm ± mm)
Width of the skull	19.0±0.1
Distance from the center of the ear-holder to the highest point on the fornix	11.5±0.3
Distance from the inion to the coronal suture	19.3±0.2
Distance from the inion to the frontal zero plane	11.5±0.2
Distance from the inion to the posterior edge of the orbit	14.0±0.3

In addition, we have worked out stereotaxic atlases of the brains of the hibernants which are most commonly used in Russian and Western neurophysiological work (the European hedgehog - Erinaceus europaeus L., the red-cheeked suslik- Citellus erythrogenus, and the golden hamster - Cricetus auratus W.). Our own work, which included a significant number of stereotactic operations on the brains of these animals, has confirmed the agreement between the coordinates shown in these atlases and the position of the principal cerebral formations*. /52

TABLE 6. STEREOTACTIC COORDINATES OF THE GABARITIC POINTS OF THE SUBCORTICAL FORMATIONS IN THE SUSLIK

Structure	F	H	L
Nucleus caudatus	13.5±0.3	3.0±0.1	3.0±0.2
Septum	13.5±0.2	4.0±0.2	0.5±0.1
Fornix	15.0±0.3	3.5±0.2	0.3±0.1
Anterior commissure	12.0±0.2	6.0±0.1	2.0±0.1
Hippocampus	9.5±0.3	3.0±0.2	2.0±0.3
Horn of Ammon	8.0±0.2	5.5±0.3	6.0±0.2
Amygdaloid nuclei	10.5±0.2	9.0±0.2	2.0±0.2
Hypothalamus lateralis	9.5±0.3	9.0=0.3	4.0±0.2
Posterior hypothalamus	8.5±0.1	9.2±0.3	0.5±0.1
Ventromedial nuclei of the optic thalamus	9.5 0.2	10.0±0.2	1.5±0.2
Lateral geniculate body	8.5±0.3	6.0±0.3	3.0±0.2
Lateral nuclei of the optic thalamus	8.0±0.2	6.0±0.3	3.0±0.1
Medial nuclei of the optic thalamus	8.0±0.1	7.0±0.1	0.5±0.1
Central gray matter	5.0±0.2	7.0±0.2	0.5±0.1
Subtegmental part of the mesencephalon	5.0±0.2	9.1±0.3	1.5±0.2
Anterior corpora quadrigemina	5.0±0.2	2.5±0.1	1.0±0.3
Posterior corpora quadrigemina	4.5±0.3	1.5±0.1	1.5±0.1

TABLE 7. WEIGHT AND BOND DISTANCES (BREGMA-LAMDA OF BL) IN THE SKULL OF EXPERIMENTAL ANIMALS

Animal	BL	Weight
European hedgehog — Erinaceus europaeus L.	11.2	750.0
	11.2	800.0
	10.5	810.0
	11.0	850.0
	Avg. 10.0	820.0
Red-cheeked suslik— Citellus erythrogenus	6.1	280.0
	6.0	310.0
	5.9	400.0
	5.8	350.0
	5.8	350.0
	Avg. 5.9	
Golden hamster — Cricetus auratus W.	3.0	100.0
	3.2	95.0
	3.0	110.0
	2.9	100.0
	3.1	108.0
	Avg. 3.0	

*Students of the Novosibirsk State University took part in compiling the atlas.

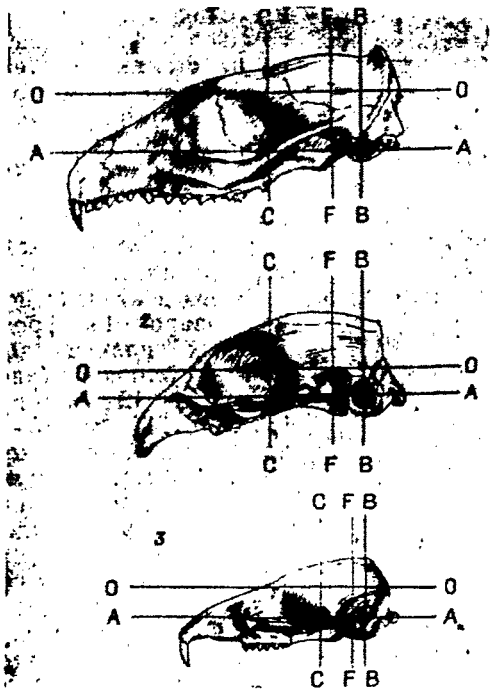


Figure 1. Principal Planes of Reference in the Brain of the European Hedgehog (1), the Red-cheeked Suslik (2), and the Golden Hamster (3). A--A Principal Horizontal Plane; O--O Horizontal Zero Plane; F--F Frontal Zero Plane; C--B Portion of the Brain used for Calculating the Stereotaxic Coordinates.

After the animals had been exsanguinated under nembutal anesthesia, the brains were fixed with equal volumes of physiological solution and 10% neutral formalin via the carotid arteries. Then, after broad trepanation of the fornix, removal of the lower jaw and opening of the accessory cavities, the skull with its contents was immersed in neutral formalin for two days.

Two days later the head was positioned in the head-holder of an EMIB-1 or SEZh-1 stereotaxic instrument. The plane passing through the centers of the acoustic ducts and the lower edge of the orbit served as the principal reference plane. The horizontal zero plane was taken at 10 mm above the principal plane in the hedgehog and 4.5 mm above the principal plane in the marmot and hamster. The skull of the hamster was positioned in such a way that the center of the ear-holder was two millimeters below the edge of the orbit (Fig. 1). /53

With the aid of the horizontal head of the stereotaxic instrument, steel needles were then introduced from the side of the cerebellum, parallel to the principal reference plane and at a distance of 4.5 mm from the sagittal line in hedgehogs and susliks and 3 mm in hamsters. Vertical marking needles were placed at a distance of 3-3.1 mm to the right and left of the sagittal line, perpendicular to the principal horizontal plane and parallel to the frontal plane. The first pair of vertical needles was introduced at a distance of 4.5 mm from the frontal zero plane and the second at 14.5 mm from the frontal zero plane (in hedgehogs and susliks), while in hamsters the corresponding distances were 2 and 7 mm (see Fig. 1).

Thus, the frontal recording plane was taken as a vertical plane situated 4.5 mm (for hedgehogs and susliks, 3 mm in hamsters) in front of the usual /54 frontal zero plane (a plane passing through the centers of the ear-pins perpendicular to the horizontal reference plane).

After introduction of the needles the brain was again fixed for 5 days. Then, the needles were withdrawn and, by passing a blade parallel to the frontal plane, a section 10 mm thick (in hedgehogs and susliks, 5 mm thick in hamsters) was excised and fastened onto the stage of a freezing microtome. Frontal slices 90 microns in thickness were then prepared from this section, after which every third slice was photographed and graphically reconstructed.

A total of 14 frontal planes in the susliks brain (Fig. 2-8), 12 in the hamster (Fig. 9-14) and 13 in the European hedgehog (Fig. 15-21) were used to compile the atlases. The agreement between these coordinates and the location of the intracerebral nuclei, as well as their cellular composition, was controlled in slices stained by the method of Nissl. In addition, in a special series of experiments, we studied the electrode tracks marked by means of a direct current after orientation of the electrodes in the brain on the basis of the described coordinates. The weight and bone distances of the experimental animals are shown in Table 7.

In order to verify the structures we used data available in the literature, especially Bauchot's cytoarchitectonic diagrams of the brains of insectivores (289), the stereotaxic atlas of the golden hamster compiled by Smith and Bodemer (579), and the diagrams published by I. N. Filimonov (216), V. V. Amunts (2) and Petrovicky (533). The Latin abbreviations used are taken from the atlases of Jasper and Aimon-Marsan and Marshall and Firkova (37), which are in common use among neurophysiologists.

KEY TO THE ATLASES /55

A

AAA — area amygdalaris anterior
AB — nucleus basalis amygdalae
AC — nucleus centralis amygdalae
AD — nucleus antero-dorsalis
AL — nucleus lateralis amygdalae
AM — nucleus anteromedialis thalami
AHA — area hypothalamica anterior
AMYG — amygdala
AHL — area hypothalamica lateralis
APL — area praeoptica lateralis
APM — area praeoptica medialis
AV — nucleus anteroventralis thalami

C

CA — commissura anterior
CC — corpus callosum
CCS — commissura colliculi superior
CD — nucleus caudatus
CDP — caudate-putamen complex
CE — capsula externa
CF — commissura fornicis
CHO — chiasma opticum
CI — capsula interna
CL — claustrum
CM — centrum medianum
CORT — cortex
CP — commissura posterior
CPYR — cortex pyriformis
CS — colliculus superior

D

DIAG — regio diagonalis
DTV — decussatio tegmenti ventralis

E

EP — regio entorynalis
EC — capsula externa

F

FF — fimbria fornicis
FD — fascia dentata
FX — fornix
FO — fasciculus opticus

G

GL — corpus geniculatae laterale
GLD — corpus geniculatae laterale (pars dorsale)
GLM — corpus geniculatae mediale
GLV — corpus geniculatae laterale (pars ventralis)
GP — globus pallidus

H

H — habenulae
HDM — nucleus dorsomedialis hypothalami
HIPP — hippocampus
HL — nucleus habenulae lateralis
HM — nucleus habenulae medialis
HP — hypothalamus posterior
HVM — nucleus ventromedialis hypothalami
H_{1-5} — area of Ammon's horn

J

JP — nucleus interpeduncularis

L

LA — nucleus lateralis anterior
LM — lemniscus medialis
LP — nucleus lateralis posterior

M /56

MD — nucleus medio-dorsalis
MM — nucleus mammilaris medialis
ML — nucleus mammilaris lateralis

N

NCAST — nucleus commissurae anterior et striae terminalis
NCM — nucleus centralis medialis
NFS — nucleus fimbrialis septi
NHP — nucleus hypothalamicus posterior
NP — nuclei pontis
NRP — nucleus reticularis pontis

N

NRT — nucleus reticularis tegmenti
NSL — nucleus septalis lateralis
NSM — nucleus septalis medialis

P

P — nucleus posterior
PC — pedunculus cerebri
PF — nucleus parafascicularis
PR — praetectum
PSB — regio praesubiculum
PTH — nucleus posterior thalami
PUT — putamen
PV — nucleus paraventricularis
PVA nucleus paraventricularis — anterior

R

R — nucleus reticularis
RAMYG — regio amygdalae
RE — nucleus reuniens
RET — nucleus reticularis thalami
RH — nucleus rhomboidalis
RPO — nucl. retic. pontis oralis
RPYR — regio pyriformis
RV — nucleus reticularis ventralis

S

SGC — stratum griseum centrale
SN — substantia nigra
SO — nucleus supraopticus
SPT — septum
ST — stria terminalis

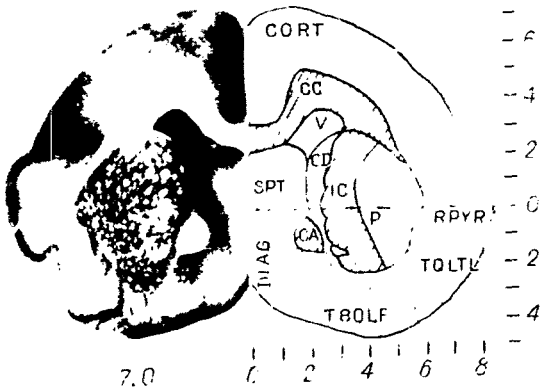
T

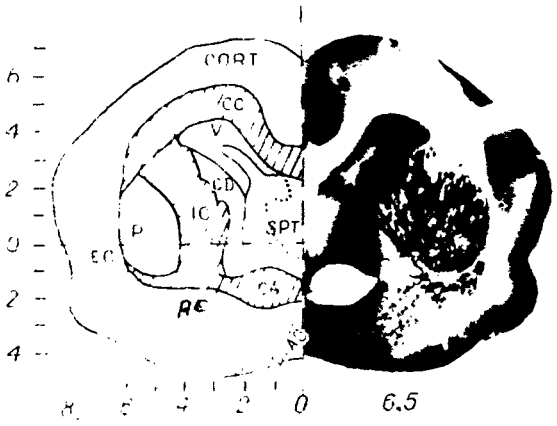
TBLF — tuberculum olfactorium
THP — tractus habenulae-peduncularis
TO — tractus opticus
TOLF — tractus olfactorius
TMG — tractus mammillotegmentalis
TT — taenia tecta
TMT — tractus mammillothalamicus
THP — tractus habenulae—peduncularis

V

V — ventriculus lateralis
VA — nucleus ventralis anterior
VD — nucleus ventrodorsalis
VDM — nucleus ventralis (pars dorsomedialis)
VE — nucleus ventralis thalami
VL — nucleus ventralis lateralis
VM — nucleus ventralis medialis
VPM — nucleus praemammillaris ventralis

Stereotaxic Atlas of the Brain
of the European Hedgehog





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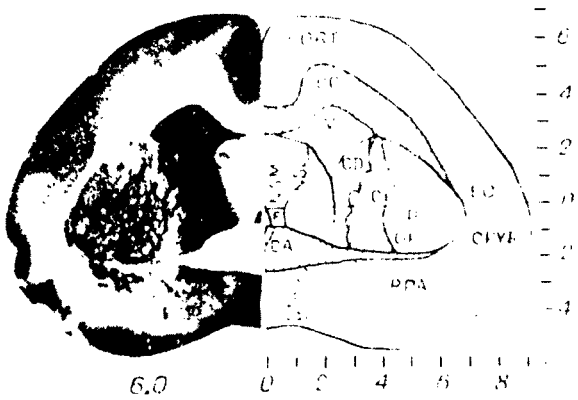

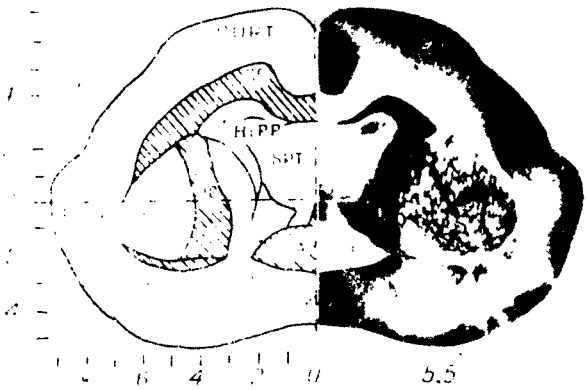

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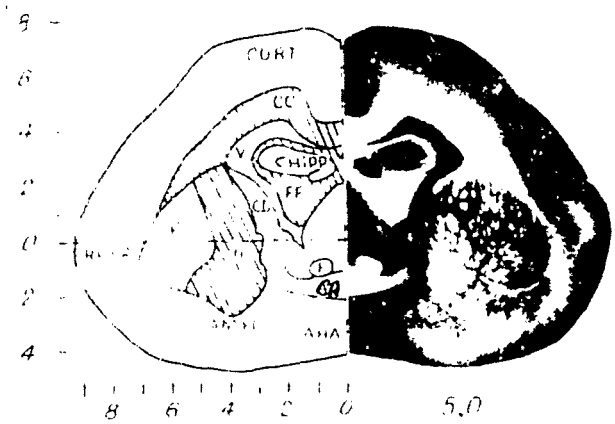

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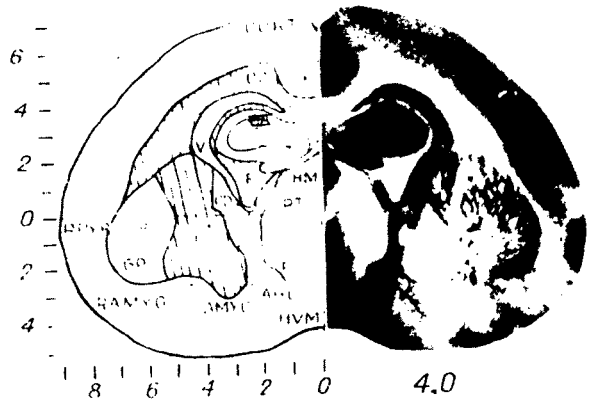

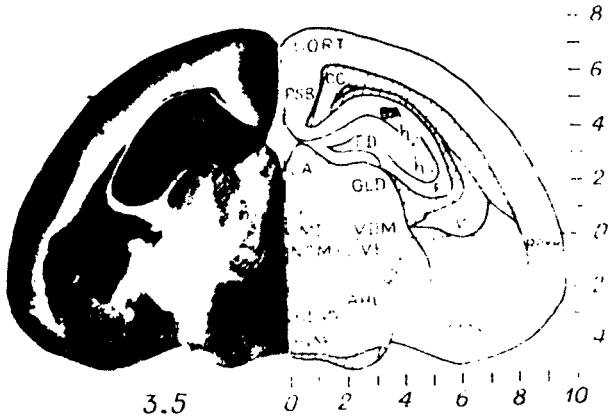

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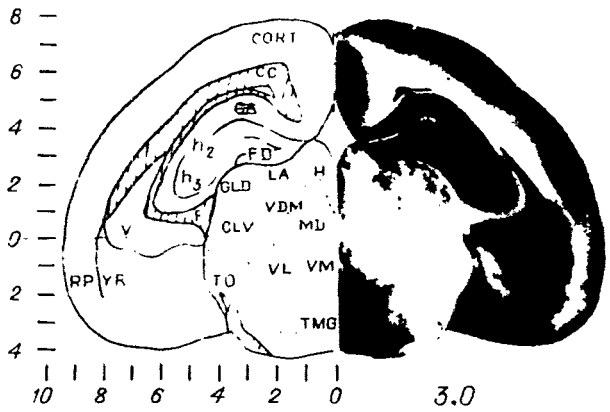
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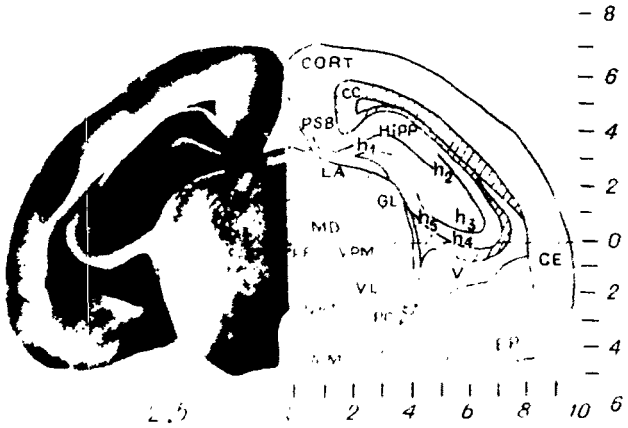
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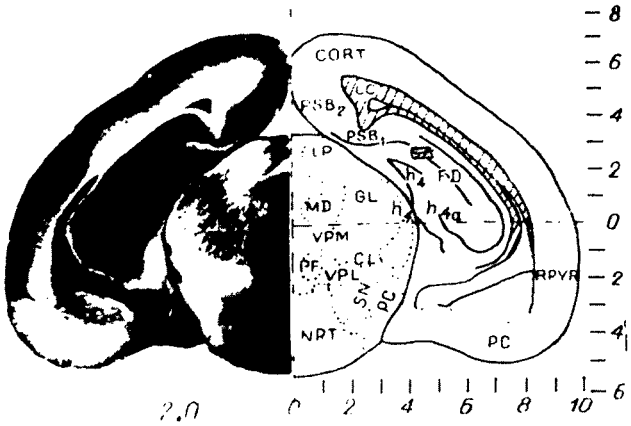
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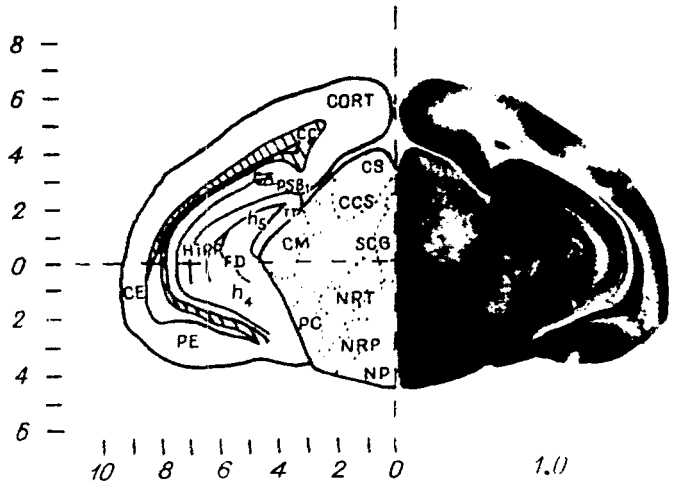
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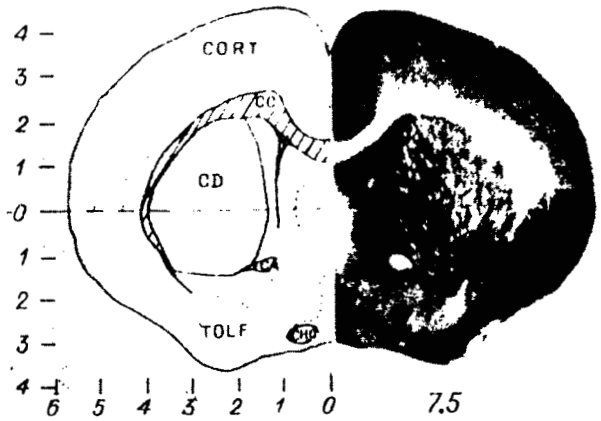
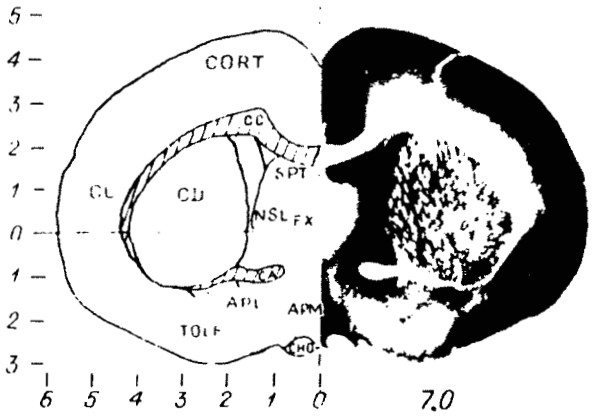
Figure 8.

Stereotaxic Atlas of the Brain
of the Golden Hamster





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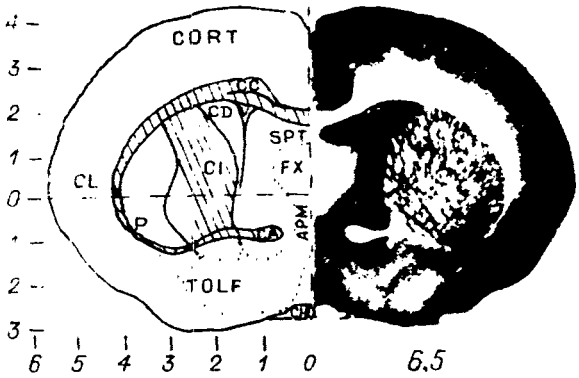
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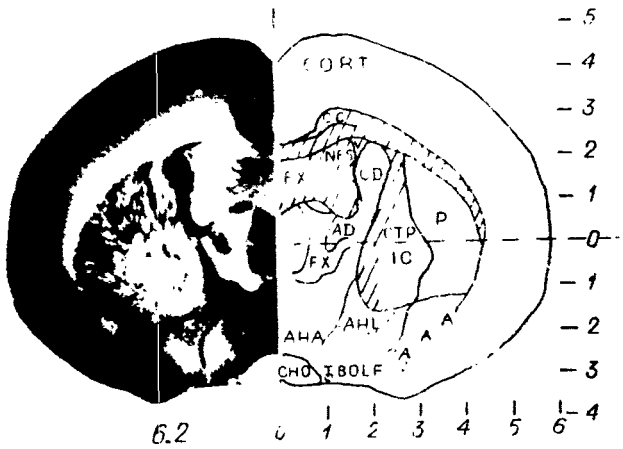
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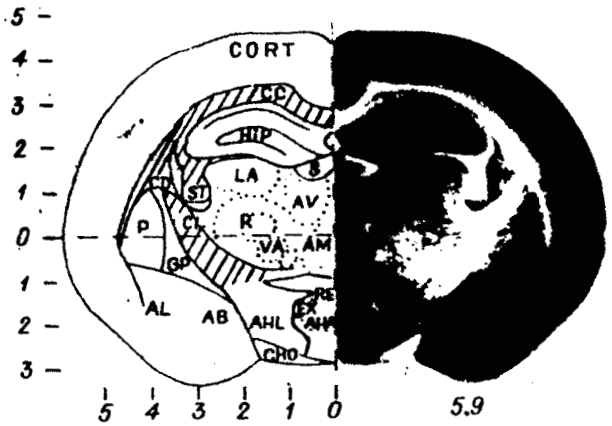
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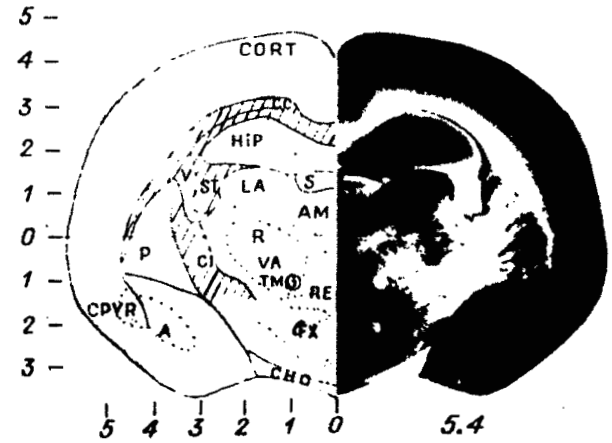
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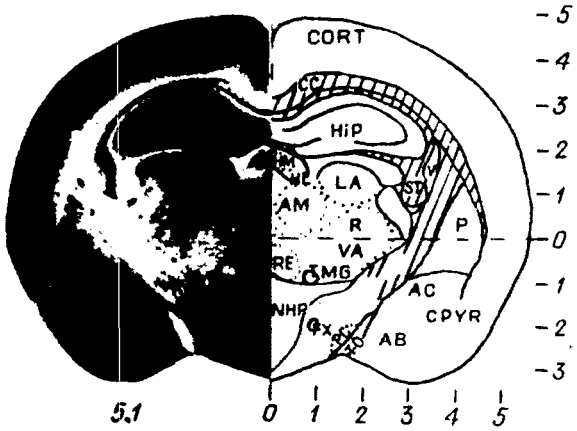
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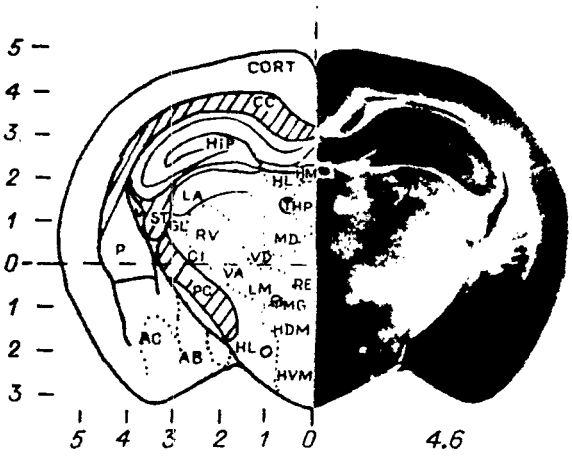
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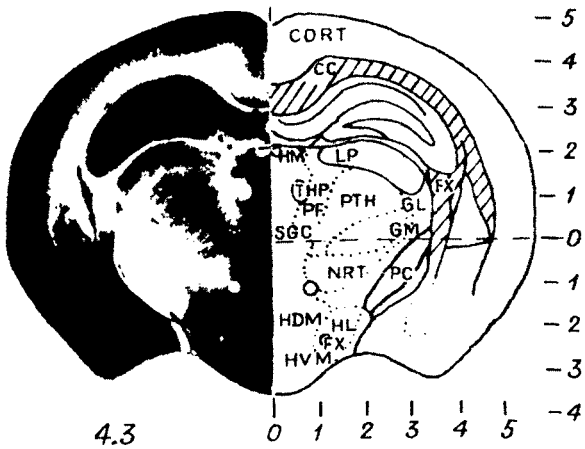
Figure 12.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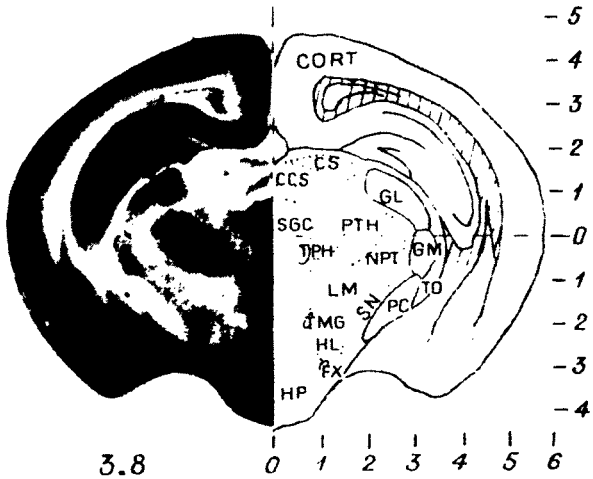
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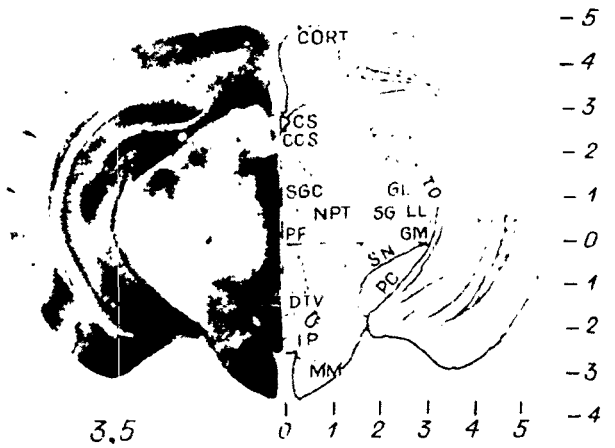
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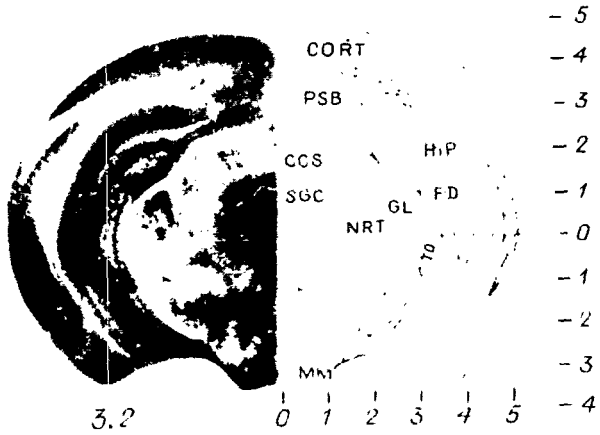
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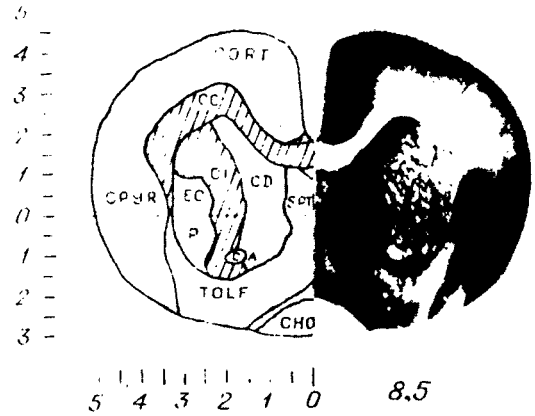
Figure 14.



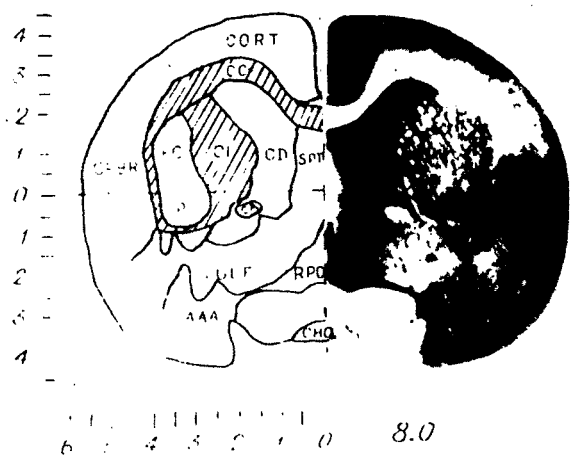


Figure 15.

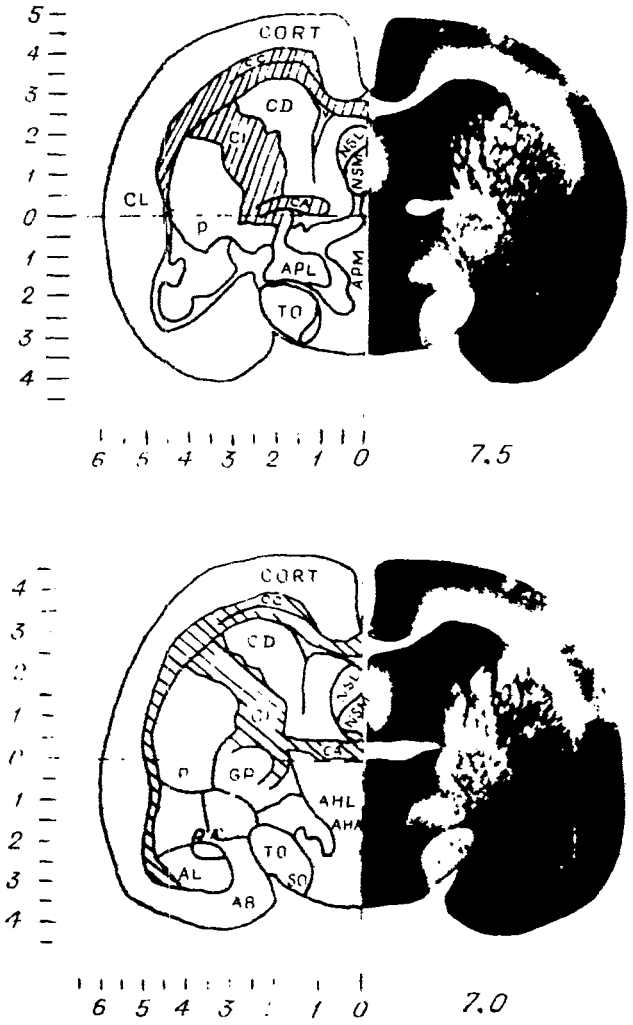

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Figure 16.

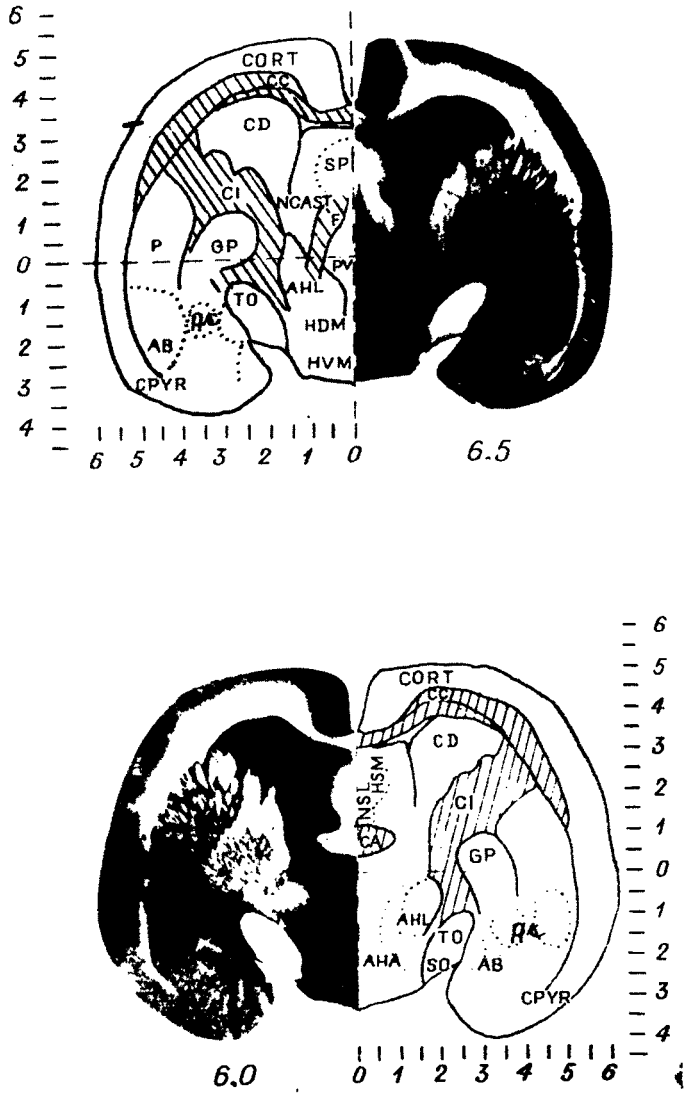
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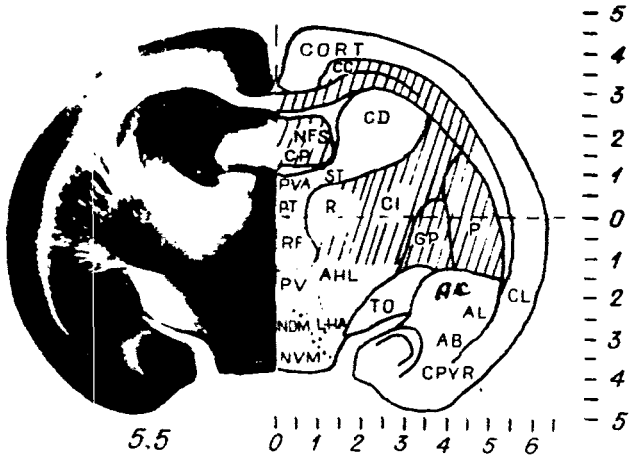
Figure 17.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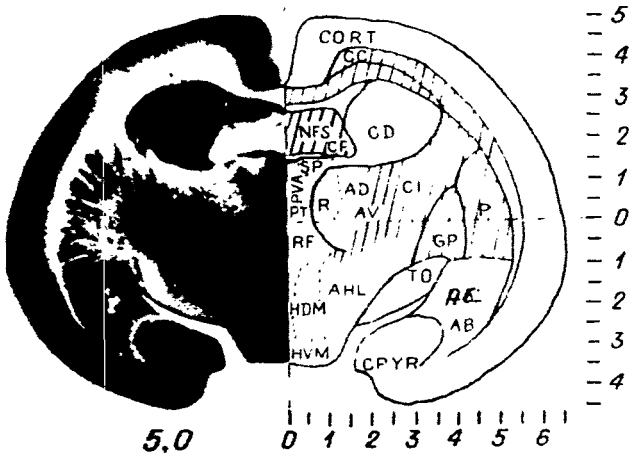
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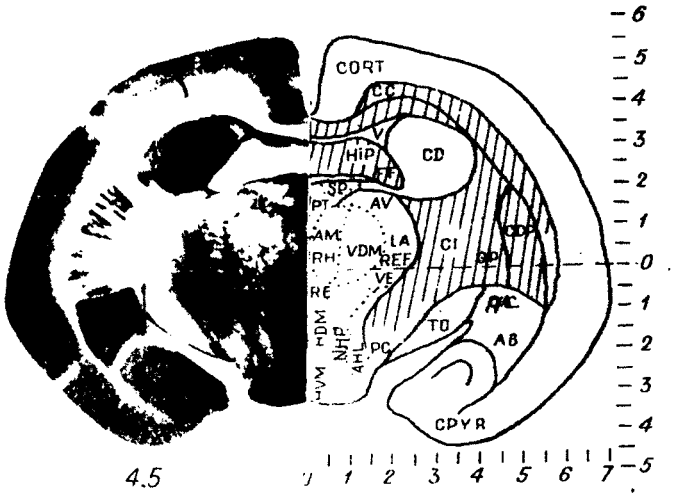
Figure 18.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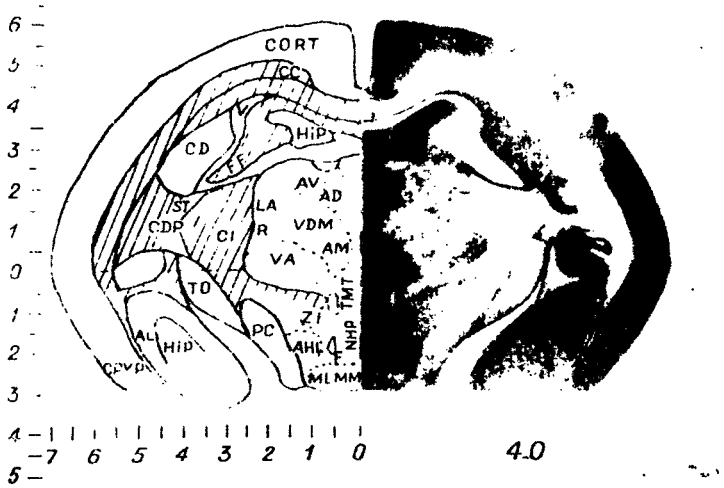
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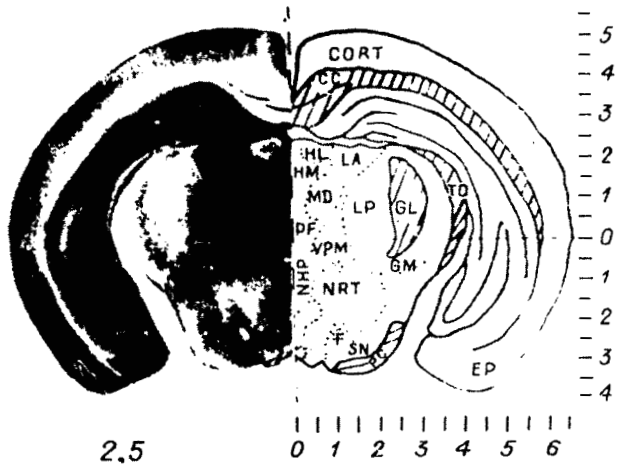
Figure 19.

Figure 20.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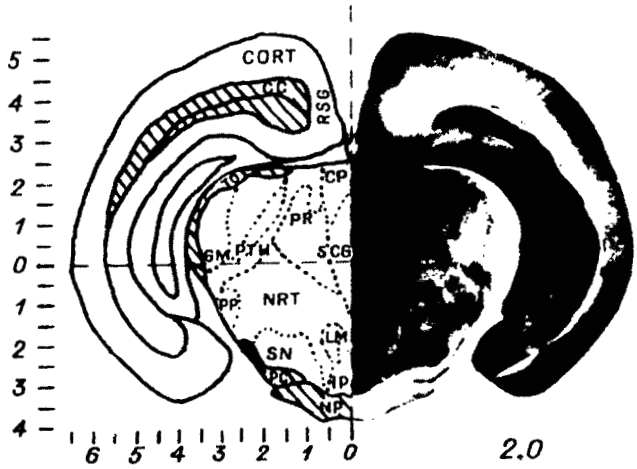
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Figure 21.

During the period from October to March in 1959-63, 48 experiments /57
varying in duration from 2 days to 2.5 months were carried out on 39 European hedgehogs and 9 susliks; the experiments were planned so as to cover the principal period of hibernation of these animals (94, 346). Some of them (28 experiments) were carried out in the Western Urals (Perm), while the others were carried out in the southwestern Ukraine (Odessa). Since the latitude had a definite effect on the time of hibernation, its beginning and end, it also had an effect on the cyclic nature, continuity and duration of torpor.

In the first, operative stage the animals were positioned in the head-holder of the stereotaxic instrument and electrodes were inserted into the various subcortical formations on the basis of the stereotaxic coordinates. In the overwhelming majority of the experiments chloralose was used as the anesthetic (not more than 80 mg for an animal weighing 600-750 g, and an average of 50-65 mg). The choice of anesthetic was made during control recordings of the EEG, respiration and ECG, after which all of the subsequent animals were operated on successfully. For long-term experiments we generally inserted, on the basis of the stereotaxic coordinates, platinum or nichrome electrodes covered with liquid plexiglass and having a diameter of at most 100 microns. When bipolar microelectrodes were used, the distance between the electrodes did not exceed 0.5 mm in the vertical direction (resulting in an interelectrode resistance of at most 40 kΩ). Platinum screws and wires having a diameter of 600-800 microns were used as cortical electrodes.

The electrodes were connected to the input of the amplifier by means of multiple soft conductors which guaranteed complete freedom of movement to the animal in the chamber, intake of food, etc. At the sites of insertion of the electrodes the surface of the skull was covered with a quick-drying plastic of the "Stirakril" type which guaranteed a firm fixation of the armature of the two independent socles linking the experimental subject with the input of the amplifier and preventing artefacts during the recording of the electrocorticogram.

Depending on the design and purpose of each experiment, the animals were either maintained under free conditions at room temperature or placed in a refrigerator where the environmental temperature could be regulated within the limits from -4 to +18-20°C. After the animals had been placed in the cold room, the temperature was decreased gradually, reaching 2 ± 2° C within 7-12 days
after the operation; this temperature range was found to be optimal for the on- /58
set of torpor.

The refrigerator was divided into communicating compartments (150 × 150×280 mm). In some cases the dividing walls were taken out and several animals were then placed in these cages at one time. Each of these compartments was equipped with an individual sound system, an independent oxygen supply and an electrode-distribution stand. After the temperature in the rooms reached 2-4 °C, feeding of the animals was stopped and then the water supply was also interrupted.

The intracerebral temperature was recorded by means of copper-constantan thermocouples of MT-54 low-temperature microthermoresistors covered with liquid plexiglass dissolved in chloroform and connected to a mirror galvanometer.

A train oscillograph of the MPO-2 type with a rheostat-capacitance amplifier at the input, and the six-channel recording electroencephalograph of the Experimental Plant of the USSR Academy of Medical Sciences and the "Alvar-Electronics" polygraph company were used at various stages of the investigation to record the biopotentials. At the end of each experiment the electrodes were either marked with a direct current or their position was visually evaluated.

Recording of the EEG was naturally begun immediately after the animals were placed in the refrigerator, or even immediately after the operation when they were still outside the chamber. During the first 5-7 days the recording was continued around the clock at intervals of 40-90 minutes. Thereafter, in connection with the stabilization of the EEG, it was only recorded 2-4 times a day.

The duration of each experiment was determined by a combination of the following factors: the depth and rate of onset of hibernation, the frequency of "spontaneous" arousal, the nature of the electroencephalographic findings, and the activities planned after the experiment was terminated (histochemical studies, marking of the electrode placement by a direct current, etc.).

Out of 121 animals operated on during the period 1959-64, only 48 were selected for a long-term experiment and in only 18 of these did we succeed in observing a full cycle including all the stages of natural adaptation: onset of hibernation, torpor, so-called tentative awakening and final arousal.

We are specially emphasizing this problem since it seems to us that the extreme difficulty of the study was in most cases unrelated to the essence of the experimental task or the methodological and technical capacities of the investigator, being caused exclusively by the characteristics of the physiological /59 activity of the experimental subjects under natural conditions. Success or failure of an experiment was most commonly related to the peculiarities of the vital functions of the hibernating mammals, on which the possibility of exerting an external effect was quite limited. This is also the reason for the extremely small number of studies devoted to an analysis of the bioelectric phenomena in hibernating animals under natural conditions of life. Kayser (441) has also pointed out analogous difficulties.

If the publications of the last decade which have been devoted to the electroencephalographic study of hibernants are approached critically, if is easy to note that the nature of the study was limited only by the use of the hibernants as one-time subjects of an electrophysiological experiment.

In the work of Chatfield, Kayser, Suomalainen et al., Strumwasser and Johnson (320, 417, 441, 592) which was reviewed above, there is no electroencephalographic description of all the successive stages of hibernation. The period of torpor, when the brain temperature reaches its lowest values, and the stages of active wakefulness have been the most frequently studied. It is obvious that this was caused not by the special problems of the experiment but by the impossibility of following all stages of hibernation in a relatively small number of animals. We have tried to eliminate these deficiencies to a certain extent: out of a large number of experiments, we have selected those in which

we succeeded in observing the detailed electroencephalographic changes during the principal successive stages of mammalian hibernation. In this way, the study of the hibernating animal was replaced by a study of hibernation itself.

The temperature of the cerebral cortex and the subcortical formations; its changes in connection with the onset of hibernation and torpor. The temperature of the brain undergoes significant fluctuations during the first 24 hours after the animal is placed under low-temperature conditions. These fluctuations also take place in the waking animal, but they are most marked during the one or two days just before onset of hibernation. In general, the fluctuations in brain temperature during hibernation are one of the most important indications of its discontinuous course; this has been described quite clearly in the work of N. I. Kalabukhov (93, 94) and has been the subject of special studies carried out /60 by both Western and Soviet authors (87, 437, 494, 528, 529, 531).

The onset of hibernation is also characterized by significant fluctuations in the temperature of the cerebral cortex and subcortical formations. The cortico-subcortical temperature gradient frequently exceeds 7-10°C, since the temperature of the cortex drops significantly more rapidly than the temperature of the hypothalamus or the reticular formation of the brainstem. After the onset of hibernation has been completed, the lowest temperatures are found in the cerebral cortex while the highest are recorded in the hypothalamus.

"Spontaneous" temperature fluctuations, about which a few words have been said earlier, appear most commonly in the formations of the brainstem. They may attain 5-6°C in the course of a few hours, at the same time that the temperature in other parts of the brain remains relatively stable. These fluctuations attain their maximal amplitude when the animal is aroused from hibernation or (even more markedly) under conditions of the so-called "tentative" arousal which will be described below. Each successive lapse back into the hibernating state is characterized by the gradual disappearance of this temperature gradient. This fact acquires considerable importance in evaluating the mechanisms of the maintenance of cerebral homeostasis in the hibernating animal.

We were unable to observe decreases in temperature below 2°C for the cerebral cortex and 3°C for the subcortical structures. A more significant drop in brain temperature was inevitably accompanied by either complete or "partial" arousal of the animal.

Regardless of the factors causing it, arousal of the hibernating animal was most commonly characterized by a very rapid return of the brain temperature to its initial values; arousal became irreversible after the temperature in the subcortical structures, particularly in the hypothalamus and the reticular formation of the mesencephalon, reached 19-20°C. This increase in brain temperature during arousal is characterized by its exponential form and by the absence of the periodic fading which is so characteristic of the onset of hibernation and the period of torpor.

Background bioelectric activity in the various parts of the brain of hibernating mammals in the waking state. Before describing the findings relating to

the electrical phenomena of the waking state in hibernants, attention should be called to the fact that the rhythmic picture of the EEG is quite different in form in the different parts of the brain and includes most of the known forms of bioelectric activity. This contradicts to some extent the hypothesis of Jerme (409), the essence of which is that the complexity of the EEG picture is determined primarily by a functional load on some portion of the brain (thus explaining why the "richest" electrical activity in the brain of the European hedgehog was found in the olfactory lobe). This was not confirmed in our experiments. /61

The background activity in the cerebral cortex of the waking animal is characterized by the presence of regional differences: the sensorimotor region of the cortex is characterized by mixed dysrhythmic waves having a frequency between 3-6 and 20-30 cycles/sec. The amplitude of these waves usually does not exceed 30-40 microvolts. In the anterior portions of the cerebral cortex of hibernating animals, against the background of the dysrhythmic activity described above, slower waves exceeding 80-100 microvolts in amplitude are also sporadically recorded in the waking animal.

The parietooccipital areas of the cerebral cortex are characterized by slower grouped waves which are quite similar to the "burst activity" described and studied in detail in homoiothermic mammals (61, 127, 184, 185, 187, 642).

The analysis of the statistical distribution of the waves in the EEG performed by Faure (362) showed that the dominant form of bioelectric activity in the cerebral cortex in the waking state, at a cortical temperature of 32-34°C, consists of low-voltage waves having a frequency of 15-20 cycles/sec. and an amplitude of no more than 50-60 microvolts. This type of activity also predominates in the subcortical structures, particularly in the hypothalamus and hippocampus.

Thus, one can provisionally speak of three types of "spontaneous" activity that can be recorded in the brain of hibernating mammals (hedgehogs and ground squirrels): a) synchronized rhythms, which are most commonly encountered in the hypothalamus and hippocampus; b) paroxysmal forms having a frequency range from 6 to 8 cycles/sec., and c) "burst activity" which is similar to the alpha rhythm. Finally, the EEG also shows rapid dysrhythmic patterns serving as a background for the slow generalized rhythms.

As a result, the "spontaneous" activity of the various parts of the hibernant brain in the waking state consists of a broad spectrum of fluctuations in biopotential, due apparently to the multiplicity of the functional states of these structures and the characteristics of their morphofunctional connections. /64

Electrical activity of various parts of the brain of hibernating mammals during onset of hibernation and torpor. The initial onset of hibernation, which is accompanied by an insignificant drop in brain temperature to 29-31°C, is characterized by the appearance of episodic synchronized forms of bioelectric activity which are especially pronounced in the subcortical areas of the brain. These waves reach amplitudes of 65-120-150 microvolts in the subcortical areas and 40-60 microvolts in the cortex. They can be recorded simultaneously with elements of the "burst activity" which also appears in the EEG during this period.

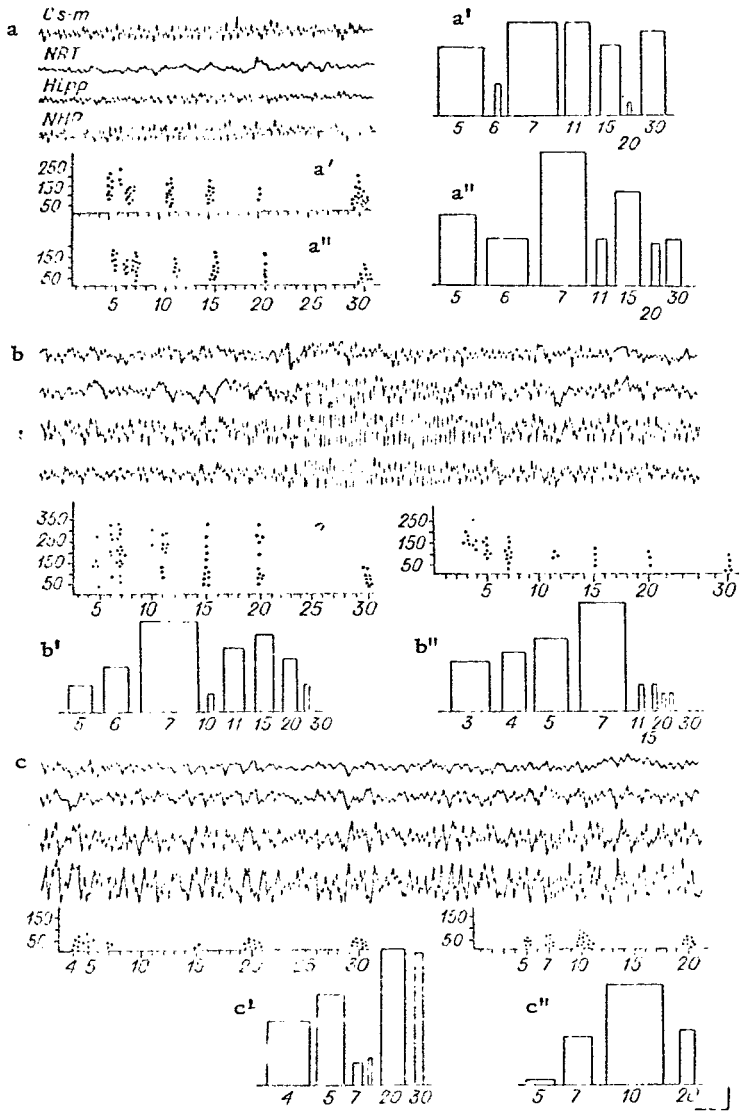

Figure 22.

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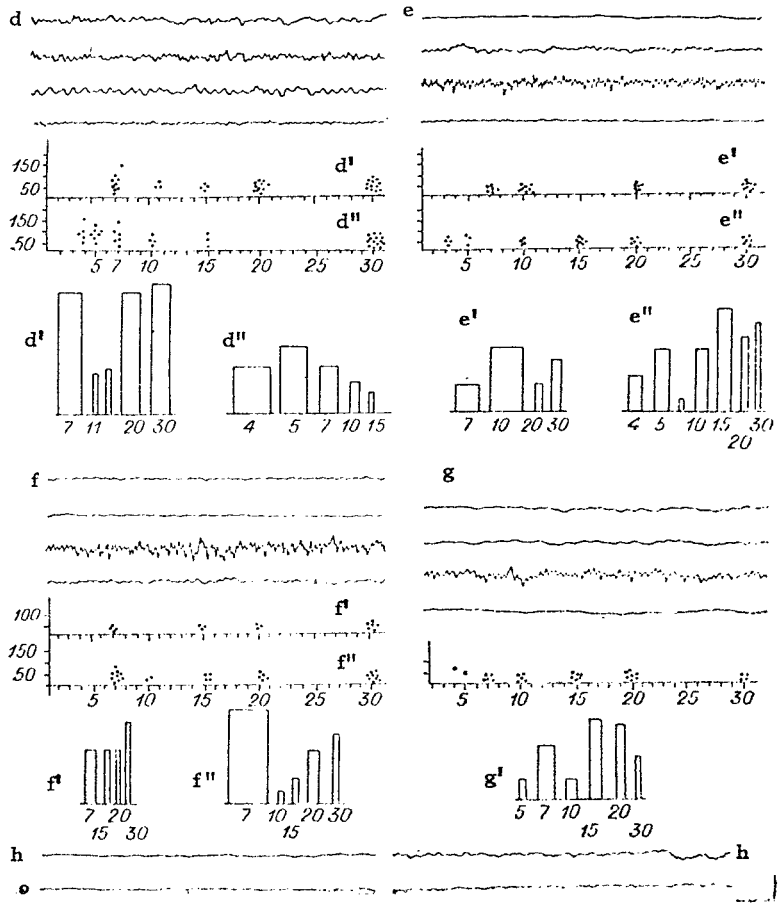

Figure 22. Changes in Electrical Activity in Various Parts of the Brain of the European Hedgehog during (a) the Waking State, (b-e) the Onset of Hibernation, and (f-h) Torpor. Experiment 41, Feb, 28 - March 13, 1961. Histograms of the EEG of the Cerebral Cortex (a') and Hippocampus (a''). Each Point on the Graph Represents One Fluctuation in the EEG (a 10-second segment). The Base of Each Rectangle Shows the Percentage of Time During which the Given Frequency was Recorded in the EEG, while the Height Shows the Relative Contribution of This Frequency to the Entire EEG Spectrum. The Calibration is to 100 Microvolt-seconds.

The appearance of these two forms of electrical activity may indicate that they have different origins. Statistical analysis of the distribution of the waves in the EEG during this stage of hibernation most often reveals significant changes in the frequency spectrum (in the presence of relatively insignificant changes in the temperature of the cerebral cortex). In the cortex and subcortical layers of the dominant form of activity becomes potentials having a frequency of 5-7 cycles/sec. (Fig. 22, a, b).

The final onset of hibernation and the drop in brain temperature to 24-26°C are accompanied by the appearance of synchronized slow waves having a frequency of 4-6-10 cycles/sec. and amplitudes of up to 50 microvolts in all parts of the brain. Here again, both types of synchronized activity can be recorded simultaneously in the EEG (Fig. 22, c, d).

Initial torpor and the drop in cortical temperature to 19-22°C are accompanied by the predominance of synchronized waves having a frequency of 4-5 cycles/sec. and an amplitude of 80-120 microvolts in the hippocampus. In the cerebral cortex the dominant form of activity consists of low-voltage waves having a frequency of around 20-30 cycles/sec. Thus, a significant drop in the amplitude of the biopotentials is noted in the presence of only moderate changes in the frequency spectrum of the EEG.

The final onset of torpor when the temperature of the cerebral cortex has fallen to 14-18°C is accompanied by a significant decrease in the amplitude of all types of activity along with the appearance of a persistent mixed dysrhythmia in the hippocampus (Fig. 22, d, e). In the mesencephalic reticular formation /65
and the hypothalamic region, one observes episodic, rhythmic damping waves having an amplitude of no more than 20-40 microvolts. The changes in bioelectric activity associated with torpor thus consist basically of a drop in amplitude with temporary maintenance of the frequency spectrum except for loss of the 10-15 cycles/sec. band in the cerebral cortex and hippocampus.

Deep torpor generally sets in about 7-14 days after the beginning of the experiment and is accompanied by a drop in cortical temperature to 7-13°C. Under conditions of deep torpor the background bioelectric activity, its regular /66
and mixed dysrhythmic waves as well as the paroxysmal types of electrical activity can be recorded mainly in the structures of the limbic complex, especially in the hippocampus and hypothalamic nuclei. There are no changes in the variety of the biopotentials in the cerebral cortex (Fig. 22, f-h). Analogous changes in bioelectric activity are shown in Fig. 23.

Thus, torpor is characterized by a gradual suppression of activity, first in the cerebral cortex and then in the subcortical layers. At first, these changes are recorded in the absence of any significant change in the frequency spectrum, and only later is the synchronization of activity also affected. The onset of hibernation is accompanied by an unavoidable "impoverishment" in the frequency spectrum of the EEG, particularly in the cerebral cortex. Over the entire frequency spectrum represented in the EEG, the proportion of slow waves inevitably increases with increasing depth of torpor.

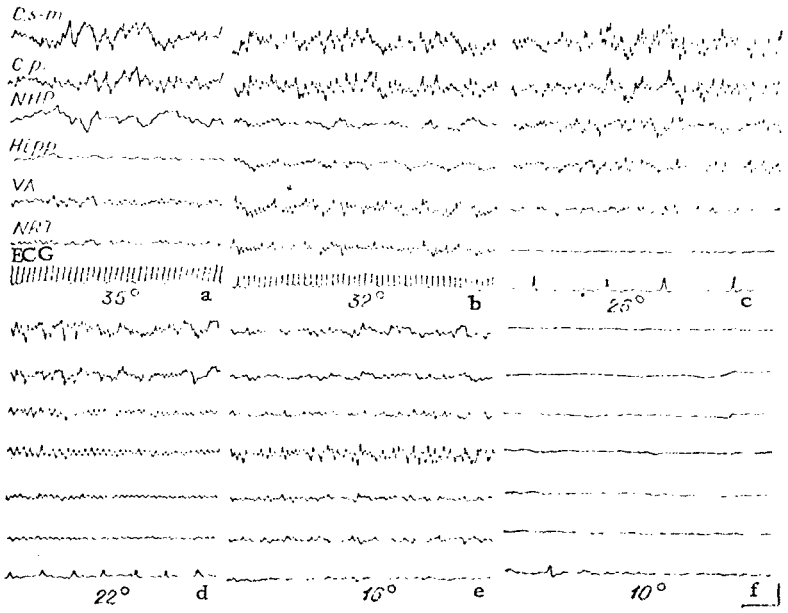

Figure 23. Electrical Activity in Various Parts of the Brain of the European Hedgehog during (a, b) the Waking State, (c, d) the Onset of Hibernation, and (e, f) Torpor. Experiment 16, Feb. 17, 1961 to Jan. 10, 1962. The Brain Temperature is Shown Below Each EEG. For the Meaning of the Abbreviations, See the Atlas of the Hedgehog Brain (Figs. 2-8). ECG Stands for Electrocardiogram.

It can be seen in Fig. 23 that the onset of hibernation and a definite drop in cortical temperature to 28-29°C are accompanied by a distinct drop in amplitude of the electrical activity and by a shift to the left in the frequency spectrum, especially in the cerebral cortex and the reticular formation of the brainstem. All possible frequencies appear in the EEG of the hippocampus during this period. Later, as the depth of hibernation increases and the cortical temperature drops to 22-26°C, the shift to the left in the frequency spectrum is accentuated. During this period, the changes in frequency in the electrocorticogram are accompanied by a drop in the amplitude of the biopotentials. There is also a decrease in activity in the anteroventral parts of the optic thalamus. Depression of the biopotentials is recorded at 10°C (see Fig. 23, f).

The normal course of hibernation is most commonly interrupted by periods of so-called "tentative" arousal, shown electrographically in the figures which follow. "Tentative" arousal is not accompanied by any significant change in cortical temperature, the depression of which continues essentially unchanged despite the significant changes in electrogenesis in all parts of the brain. In the EEG of the subcortical layers, clear regular waves appear having a frequency of 4-7 cycles/sec. and uniformly distributed over all parts of the brain. Regular and persistent forms of bioelectric activity continue to be recorded in the hippocampus during this period, and electrogenesis in this part of the brain

becomes even more marked during "tentative" reestablishment of the biopotentials. As the temperature of the cortex drops towards 20-21°C, slow waves having a frequency of 3-5 cycles/sec. begin to predominate in the records from all parts of the brain and the rapid forms of electrical activity gradually disappear (Fig. 23). /67

Electrical activity of various parts of the brain in hibernating mammals during natural arousal. By analyzing the changes in bioelectric activity in various parts of the brain during the onset of hibernation and torpor, we were able to show that the "spontaneous" rhythm first disappears or becomes weaker in the cerebral cortex. The rhythmic pattern of the electrocorticogram tends to be levelled out, the endogenous activity of the cortex is suppressed, and the EEG is characterized by "synchronized" waves which appear most clearly in the structures of the mesencephalon, the mesencephalic portion of the reticular formation and the hippocampus (Fig. 24, b, c). This synchronization of the bioelectric activity is initially paroxysmal in nature, being quite similar to convulsive activity (Fig. 24, d). These convulsive forms of electrogenesis are particularly pronounced in the mesencephalic reticular formation and the hypothalamic region. Subsequently, synchronized waves resembling "burst activity" and represented by spindles consisting of waves with a frequency of 5-9 cycles/sec. and a modulated amplitude of 70-100 microvolts appear with considerable consistency in the records from the subcortical formations and especially in the mesencephalic portion of the reticular formation and the structures of the mesencephalon (see Fig. 24, d). Attention should also be called to the particular variant of altered electrical activity shown in Fig. 24. The convulsive discharges which were noted at the very beginning of the study (during the early onset of hibernation) appear periodically in the mesencephalic reticular formation and the hypothalamic region (Fig. 24, e).

When the temperature of the cerebral cortex reaches 22-24°C, the dominant form of electrical activity becomes the monophasic generalized waves which have been described earlier and which are found in practically all parts of the brain except the cerebral cortex, which already shows depression of activity at this stage of hibernation (see Fig. 24, b-e). Since these synchronized waves are particularly characteristic of the hypothalamic region, followed by the hippocampus and the reticular formation (Fig. 24, d, e), it can be concluded with some assurance that these parts of the brain represent the source of this type of bioelectric activity.

Independently of this synchronization of bioelectric activity, an increase in the depth of hibernation leads to suppression of the "spontaneous" rhythms in the cerebral cortex. Then, against a background of this depression, which is usually recorded at a cortical temperature of 14-18°C, relatively constant and regular biopotential waves appear in the structures of the hippocampus (Fig. 24, h). Attention should also be called to the phenomenon of generalization of the cardiac rhythm, which is consistently observed under conditions of deep torpor (Fig. 24, h, i). /70

We have thus been unable to observe the complete and prolonged depression of activity seen in the experiments of Kayser (441) and Johnson (417). Constant biopotential waves are maintained in the structures of the limbic complex

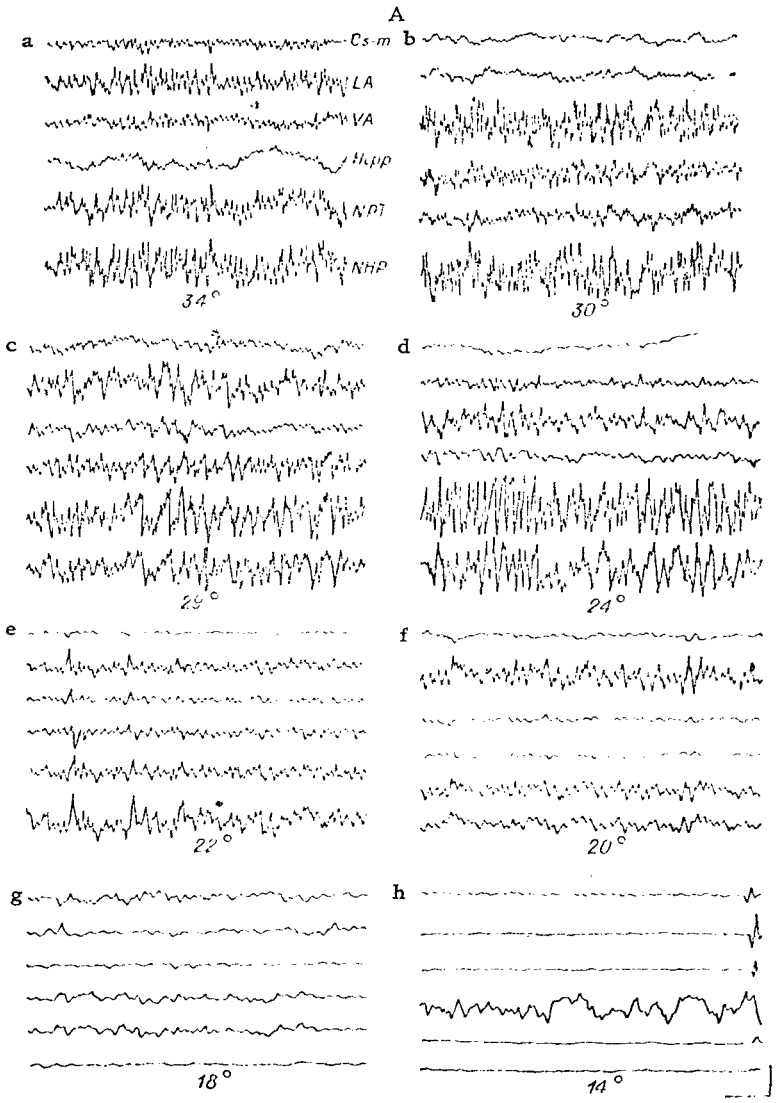

Figure 24.

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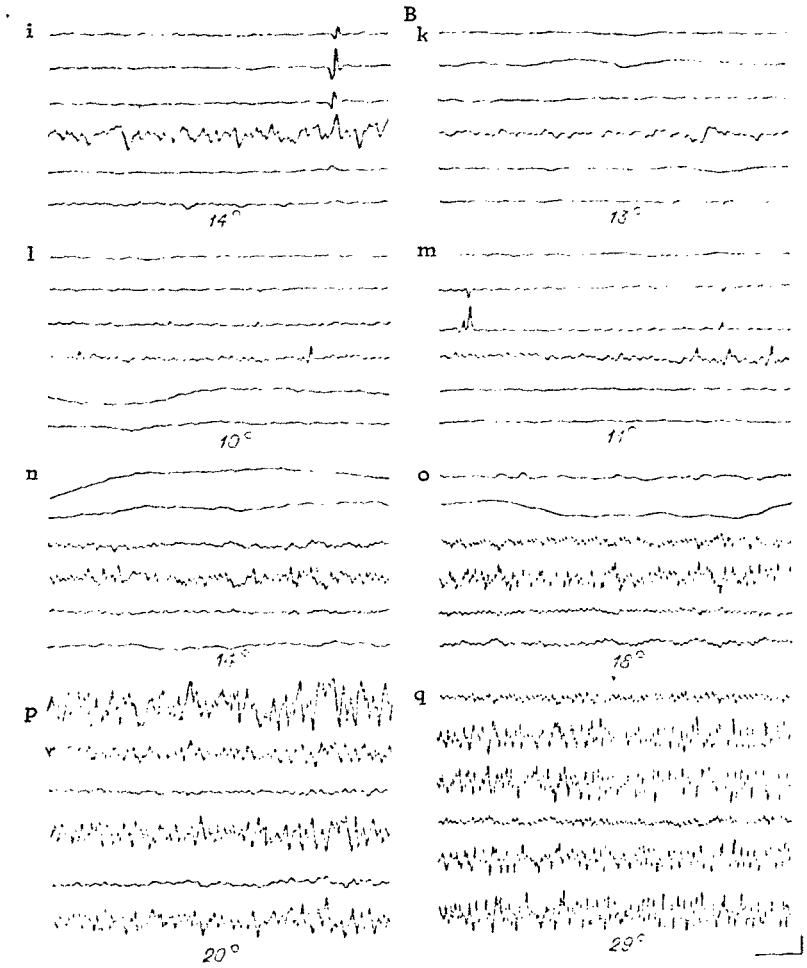

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Figure 24. A — Early Onset of Hibernation and Torpor in the European Hedgehog. Experiment 19, Feb. 18, 1962 to Jan. 21, 1963. Synchronization of Activity and Appearance of a "Burst Component" (d). B — Torpor (k-m). Maintenance of Background Activity in the Hippocampus and Its Suppression (k, l). Natural Arousal (p, q).

(Fig. 24). The deep depression seen at a cortical temperature of 9-11°C is accompanied by the appearance of individual spikes having amplitudes of up to 50-60 microvolts, which are particularly pronounced in the hippocampus and the mesencephalic structures (Fig. 24, i-m).

It might be suggested that the "residual" electrogenesis under very low temperature conditions is maintained only in these parts of the brain in order to guarantee, on the one hand, the possibility of minimal homeostatic responses and, on the other hand, the onset of arousal. The experiment which we are discussing at the moment represents the clearest illustration of the correctness of this hypothesis. Particular attention should be directed at the constancy of the frequency and amplitude characteristics of the records from the hippocampus (see Fig. 24, n-p). The relationship between the successful reestablishment of electrogenesis during natural arousal and the nature of the bioelectric changes in this portion of the brain is quite clear.

Biopotentials having a reduced amplitude may be observed at the very beginning of arousal in the region of the mesencephalic reticular formation and the medial parts of the optic thalamus (Fig. 24, n, o). Then, while becoming weaker in these areas, biopotentials reappear and become stronger in other subcortical areas, radiating into the cerebral cortex (see Fig. 24, p). Persistent signs of desynchronization appear in the EEG, particularly in the subcortical layers. At a certain stage of arousal, the nature of the activity in the hippocampus is precisely the opposite (as far as the frequency spectrum is concerned) of the changes in bioelectric activity in the mesencephalic reticular formation (see Fig. 24, q).

The fact that the cardiac complex is generalized in the EEG has already /71
been pointed out many times. It is also appropriate to call attention to the generalization of the respiratory activity which is reflected in the electrogram of the skeletal muscles. This phenomenon is most typically seen during arousal and seems to be quite similar to the phenomenon of Orbeli and Kunstman (117). As we pointed out in an earlier paper (231), the generalization of respiratory activity in the EMG and EEG in animals undergoing arousal is apparently related to the increase in proprioceptive afferentation. The afferent impulses originating in the proprioceptors are summated, in this stage of hibernation, with the high activity of the respiratory neurons in the reticular formation of the medulla oblongata. From here, with a view towards synchronization with the respiration, they proceed along the descending pathways to the motor neurons of the spinal cord innervating the respiratory musculature and the muscles of the extremities, where they are also reflected in the EMG (231, 385).

This study of the bioelectric changes in the brain of hibernating animals during arousal has shown that it is not always possible to study these changes in such a differentiated way as has just been described. Under conditions of deepening hibernation, elements of synchronized activity may be recorded simultaneously from all parts of the brain, both when the temperature of the cerebral cortex is still 16-20°C and when arousal has begun. The reestablishment of electrical activity during arousal proceeds by way of generalization of the "burst activity", which can also be recorded simultaneously from all parts of the brain. Then episodic spike discharges and groups of rapid waves appear,

uniformly distributed over all of the structures studied. In this case, during all stages of arousal, the various parts of the brain seem to lack any electrographic specificity. The characteristic stepwise course which is found during the onset of hibernation is absent here (analogous results have been obtained during the study of induced bioelectric responses; as will be shown in the discussion below, these are characterized during arousal by the simultaneous appearance of all parameters). Final arousal is characterized by generalization of the high-voltage spike discharges and is accompanied by marked respiratory activity. These are the variations in the reestablishment of electrical activity which are typical of arousal in hibernating animals.

* * *

It was shown earlier that the onset of hibernation, the transition from the waking state to torpor and, on the other hand, the arousal from hibernation are all inevitably characterized by the formation (and subsequently by the predominance in the records from various parts of the brain) of the so-called "burst activity" which is phenomenologically and functionally similar to the alpha rhythm. In this case, the biopotentials forming the "spindle" of this type of activity are preceded by regular groups of waves having a modulated amplitude of 60-100 microvolts and a frequency of 7-14 cycles/sec. (Fig. 25). This type of electrical activity can be recorded most clearly during the transitional periods in the vital activity of the hibernating mammals. The first groups of "burst activity" most commonly appear simultaneously in the ventral parts of the optic thalamus and in the cerebral cortex (Fig. 25, b), reaching a maximum when the cortical temperature reaches 24-29°C. At this point this activity is represented by regularly alternating, persistent, synchronized and monorhythmic groups. When the onset of hibernation is complete and during the early stages of torpor, these "spindle bursts" usually lose their clearly modulated shape, becoming irregular, and the rhythmic nature of their appearance is disrupted (Fig. 25, e). /72

The decrease in amplitude of the waves making up the "burst activity" is accompanied by disruption of the regular appearance of the spindles and a decrease in the number of potentials becoming a part of each spindle. In the temperature range from 22 to 17-18°C, the "burst component" of the EEG gradually disappears, and even at a temperature of 20-22°C it is recorded primarily in the ventral portions of the optic thalamus and the sensorimotor region of the cerebral cortex (Fig. 25, e). Complete suppression of this alpha-like activity is usually observed below 17°C (Fig. 25, f).

Characteristics of the electrical activity in some parts of the limbic complex. The most interesting aspect of the functional activity of the limbic complex has been the laws governing electrogenesis in the hippocampus. As can be seen from the above descriptions of the changes in electrical activity in various parts of the brain of hibernating animals, the hippocampus and some of the other structures of the "limbic complex" (the amygdaloid nuclei, the olfactory lobe) retain the ability to generate biopotentials under conditions which are very close to the "electrical zero point". Against a background of depression, when the records from the cortex and the subcortical formations /76

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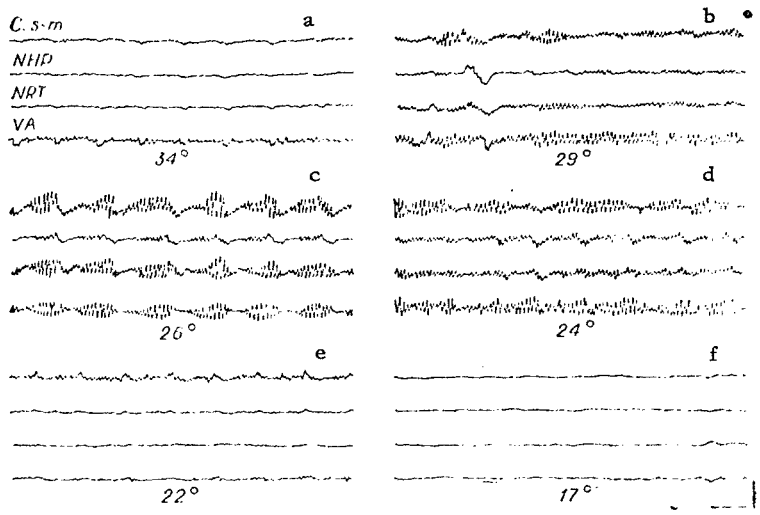

Figure 25. The "Burst Component" of the EEG in Various Parts of the Brain of the European Hedgehog. Onset of Hibernation (b-d) and Torpor (e, f). Experiment 24, March 1-18, 1962.

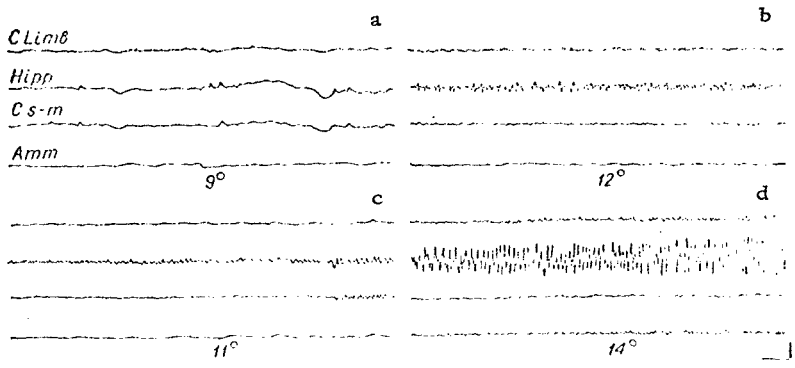

Figure 26. Changes in Electrical Activity in the Hippocampus, Limbic Cortex and Amygdaloid Nuclei during Torpor. Experiment 25, April 2-14, 1963.

show only scattered damping waves and generalized rhythms related to the activity of the heart and respiratory system, those from the hippocampus still show monomorphic discharges in the form of relatively regular waves, high in frequency and low in amplitude, which are not accompanied by any outward signs /76

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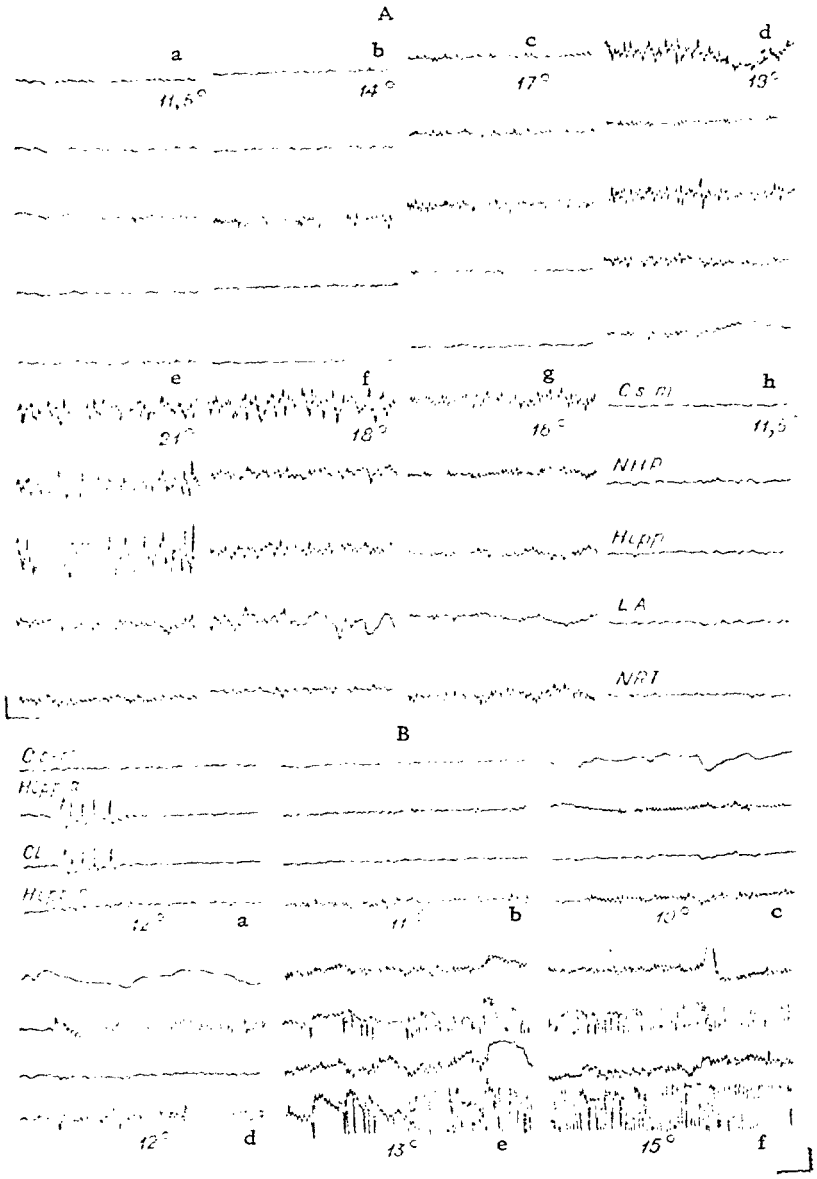

Figure 27.

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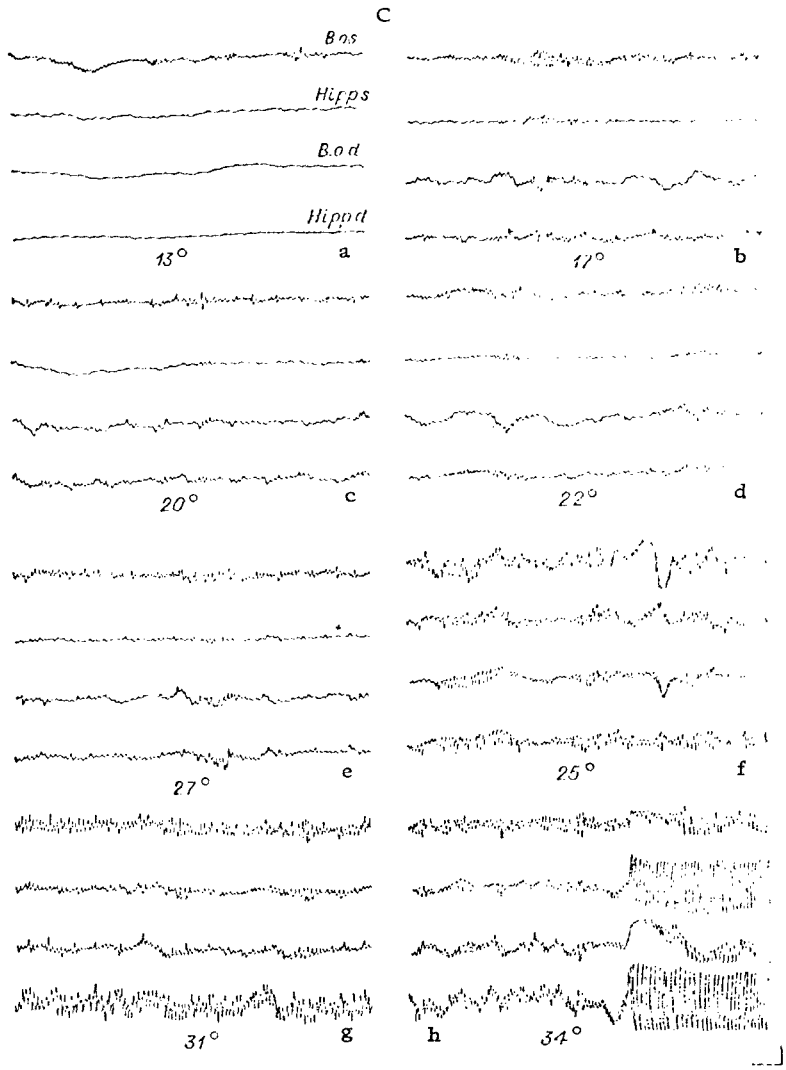

Figure 27. Changes in Electrical Activity in Some Limbic Structures during Various Stages of Hibernation in the European Hedgehog. A —Tentative Arousal. Experiment 17, Feb. 1-18, 1962. (See Text). B — Generalized Changes in Electrical Activity in the Hippocampus and Cerebral Cortex, Torpor. Experiment 18, April 18-29, 1962. C — Changes in Electrical Activity in the Olfactory Lobe and Hippocampus during the Initial and Final Stages of Arousal (C, a-d; C, e-h). Experiment 16, Feb. 1-16, 1962. The Letters B. O. Stand for the Olfactory Lobes (Bulbus Olfactorius).

of respiratory activity. It should be emphasized that during deep torpor, when the electrocorticogram (and the records from some subcortical structures) shows scattered sporadic waves, the bioelectric changes in the hippocampus are relatively regular ("ordered") in character (Fig. 26).

When the brain temperature is undergoing only insignificant changes, around the "electrical zero point", the EEG from the hippocampus first shows regular, low-voltage waves which then increase in amplitude and are transformed into a regular rhythm having a frequency of 18-22 cycles/sec. and an amplitude of up to 80-100 microvolts (see Fig. 26, d). These changes in hippocampal electrogenesis take place against a background of extremely poor, low-voltage activity in the cerebral cortex and other parts of the limbic system.

The development of a hippocampal rhythm may be limited exclusively to the limbic structures, without radiation to the other parts of the brain (see Fig. 26, d); under these conditions these rhythms may be quite stable and prolonged.

The regular activity in the hippocampus, which is quite similar to the convulsive forms of electrogenesis, is sometimes synchronous and bilateral in nature, but in other cases this form of activity may be preserved in the structures of only a single hemisphere. Attaining a significant amplitude and regularity of appearance, the bioelectric changes in the hippocampus and some other parts of the limbic system then become generalized, being accompanied by brief periods of so-called "tentative" arousal and the temporary reappearance of cerebral electrogenesis. Sometimes this is accompanied by an increase in brain temperature, although quite often the temperature fluctuates only within rather insignificant limits.

Despite their relatively limited character, the biopotentials here are also paroxysmal and "regular" in nature. This form of activity is characterized by /77 rather prolonged after-discharges which lead in turn to the appearance of new potentials. If the after-discharges are "potentiated", this leads to generalized activity which is quite similar to the phenomena of hypersynchronization and irradiation (see Fig. 27, B).

Despite the periodic suppression of activity in the hippocampus, accompanied by a decrease in cortical temperature, this is always followed by generalization of the bioelectric changes with the development of a regular bilateral rhythm, convulsive group discharges and finally significant motor excitation on the part of the animal (see Fig. 27, B, d-f). Attention should be called to the fact that the appearance of hypersynchronization followed by generalization of these types of activity in the hippocampus may be observed at very low cortical temperatures. Here the hippocampus plays the role of a "trigger", potentiating the existing spontaneous activity and guaranteeing its subsequent generalization; such an increase in activity via the hippocampus most often "leads" to the development of so-called "tentative" arousal, which is a rather characteristic stage in mammalian hibernation (see Fig. 27, A).

* * *

As a rule, the onset of hibernation and the stage of final torpor are preceded by several stages of brief, preliminary, "tentative" arousal; in our opinion, this is one of the most convincing proofs of the interrupted nature of hibernation. In any case, the electrographic changes during "tentative" arousal can serve as a better criterion of the interrupted nature of hibernation than the methods which are often used in ecological physiology (363). The duration of "tentative" arousal usually fluctuates within rather broad limits and may reach 4-20 hours. As was mentioned earlier, the brain temperature rises from 7-11 to 21-29°C during this period and then falls back to the original levels.

At the beginning of "tentative" arousal, the first signs of alterations in bioelectric activity appear in the structures of the limbic system, especially /78 the hippocampus (Fig. 27, a, b). At a cortical temperature of 9-14°C, the hippocampal EEG shows low-voltage, high-frequency waves having an amplitude of no more than 20-40 microvolts. Episodic damping waves are also recorded during this period in the hypothalamus, but they are not as pronounced, of as long duration or as regular here as in the hippocampus (Fig. 27, A, a-c). As the brain temperature rises further, regular bioelectric changes appear in the cerebral cortex and the lateral nuclei of the optic thalamus (Fig. 27, A, d). Dysrhythmic activity appears in the cerebral cortex and the subcortical layers at a temperature of 19-21°C (Fig. 27, A, e). Regular types of activity in the form of rhythms having a frequency of 12-14 cycles/sec. and an amplitude of 40-80 microvolts are most commonly recorded after the appearance of the first signs of electrogenesis in the mesencephalic reticular formation, usually above 19-20°C. The endogenous electrical activity of the hippocampus remains at a rather high level during the entire tentative arousal and is suppressed only after the decrease in brain temperature and the increase in the depth of hibernation (Fig. 27, A, g, h).

As we saw earlier and as is illustrated in Fig. 27, the temperature of the cerebral cortex begins to rise immediately after the appearance of the initially sporadic but then regular bioelectric changes in the hippocampus. The temperature usually does not reach the starting levels, rising only to 20-24°C. Nevertheless, the appearance of regular, rhythmic, "spontaneous" activity can be observed at lower cortical temperatures within the framework of tentative arousal than would generally be the case in the waking animal. This fact must not be ignored, but it is also impossible to interpret it satisfactorily at the present time. An increase in depth of hibernation, a relapse back into hibernation or the onset of torpor generally take place at 11-16°C (Fig. 27, A, g, h). As in the case of complete torpor, a complete suppression of activity is recorded at 7-11°C.

The structures of the limbic system (the hippocampus and, in part, the amygdaloid nuclei and the limbic cortex) are thus characterized by a specific type of change in electrical activity; this is apparently connected with the characteristic function of these parts of the brain in hibernating mammals, with their role in the onset of hibernation and torpor.

By way of conclusion to a section which has been devoted to an analysis of the bioelectric changes in the formations making up the limbic functional complex, it seems appropriate to say a few words about the nature of electrogenesis in the olfactory lobe of hibernants. The information which is processed

within the limits of the olfactory analyzer in hyperosmic animals (one example of which are the insectivores such as the European hedgehog) is connected to /79 a definite extent with the control of behavioral responses in the hibernants (167).

The electrical activity in the olfactory lobe of hibernating mammals also undergoes definite changes in connection with periods of adaptation. In the European hedgehog and suslik, the spontaneous electrical activity is characterized by rather typical bursts of regular activity having a frequency of 40-60 cycles/sec. and an amplitude of 50-60 microvolts (see Fig. 27, C, b). These bursts of activity, which are quite characteristic of the mammalian olfactory lobe, are most commonly bilateral and synchronous, although the asynchronous appearance of biopotentials in this part of the brain is also possible (see Fig. 26). As the brain temperature falls and the depth of hibernation increases, the amplitude of the waves constantly decreases without any significant changes in the frequency spectrum (see Fig. 27, C, d). The mean amplitude, calculated on a segment of the electroolfactogram in 7 experiments, was 15 seconds. Statistical analysis of the records from the olfactory lobe showed that the mean amplitude decreases progressively in parallel with the decrease in cerebrocortical temperature below 24-26°C, and is not subject to significant "spontaneous" fluctuations. The frequency spectrum of the EEG from the olfactory lobe begins to change below 24°C, at which point the periods of electrographic silence become longer and the number of waves composing each "burst" of activity is reduced. Depression of activity in the olfactory lobe sets in between 14 and 11°C.

It should be pointed out that the electrical activity which is characteristic of this part of the brain both in the waking state and during torpor does not spread far beyond the limits of the olfactory lobe. Generalization of these "bursts" of activity is most commonly observed during arousal.

Even at the very beginning of arousal, when the temperature of the olfactory lobe does not exceed 13-17°C (Fig. 27, C) and the endogenous activity is limited to scattered "bursts", moderate radiation of bioelectric activity into the other structures of the limbic complex, particularly the hippocampus, may be observed (see Fig. 27, C, b).

Subsequently, this radiation of bursts of sinusoidal activity becomes more pronounced and at 26-31°C they become generalized. Definitive arousal is accompanied by a widespread radiation of olfactory-lobe activity into the structures of the hippocampus and by the development of typical bioelectric activity /80 (which we have described previously) in the form of convulsive paroxysmal discharges in this part of the rhinencephalon (see Fig. 27, C, h). Analogous changes in the endogenous electrical activity of the olfactory lobe of the European hedgehog have been described in (263) and (553).

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Thus, the brain of hibernating mammals serves as an "organized system" over the temperature range from 36 to 21°C. A definite impression has been obtained that the cerebral cortex lacks the capacity to generate "spontaneous" biopotentials below 19-21°C. At this point, or sometimes a little later, the regular activity in the mesencephalic reticular formation also disappears. Consequently, the ability of these segments of the brain to interact also apparently disappears in this temperature range. In any case, interrelationships of this type play a role in the onset of hibernation and torpor.

The broadest possible approach to an analysis of the facts presented in this part of the present monograph shows that the dynamics of the suppression of "spontaneous" activity during the onset of hibernation and torpor most commonly follow the following gradient: cerebral cortex — mesencephalic reticular formation — medial thalamus — lateral thalamus — olfactory lobe — hypothalamus — hippocampus and other formations of the limbic complex (particularly the amygdaloid nuclei). The reestablishment of electrogenesis in the brain of the animal undergoing arousal involves the same structures in reverse order.

Even a preliminary analysis of the factual material shows that the onset of hibernation and torpor are characterized by some kind of successive inhibition of individual cerebral systems. This creates the possibility of evaluating the role of each individual structure in the general neurodynamics of the mammalian hibernant brain.

Arousal usually abolishes the possibility of a differentiated analysis of the structures playing a successive role in the emergence from hibernation. On the basis of the factual material, the definite impression has been obtained that during arousal of an animal from a state of torpor, the principal systems of the brain may not be activated in strict succession but more in parallel. The functional outcome of this phenomenon is the simultaneous excitation of several /81 cerebral formations.

Particular attention has been devoted in this section to a description and analysis of the electrical activity in the hippocampus and other formations making up the limbic functional complex. These parts of the brain are more resistant than others to cerebral hypothermia and retain the ability to generate biopotentials under conditions of deep torpor. This peculiarity of the hippocampus as a functional system can obviously be used by the brain as a "launching mechanism" for arousal. This hypothesis is most clearly illustrated by the phenomena of so-called "tentative" arousal, when the hippocampus is excited at the very beginning of the active arousal process and is the last to cease functioning after the cerebral temperature has again fallen.

It seems likely that the conditions of torpor create the possibility for the local excitation of either a single formation or a complex of formations which are anatomically and functionally interrelated. Such excitation may be limited to the hippocampal neurons, but may also become generalized. The generalization of hippocampal activity in hibernants under conditions of very low cerebral temperatures is apparently connected with the electrical and morphochemical characteristics of the neurons of this part of the limbic system.

An analysis of the changes in background bioelectric activity in various cerebral formations has thus made it possible to postulate the existence of several functional systems which determine the cerebral relationships during hibernation. These are undoubtedly connected both with the specific characteristics of the individual neurons of these systems and with their functional organization. Our further experiments have been devoted to the study of, on the one hand, the dynamics of individual neurons of the neo- and archicortex, and on the other hand, the functionally connected cells of the projection center during hibernation.

CHAPTER III. /82
BIOELECTRICAL RESPONSES PRODUCED IN THE CEREBRAL CORTEX OF HIBERNATING MAMMALS

CHARACTERISTICS OF THE TRANSMISSION OF IMPULSES ALONG SPECIFIC AND NONSPECIFIC PATHWAYS DURING VARIOUS STAGES OF HIBERNATION

General comments. Localization of the primary and secondary bioelectric responses in the cerebral cortex of hibernating mammals. Dynamics of the induced potentials in connection with the stages of hibernation. Temperature "resistance" of the various components of induced responses. Characteristics of the excitable properties of the pyramidal neurons of the third and fourth strata. Transmission of excitation in the upper layers of the cerebral cortex. Secondary responses. "Neuronal" contour in relation to the stages of adaptation. Development of inhibitory and excitatory synaptic effects in the upper layers of the cortex in hibernating animals.

The description of the background bioelectric activity during the onset of hibernation, torpor, and natural arousal has shown that the initial changes appear in the cerebral cortex, followed by the subcortical layers and the brainstem, and arousal is characterized by the primary reestablishment of electrogenesis in the structures of the archicortex, followed by the cortical neurons. The following question which requires an answer is then: what are the endogenous electrical properties of the cortical elements (the neurons and dendrites) making up the first cortical layer ? It was natural to want to determine whether both inhibitory and excitatory processes could be primary in the cerebral cortex, or whether the latter are merely "coupled", being the result of the interruption of ascending activating influences. The following two chapters, which deal with a study of induced responses in the cerebral cortex, are devoted to testing these hypotheses. /83

In addition, under the conditions of natural adaptation accompanying the onset of hibernation, torpor and arousal in hibernating animals, the characteristics of the transmission of excitation along specific and nonspecific pathways would seem to acquire particular importance. It could certainly be postulated that it is precisely those mechanisms which are connected with the facilitation and inhibition of afferent impulses entering the cerebral cortex and other parts of the brain which must be intimately connected with the

periodic changes in cerebral homeostasis that are characteristic of hibernants. The question of the temperature limits for the transmission of afferent impulses, their amplification, facilitation or inhibition and the transformation of excitation in the central pathways seems to us to be of the greatest importance in studies dealing with the characteristics of natural adaptation in hibernating mammals.

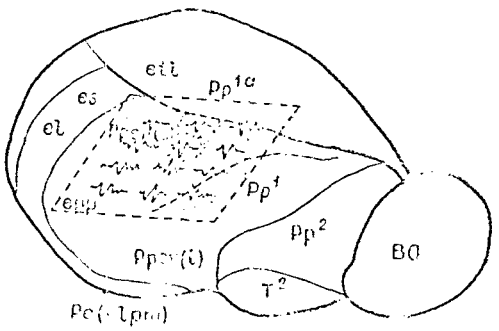

Figure 28. Topography and Structure of Induced Potentials in the Somatosensory Projection Zone of the Cerebral Cortex of the European Hedgehog. Electrical Stimulation of the Contralateral Sciatic Nerve. Unanesthetized Animal. Cortical Temperature of 29°C. Here and in Later Figures, an Upward Deviation of the Beam Corresponds to a Negative Potential under the Active Electrode.

There is no information in the literature on this question, and we believe that the following sections of this monograph will fill a gap in the information relating to the cerebral mechanisms of mammalian hibernation. /84

This series of experiments was preceded by a search for the limits of cortical projection and the focus of maximal activity of potentials induced by electrical stimulation of the peripheral nerve or the thalamic relay nucleus. This search was carried out on the basis of the cytoarchitectonic diagrams of I. N. Filimonov (216) (Fig. 28) and with the aid of a mobile active electrode, as has been described in numerous publications devoted to induced bioelectric responses in the cerebral cortex (116, 172). In studying potentials induced by means of auditory stimulation, we used the photo-phonostimulator of the Alvar Electronics Co. In experiments on the bioelectric responses induced by electrical stimulation of the thalamic relay nucleus or a peripheral nerve, the stimulator designed by N. V. Nyrova, an engineer in our laboratory. This stimulator is able to produce a broad range of rectangular impulses having

an amplitude from 0 to 25 volts and a duration of 0.1-0.8 milliseconds. Stimulation was applied to either the ipsi- or contralateral sciatic nerve, and in some experiments the anterolateral nucleus of the optic thalamus was stimulated. In this case we used bipolar nichrome electrodes with an electrode gap of 0.5-0.8 mm.

After a stable signal had been obtained in the projection zone, the electrode was fixed in place by means of quick-drying plastic (in order to permit a long-term experiment to be continued at a later date) and the animal was placed in the refrigerator as described in the previous chapter. In short-term experiments, the animal was placed in the stereotaxic device and was then cooled in a special cuvette under constant temperature control.

After the scanner had been set in motion by means of a starting stimulus, a second stimulus was given which led to the development of a bioelectric potential. The induced bioelectric responses were recorded photographically by the method of sequential superposition on immovable film. The parameters of the induced potentials were then evaluated statistically ($M \pm m$ for 10 superpositions of the track of the beam).

Topography and structural characteristics of the induced potentials in the cerebral cortex of hibernating mammals. Electrical stimulation of the sciatic nerve or the ipsilateral thalamic relay nucleus in the European hedgehog produces local and generalized bioelectric responses of the mono- and polyphasic type (see Fig. 28) along the boundaries of the $P_{per(e)}$ and $P_{per(i)}$ fields of the cerebral cortex (216). /85

Figure 27 shows the distribution of the induced bioelectric responses having a minimal latent period, a maximal clarity and minimal duration of each component of the potential, and a minimal resistance to repeated stimulation of the afferent pathway. The stability of the parameters and phases of the induced responses, which are similar to those in homoiothermic mammals, is clearly demonstrated. The focus of maximal activity (FMA) was localized to a point half way between the transitional poles.

The induced potentials were recorded under local or light chloralose anesthesia in the conscious or lightly anesthetized animal at a cortical temperature of 28-32°C (under the conditions used to search for the FMA or to determine the zone of optimal characteristics of the signal). The following components of the induced responses could be detected:

1. A primary positive wave appearing in the projection zone with a latent period of 6.8-9.4 milliseconds ($M \pm m = 8.1 \pm 1.3$ msec.). The amplitude of this wave varies considerably, between 40 and 80 microvolts, while the duration is 18-35 milliseconds;

2. A primary negative wave having an amplitude of 100-180 microvolts and a duration of 28-45 milliseconds (Fig. 28);

3. The early negative response (ENR) which was first described by K. M. Kullanda and V. N. Chernigovskiy (116). The zone in which this type of induced response can be recorded is usually in the area of the $P_{per(e)}$ field and merges smoothly with the zone in which the secondary responses of the epp, Pp' and Pp fields are recorded, bordering on the FMA;

4. A secondary positive wave appearing with a latent period of 40-60 milliseconds and a duration of 50-80 milliseconds. The amplitude of these secondary discharges is 100-220 microvolts. A deep positive wave could also be recorded in the FMA (see Fig. 28). The zone in which the secondary bioelectric responses are found in the cortex of hibernating mammals usually overlaps considerably with the boundaries for the recording of the primary responses. The amplitude of the secondary positive waves is usually 3-5 times that of the primary components of the induced bioelectric responses. This /86
finding, about which more will be said later, is particularly striking during the onset of hibernation and torpor. Even in the waking state in the unanesthetized animal, however, the magnitude of the secondary responses seems to be one of the principal characteristics of the induced biopotentials in the cerebral cortex of hibernants.

Rhythmic stimulation of the peripheral nerve or thalamic relay nucleus produces a succession of changes in the secondary responses which is quite similar to that in homoiothermic mammals: even on the first attempt, application of a stimulus having a frequency greater than one per second to the sciatic nerve abolishes the secondary induced response; thus, an increase in the frequency of stimulation leads to a decrease in amplitude of the secondary potentials and their gradual disappearance;

5. A secondary negative wave, this being a variable component of the generalized bioelectric responses in the associative zones of the cortex in hibernants. Nevertheless, this component of the secondary response may be analyzed quite accurately; it has a duration of 60-70 milliseconds and an amplitude which varies considerably but may reach 50-120 microvolts. In these zones the secondary responses can be recorded independently, with no connection to the primary potential. They appear clearly in the form of complexes having two or three components and a latent period of 80-100 milliseconds. The secondary responses in these zones may vary in form but they are always recorded outside of the FMA and the zone in which the primary potentials appear;

6. Trace waves - these are multicomponent polyphasic groups of waves which are also often seen after electrical stimulation of the specific projection pathway. These waves most commonly fall within the range of the alpha rhythm and are generally extremely sensitive to a decrease in brain temperature or to repeated electrical stimulation of any part of the projection pathway.

The secondary waves usually become more pronounced with a decrease in cortical temperature: the latent period remains the same, but the amplitude of the positive and negative components is increased and the duration is decreased. The "spontaneous" accentuation of the secondary responses following a brief "preliminary" decrease in cortical temperature is so pronounced in

animals in a state of incipient hibernation that the potentials arising after such a metamorphosis recall the secondary discharge described by Forbes and Morisson (372) in homoiotherms under barbiturate anesthesia. In hibernating /87 mammals this phenomenon can be recorded in the waking state or after premedication with chloralose.

Such are the general properties of the induced bioelectric changes arising in the FMA and transitional poles of the cerebral cortex in the conscious or lightly anesthetized European hedgehog.

Dynamics of the primary bioelectric responses in the cortex of hibernating mammals during the onset of hibernation, torpor and arousal. At a cortical temperature of 29-34°C, the induced potentials are characterized by relatively stable parameters. Repeated sequential superposition of the light- /88 beam demonstrates that the mean amplitude and duration of the components are quite constant during this period and show no statistically significant fluctuations. In this temperature range, the amplitude of the positive and negative component of the primary response is 70±5 and 100±8 microvolts, respectively (Fig. 29); the latent period of the response does not exceed 8 milliseconds.

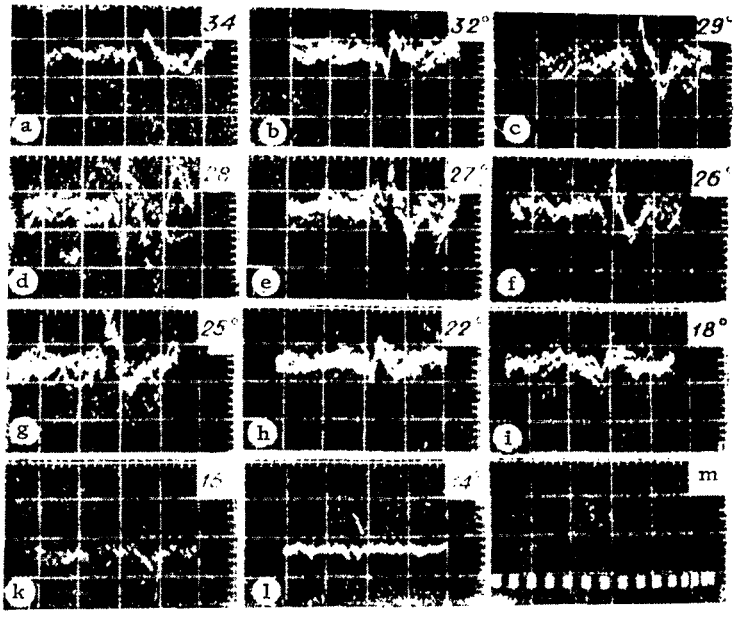

Figure 29. Dynamics of the Primary Responses during Onset of Hibernation and Torpor in the European Hedgehog. Electrical Stimulation of the Sciatic Nerve. The Temperature of the Cerebral Cortex in °C is shown in the Right Upper Corner. Experiment 30, Feb. 6, 1962.

The initial stages of the onset of hibernation (at a cortical temperature of 26-28°C) are characterized by a 10-14% increase in the amplitude of both components of the primary response and by extremely frequent and constant fluctuations in the parameters of the components. In a later section we will pay special attention to an analysis of the changes which are seen in the secondary generalized responses. At this point, however, we can already call attention to two findings: 1) the predominant appearance of these responses during the onset of hibernation, and 2) the "concordant" changes which are seen in the negative component of the primary response and in the secondary complex (an increase in amplitude of the secondary wave is inevitably accompanied by an increase in negativity of the primary response). These changes are the most characteristic for the phase of deepening hibernation when the cortical temperature drops to 25-28°C (see Fig. 29, d, g). During this period the amplitude of the negative wave of the primary response increases to 120±12 microvolts.

This increase in amplitude of the negative component of the primary response is not accompanied by any significant change in the latent period or the parameters of the positive wave. The increase ceases by the beginning of the following stage of hibernation — torpor, at the very beginning of which the brain temperature usually decreases to 20-22°C (see Fig. 29, f, h).

This stage of hibernation is already accompanied by marked changes in the latent period of the primary response, although the parameters of its positive component remain stable. Around a brain temperature of 23°C the principal changes in the induced bioelectric responses are a decrease in amplitude of the negative potential, a significant increase in the latent period, and in some cases an increase in the duration of all phases of the response. A 29-34°C the latent period is 6-12 milliseconds, but by the beginning of torpor it increases to 13±1 milliseconds and then eventually reaches a value of 36±1.5 milliseconds. The latent period of the primary bioelectric response thus increases by an average of 400-500% in connection with the development of torpor (calculations were carried out with the numerical values from 10 /89
experiments using sequential superposition of the potentials at each stage of hibernation, within the limits of a definite temperature range).

The secondary components of the induced bioelectric responses generally disappear in the temperature range 19-22°C (see Fig. 28, h, k and later in the section on "Secondary responses"). The positive component of the primary response is the most stable and highly resistant to a drop in temperature of the cerebral cortex, persisting even at a cortical temperature of 9-14°C.

The behavior of the primary bioelectric responses in the FMA described above are quite typical for all of the experiments of this group. The course of these changes can be more or less prolonged (from 180 to 600 minutes), but they always fall into the pattern described above.

The entire cycle of changes may also be followed during a shorter time interval: in the artificially cooled animal which has first been superficially anesthetized with chloralose, the stages of hibernation are significantly shortened. In such a case, the increase in the negative component of the primary

response and in the amplitude of the secondary wave are already noted 30-40 minutes after the onset of cooling, in a temperature range of 27-30°C. The reduction in the secondary wave and then later the decrease in amplitude of the negative component of the primary response are observed 40-45 minutes after the development of the first signs of torpor (changes in muscle tone, cardiac activity, respiration, etc.). At a cortical temperature of 24-26°C the negative component decreases in amplitude but increases in duration: at 24°C it has an amplitude of 50±5 microvolts while at 19°C the induced response consists of a primary positive wave with an amplitude of 28±3 microvolts and an insignificant negative deviation. At a cortical temperature of 9-14°C, electrical stimulation of the projection pathway no longer produced local bioelectric potentials in the projection zone (see Fig. 29).

This behavior is not observed with the extreme clarity shown in the illustrations in all experiments devoted to a study of induced bioelectric responses in the cerebral cortex. Although the principal tendencies described above are maintained, the changes may not be quite as pronounced. The transformations in the primary response may be reduced to a minimum; the changes in the parameters of the negative potential, and then also in the positive components, as well as the increase in the latent period may be "uncoupled" in time from the changes in the secondary responses. In such cases a drop in cortical /90 temperature from 36 to 26°C may not lead to any decrease in amplitude of the positive and negative potentials, but may still be accompanied by a complete cycle of changes affecting the secondary responses.

Nevertheless, below 17°C the parameters of the induced responses become unstable and at a cortical temperature of 9-11°C electrical stimulation no /91 longer produces any change in the properties of the neurons of the projection zone.

Arousal of the hibernating animal, with reestablishment of cardiovascular and respiratory activity and muscle tone, is accompanied by successive phases of reestablishment of the parameters and nature of the induced potentials (Fig. 30). The entire cycle of changes described above, which are typical of the induced responses in an animal falling into a state of hibernation, is essentially recorded in reverse order and in rather close temporal agreement with the principal temperature stages of arousal: its initiation and development and then the establishment of active wakefulness.

In the course of arousal, the first signs of induced bioelectric responses in the projection zones during electrical stimulation of any segment of the projection pathway are seen at a cortical temperature of 9-14°C (see Fig. 30). In the overwhelming majority of experiments (28 out of 34 in this series), these consist of the appearance of a complex response: the primary response in this case is immediately produced in final form, but with a negative component which is significantly reduced in amplitude, while the secondary responses appear inconsistently and are very sensitive to rhythmic stimulation. During the subsequent course of arousal from hibernation, the polyphasic bioelectric response is characterized by stable parameters for a relatively long period of time (see Fig. 30, d-i). This period covers the temperature range from 16 to 25°C, which corresponds to the stage of definite arousal of the animal. The primary waves and secondary components of the induced responses are now quite stable. When

arousal is complete and the cortical temperature has reached 28-32°C, then there is a clear increase in amplitude of the negative component of the primary reaction, generally accompanied by a deepening of the secondary response (see Fig. 30, m-p).

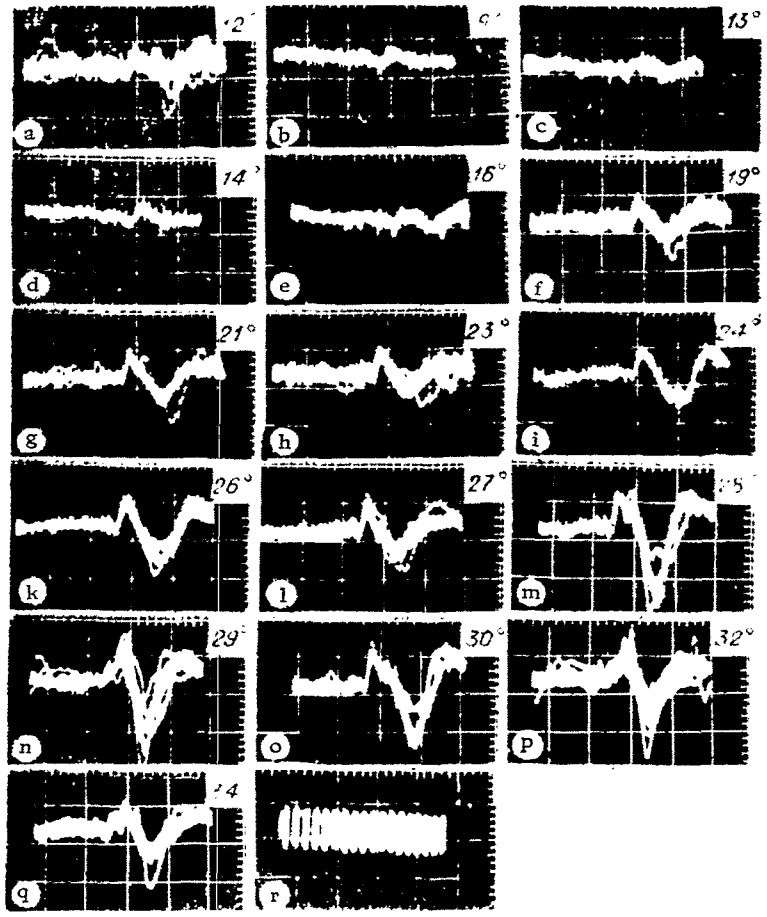

Figure 30. Dynamics of the Induced Potentials in the Cerebral Cortex during Arousal. Electrical Stimulation of the Sciatic Nerve; r - Calibration Record at 250 Microvolts, 20 Milliseconds. Experiment 31, Feb. 9, 1962.

The stability of the amplitude and temporal parameters of the secondary bioelectric responses in the face of a constant increase in the amplitude of the primary complex is quite interesting (see Fig. 30): in the temperature range from 19 to 25°C the primary complex is doubled, reaching 180±12 microvolts compared to an original amplitude of 100±15 microvolts.

The stage of rapidly developing arousal and the establishment of active wakefulness, which are observed in the course of the next 90-180 minutes under the conditions of the present experiments (at a cortical temperature of 21-23°C), are characterized by simultaneous accentuation of both the secondary responses and the negative component of the primary response. The amplitude of the primary positive wave at this time reaches 70±8 microvolts, compared to 100±15 microvolts for the negative component and 180±12 microvolts for the secondary positive wave. A record of the induced potentials in the awakened animal at a cortical temperature of 31-34°C demonstrates clearly that the entire complex returns to the starting values at precisely this stage of hibernation (see Fig. 30, m). /92

In 8 of the 34 experiments in this series, we were able to observe a very rapid, "lightning-like" onset of hibernation and a very rapid disappearance of the induced responses. In the course of 40 minutes we were able to observe first accentuation of the secondary discharges and the primary negative wave, then the disappearance of generalized secondary responses, and finally the complete absence of induced responses. It should be pointed out that this entire cycle of changes took place at a higher cortical temperature than the temperatures cited previously for the onset of hibernation and torpor. If the usual critical level for the formation of a superficial positive wave is a temperature of 9-11°C, in this case suppression of induced activity was already observed at 16-17°C. The amplitude of the induced bioelectric responses underwent the same variations as described in the previous illustrations.

Thus, in this case, the first change was an increase in the duration and amplitude of the secondary discharges. This usually takes place at a cortical temperature of 29-36°C. In the case of a "lightning-like" onset of hibernation, this period of accentuation was only a few minutes away from the complete suppression of the secondary responses. The parameters of the primary components of the induced response remained constant in this case despite the suppression of the secondary discharges. In these experiments, suppression of the primary positive waves was also seen in the temperature range from 14 to 11°C.

The secondary responses which are recorded in the cerebral cortex during electrical stimulation of various portions of the projection pathway are characterized, as a rule, by a polyphasic structure (Fig. 31).

In the waking state, these induced responses reach an amplitude of 120-180 microvolts and appear with a latent period of 40-60 milliseconds. During low-frequency stimulation of the peripheral nerve or the thalamic relay nucleus, these responses are comparatively stable (see Fig. 31, c-g). The initial stages of the onset of hibernation are characterized by a marked /93
and prolonged accentuation of the secondary waves, which reach amplitudes of 200±18, 240±8 and 120±6 microvolts, respectively, at a cortical temperature of 27-29°C; i.e., they are increased 150-200% (see Fig. 31, i-l). This is usually accompanied by an increase in the regularity of appearance of the secondary responses and by a shortening of the latent period and the duration of the individual phases; a so-called "true" facilitation of the induced potentials is observed (142).

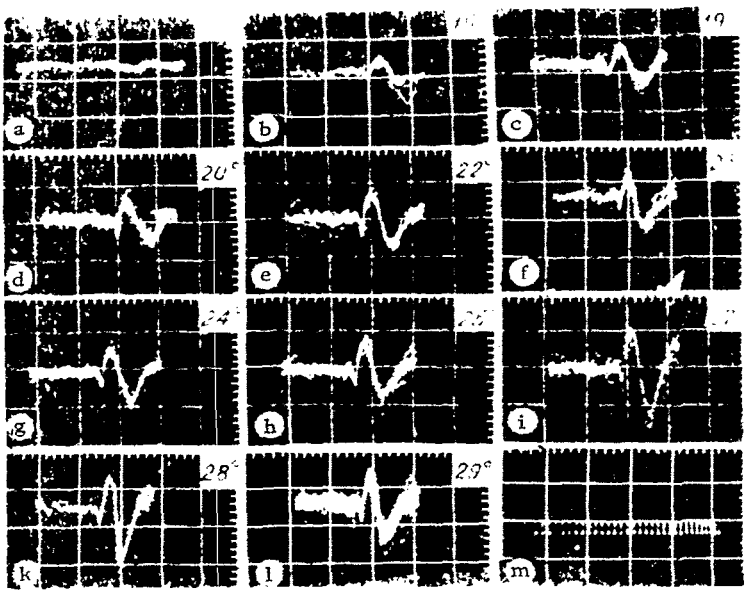

Figure 31. Dynamics of the Induced Potentials in the FMA of the Somatosensory Projection Zone of the Cortex of the European Hedgehog. Electrical Stimulation of the Sciatic Nerve. Experiment 29, Oct. 25, 1962.

As was briefly mentioned earlier, the onset of hibernation and torpor are inevitably associated with a decrease in amplitude and increase in duration of the secondary responses (Fig. 32). Thus, at 23°C their amplitude is reduced by 75-80% and at a cortical temperature of 17°C no secondary bioelectric responses can be recorded after stimulation of any portion of the projection pathway (see Fig. 31, g). /94

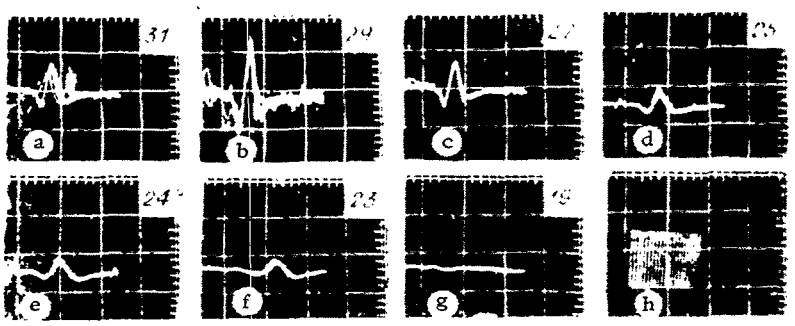

Figure 32. Secondary Bioelectric Responses in the Cerebral Cortex of the European Hedgehog. Stimulation of the Sciatic Nerve at a Frequency of 1 cycle/sec.

The same type of changes in the secondary bioelectric responses in the cerebral cortex were preserved in those experiments in which the potentials showed an uncharacteristic resistance to low temperatures. In these few experiments (6 out of 34), the secondary responses were characteristically constant at brain temperatures of 18-19°C. As a rule, this was accompanied by prolonged and stubborn wakefulness, about which more will be said later. At this point we will limit ourselves to merely stating the facts. It seems most likely that the so-called "anti-hibernation" mechanisms came into play and found an electroencephalographic reflection in these experiments. Nevertheless, phases in which the above-mentioned responses were accentuated were observed even in these cases.

On the other hand, in 7 experiments out of 34, we observed accelerated changes in the induced potentials resembling those which we called "lightning-like" in the studies of the primary responses. In these cases, all phases of the transformation of the secondary responses described above could be observed within 10-45 minutes after the beginning of the onset of hibernation. It should be emphasized that the disappearance of the secondary responses usually coincides in time with a significant drop in the temperature of the cerebral cortex and with the rapid development of torpor (Fig. 32).

After the brain temperature reached 20-21°C, the secondary responses disappeared in the course of the next 10-12 minutes. In these experiments, disappearance of the secondary potentials occurred at higher cerebral cortical temperatures. /95

Arousal produced by physical warming of the hibernating animal or by administration of an excitatory stimulant under conditions of either a short-term or a long-term experiment, as well as arousal connected with the natural development of an adaptive process, is accompanied by reestablishment of the capacity for impulse formation in the neurons of the associative zone. Initial secondary waves in the form of a slow, low-voltage wave which is highly sensitive to repeated stimulation is already recorded at a cortical temperature of 19-20°C. The amplitude of this response does not exceed 25±6 microvolts for the positive component and 45±3 microvolts for the negative component (in those cases in which the latter is already established at this stage of arousal). The subsequent alterations in the secondary bioelectric responses can clearly be divided into two stages: before the period of accentuation of the secondary potentials and after this period. All of these changes in the induced secondary bioelectric responses result essentially in the formation of a polyphasic complex, a gradual decrease in its duration, and finally its stabilization to repeated electrical stimulation of the projection pathway. At 24°C sequential superposition reveals some constancy of the potentials even during stimulation of the peripheral nerve at a frequency of 2 per second (see Fig. 31, i). When the cortical temperature is increased to 25-27°C, the parameters of the secondary bioelectric responses are finally stabilized. Above 26-27°C there is a marked accentuation of the secondary responses following both single (see Fig. 31, I) and rhythmic stimuli.

Reestablishment of the secondary bioelectric responses during arousal of an animal from hibernation is thus characterized mainly by the presence of all those transformations which we described earlier for the case of an animal falling into torpor.

Induced bioelectric responses in the auditory projection zone. In some experiments which we carried out to study the changes in the EEG in hibernants during various stages of natural adaptation, we succeeded in observing induced responses in the auditory projection zone of the cerebral cortex; the dynamics of these responses differed only insignificantly from what has been described above.

An auditory stimulus led to the appearance of a polyphasic complex consisting of a primary positive and negative wave as well as of secondary and trace discharges in the auditory projection zone of the cerebral cortex. The onset of hibernation, accompanied by the changes in respiration and cardiac activity which have been described above, was characterized by a gradual accentuation of the primary response, reflected especially in an increase in amplitude of the positive component. As before, a drop in cortical temperature and the onset of hibernation were accompanied by a decrease in amplitude of the biphasic primary complex, an increase in its duration, and the appearance of trace discharges. At a temperature of 6-8°C in the cerebral cortex an auditory stimulus produces only a constant positive potential. /96

The development of excitation in the cortical neurons responsible for the formation of the positive component of the primary response can thus be recorded in the auditory projection zone in a temperature range which is somewhat lower than that described earlier for experiments dealing with the bioelectric responses in the somatosensory projection zone. During arousal, reestablishment of the structure of the induced response is already observed at 17°C. The subsequent changes in the induced potentials consist of an increase in amplitude of the primary complex, shortening of its duration, and the appearance of trace waves. This period generally lasts for 70-160 minutes if arousal continues without interruption, but is prolonged if the rewarming process is delayed.

Reestablishment of the nature of the induced potentials is observed at a cerebral cortical temperature of 29-31°C. It should be pointed out that the "enriched" basal activity in the electroencephalogram at a cortical temperature of 29-32°C does not prevent the detection of a clear local potential in the projection zone.

* * *

The onset of hibernation and torpor are thus accompanied by regular changes in the induced bioelectric responses in the somatosensory and auditory projection zones. These changes can be quite clearly separated into several stages, the first of which is characterized by periods of accentuation of the induced bioelectric responses, particularly the secondary potentials. In the experiments of this series, true accentuation of the induced bioelectric responses was detected most clearly at a temperature range between 26 and 29°C. /97

The second stage is characterized by a gradual reduction in the secondary bioelectric responses, coinciding in time with a deepening of hibernation and incipient torpor. In any case, disappearance of the secondary potentials is generally accompanied by a significant increase in depth of torpor, decreases in heart rate and respiration, and depression of the "spontaneous" electrical activity of the cerebral cortex.

This is followed by depression of the primary negative waves, which are closely linked under conditions of incipient hibernation with the secondary positive wave. In the temperature range from 19 down to 12°C, the induced bioelectric response consists of a primary positive wave — the component which is most resistant to low temperatures. All of the changes described above for the induced potentials in the projection zones are to a certain extent "universal" and are observed in each area representing groups of neurons of varying function.

Arousal is characterized by reappearance of the structure and parameters of the induced bioelectric responses. However, this process does not fall as clearly into definite stages. Reestablishment of the entire complex of the induced response proceeds significantly more rapidly during arousal than its "differentiation" during the onset of hibernation and torpor.

CHAPTER IV. /98
ELECTRICAL PROPERTIES OF THE APICAL DENDRITES OF THE CEREBRAL CORTEX OF HIBERNATING MAMMALS

Temperature Range of the Activation of the Postsynaptic Dendritic Membrane.
Electroencephalographic Effects of Strychnine and GABA in the Cerebral Cortex.
Temperature Gradient of the Effectiveness of Hyper- and Depolarizing Agents in Various Stages of Hibernation.
The Hypothetical Mechanism of Cortical Inhibition in Hibernating Mammals.

Experiments devoted to an analysis of the primary and secondary bioelectric responses in the cerebral cortex of hibernating mammals have shown that the transmission of excitation along specific and nonspecific conducting pathways undergoes regular changes in all stages of natural adaptation. It is only natural that these changes should be determined, first of all, by the excitability of the cortical neurons taking part in the formation of the induced responses, particularly the pyramidal cells of the third and fourth layers and the intermediate neurons of the second layer, and then secondarily by the characteristics of transmission in the upper layers, in the direction of the apical dendrites.

The present chapter will be devoted to the regular changes which characterize the postsynaptic activity of the apical dendrites of the cortical neurons. This phenomenon was investigated by studying the dendritic potentials arising in the cortex during its direct stimulation by means of an electric current (19, 148, 172, 175, 22, etc.).

Direct electrical stimulation of the cortex in order to produce dendritic potentials (which are known to depend on post-synaptic activation of the apical dendrites of cortical neurons) was carried out under conditions analogous to /99
those described above. The stimulating and recording electrodes and a sensor for measuring the temperature of the cortical zone were implanted along the boundaries of the $P_{per(e)}$ and $P_{per(i)}$ fields. The bipolar stimulating electrodes (with a distance of no more than 0.8 mm between them) were connected to the radiofrequency output of a stimulator, while the recording electrode was connected to the input grid of an amplifier tube. Stimulation was carried out with rectangular impulses having the parameters described above. The statistical

processing of the data and the technique for recording the biopotentials remained the same as before.

The electrical effects of the application of strychnine and gamma-aminobutyric acid (GABA) to the surface of the cortex were studied both in an independent series of experiments and during experiments on the induced potentials in the projection zone. The drugs were applied either in crystalline form or with the aid of filter paper, and the effects produced were recorded either on the screen of an ENO-1 oscilloscope or on an recording electro encephalograph. Strychnine was used either in crystalline form or as solutions having concentrations of 1:100 or 1:25. The need for high doses of strychnine can be ascribed entirely to the low sensitivity of the cerebral cortex of hibernating animals to this depolarizing agent. GABA was used in the form of a 0.5-2% solution.

General properties of the apical dendrites of the pyramidal neurons of the hibernant cerebral cortex. In a state of active wakefulness or superficial chloralose anesthesia, at a cortical temperature of 29-34°C, the dendritic potentials are characterized by a negative-positive complex having the following parameters: they appear with a latent period of 1-1.5 millisecond, their negative component attains 800-1000 microvolts at a duration of not more than 50-70 milliseconds, and the superficial positive wave usually does not exceed 120-250 microvolts.

Thus, the starting values for the dendritic potentials in the waking animal or the superficially anesthetized hedgehog or suslik do not differ from those in homoiothermic mammals (148, 175, 222).

The way in which the amplitude characteristics of these potentials depend on both the temperature of the cortex and the parameters of the stimulating current is illustrated in Fig. 33 and in the curve (Fig. 33, m) reflecting the distribution of the mean values of the negative components of the dendritic potentials as a function of changes in cortical temperature and the voltage of /101
the stimulating current. Each point on the curve is based on a statistical evaluation of the results of 10 similar experiments. At a cortical temperature of 28-32°C, if stimulation with a 6-volt current results in the appearance of a dendritic potential having the parameters described above, then increasing the voltage to 15 volts regularly produces a 200-300% increase in the amplitude of the potential, from 400±10 to 1200±60 microvolts (Fig. 33, b). A subsequent decrease in brain temperature such as that shown on the T° axis of the curve (Fig. 33, m), which may be observed during the onset of hibernation, prevents such a marked increase in the amplitude of the dendritic potential in connection with an increase in voltage of the stimulating current: within the temperature limits indicated, if the cortical temperature is decreased to 22-26°C, then the effect does not exceed 20-40% and is characterized by an increase in the negative dendritic potential from 480±10 to 660±20 microvolts (see Fig. 33, d-f). At a cortical temperature of 20-21° C, this effect disappears altogether: an increase in stimulus voltage no longer leads to any significant change in dendritic potential (291±10 compared to 318±6 microvolts, for example). This relationship is then maintained despite the "spontaneous" fluctuations in potential amplitude, which usually lie outside the limits of statistical significance.

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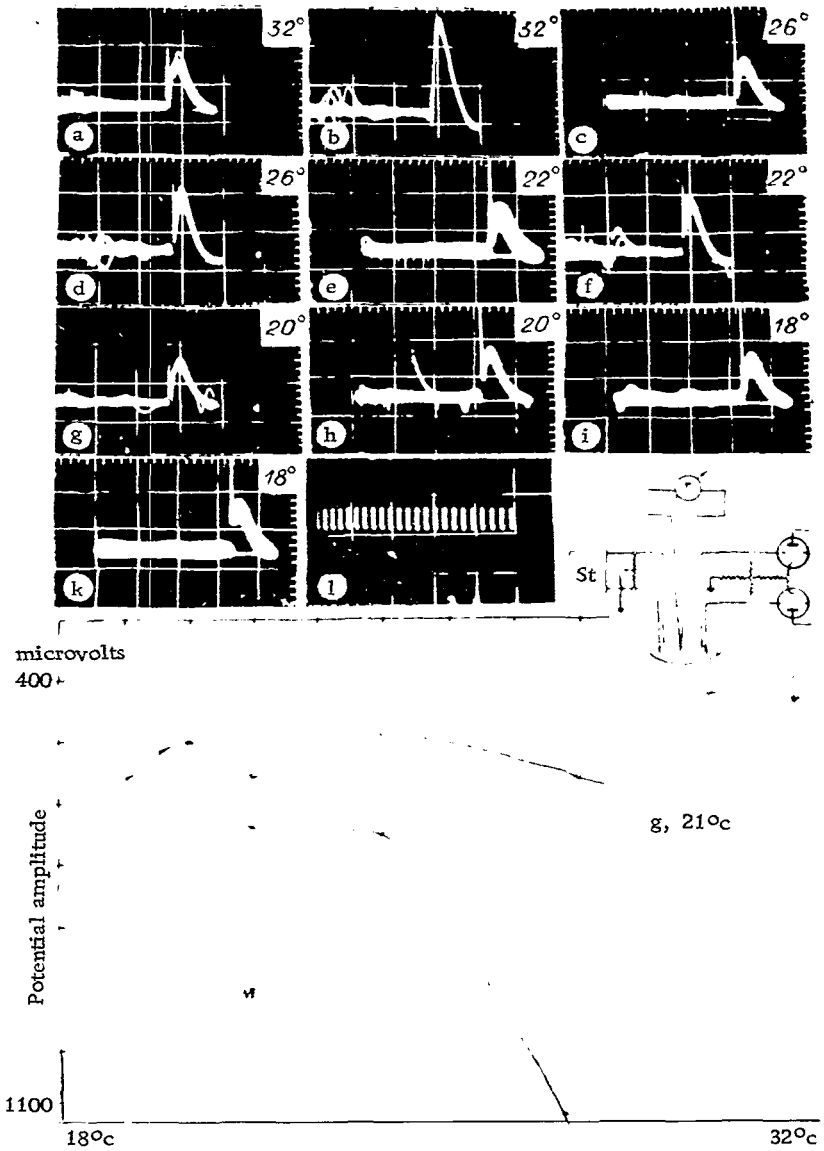

Figure 33. Effect of Temperature and the Characteristics of the Stimulus on the Dendritic Potentials in the Cortex of the European Hedgehog: a, c, e, g, i — Negative Components of the Dendritic Potentials at an Output Voltage of 6 Volts; b, d, f, h, k — 15 Volts; l — Calibration with 250 Microvolts at 20 Milliseconds; H — Conditions of the Experiment, St — Stimulator, T — Temperature Sensor. Experiment 38, Oct. 10, 1963. Chloralose, 3 mg/100 g body wt.

Thus, during the onset of hibernation and in the first stage of torpor (when the brain temperature decreases to 19-20°C), a definite relationship is preserved between the amplitude of the stimulus and the direct response in the cerebral cortex. This phenomenon is apparently related to changes in excitability of the post-synaptic membrane of the apical dendrites in the cortex and the simultaneous excitation of a large number of synaptic contacts converging on the dendrites.

Changes in dendritic potentials during the onset of hibernation and torpor. /102
The onset of hibernation is accompanied by regular changes in the parameters of the dendritic potentials. In the range between 34 and 29°C, the "spontaneous" fluctuations in these potentials do not exceed the mean value of 196±8 microvolts (Fig. 34, a). At a cortical temperature of 26-29°C (and even more clearly if the temperature has been decreased to 24-25°C), there is some increase in amplitude of the negative component of the dendritic potential (without any increase in duration) and a deepening of the positive wave (Fig. 34, c). During

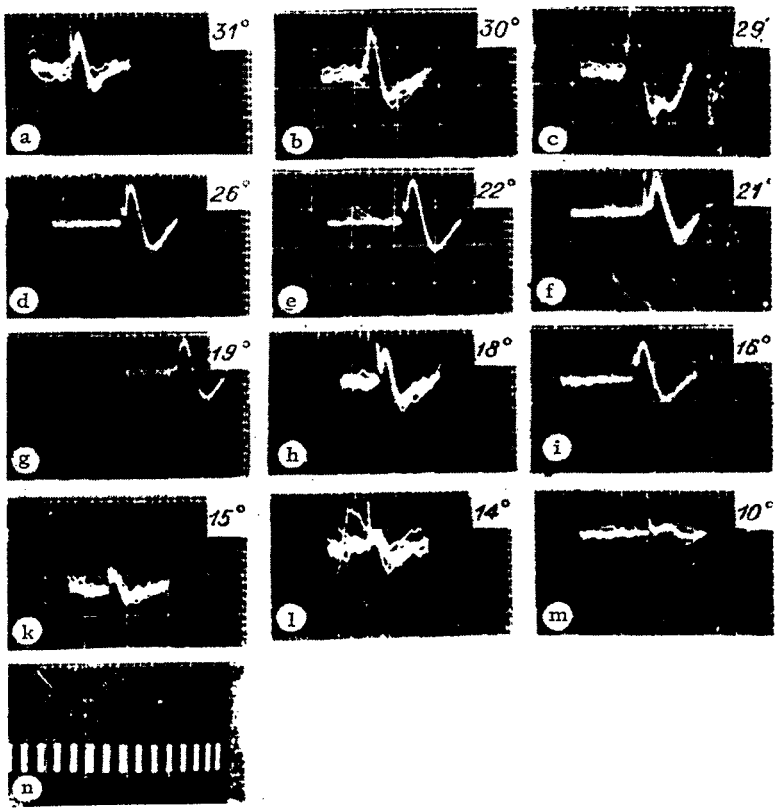

Figure 34. Changes in Dendritic Potentials in the Cortex of the European Hedgehog. The Various Letters have the Same Meaning as in Fig. 33. Experiment 41, Oct. 26, 1963. Chloralose, 3 mg/100 g body wt.

this period the dendritic potential increases from 196±8 — 225± to 357±13 microvolts. The changes in dendritic potential in the temperature range from 24 to 18°C, which coincides with the beginning of torpor, are quite interesting. /103 It is during this period that a progressive decrease in the amplitude of the negative component sets in: during the course of 40-80 minutes it drops from 357±13 to 245±10 microvolts. This is followed by a brief period of stabilization of the parameters, after which the amplitude drops rapidly until a temperature of 11°C, reaching 88±6 microvolts (Fig. 34, l). Below 10-11°C stimulation of the surface of the cortex produces only a slow prolonged wave (lasting 50-70 milliseconds), the amplitude of which does not exceed 33±4 microvolts (Fig. 34, m). Suppression of the negative component of the dendritic potentials is seen at a cortical temperature of 7°C.

Changes similar to those described above are quite typical and were recorded in 21 of the 28 experiments in this series. Accentuation of the dendritic potentials could be recorded most clearly at a cerebral cortical temperature of 26-29°C. Sometimes, this accentuation of the dendritic potentials was preceded by a brief period of fluctuation; nevertheless, these fluctuations remained outside the limits of statistical significance.

The period of accentuation of the dendritic potentials is followed by stabilization of the amplitude of the negative and positive waves, which usually takes place over the course of the next 30-40 minutes. Actually, even in this temperature range (from 23 to 18°C), there is a smooth gradual decrease in the amplitude of the potentials (from 210±16 to 203±16 microvolts). As before, a drop in cortical temperature below 12°C still does not prevent the appearance of dendritic potentials, the amplitude of which reaches 46±4 microvolts during this period (Fig. 35, i).

Arousal is accompanied by a rather rapid reestablishment of the structure of the dendritic potentials. This takes place extremely rapidly and lacks the stepwise character which is particularly typical of the onset of torpor. This also makes it considerably more difficult to study the phenomena of arousal in hibernating animals in detail. At 11°C, stimulation of the cortex produces a dendritic potential having a slow negative wave with an amplitude of 80±5 microvolts, but already at 15°C the amplitude of the potential is doubled (around 15°C the amplitude equals 170±3 microvolts while at 18°C it equals 250±8 microvolts) (Fig. 36, a-e). During the temperature interval between 19 and 28°C, just as when the animal is falling into a state of hibernation and torpor, the potentials are stabilized to a certain extent within the limits from 220±6 to 250±18 microvolts (Fig. 36, f, k). This is followed by a phase of accentuation, during which the amplitude of the potential rises to 380±14 microvolts (Fig. 36, h), and by 30°C the parameters of the dendritic potentials have /104 returned to the initial values which are characteristic of the waking state (Fig. 36, i-l).

Electroencephalographic effects of strychnine and GABA on the cerebral cortex of hibernating mammals. The experiments which were described above and which were designed to study the electrical properties of the neurons of the primary projection zone and their dendrites showed that the functional characteristics of these structures undergo regular changes during the onset of

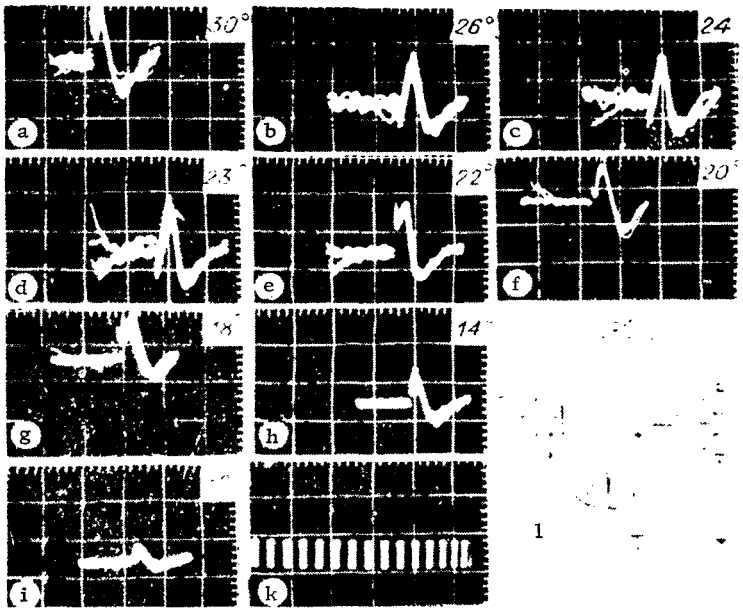

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Figure 35. Changes in Dendritic Potentials in the Cortex of the European Hedgehog. Unanesthetized Animal. Experiment 42, Oct. 30, 1963.

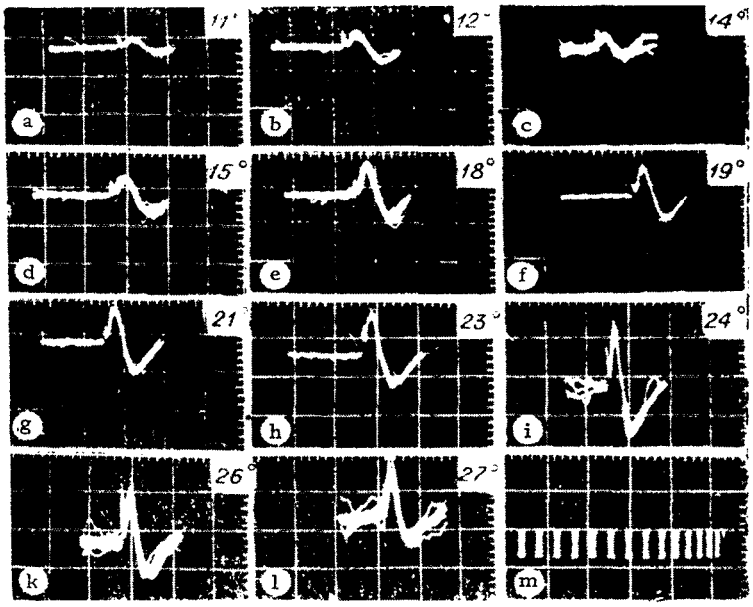

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Figure 36. Changes in Dendritic Potentials During Arousal Produced by Rewarming. Experiment 42, Oct. 30, 1963.

hibernation, torpor and arousal. At various stages of hibernation, excitation was found to be blocked in various links of the primary "neuronal" circuit. However, our analysis of the functional organization of the cerebral cortex would be incomplete if we did not attempt to clarify the dynamics of the inhibitory and stimulatory synaptic effects in the cortex during the development and deepening of cortical inhibition. In order to solve this problem, experiments were carried out in which we studied the EEG effects of strychnine nitrate and GABA following their topical application to the cerebral cortex.

Strychnine is well known to act specifically against the hyperpolarizing synapses. On the other hand, GABA is able to block the depolarizing contacts, while also exerting a general depressant effect on the central neurons (148, 200, 551, 552). Following their parallel or successive application to the surface of the cerebral cortex, the interaction between strychnine and GABA must take place at the level of the post-synaptic membrane of the cortical neurons and their dendrites in the upper layers (148).

Nevertheless, this well-known hypothesis of Purpura and Grundfest is currently being subjected to a certain amount of reexamination. On the one hand this is due to the fact that new experimental data have been obtained, while on the other hand it is a reflection of new interpretations of the data obtained originally by the American neurophysiologists. It has been suggested that the inhibitory effect of GABA may be due to a nonspecific effect on the cortical neurons (200). Yu. B. Manteifel' has rightly pointed out that the obligatory effect of the application of GABA to the surface of the cortex (i.e., the positivization of the negative components of the induced responses) may be looked upon as the result of a complete deactivation of the neurons in the area in which the substance is applied and the unmasking of the activity of the neighboring structures. GABA suppresses the reactive potentials of the optic tegmenta (which are formed on the basis of the post-synaptic potentials of the dendrites), interrupting the transmission of the stimulus to the body of the neuron. This comparatively physiological approach to the solution of the problem of the nature of the functional and electrographic effect of GABA has been found to be quite suitable here. /106

Regardless of the mechanisms of the central inhibitory effect of GABA, we used this compound as a strychnine antagonist (148, 355, 364 and others). It was necessary to examine the possibility of producing stimulatory and inhibitory effects in the cerebral cortex of hibernating mammals during various stages of adaptation, i.e., during the natural or artificial development of a state of either inhibition or activity in the greater part of the central neurons.

The experiments of Chatfield, Lyman and Purpura had already shown that the discharges arising in the hamster cortex following the local application of strychnine are only produced over a definite temperature range during cooling of the animal. The strychnine peaks (SP) are suppressed during torpor and reappear when the hibernant is rewarmed (318).

This series of experiments can be divided into three groups. In the first group the substances were applied locally to the cortex of waking animals at a

temperature of 30°C or higher. Later, after effects had appeared in the EEG, the animals were either placed in a refrigerator or physically cooled in a special cuvette. In the second group of experiments, strychnine and GABA were applied to the cortex of animals which were already in a definite stage of torpor. In the third group, we studied the effects of these substances on induced potentials (dendritic potentials and primary responses) during various stages of /107 adaptation. Naturally, the respiration and cardiac activity of the animals were controlled at the same time, both visually and electrographically, and the background electrical activity of the cerebral cortex was recorded.

Strychnine was used in the form of a 0.1-0.25% solution by the generally accepted technique of Baloni and Amantea, or in the form of crystals, while GABA was used as a 0.5-2% solution. The temperature and electroencephalographic effects were recorded as in the earlier experiments. Visual evaluation was made possible by the screen of an ENO-1 oscilloscope. Some of the experiments dealing with the effect of strychnine on the cerebral cortex at various tempeartures were carried out with an recording electroencephalograph.

A strychnine discharge already appears 2-4 minutes after application of a crystal of strychnine or a piece of filter paper moistened with a solution of the compound. Pronounced, stable strychnine peaks were observed in every experiment in this series in which the usual concentrations of strychnine were applied (1:250, 1:1000). The strychnine peak is characterized by a triphasic structure: the first positive wave has a duration of 12-15 milliseconds and reaches an amplitude of 100-200 microvolts; the negative component of the strychnine peak and the second positive complex reach amplitudes of 500-1000 and 350-600 microvolts, respectively, with a duration of 30-35 and 40-45 milliseconds.

In the waking state or under very light chloralose anesthesia (30-40 mg per animal) at a cortical temperature of 29-30°C, there is a gradual increase in the frequency and amplitude of the strychnine peak, especially in the primary positive and negative components of the discharge. As the temperature of the cortex drops further and the frequency of the discharges decreases, the amplitude of the strychnine peak remains unchanged or may even increase. Thus, if discharges having a frequency of 2-4 per second can be recorded at a cerebral cortical temperature of 28-31°C, then at a temperature of 18-21°C the frequency does not exceed 0.3-0.5 cycles/second.

The changes in the strychnine potentials in the cerebral cortex of susliks show essentially the same pattern. There is an initial development of episodic strychnine peaks, followed by an increase in their frequency until the cortical temperature has fallen below 26-28°C (Fig. 37). Then, at a temperature of 17-18°C, the amplitude of the discharges decreases while the duration increases. The strychnine peak suddenly disappears from the EEG of hibernating animals at a cortical temperature of 15-17°C (Fig. 37). At low cerebral cortical temperatures and in a state of established torpor, repeated application of strychnine /108 does not produce any effects in the EEG and does not "potentiate" a strychnine peak which may have appeared earlier.

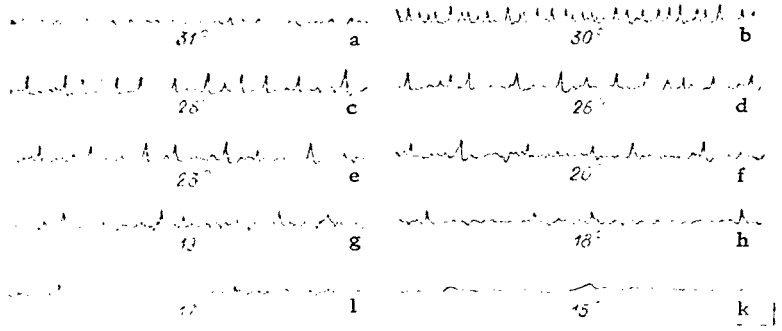
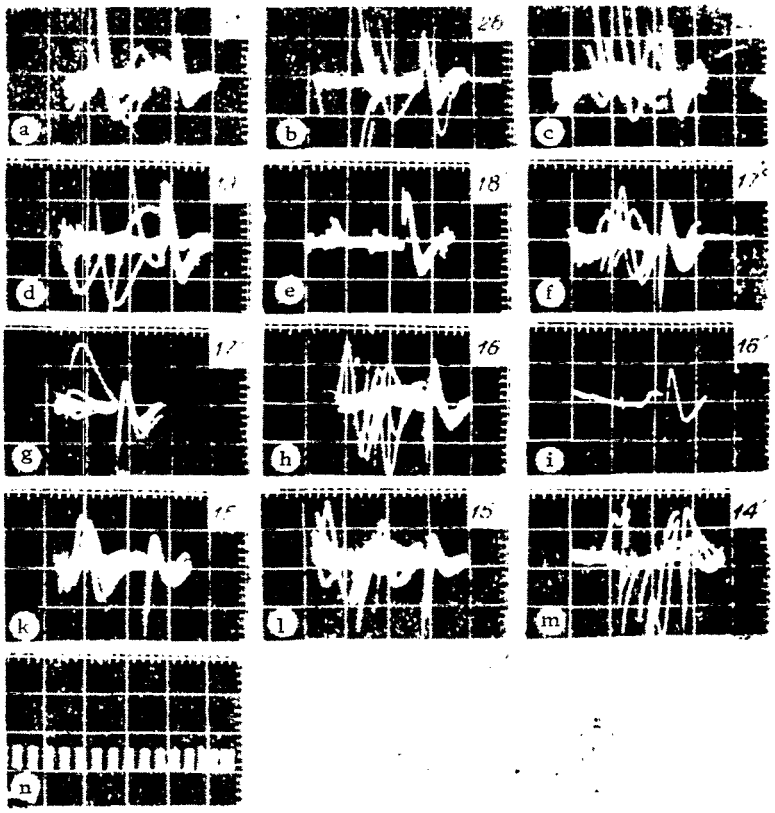


Figure 37. Changes in the Strychnine Peaks in the Cerebral Cortex of the Suslik during Onset of Hibernation (b-e) and in Torpor (f-k). Experiment 15 (long-term, March 30, 1960. Unanesthetized Animal.



/109

Figure 38. Effect of Strychnine on the Induced Potentials in the Cerebral Cortex of the Suslik at Various Temperatures.

Application of a 1-2% solution of GABA to the surface of the cortex in an animal already showing the effects of strychnine produces an immediate drop in the amplitude of the negative component of the strychnine peak, followed by its inversion. The inverting effect of GABA persists for a fairly long time in the cortex of hibernating mammals during the onset of hibernation and incipient torpor. It also persists if the surface of the cortex is washed with Ringer's solution. As the level of hibernation deepens, accompanied by a drop in cortical temperature and the onset of torpor, this effect is not abolished by a repeated application of strychnine nitrate. During this period even large concentrations of depolarizing agent are unable to produce motor disturbances of the type of generalized and focal convulsions. If the inversion of the negative component of the strychnine peak which is produced by GABA in the cerebral cortex of the waking animal is reversed by strychnine, this usually does not become possible until 15-25 minutes after the application of GABA. Spontaneous reversion of the negative potential (without application of strychnine or another depolarizing agent) does not occur in the hibernating animal in a state of torpor.

When the effects of strychnine are studied against a background of electrical stimulation of the brain surface, then these effects, which usually develop 1-2 minutes after application of the compound to the surface of the cortex in the waking animal (or an animal under very light chloralose anesthesia), are not /110 limited to an increase in the negative component of the dendritic potential. Strychnine usually "facilitates" (accentuates) the appearance of the positive wave which is related to the electrotonic effect of the dendrites on the body of the pyramidal neurons. This "potentiating" effect of strychnine is also observed at low cortical temperatures. In any case, it prevents the usual suppression of the dendritic potential which develops during this period in the hibernant brain (Fig. 38).

An analysis of the effect of strychnine on the dendritic potentials also reveals the following extremely interesting phenomenon: in the presence of a strychnine potential having a high frequency and amplitude, the dendritic potentials do not alter their parameters as the cerebral cortical temperature drops to 16-18°C (see Fig. 38, f-i). The strychnine discharges can still be recorded after the initial suppression of the negative component of the dendritic potentials. There is thus a relationship between the amplitude of the induced dendritic potential and the strychnine discharges preceding the appearance of the postsynaptic potential of the dendrites.

Direct stimulation of the cerebral cortex by an electric current (if carried out after application of strychnine) usually produces a high-voltage synchronous discharge, the origin of which is related to the depolarization of the intermediate neurons of the second layer (174). This discharge is still seen in the cortex at a temperature of 19-21°C (see Fig. 38, k). It undergoes a clear inversion following application of GABA in this temperature range, while the dendritic potential itself remains unchanged under the influence of strychnine.

The inverting effect of GABA is consistently preserved at low cortical temperatures. Inversion of the negative potential, which is related to postsynaptic activation of the apical dendrites of the pyramids of the third and

fourth layers, is observed at a brain temperature of 24-26°C. Despite a subsequent drop in brain temperature, the inverted negative potential can be accentuated still more.

The changes in the primary responses of the somatosensory projection zone following application of strychnine recall the changes described in the section on the dendritic potentials: only 2 minutes after application of a 1% strychnine solution to the cortex, strychnine potentials appear having a polyphasic configuration. As the cerebral cortical temperature drops to 27-28°C and the strychnine potential grows, there is a significant increase in the amplitude of both phases of the primary response, especially in the positive wave; /111
the regularity of the potentials also increases, while their duration and latent period are shortened. This is also observed after a decrease in cortical temperature to 25-26°C. Then the amplitude of the negative component of the primary response increases and the entire complex acquires the typical configuration of a strychnine discharge. Thus, in the temperature range between 28 and 24°C, the appearance of a synchronous pyramidal discharge and the transmission of excitation to the upper layers of the cortex are regularly "facilitated". Below 20-21°C there is a rapid decrease in the frequency and amplitude of the strychnine potential; when the cortical temperature reaches 16-18°C, there is a simultaneous increase in the duration and decrease in the amplitude of the primary response.

* * *

On the basis of the results described, it can be concluded that the various stages of hibernation are characterized by a definite pattern of changes in the post-synaptic activity of the apical dendrites of the cerebral cortex. The first stage is characterized by spontaneous fluctuations in potential amplitude arising in the temperature range from 34 to 28°C. Despite the significant temperature gradient, the parameters of the dendritic potentials are unchanged.

This is followed by a period in which the potentials are first accentuated and then depressed. The period of accentuation of the dendritic potentials sets in in the temperature range between 26 and 29°C and is "synchronized" with the onset of hibernation; depression of the potentials is observed at a cortical temperature of 17-19°C and coincides with the onset of torpor.

Arousal of hibernants is characterized by a reverse order of changes in the dendritic potentials. However, these cannot be divided into definite stages quite as easily (we refer here to the stepwise changes in the electroencephalographic phenomena). Their transformations can be readily overlooked under experimental conditions. Nevertheless, arousal is also characterized by an accentuation and stabilization of the parameters of the dendritic potentials which develop in a very short space of time.

Application of strychnine and GABA to the surface of the cortex of animals in the waking state or under superficial anesthesia leads to the appearance of the well-known electroencephalographic effects: strychnine discharges and /112
inversion of the negative component of the dendritic potentials. However, these effects differ in a number of essential details from the effect which is usually observed in the cerebral cortex of homoiothermic mammals.

Attention should be called first of all to the need for a high concentration of strychnine nitrate before a strychnine peak can be produced in the hibernant cortex; as has been shown, the depolarizing effect of this compound can be demonstrated successfully only by the use of crystalline strychnine. This fact alone already indicates a decreased sensitivity of the post-synaptic dendritic membrane of the central neurons to stimulatory agents in hibernating mammals.

Attention should also be called to a number of findings relating to the transformations of the induced potentials — the dendritic potentials and primary responses. These findings indicate that under conditions of low brain temperatures (16-18°C) corresponding to a state of hibernant torpor, all of the changes in the induced potentials normally produced by application of strychnine are abolished and it is no longer possible to reproduce the depolarizing effect of this compound in the cerebral cortex. On the other hand, the effect of GABA is intensified even under conditions when the cortical neurons are no longer able to process excitatory stimuli.

We have already briefly evaluated the effectiveness of strychnine during torpor and spoken of the relatively low sensitivity of the post-synaptic membrane of the cortical neurons to depolarizing agents. There is some experimental basis for continuing along these lines and hypothesizing that the somatodendritic membrane of the central neurons in hibernating mammals may be highly sensitive to inhibitory agents such as GABA. From this it can be concluded that more favorable conditions are created for the manifestation of the deactivating inhibitory effect of GABA during the onset of hibernation and torpor in the cerebral cortex of hibernants, and that there are no factors favoring the manifestation of the stimulatory effects of strychnine.

CHAPTER V. /113
SOME HISTOCHEMICAL CHANGES IN THE BRAIN OF HIBERNATING ANIMALS

Histochemical Localization of Biologically Active Compounds and the Activity of the Central Neurons.
Distribution and Intracellular Localization of Succinic Dehydrogenase in Various Parts of the Brain.
Role of Glycogen in the Activity of the Central Nervous System.
Distribution of Glycogen in the Brain of Hibernating Mammals during Various Stages of Hibernation.

The present chapter will be devoted to the results of histochemical studies of the distribution of some biologically active substances in the central neurons of hibernating animals; an attempt will also be made to compare their localization and activity with the characteristic electrical activity during the various stages of hibernation. As has been shown in the earlier parts of this monograph, the characteristic function of the brain of these animals results in "programmed" excitation and inhibition of the principal cerebral regulatory systems in the various stages of adaptation. Thus, under these conditions, the state of a group of neurons responsible for carrying out a particular functional task can be evaluated quite accurately by the use of complex electrophysiological and histochemical methods. Our experimental conclusions have been confirmed by non-Soviet publications containing analogous material (561, 570, 623). There is no doubt that it would be desirable to verify the correctness of the experimental findings by comparing them with the results obtained in homoiothermic animals; some such experiments have already been carried out (105-107, 180, 374), and the results obtained in this way do not seem to contradict one another. /114

The purpose of our histochemical studies was to investigate the distribution of some of the active substances in the central neurons of the brain of hibernating mammals in the hope, at a later stage, of being able to relate these findings to concrete functional phenomena such as are reflected in the electrical activity of the cerebral structures. Out of all our material on the histochemical changes in the cerebral structures of hibernating animals, only the following two questions will be evaluated in the present section:

1. The activity of the oxidative enzymes, with as example the distribution of succinic dehydrogenase (SDH), in relation to the functional state of the central neurons in hibernating animals;

2. The distribution of glycogen in various parts of the brain in relation to the electroencephalographic forms of some systemic adaptive responses.

As a rule, material for the histochemical studies was taken from a live animal in a definite stage of hibernation (at a strictly determined brain temperature).

ROLE OF THE SUCCINOXIDASE SYSTEM IN THE ACTIVITY OF THE NEURONS OF THE CENTRAL NERVOUS SYSTEM. SDH AND THE FUNCTIONAL STATE OF THE NERVE CELL.

By now it has been demonstrated quite conclusively that the activity and distribution of SDH in the mitochondria of the nerve cells, the synaptic termini and the dendrites are an adequate cytochemical indicator of the state of activation or inhibition of the elements of the central nervous system. Excitation of the central neurons is accompanied not only by an unquestionable increase in SDH activity but also by a true increase in its concentration. In view of the role of this enzyme in the transfer of electrons, these results may indicate that increased neuronal activity is accompanied by increased energy metabolism. The study of the distribution of SDH in the nerve cell may provide an answer to the /115 question of the connection between the processes of nervous excitation, and the transmission of excitation in the form of an action potential, and the enzymes of the Krebs cycle, oxidative phosphorylation and electron transport, processes which are localized in the mitochondrial apparatus and which supply the principal energy requirements of the cell. Changes in energy metabolism which have been localized cytochemically to the neuronal mitochondria may be connected with both the transneuronal and the transsynaptic transfer of excitation. Reports in the literature (36, 46, 47, 105-107, 161, 163, 164, 375) indicate that the rate of oxidative metabolism in the brain is extremely high. The energy which is constantly being liberated is consumed for synthetic processes which are necessary for the regeneration of substances entering into the structural formations of the cell and for the functional activity of the brain as a whole.

In studying the cerebral activity of hibernants, all of these considerations acquire particular significance. It is well known that, during hibernation, the European hedgehog can tolerate a hypoxia for many hours without harm, which would be absolutely fatal for the same animal in the waking state (295). As was already mentioned earlier, evisceration and the resultant exsanguination of the brain do not suppress the automatic activity of the respiratroy center in susliks, although being fatal in normothermic animals (119). This has led some investigators to classify the hibernating mammals among the aerobic organisms which are able to tolerate the absence of oxygen for many hours, such as the amphibia, some arthropods, and the mollusks (425, 585). In this connection, the characteristics of the cerebral respiration in hibernants has led to a study of the indicators of oxidative metabolism in the cerebral tissue.

What is currently known about the oxidative metabolism of the hibernant brain, studied in vivo or in vitro? Some information has already been given in this regard at the beginning of this monograph. When the air temperature is decreased from 30 to 5°C, the oxygen consumption in the hamster and marmot drops by 60-67%, either suddenly or gradually. Respiratory activity in these animals is preserved at a body temperature of 3.5-5°C, while nonhibernating homoiotherms stop breathing within the limits of 19-21°C (268-270). The onset of hibernation leads to a sharp decrease in oxygen consumption: from 2.179 ml/g tissue/hr. to 0.071, while the CO_2 output falls from 2.295 ml/g in the waking animal to 0.043 in the hibernating subject. Arousal is accompanied by a stepwise increase in oxygen consumption, which reaches 6.436 ml/g in the course of an hour.

The respiratory coefficient drops to 0.63 during hibernation, indicating that fat is being consumed as a source of energy, and rises to 0.89 during /116 arousal indicating the mobilization of heat at the expense of the glycogen of the muscle, liver and possibly even the brain (67, 68). Analogous conclusions were expressed by Kayser and his associates on the basis of the results of experiments carried out on marmots (443).

In vitro studies indicate the following: the oxygen consumption by brain slices at 10-36°C is highest in the case of the rat, followed by the waking and then by the hibernating hedgehog. The energy of activation is 14.3, 13.2 and 13.1 kcal• mol., respectively. Electrical stimulation of brain slices produces an increase in oxygen consumption, a response which is preserved even at 10-15°C.

Thus, the onset of hibernation and deep torpor are characterized by a 97-98% decrease in oxygen consumption. Nevertheless, even at a brain temperature of 7-9°C in some segments (the hippocampus, limbic cortex, amygdalus, and olfactory lobe), prolonged periods of electrical activity and responses produced by electrical stimulation of the cortical surface can be recorded. We would like to emphasize the latter finding, since an experimental explanation for it will be provided later. All of these facts lead to the hypothesis that torpor is characterized by an economical and optimal level of oxidation, a hypothesis which can be evaluated by studying the level of activity of the oxidative enzymes taking part in the Krebs cycle.

However, a sharp and significant drop in the oxygen supply to the hibernant brain also apparently activates the mechanisms of anaerobic glycolysis (305, 307); such activation may also depend on the carbohydrate reserves of the brain and particularly on the reserves of glycogen, the intensity of the metabolism of which in the hibernant brain apparently also determines the rate of utilization of these reserves and the energy yield of anaerobiosis. Since this is directly related to the reserves and accumulation of glycogen, the second problem was to determine glycogen by means of histochemical techniques. The following section will be devoted to a brief description of the results of histochemical studies on the localization of succinic dehydrogenase and glycogen in the central neurons of hibernating animals.

For the histochemical localization of SDH activity we used a modification of the method of Seligman and Rutenberg (161). Slices of brain tissue taken from a live animal were incubated in a mixture of NST and sodium succinate for 20 hours at a temperature of 20, 28 or 37°C. The reduction of NST was then /117 stopped by formalin fixation, after which the tissue slices were embedded in a glycerol-gelatin gel. Incubation was carried out under anaerobic conditions at a pH which was controlled within the range 7.5-7.8.

Experimental and control preparations were then fixed on a single microscope slide and photographed, taking care that the following standard conditions were maintained for each slide: stabilization of the illumination, the use of the same filters, the use of flat negative photographic film having a constant amount of emulsion, and simultaneous development of the negatives of the control and experimental preparations. The entire "system of precautions" described above was directed at decreasing the percentage error which could arise during the process of obtaining a negative image and which could affect the subsequent photometric determination (31). The photometric determination was preceded by construction of the so-called characteristic darkening curve of the film (for each amount of emulsion). This curve reflects the relationship between darkening of the negative and transparency of the object (assuming a constant definite exposure time).

We constructed a characteristic curve for a particular amount of emulsion on the basis of preliminary data obtained on an MF-2 microphotometer for a so-called stepwise reducer (photometric wedge), each "step" of which is characterized by a known constant transparency. In this way a curve was obtained showing the relationship between darkening of the negative and transparency of the reducer. Since the "degrees of darkening" (individual portions of the wedge) were recorded on the same film as the experimental object, and since everything was exposed for the same length of time and then developed simultaneously, the relationship between darkening and transparency obtained for the characteristic curve could be extrapolated not only to the stepwise reducer but also to the experimental object — in this case the neuron. The photometric data obtained in this way, which depend on both the distribution of the substance (SDH) and its content in the preparation, were then processed statistically using Student's tables.

Distribution of SDH activity in various parts of the brain of hibernating animals in various stages of natural adaptation. The enzymatic activity, which is related to the degree of impregnation of the neuronal protoplasm with formazan, shows regular alterations which depend primarily on the state of vital activity of the animal (active wakefulness with a cerebral cortical temperature /118 of 30-36°C, onset of hibernation with a temperature of 20-29°C, and torpor with a temperature of 7-19°C).

Cerebral cortex. An analysis of the distribution of formazans in the cytoplasm of the neurons and dendrites of various parts of the brain showed the lowest concentration in the cortical neurons. This is in essential agreement with the distribution of enzymatic activity in the cortical cells of homoiothermic animals (35, 36). In a state of active wakefulness the picture of the mitochondria

is directly related to the distribution of the enzymatic activity and clearly reveals the outline of the perikaryon of the neuron and dendrites, which can be traced for considerable distances from the body of the cell. In some neurons one can also see an outline of the nucleus, which is free of precipitated formazans. The dendrites are intensely impregnated with formazans.

An analysis of the distribution of SDH in the neurons of the cerebral cortex shows that the maximal enzymatic activity is found in the dendrites, with decreasing amounts in the direction of the neuronal body and the monticulus of the axon. The axon itself does not contain measurable amounts of SDH, the enzyme being detectable only within the initial segment of the nerve process.

When the SDH activity in the neurons of the cerebral cortex is high, then the synaptic accumulations which become noticeable during a gradual transition to a state of neuronal inhibition cannot be detected. The surrounding neurons of the glial cell show relatively little enzymatic activity, forming a soft pale-rose background against which the bodies of the ganglion cells become clearly visible. It should be emphasized that a state of active wakefulness is characterized by a relatively uniform distribution of SDH activity in the various neurons of the brain, while during the onset of hibernation there is a "separation" of the ganglial cells into weakly- and intensely stained cells showing statistically significant differences in enzymatic activity.

During the onset of hibernation and the accompanying decrease in cerebral cortical temperature, there is a variable decrease in the staining intensity of the neuronal elements of the cortex, leading to a less marked decrease in the formazan impregnation of the dendrites, while the actual "content" of SDH in the cells decreases significantly (Fig. 39). This process of redistribution of the SDH activity in the neuron is accentuated as the cortical temperature drops /119
further and a state of definitive torpor is reached (Fig. 39, c).

During torpor, the presence of cell bodies was most commonly revealed by the outlines produced by the distribution of formazans in the perikaryon of the neurons (Fig. 39, e). However, a progressive and significant decrease in enzymatic activity in the cytoplasm of the neuron was seen to accompany the persistence of a rather intense formazan impregnation in the dendrites. This /120
finding will be encountered repeatedly, emphasizing its constancy for certain groups of neurons and establishing its relationship with definite functional properties of the cells and their processes.

Statistical evaluation of the photometric data characterizing the level of SDH activity in the neurons confirms the validity of the general picture given on the basis of the SDH distribution: in the waking state the enzymatic activity in the body of the neuron and in the dendrites is the same. During the onset of hibernation the enzymatic activity decreases markedly, but this decrease is not at all uniform: in the cytoplasm of the neurons there is a pronounced decrease, while in the dendrites the enzymatic activity remains quite high even during torpor when the cerebral temperature has reached low values (see Fig. 39, e).

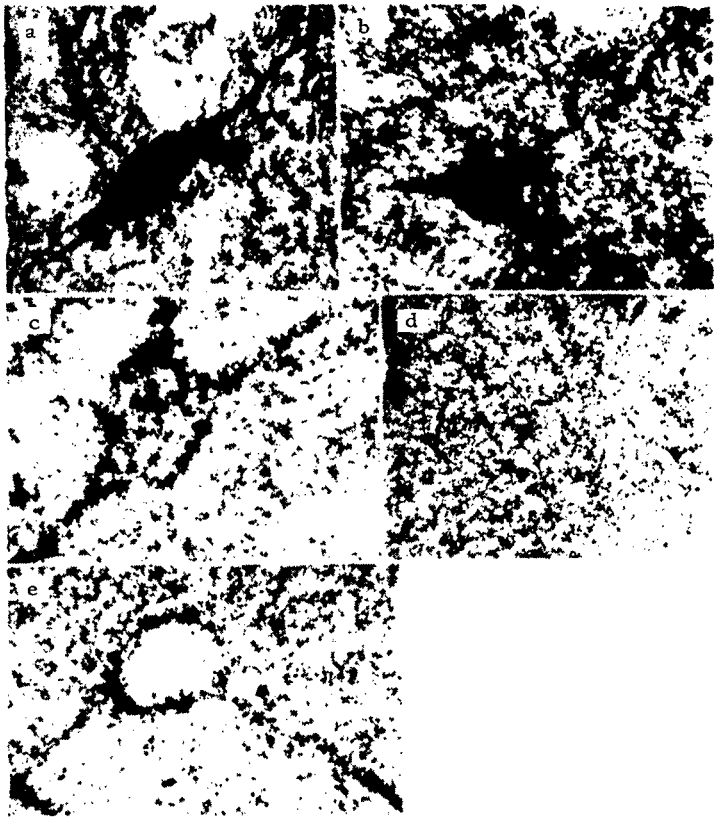


Figure 39. Succinic Dehydrogenase in the Cortical Neurons of the European Hedgehog. Waking State. Brain Temperature of 34°C (a), 21°C during Onset of Hibernation (b-d), 9°C during Torpor (e). Method of Seligman and Rutenberg. NST. Ocular 14x, Objective 90x, Apochromatic Immersion Lens; d - Ocular 7x, Objective 20x, Apochromatic.

Hippocampus. The neurons of this part of the archicortex are characterized by stable levels of enzymatic activity as revealed by an intense formazan impregnation of the cytoplasm of the cell body and the dendrites. This characteristic histochemical localization of the SDH activity also persists at very low cerebral temperatures. It is still observed when the "spontaneous" bioelectric activity is generally suppressed in all parts of the brain, retaining an episodic and irregular character only in the region of the hippocampus and some nuclei playing a role in the functional limbic complex. Even in a state of deep torpor when the brain temperature has reached 5-11°C, a significant number of hippocampal neurons are still characterized by an "initial" activity normally found in the waking state.

Diencephalon. The neurons of this part of the brain, and particularly the ventromedial nuclei of the optic thalamus, are characterized by high levels of SDH activity as revealed by a significant deposit of formazan granules in the cytoplasm. The characteristic SDH distribution enables the outlines of the cell body, its dendrites, and the precipitate-free nuclear area to be clearly distinguished. The glial elements also show a high enzyme content in these parts of the brain, and during the onset of hibernation when there is a drop in intracellular SDH activity, these elements show a significant accumulation of formazan granules. This is followed by a decrease in SDH activity in all cellular groupings in this part of the brain. The formazan distribution in the cell bodies and dendrites of the thalamus and hypothalamus differs from that in other parts of the brain, particularly from that in the cerebral cortex. In this area we were /121 unable to detect a marked "redistribution" of enzymatic activity from the cell body into the dendrites as a result of a decrease in brain temperature and the onset of torpor.

Mesencephalon (reticular formation and corpora quadrigemina). The neurons of the mesencephalic portion of the reticular formation of the brainstem are characterized by an intense formazan impregnation of the cytoplasm of the cell bodies and dendrites, in which the enzyme is distributed in the form of small chains or individual granules, sometimes forming "intercepts" which are devoid of SDH. There is no marked or progressive decrease in enzymatic activity during onset of hibernation in the neurons of this part of the brain, which continue to show extremely high SDH activity even at a brain temperature of 17-21°C, coinciding in time with the initial drop in electrical activity in this area. Only after hibernation and torpor have reached deeper levels does a progressive decrease in enzymatic activity set in in all cellular populations of the mesencephalic reticular formation.

Pons varolii. The neurons of the reticular nucleus of the pons varolii show extremely high enzymatic activity in the waking state as well as during the onset of hibernation and torpor. In the waking state SDH is uniformly detected in all elements of the cell. Within the limits of the nucleus, the mitochondria of all cellular groups actually show an extremely regular formazan impregnation. In most preparations the distribution of formazan granules enables the branching course of the dendrites to be followed for a considerable distance from the body of the cell.

During the onset of hibernation the SDH activity in the cytoplasm remains quite high in the neurons of this part of the brainstem. In most preparations the formazan distribution in the cell bodies and dendrites is uniform, suggesting that the various cellular groupings in the pons varolii are functionally identical in animals during the onset of hibernation. Definitive hibernation and torpor are accompanied by a statistically significant drop in enzymatic activity. Then the SDH activity also decreases here, while remaining relatively high in the cell bodies and the proximal portions of the dendrites. Nevertheless, the enzymatic activity in the cellular groups of the pons varolii remains quite high during torpor, the formazan distribution being quite constant and uniform.

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Figure 40. Succinic Dehydrogenase in the Efferent Neurons of the Medulla Oblongata in the European Hedgehog. Waking State (a, b), Onset of Hibernation (c, d), Torpor (e, f). Method of Seligman and Rutenberg. Ocular 15x, Objective 90x, Apochromatic Immersion Lens (a-c, e); d, f — Ocular 7x, Objective 20x, Apochromatic.

The large neurons of the medulla oblongata in waking animals are characterized by intense formazan impregnation, with large clumps filling the entire cell body and its dendrites, which can be followed for a very long distance from the center (Fig. 40, f). The accumulation of formazan granules in the neurons of the medulla oblongata is not uniform: in some neurons these granules are concentrated mainly in one part of the cell body, while in other neurons they are distributed comparatively uniformly over the entire "territory" of the cell. This may be related to the irregular distribution of the synaptic termini on the cell surface, which leads to local excitation of the neurons at the sites at which the formazan granules are also concentrated. /123

A decrease in SDH activity in the neurons of the medulla oblongata is observed only under conditions of deep torpor when the electrical activity of the brain is depressed. Even at a brain temperature of 4-7°C, large numbers of neurons can still be detected in the motor nuclei of the medulla oblongata which are characterized by high enzymatic activity (Fig. 40, c, e).

* * *

The initial SDH activity in the various parts of the brain of the waking animal can be arranged in the following descending order: medulla oblongata, mesencephalon, pons varolii, hippocampus, hypothalamus, thalamus, and the cerebral cortex. The onset of hibernation and subsequent torpor are accompanied by a true decrease in enzymatic activity and by an intracellular redistribution of SDH. Under conditions of torpor and a maximal decrease in brain temperature, certain parts of the brain are characterized by relatively high and constant levels of enzymatic activity. This is especially true of the neurons of the medulla oblongata, the pons varolii and the hippocampus. A sharp decrease in enzymatic activity is noted under these conditions in the neurons of the diencephalon and the cortex. The distribution of SDH activity described above is confirmed by evaluation of the photometric data (Fig. 41, a-f, Fig. 42). /124

Intracellular redistribution of the SDH activity is a constant characteristic for some parts of the brain and results in a "transfer" of the region of maximal SDH activity from the body of the neuron into its dendrites. The histophysiological differentiation of the heterogeneous structures of the neuron is obviously a typical phenomenon for the histochemical characterization of the changes in oxidative metabolism in the neurons of the cortex and hippocampus. /126

The state of active wakefulness is generally characterized by histophysiological uniformity of the neurons of all cerebral structures. The onset of hibernation, on the other hand, is accompanied by the histophysiological redistribution within the neuron population which has been described above: the mitochondria of some neurons show a high degree of formazan impregnation while in others the intensity of oxidative metabolism is apparently relatively low. Histophysiological differentiation is thus a universal phenomenon which is characteristic not only of cell groups which are joined for the performance of various functional tasks but also of individual neurons.

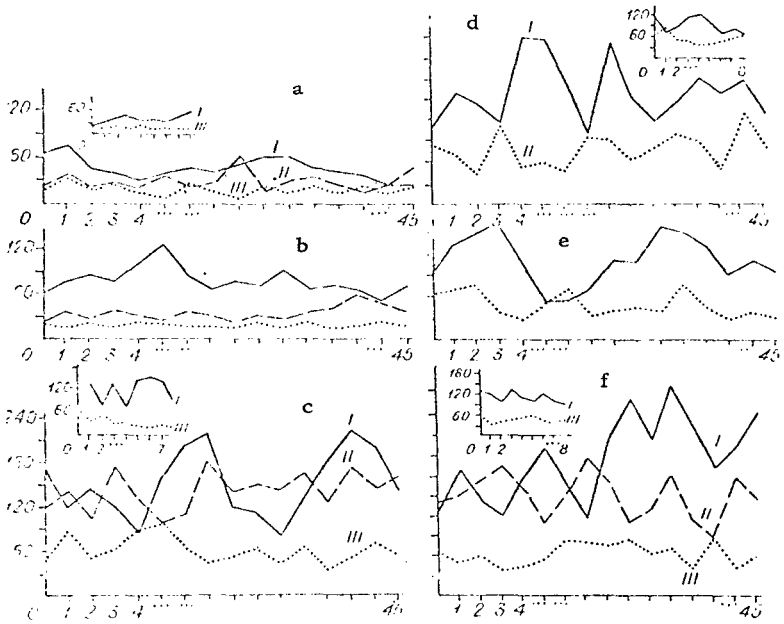

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Figure 41. Distribution of SDH in Some Parts of the Brain of the European Hedgehog during Various Stages of Adaptation. Each Curve Represents the Mean Values from 50 Neurons. Photographic Integration of Photometric Data. The Solid Lines Represent the Waking State, the Dashed Lines Represent the Onset of Hibernation, and the Dotted Lines Represent Torpor. a - Cerebral Cortex, b - Hippocampus, c - Corpora Quadrigemina, d - Tegmental Nucleus of the Reticular Formation, e - Pons Varolii, f - Medulla Oblongata. The Abscissa Shows the Points at Which the Preparations were Scanned, while the Ordinate Shows the Readings on the Linear Scale of the Photometer. The Upper Curves Show Photometric Data on the Dendrites.

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The relatively high rate of dehydrogenation in the mitochondria of individual groups of neurons and, equally important, in their dendrites suggests that dehydrogenation plays a role in the formation and maintenance of electrical activity in these structures under conditions of profound torpor. The ideas expressed here are quite similar to the conclusions regarding the role of the dendrites in the oxidative metabolism of the neuron as a whole (653, 676).

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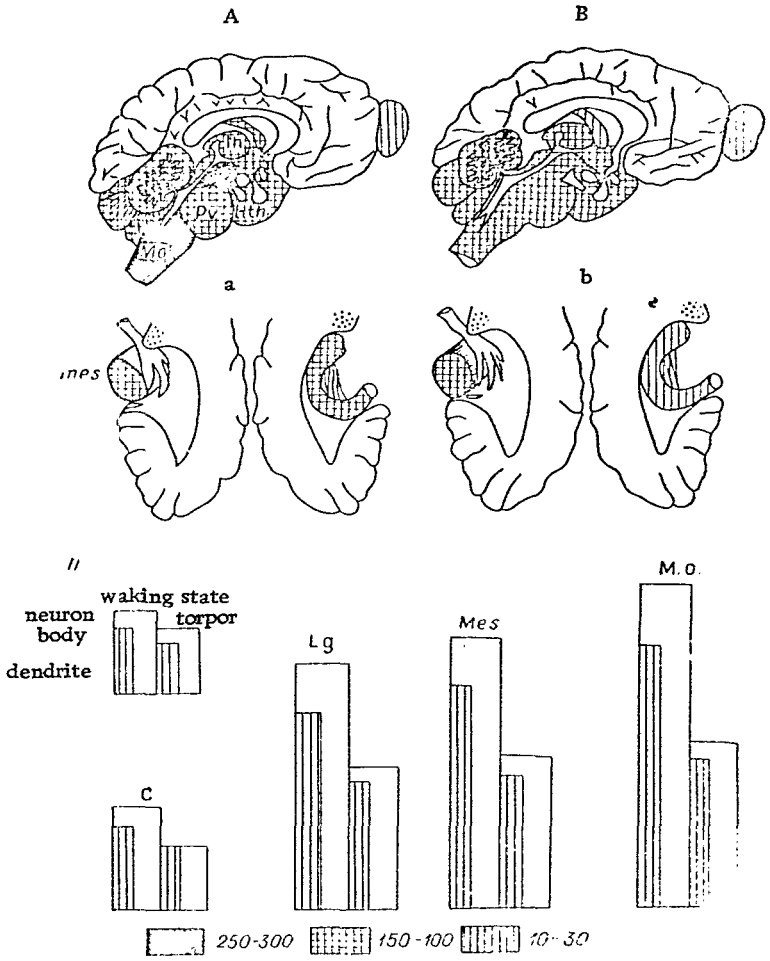

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Figure 42. I. Schematic Distribution of SDH in Various Parts of the Brain. A, a - Waking State, B, b - Torpor. Compiled on the Basis of a Statistical Evaluation of the Photometric Data on 420 Neurons. C - Cerebral Cortex, Th - Optic Thalamus, Hth - Hypothalamus, Mes - Mesencephalon, M.o. - Medulla Oblongata, PV - Pons Varolii, HIPP - Hippocampus, a, b - Sectional View from the Base of the Brain.

II. Distribution of SDH Activity in the Bodies and Dendrites of the Neurons of the Cerebral Cortex (C), Lamina Quadrigemina (Lg), Mesencephalon (Mes) and Medulla Oblongata (MO). Readings from the Linear Scale of the Photometer are Shown Underneath.

Histochemical localization of glycogen in the hibernant brain during various stages of adaptation. Carbohydrate metabolism occupies an important place in the metabolism of the mammalian central nervous system. Glycogen actually represents the most important food reserve making possible the mobilization of energy for the working neuron. To a large extent, the direction and rate of glycogen metabolism determine the ability of the regulatory systems of the brain to carry out both "systematic" and "special" reactions directed towards the maintenance of cerebral homeostasis. Nevertheless, the role of cerebral glycogen in the mechanisms of adaptation is still largely uninvestigated.

In view of this, we felt that it would be appropriate to study the histo- and cytochemical dynamics of glycogen in the various parts of the hibernant brain during various stages of adaptation.

In undertaking this series of histochemical investigations, we proceeded /127 on the basis of the biochemical data obtained in the laboratory of A. V. Palladin, who demonstrated the special nature of carbohydrate metabolism in the brains of animals falling into a state of hibernation (153, 218), as well as some of the latest histochemical findings of non-Soviet investigators (516, 572).

The technique for obtaining the study material differed only in some cases in which the brain was fixed via the carotid arteries using the neutral mixture of A. L. Shabadash. However, a comparison of the preparations obtained in this way with those stained after the usual fixation in solution showed that they were essentially identical. In most experiments, the brain was therefore frozen as a whole and small parts of it (taken with reference to the atlas of the brain of the animal) were fixed in neutral mixture and stained by the methods of A. L. Shabadash (224) or Mitchell and Wislocki (655), using silver.

The initial histochemical changes in the distribution of glycogen in various parts of the brainstem are observed during the transitional period preceding the onset of hibernation. The clearest localization of polysaccharide occurs in the large neurons of the mesencephalic reticular formation and in individual ganglion cells of the medial nuclei of the optic thalamus. Glycogen is concentrated in the cytoplasm in the form of condensed granules which are associated with the tigroid masses. In the neurons of the mesencephalic reticular formation, it is also found in the proximal portions of the dendrites; polysaccharide cannot be detected by histochemical methods in the protoplasm of the dendrite or the monticulus of the axon.

In the cerebral cortex of the waking animal there is only an insignificant accumulation of glycogen, incorporated in the perikaryon of the neuron in the form of small granules. Statistical evaluation of the data on the comparative distribution of polysaccharide in the neurons of the reticular formation of the brainstem and the cortex have shown statistically significant differences in glycogen content between these two areas in the waking state.

The onset of hibernation is characterized by "condensation" of glycogen in the form of large granules which are associated with the tigroid bodies in the

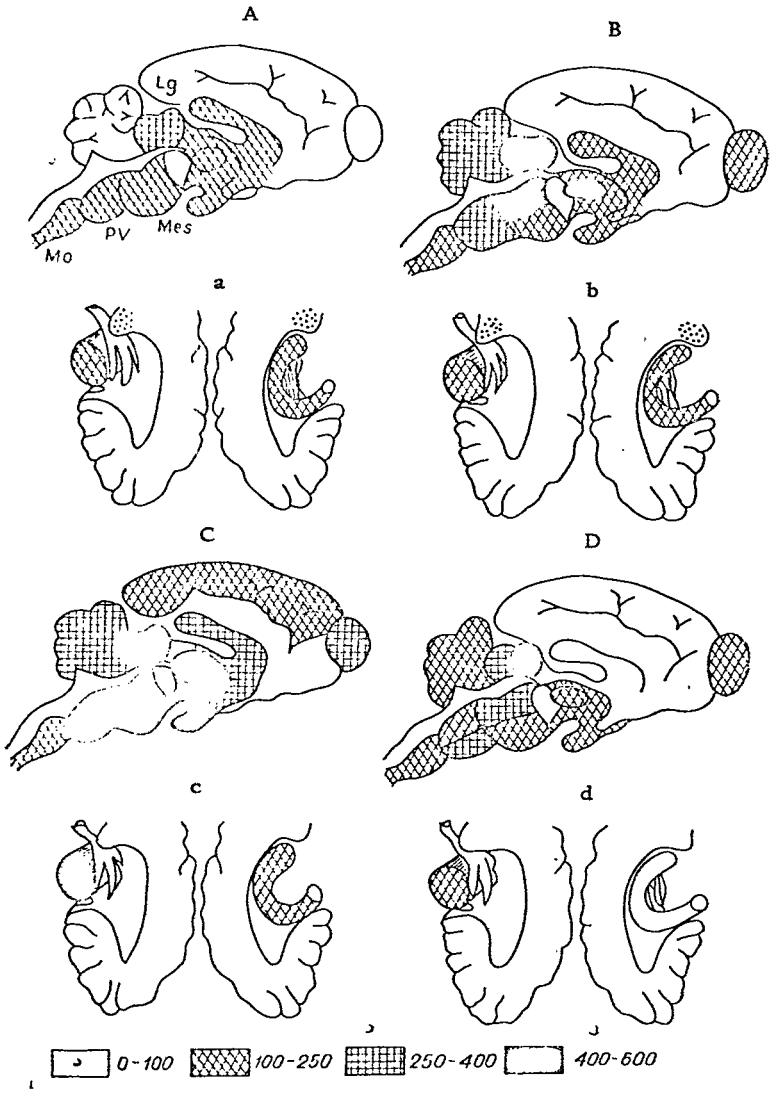

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Figure 43. Schematic Distribution of Glycogen in the Neurons of Various Parts of the Brain of the European Hedgehog. A, a - Waking State; B, b - Onset of Hibernation; C, c - Torpor; D, d - Arousal. The Diagrams were Compiled on the Basis of Photometric Data, the Scales for Which are Shown in Arbitrary Units in the Lower Portion of the Figure (the Linear Scale of the Photometer).

cytoplasm of the neurons of the mesencephalic reticular formation, the medial nuclei of the optic thalamus and the hypothalamus. Nevertheless, the difference in glycogen content between the neurons of the cortex and the brainstem remains /127 significant. It is precisely the onset of hibernation, characterizing the transition of the animal from the waking state to torpor, which is marked by the most significant redistribution of polysaccharide in the neurons of the nonspecific structures of the brainstem and thalamus.

During torpor, when the temperature of the cerebral cortex reaches 7-19°C, the largest accumulations of polysaccharide granules are seen in the neurons of the mesencephalic reticular formation and the medial nuclei of the optic thalamus. The glycogen content also increases markedly in the cortical neurons compared to the initial level in the waking state. Arousal leads inevitably either to a complete disappearance of glycogen from the cytoplasm or to a significant decrease in glycogen content. Nevertheless, during this period of maximal mobilization of brain glycogen, significant accumulations of polysaccharide granules, associated with the tigroid substance of the cytoplasm, persist in the large neurons of the mesencephalic reticular formation and the medial nuclei of the optic thalamus.

The distribution of brain glycogen is thus characterized by the following descending scale: the mesencephalic reticular formation, the pons varolii, the medial nuclei of the optic thalamus, the hypothalamus, and the cerebral cortex (Fig. 43). The onset of hibernation is characterized by the ability to condense and apparently also to synthesize polysaccharide in the neurons of the reticular formation and the medial nuclei of the thalamus, i.e., in those structures belonging to the so-called nonspecific projection system. Glycogen accumulation is most typical of the cytoplasm of the neurons and to a significantly smaller degree of the dendrites.

Arousal is unavoidably accompanied by exhaustion of the polysaccharide reserves in the cortical neurons and by maintenance of significant amounts of glycogen in the cells of the mesencephalic reticular formation and the medial nuclei of the optic thalamus.

CHAPTER VI.
IMPULSE ACTIVITY AND ULTRASTRUCTURAL CHANGES IN THE HIPPOCAMPAL NEURONS OF HIBERNATING ANIMALS /130

Basal Activity in the Hippocampal Neurons;
Classification and Statistical Analysis.
Effect of Anesthesia and Physical Cooling on the Parameters of Impulse Activity.
Comparative Ultrastructural Changes in the Synapses of the Hippocampus and the Neocortex of Hibernants during Various Stages of Hibernation and in the Waking State.
Correlations between Ultrastructure and Physiological Function in the Cellular Elements of the Hippocampus and Neocortex in Hibernating Mammals.

In the preceding chapters, we have attempted to show that rather complex interrelationships between the various parts and nuclei of the hibernant brain arise during the onset of hibernation, torpor and arousal, when first one and then the other part may play a leading role. Besides controlling the brain temperature at an optimal level, they fulfill the function of "search systems" (cybernetic systems) and guarantee resumption of brain function in a state of deep torpor by creating the conditions for arousal. It can be seen from the experimental data presented that the above pertains mostly to the limbic structures, especially the hippocampus.

Even on the basis of the American publications discussed previously, it could already be concluded that electrical activity in the limbic structures is "resistant" to low cerebral temperatures (318, 320). This has been most clearly demonstrated by our own work and that of Strumwasser (232-234, 590). It could be concluded, first of all, that "tentative" and definitive arousal are both characterized by an initial resumption of electrical activity /131
in the hippocampus, and then in the hypothalamus, amygdalus and medial cortex (236, 238). The reappearance and regularization of electrical activity during arousal take place at a temperature 8-10°C lower in the hippocampus than in the mesencephalic reticular formation, the optic thalamus or the neocortex.

Since we had not done any personal studies with microelectrodes on the cellular elements of the hippocampus and other limbic structures, our review of the existing data was limited to a review of the existing literature on the electrophysiology of the hippocampus in homoiotherms (639, 640, 658, 677). This obviously did not enable us to make any kind of a direct characterization of the physiological properties of the neurons of the hibernant archicortex. Some ideas can be expressed, however, on the basis of the results described in the preceding chapter, which indicated that the hippocampal pyramidal neurons, like the respiratory motor neurons of the medulla oblongata, are characterized by highly active processes of dehydrogenation, not only during the onset of hibernation and in the waking state but also, and most importantly, in a state of profound torpor. These facts, along with the data obtained from the literature, have enabled us to formulate several questions for which we will attempt to find at least a partial solution in the present chapter. No final conclusion can yet be reached on these questions, since significant number of them are now being worked on and tested experimentally. Nevertheless, by way of introduction to the problem of the special properties of the cellular elements of the hibernant hippocampus, we felt that it would be possible to describe and evaluate the experimental data which have been obtained on this problem to date.

We therefore undertook to give a qualitative and statistical description of the basal electrical activity in the neurons of the hibernant hippocampus during artificial physical cooling of animals which were either under nembutal anesthesia or immobilized with curare. Although we first evaluated the temperature gradient for changes in electrical activity in these cells, it seemed equally important to show what kind of changes take place in the electrogenic membranes and the synaptic apparatus of the hippocampal neurons under conditions of active wakefulness, torpor or arousal, as well as during artificial experimental conditions. The first problem was solved by the use of microelectrode techniques and statistical studies of impulse activity in the hippocampal neurons of the red-cheeked suslik (Citellus erythrogenus) during various stages of physical cooling (with or without anesthesia). The second problem was solved with /132
the aid of electron microscopy. In the last analysis it seemed extremely important to compare the characteristics of electrical activity and the submicroscopic organization of the cellular elements of the hippocampus and neocortex, which show such fundamentally different changes in electrical activity during the onset of hibernation, torpor and arousal, by means of different investigative techniques.

A. Techniques of the physiological experiments. A microphysiological experiment in which we studied the electrical activity of the hippocampal neurons (the CA_2 field) was carried out on 28 red-cheeked suslik. In some of the experiments the animals were first anesthetized with nembutal (5 mg per 100 g body weight) and after they had been positioned in the head holder of the stereotaxic device part of the parietal cortex was removed in order to permit introduction of the microelectrodes into the dorsal hippocampus under visual control. A second series of experiments was carried out on conscious animals immobilized with curare (d-tubocurarine, 0.02 mg per 100 g) and then respirated artificially. The opening for insertion of the microelectrodes was drilled in the region of the CA_2 projection field on the basis of the stereotaxic

coordinates given in our atlas of the suslik brain. The dura mater was then removed and the animals were cooled in a special, hermetically-sealed, metallic chamber in which the spaces between the walls were filled with a mixture of ice and KCl. The temperature of the brain was measured by means of an electrical thermometer or a low-temperature microthermocouple.

Pyrex glass micropipettes filled with 2.5M KCl and having a tip diameter of 1-1.5 micron were used as microelectrodes. The resistance of these microelectrodes was within the range of 4-12 megohms. Via a cathode follower the microelectrode was connected to the input cascade of a UBP1-02 amplifier. The activity of the neurons was checked visually on the screen of an I-6 indicator tube and was recorded on magnetic tape (Yuza-10 tape recorder) after preliminary amplitude discrimination with the aid of a pulse shaper. At the same time, the process could be recorded photographically from the screen of a type D-561 double-beam oscillograph using a "Meopta" photorecorder.

In the present part of this monograph, we will describe the results of studies on 84 neurons from suslik hippocampus (32 under barbiturate anesthesia, 21 from the conscious animal and 31 from experiments in which the animals were cooled physically to a brain temperature of 4-6°C). /134

B. Method for the analysis of impulse activity. The previously shaped pulsate activity of the hippocampal neurons was fed into the operative memory of a "Dnepr" computer in such a way that each interval between impulses was represented by the corresponding binary number (648). The block diagram of the system developed at the Mathematics Institute of the Siberian Division of the USSR Academy of Sciences for processing the experimental data is shown in Fig. 44, a. The input of information was continued automatically in a cyclic rhythm until the memory was filled. Visual control of the impulse activity (before and after amplitude discrimination) with the aid of a double-beam indicator tube of the "Krizik" type made possible the choice of optimal conditions for the shaping of the impulse flux from the point of view of the optimal signal: noise ratio. The results of computer processing were presented in graphic form on a type ORD-250 indicator tube and were tabulated in numerical form using a "Soemtron" digital printer. The image on the cathode-ray screen was also recorded on motion picture film in order to create the conditions for input of information by means of punched paper tape.

For the construction of the statistical tables, we generally used blocks of no more than 1024 numbers. The choice of blocks of this length was determined in the first place by the capabilities of the digital computer (an upper limit being set mainly by the capacity of the operative memory units) and in the second place by the demands usually made on mathematical statistics with regard to guaranteeing a sufficiently close approximation between the true and the calculated values of the statistical parameters.

As the simplest characteristic of the temporal structure of the processes under investigation, we selected the intensity of the impulse flux $\mu = \frac{m}{\Delta t}$ where m is the number of impulses per fixed time interval t (=12.5 sec.). In the

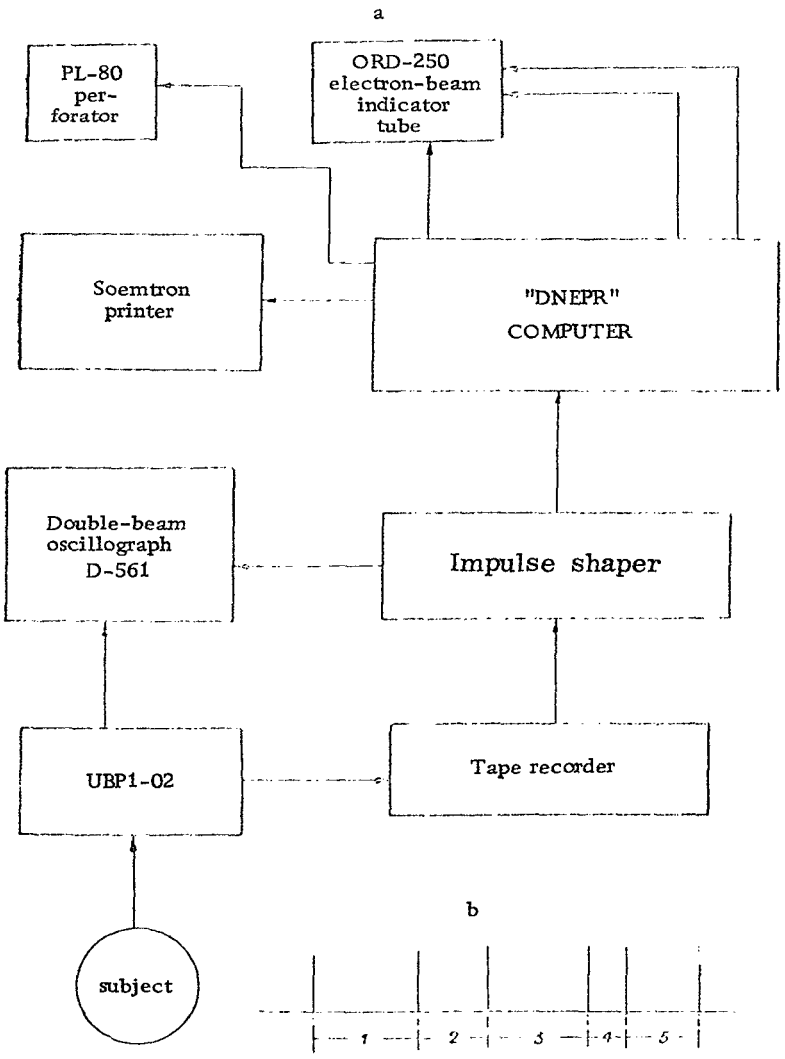

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Figure 44. a - Block Diagram of the System for Studying the Pulsate Activity of the Central Neurons; b - Principle Underlying the Construction of Correlation Fields for Intervals of the First Order Between Impulses (See Text).

experiments to be described later, the inequality $T \geqslant \Delta t$ where T is the duration of the record being analyzed was always fulfilled. In this way, it was possible to follow the changes in intensity over the course of the whole experiment, which is extremely important for evaluating the effect of hypothermia on the activity of the cellular elements of the brain.

Since the most complete characterization of any chance value is the probability distribution of its magnitude, we constructed unidimensional differential and integral histograms showing the distribution of the length of the interval between impulses of neuronal activity for intervals of the first order. /135

In addition, we calculated the following statistical parameters:

1. The first initial moment $V_1 = \sum_{i=1}^{n} \frac{xi}{n}$,

where xi is the duration of i, the interval in the block of numbers being analyzed, and n is the total number of intervals.

2. The second central moment $\mu_2 = \sum_{i=1}^{n} \frac{(x_i - V_1)}{n}$.

3. The standard deviation $\sigma = \sqrt{\mu_2}$.

4. The coefficient of variation $\theta = \frac{\sigma}{V_1}$.

5. The coefficient of asymmetry $A = \sum_{i=1}^{n} \frac{(x_i - V_1)^3}{n\sigma^3}$.

6. The excess in form $K = \sum_{i=1}^{n} \frac{(x_i - V_1)^4}{n\sigma^4} - 3$.

In calculating these parameters, no correction was introduced for bias in the values in view of the large amount of data in each block being analyzed.

The statistical relationship between adjacent intervals between impulses was analyzed by the construction of first-order correlation fields. The procedure for this can be outlined as follows: the duration of the first of two adjacent intervals is recorded on the abscissa while that of the second is recorded on the ordinate (Fig. 44, b). A point is then placed at each intersection of two coordinates on the graph. Then the duration of the second interval is recorded on the abscissa while that of the third interval is recorded on the ordinate. The correlation field or "correlation lattice" constructed in this way describes the qualitative characteristics of the correlation between adjacent intervals between impulses.

The errors arising during the analysis of the neuronal impulse activity on a "Dnepr" computer are due mainly to the errors introduced by the impulse shaper and by the magnetic recording. The maximal relative error in impulse shaping is given by an equation taking the form

$$A = \frac{1}{M} \cdot 3\sigma \cdot 100\% = 3 \cdot \theta \cdot 100\%,$$

where σ is the mean square deviation, θ is the coefficient of variation, and V_1

is the first initial moment. In order to evaluate this error, the periodic sequences of impulses with varying intervals between them were fed into the computer and the coefficient of variation θ was calculated. The results of this analysis showed that the error introduced into the measurement of the duration of the intervals between impulses by the impulse shaper did not exceed 5% for intervals of the order of 3 milliseconds and was less than 1% for intervals over 10 milliseconds, which is quite acceptable for statistical analysis. For intervals of longer duration, the error is due mainly to the magnetic recorder and does not exceed 6%. /136

C. Results of an analysis of the impulse activity of the hippocampal neurons. a) Anesthetized normothermic animals. The problem of classifying the background activity of the pyramidal cells of field CA_2 of the hippocampus was solved on the basis of a general review of the statistical parameters of 32 cells. The most characteristic unidimensional, differential and integral, histograms showing the distribution of intervals of the first order between impulses are presented in Figs. 45-47. A quasi-exponential form was recorded in 55% of all cellular elements (Fig. 45, a, b); the histograms differ from one another only in the constant time of decline of the exponential part of the curve (Fig. 45, a - differential histogram; 45, b - integral histogram). The time course of the intensity for this same neuron is shown in Fig. 45, c. The values of the coordinates are shown in the rectangle placed in the upper right-hand corner of the figure. The zero is always at the lower left. The durations of the intervals between pulses are shown in milliseconds along the abscissa (x-axis), while the relative (for differential histograms) or cumulated (for integral histograms) frequencies of appearance of intervals having a given duration (in percent) are shown on the ordinate (y-axis). The third line of each rectangle shows the number of intervals included in the original block, while the fourth and fifth lines show the number of the neuron and of the sequence, respectively. In the case of intensity records, the duration of the observed process (in minutes) is shown on the abscissa, the number of impulses per second is shown on the ordinate, and the third line in the rectangle gives the time interval used for averaging (Δt).

A second type of histogram, having a multimodal form and recorded in 23% of all neurons, is shown in Fig. 45, d. The remaining 22% of the neurons had a impulse-interval distribution close to that shown in Fig. 47, a. /138

A classificational analysis of the forms of the correlation fields made possible the isolation of the four most characteristic types of relationship between adjacent intervals. The first of these (Fig. 47, c) is the most common form of correlation field, representing 74% of the analyzed cells. Here the /140 adjacent intervals in the impulse flux are completely independent of one another. The type of field shown next (Fig. 47, d) represents the strong negative correlation between intervals of the first order which is characteristic of the usual "bundled" type of impulse activity. Correlation fields of this type are recorded in 5% of all cases. Correlation fields of a mixed type (Fig. 47, e), in which a strong negative correlation alternates with the lack of correlation between adjacent intervals which is characteristic of the first type of field, are also recorded fairly often (12% of all observations). These are characterized by the presence of randomly scattered points between the two

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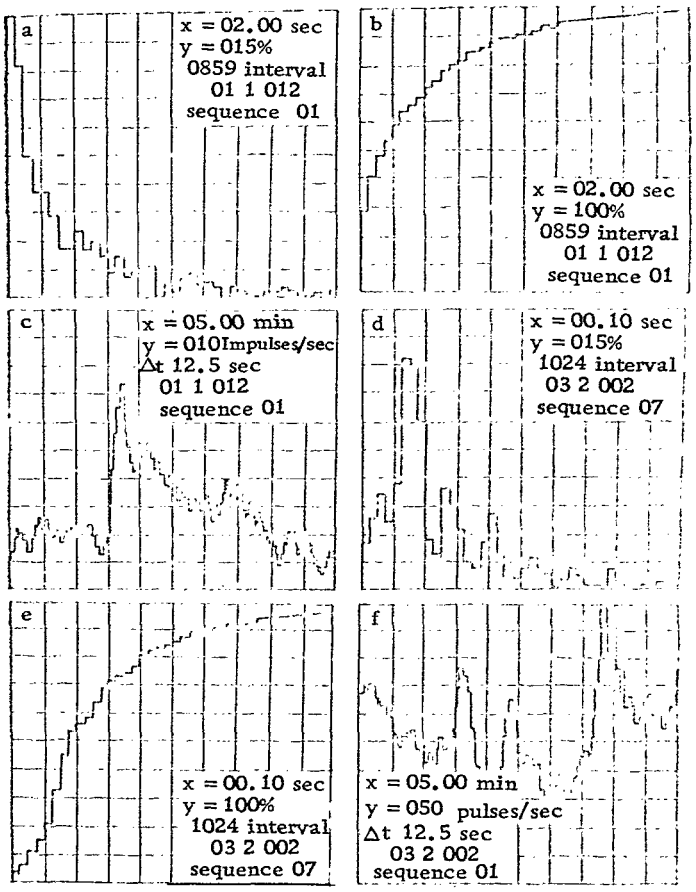

Figure 45. Differential (a, d) and Integral (b, e) Histograms of the Distribution of Intervals of the First Order between Impulses. The Information in the Rectangles Shows the Duration of the Intervals along the x-axis and the Relative (for Differential Histograms) or Cumulated (for Integral Histograms) Frequencies of Appearance of Intervals Having a Given Duration along the y-axis; the 3rd, 4th and 5th Lines in the Rectangles Show the Number of Intervals, the Number of the Neuron and the Number of the Sequence, Respectively. c, f — Time Course of the Intensity of the Impulse Flux. In these Rectangles the Duration of the Observed Process in Minutes is Shown along the x-axis, the Number of Impulses Per Second is Shown along the y-axis, the the 3rd Line Gives the Time Interval Δt over Which the Data were Averaged. Field CA_2 of the Hippocampus. Brain Temperature 32°C. Nembutal, 3 mg Per 100 g Body Weight.

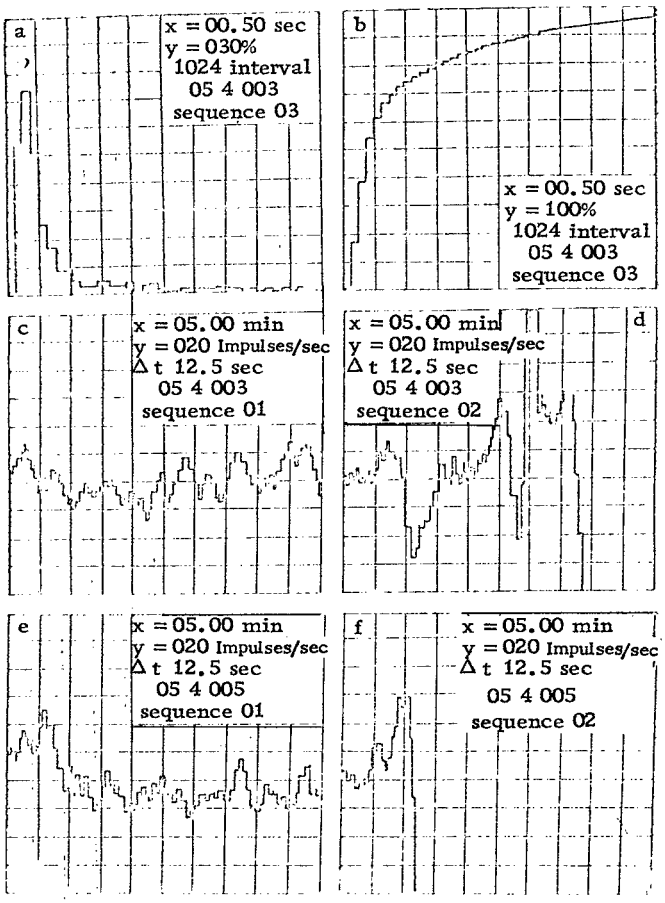

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Figure 46. Interval Histograms (a, b) and the Time Course of the Intensity (c-f) of the Impulse Fluxes of Hippocampal Neurons in Field CA_2. Nembutal Anesthesia, 3 mg per 100 g body weight. Brain Temperature 32°C.

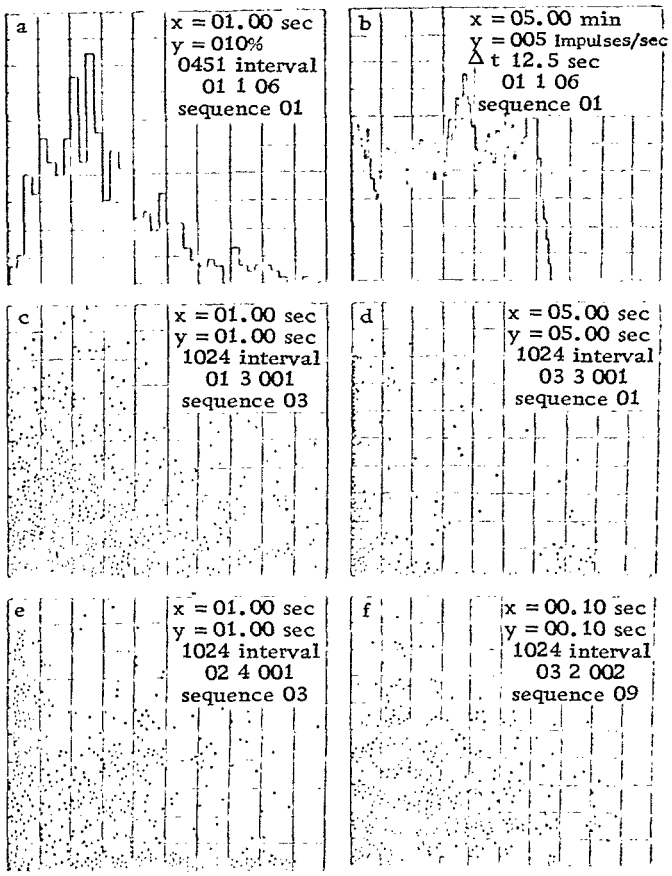

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Figure 47. Differential Histograms of the Distribution of the Impulse Intervals (a) and Time Course of the Intensity of the Impulse Flux (b). The Most Common Forms of the Correlation Fields of the Impulse Activity of the Hippocampal Neurons. Field CA_2. Nembutal Anesthesia. Brain Temperature 30-32°C. c — Non-correlated Adjacent Intervals; d - Strong Negative Correlation; e - Mixed Type; f - Multimodal Distribution (Compare Histogram 45, d).

perpendicular directions of the principal intervals. A field of this type usually indicates a transition from arrhythmic activity into bundled activity. /140

Finally, the last type of field (Fig. 47, f) represents the multimodal distribution of intervals which is also reflected in Fig. 45, d. This type of correlation field is recorded in 9% of all cells.

The values of the principal numerical parameters of the hippocampal neurons in the anesthetized animal are shown in Table 8.

TABLE 8. NUMERICAL VALUES OF THE PRINCIPAL STATISTICAL PARAMETERS OF THE IMPULSE ACTIVITY OF THE HIPPOCAMPAL NEURONS (ANESTHESIA, BRAIN TEMPERATURE 32-34°C)

Msec	Msec	Msec	Θ	K	A
609	$1.6 \cdot 10^6$	1299	2.133	11.91	239
536.1	$5.6 \cdot 10^5$	752.6	1.404	3.562	19.43
220.2	$4.5 \cdot 10^4$	213.1	0.968	1.387	2.866
491.9	$6.6 \cdot 10^5$	816.6	1.66	3.77	20.07
1847	1.10^{11}	3275	1.773	4.418	25.35
615.1	$5.3 \cdot 10^9$	729.4	1.186	2.839	10.62
266.7	$5.1 \cdot 10^9$	717.2	2.69	5.792	45.87
1094	$1.5 \cdot 10^{11}$	3890	3.555	4.845	22.87
9649	$3 \cdot 10^{11}$	5556	2.097	4.962	33.5
30.47	743.9	27.27	0.895	3.364	24.4
609.5	$2.3 \cdot 10^{10}$	1547	2.537	10.28	147.9
156.9	$4.5 \cdot 10^9$	675.1	4.302	25.77	756.5
253.8	$1.1 \cdot 10^{10}$	1054	4.152	12.78	203.4
291.4	$2.3 \cdot 10^9$	482.3	1.655	3.519	21.27
1554	$2.1 \cdot 10^{11}$	4612	2.969	8.41	97.1

b) Conscious animals (d-tubocurarine). Most cellular elements are characterized by a quasi-exponential type of distribution of the intervals between impulses (Fig. 48, a); however, this number also includes bell-shaped and weakly-dispersed distributions of a monomodal type (Fig. 48, c, d).

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Some of these show characteristic fluctuations in intensity leading to the quasi-periodic oscillations seen in Fig. 48, e, f.

When the neuronal activity is recorded in the unanesthetized curarized animal, all neurons show a characteristic independence of adjacent intervals. /142
The principal statistical parameters for this type of activity are shown in Table 9.

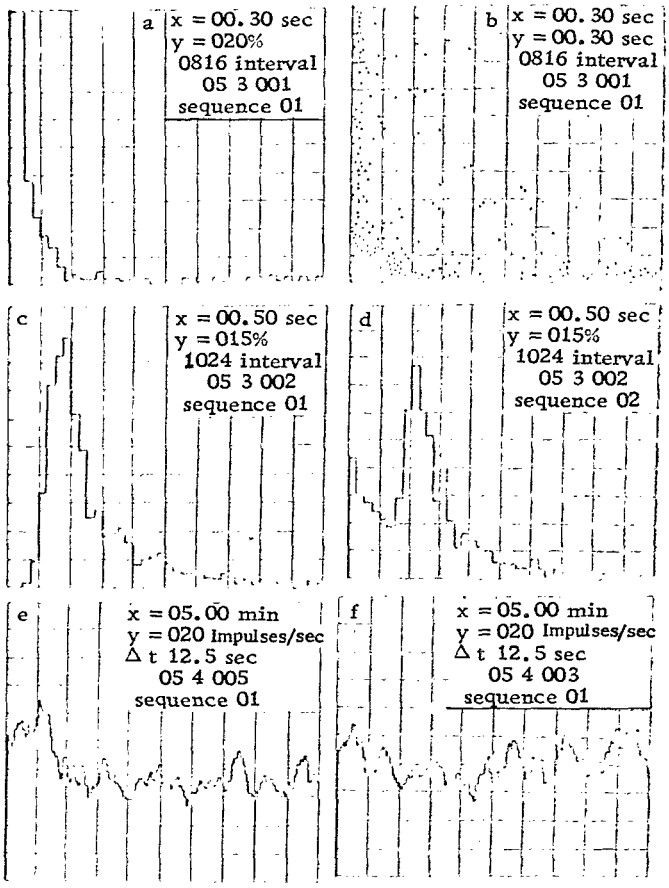

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Figure 48. Interval Histograms (a, c, d), Correlation Fields (b) and the Time Course of Intensity (e, f) for Hippocampal Neurons of Field CA_2. Brain Temperature 32-34°C. d - Tubocurarine.

TABLE 9. NUMERICAL VALUES OF THE PRINCIPAL STATISTICAL PARAMETERS OF THE IMPULSE ACTIVITY OF THE HIPPOCAMPAL NEURONS (UNANESTHETIZED ANIMAL, BRAIN TEMPERATURE 30°C) /142

Msec	Msec	Msec	Θ	K	A
99.74	$2.6 \cdot 10^8$	163.3	1.647	6.07	55.24
58.37	$6.3 \cdot 10^7$	79.69	1.325	3.220	13.95
135.4	$2.2 \cdot 10^8$	149.9	1.107	2.239	10.76
149.1	$3 \cdot 10^8$	174.6	1.171	2.179	4.789
175.2	$2.9 \cdot 10^8$	171.2	0.97	1.842	3.672
1461.7	$2.9 \cdot 10^8$	170.3	1.161	6.248	70.9
131.7	$7.3 \cdot 10^7$	85.85	0.651	1.726	5.081
219.5	$1.6 \cdot 10^9$	405.5	1.848	15.24	262.4
164.3	$2.5 \cdot 10^8$	160.2	0.975	13.85	277.1
108.5	$2.1 \cdot 10^7$	95.83	0.883	3.636	20.06
70.91	$1 \cdot 10^7$	31.8	0.448	0.829	0.582
104.7	$3.6 \cdot 10^7$	60.15	0.574	0.989	1.111

c) Effect of cooling on the statistical parameters of the pulsate activity of the hippocampal neurons (unanesthetized animals, d-tubocurarine, temperature drop from 34 to 14°C (see Table 10)). In this case the pulsate activity can be represented by a quasi-periodic process with a weakly dispersed fluctuation of the intervals between pulses (Fig. 49). Monomodal distributions which become bimodal as the brain temperature drops, but with preservation of the /143 initial independence of adjacent intervals, are also possible. The intensity is subject to periodic oscillations (Fig. 49, c, d).

In most cases there is a significant drop in the frequency of neuronal discharge to 0.1-0.2 per second in the absence of any correlation between adjacent intervals. The histograms in this case are multimodal and exponential in form (Fig. 49, a).

TABLE 10. NUMERICAL VALUES OF THE PRINCIPAL STATISTICAL PARAMETERS OF THE IMPULSE ACTIVITY OF THE HIPPOCAMPAL NEURONS (COOLING OF THE BRAIN FROM 32 TO 14°C)

Msec	Msec	Msec	O	K	A	T°
111.9	$1.3\ 10^8$	117.7	1.051	2.326	7.42	32
129.7	$3.9\ 10^9$	625.1	4.82	11.46	374.8	21
169.8	$3.5\ 10^8$	187.2	1.103	5.267	44.1	18
6250	$3.9\ 10^{11}$	6312	1.01	1.975	5.103	17
285.4	$4.4\ 10^9$	668.4	2.392	5.741	43	16
2954	$1.2\ 10^{11}$	3487	1.181	1.740	2.32	14

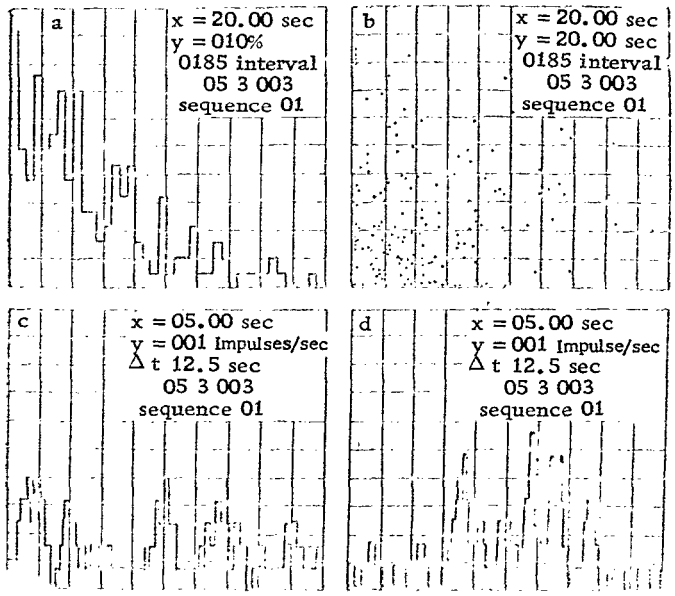

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Figure 49. Interval Histograms (a), Correlation Fields (b) and Time Course of Intensity (c, d) for the Hippocampal Neurons of Field CA_2. Physical Cooling. Brain Temperature 14-19°C. d-Turbocurarine.

TABLE 11. NUMERICAL VALUES OF THE PRINCIPAL STATISTICAL PARAMETERS OF THE INTERVALS BETWEEN IMPULSES WHEN THE BRAIN TEMPERATURE DROPS FROM 30 TO 6°C

Sec	Sec^2	A	K
2.7	42.8	6.6	177.3
17.9	393.6	2.6	8.4
5.4	111.4	1.5	0.3
4.3	25.9	2.1	12
2.1	82.3	0.2	-2.6
2.3	41.2	0.4	20.9

d) Effect of cooling on the impulse activity of the hippocampal neurons in anesthetized animals (drop in brain temperature from 34 to 6°C (see Table 11)). Results in 17 neurons were subjected to statistical analysis. A decrease in brain temperature is accompanied by decreases in spike amplitude, intensity (Fig. 50, c; Fig. 51, a-e) and dispersion. The independence of adjacent intervals is preserved even when the brain temperature drops to 6-8°C. There are no changes in form in the correlation fields. The shape of the unidimensional histograms for the intervals between impulses remains close to exponential. /144

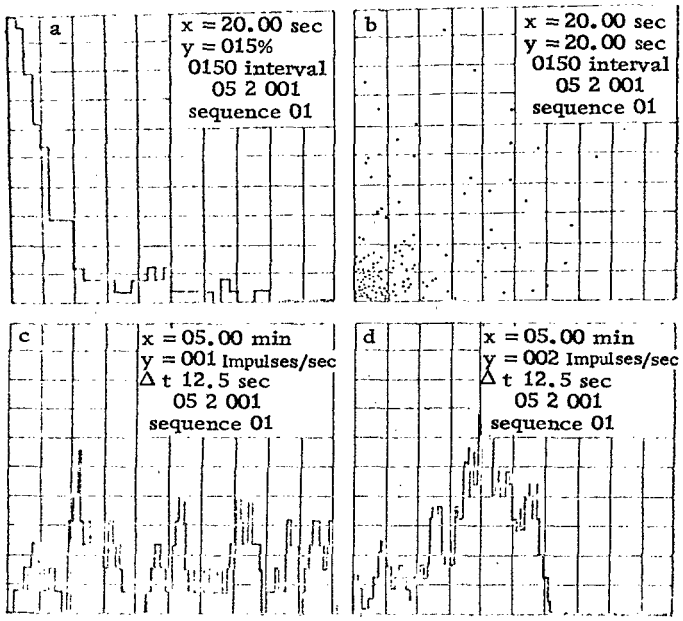
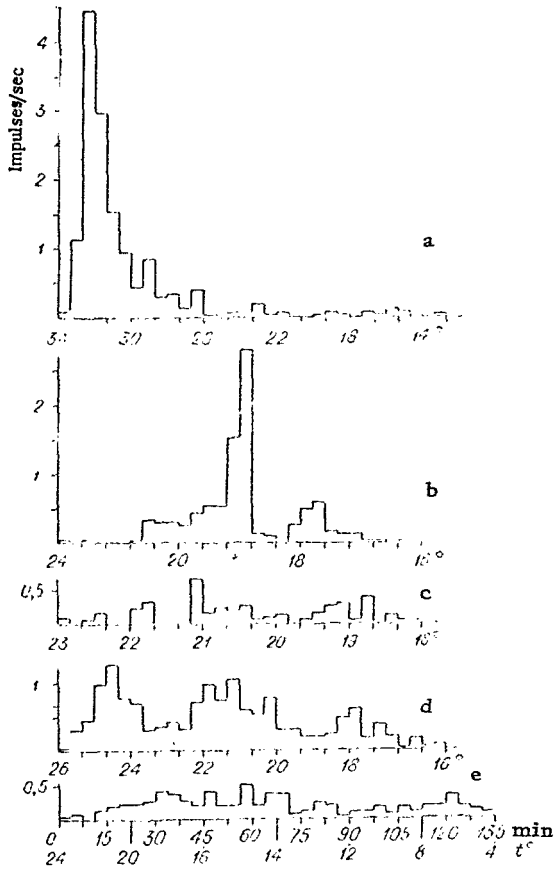


Figure 50. Interval Histograms (a), Correlation Fields (b) and Time Course of Intensity (c, d) for Hippocampal Neurons of Field CA_3. Physical Cooling. Brain Temperature 14°C. d-Tubocurarine.

* * *

The first fact which this study of the statistical parameters of the pulsate activity of the hippocampal neurons in the red-cheeked suslik succeeded in establishing relates to the isolation of the distribution types which are characteristic of the anesthetized and unanesthetized animal. The variability of the statistical parameters was found to be less in the second case. The basal activity of the pyramidal cells of the hippocampus in the waking animal is actually characterized mainly by the exponential distribution of the duration of the intervals between impulses and by the lack of correlation between adjacent intervals of the first order, while in anesthetized susliks other types of interval histograms are also encountered rather frequently (see Figs. 46, 47). In addition, the correlation fields of the cells of these animals show a definite form (see Fig. 46, c, d) which most commonly indicates the presence of bundled activity. /145 /146



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Fiqure 51. Time Course of the Intensity of the Impulse Flux from the Hippocampal Neurons, Field CA_3. Anesthesia and Physical Cooling. Brain Temperature Reduced from 34 to 6°C. The Time Course of the Brain Temperature is Shown on the Abscissa while the Neuronal Activity in Impulses/Second is Shown on the Ordinate.

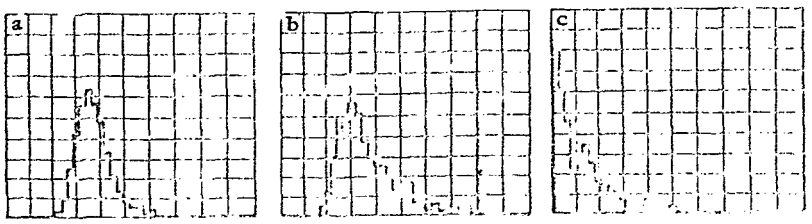

Figure 52.

A difference is also observed in the numerical values of the statistical parameters of neuronal activity. This appears particularly clearly in the first initial moments. The mean interval between impulses is 3-5 times as long for the neurons of the anesthetized animal as in the conscious animal (see Table 8).

If we could be certain that the shifts described above were not due to differences in the number of cellular elements analyzed during the various types of observations, then the increase in variability of the interval histograms and correlation fields and the increased duration of the mean interval between impulses in the anesthetized animal might be explained by the effect of nembutal on the efficiency of synaptic transmission. It is well known (664) that barbiturate anesthesia completely or partially blocks synaptic transmission due to a change in excitability of the post-synaptic membrane, which also leads to a drop in the efficiency of the transmission of a signal across the synapse. Thus, if each hippocampal neuron in the unanesthetized animal has a very large number of afferent fibers (672), then the dimensions of the polysynaptic structure of the cell are significantly reduced under anesthesia. In the first case, the characteristics of the summed post-synaptic potentials (SPSP) can be calculated by means of the limiting theorems of the theory of probability, but in the second case these theorems are not applicable since here the main condition for the operation of the limiting theorems (a large number of active synapses and a uniformly small contribution from each of them to the total process, the SPSP) has been violated. Obviously, the smaller the number of synapses the greater will be the effect of each individual synapse on the formation of the SPSP in a statistical sense, and consequently on the efferent activity of the cell.

The increase in the mean duration of the intervals between impulses may be explained by the fact that with a small number of functioning synapses, the amplitude of the SPSP would reach a threshold value more slowly than usual.

The variability of form of the interval histograms observed in the anesthetized animal, and particularly in susliks in a state of torpor, can also be explained in another way, i.e., by a change in the threshold of harmonious neuronal activity under the influence of anesthesia, keeping in mind that nembutal directly or indirectly affects the properties of the post-synaptic membrane. Taking this into consideration and postulating that the SPSP show a gaussian distribution, it is easy to explain the existence of all of the types of interval histograms observed under anesthesia (see Figs. 46, 47); the intermediate forms can also be explained on the basis that in such a case we are dealing with various thresholds of harmonious neuronal activity.

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According to the well-known paper by Rice (679), the distribution of the time intervals between points in a random gaussian process at which a previously established level is exceeded actually approaches an exponential form as this level (in the case under discussion, the threshold of harmonious neuronal activity) is increased. An experimental test of this hypothesis on an electronic model of the neuron confirmed Rice's conclusions (Fig. 52).

It thus seems possible to explain a large proportion of the types of histogram recorded experimentally. However, cases similar to those presented in Fig. 48 do not fit into this scheme: in such a case we are dealing with a heterogeneity of synaptic inputs, leading as a rule to a "bundled" type of pulsate activity and to periodic fluctuations in intensity (Fig. 48, e, f).

A decrease in brain temperature from 32 to 14°C leads, over the course of a considerable period, to a clear increase in duration of the intervals between pulses, amounting to an order of magnitude (see Table 10). The remaining parameters show no marked tendency to any significant changes. As the brain temperature drops further, to 6-8°C, the intensity of the impulse flux also continues to decrease (see Fig. 51).

No apparent changes were observed in the form of the correlation fields for the first-order intervals. However, the differential histograms showed a tendency /148 toward a transition from a monomodal to a multimodal form, while retaining their quasi-exponential shape (see Figs. 49, 50). This may indicate that the heterogeneity of the polysynaptic structure has been disrupted and that the number of active synapses is significantly decreased. It might be suggested that we are dealing, in this case, with structural deformations in the synaptic membranes and an increase in synaptic clearance. In connection with this finding it is of interest to refer to the work of Dr. and Mrs. Scheibel (cited in (660)), in which they showed that structural modifications may take place in the axodendritic synapses in hibernants, consisting mainly of indentations in and (apparently) subsequent protrusions of the spinous processes of the dendrites. A previously functioning synaptic mechanism may thus simply disappear.

Taking this fact into consideration, as well as the familiar decrease in amplitude of the local post-synaptic potentials, the SPSP and the action potentials under the influence of hypothermia (42, 43, etc.), it is comparatively easy to understand why the duration of the intervals between impulses is increased by cooling.

Physical cooling of anesthetized animals leads to a still greater decrease in the intensity of the impulse flux (see Fig. 51); this may indicate that there is a degree of similarity between the effects of cooling and of anesthesia on the statistical parameters of the impulse activity of the central neurons in hibernants.

* * *

The pulsate activity of the hippocampal neurons is thus characterized by resistance to temperatures in the neighborhood of 0°C, so that the type of statistical distribution of the impulse flux which is characteristic of the waking animal is preserved. This result (as well as the results of the electroencephalographic studies of the cortico-subcortical relationships in the hibernant brain reported in chapter II) indicates that the resistance of the cellular elements of the hippocampus to low temperatures is significantly more developed than in the structures of the neocortex and brainstem. The physiological data obtained can also serve as the basis for the hypothesis that the limbic system, and particularly the hippocampus and amygdalus, play a special role in all stages of mammalian hibernation and especially under conditions of deep torpor

and natural arousal (both the tentative and the definite arousal from hibernant torpor).

We were naturally also interested in the possible structural rearrangements which take place in the central neurons of hibernating animals during the various stages of their activity. The question of the morphological changes in the neurons has been partially answered in the preceding section and in the chapter on the neuropharmacology of arousal. At this point, it seems appropriate to present the results of electron-microscopic studies of the cellular elements of the hippocampus and neocortex in hibernating animals (the red-cheeked suslik). /149

The number of animals used for the electron-microscopic study of the hibernant brain is shown in Table 12 in relation to the functional state of the animal.

The ultrastructural changes in the neuronal elements of the hippocampus (region of Ammon's horn, field CA_2) and part of the parietal cortex of the cerebrum were studied in order to compare these two regions, which differ so markedly in their physiological relationships, at the electron-microscopic level.

TABLE 12. FUNCTIONAL STATE OF THE ANIMALS FROM WHICH THE BRAIN WAS TAKEN FOR ELECTRON-MICROSCOPIC EXAMINATION

Functional State	No. of Animals
Active wakefulness, brain temperature 37°C	3
Deep torpor, brain temperature no higher than 3°C	5
Arousal, brain temperature no higher than 24°C	6
Anesthesia + hypothermia	2
Curarization + hypothermia	2

Methodical sampling of brain tissue was carried out in the following way: after the vault of the skull had been entered, either in the intact animal or after cutting off the head (in the latter case, within 1-1.5 minutes), the areas of the brain to be studied were excised with the aid of three razor blades which were fixed in a clamp in such a way that they were separated from each other by a built-up thickness of no more than 300 microns. Using this device (see (641)), brain sections were cut out to a depth of one millimeter and tangentially to the surface of the brain. The slices obtained in this way were then separated by shaking in a 1% cooled solution of osmium tetroxide in

phosphate buffer. Fixation was continued for 2 hours at 4°C, after which the material was dried and embedded in a mixture of methyl- and butylmethacrylate /150
(1:5) and araldite (641). Slices having a thickness of 250-400Å were prepared on a KB-4800 ultramicrotome and studied with a Tesla BS-242 electron microscope. Some of the slices were first immersed in oil and examined /151
under phase contrast in order to localize the portion of the brain to be studied more accurately.

I. Figures 53 and 54 show the granular endoplasmic reticulum in the cytoplasm of a pyramidal cell from the cerebral cortex of a marmot emerging from a state of deep torpor at a brain temperature of about 7°C. The mass of reticulum consists of a series of flat cisterns bounded by membranes. The latter are surrounded on two sides by dense granules, the ribosomes, which are attached to the outer surface of the membranes in the tangential plane and have a diameter of 100-150 Å. In some sections, at the surface of the ergastoplasmic membrane, the ribosomes are distributed in rows (upper portion of Fig. 54) and a significant proportion of them are not attached to a membrane but are arranged in the form or rosettes or polysomes (see the arrow). Mitochondria with a clear matrix and with longitudinally oriented, double-walled, preserved cristae are freely distributed in the endoplasmic reticulum.

II. Mitochondria can be found along the entire perikaryon of the neuron and are frequently seen in the dendrites, axons and synaptic follicles. The cristae in the mitochondria are usually distributed longitudinally, along the long axis of the organelle, but the mitochondria often show an uncharacteristic topography of the lamellar ridges, particularly with regard to their shape and dimensions. In the myelinated axons the mitochondrial cristae can be seen in the form of thin tubules connected to the inner surface of the external limiting membrane. The quantitative relationships between the mitochondrial cristae, their structure and density, are particularly interesting since these characteristics are apparently related in a definite way to the intensity of their respiratory function. On the basis of indirect evidence, it can be stated that the number of internal membranes decreases during the onset of hibernation and torpor and increases during arousal. This relationship can be demonstrated most clearly in the neurons of the cortex and least clearly in the cellular elements of the hippocampus. This agrees to a certain extent with the results described in the preceding chapter in relation to the changes in succinic dehydrogenase activity in the central neurons of hibernants.

It is well known (46, 47, 641) that the number of mitochondria can also be related to the functional state of the neuron and that it is higher in the most /153
actively functioning parts of the cell: there are many mitochondria in the dendrites and in the parts of the synapse adjacent to the subsynaptic membrane. However, there are often only scattered mitochondria in the most active synaptic junctions, while numerous organelles with a large number of cristae are visible in the termini, which are difficult to relate to a functioning synaptic mechanism.

During torpor, the membranes of the mitochondrial cristae can be seen to move apart. Just as in the experiments reported by A. A. Manina (650), these

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Figure 53. Granular Endoplasmic Reticulum (GER) in a Neuron from the Cerebral Cortex of the Red-Cheeked Suslik. Brain Temperature 7°C. M - Mitochondria, L - Lysosome-like Body. ×50,000.

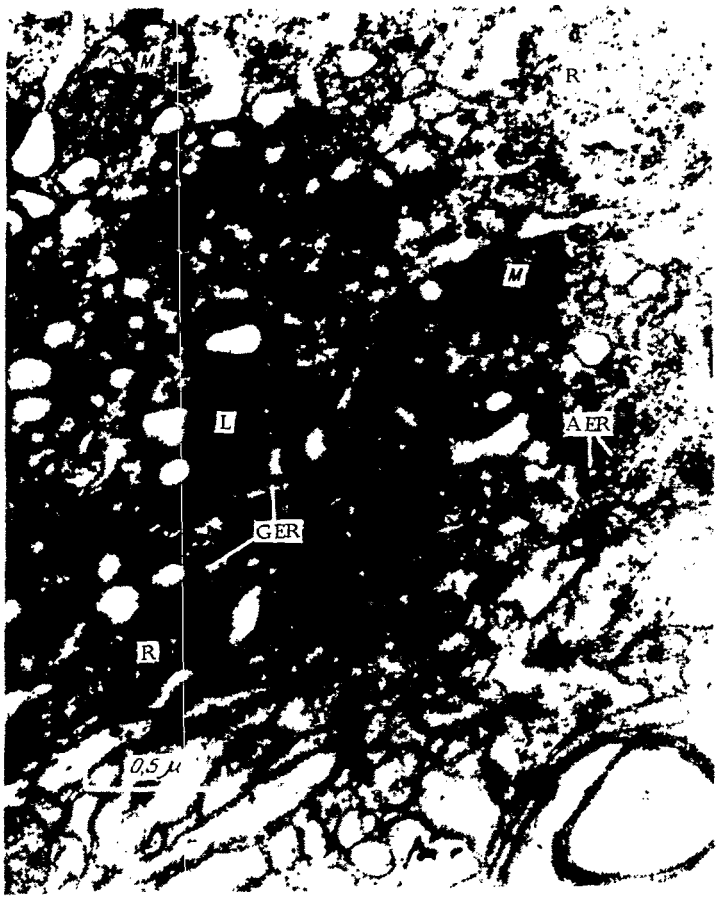

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Figure 54. Granular and Agranular Endoplasmic Reticulum in a Neuron from the Suslik Cerebral Cortex. Waking State. Brain Temperature 34°C. GER – Granular Endoplasmic Reticulum, AER – Agranular Endoplasmic Reticulum, M – Mitochondria, R – Ribosomes, L – Lysosomes, A – Axon.

submicroscopic changes (which are probably a nonspecific reaction) are also visible in our electron micrographs. Reversible disappearance of the internal mitochondria membranes is a widespread phenomenon among the hibernating animals (678).

III. As has often been described in the literature, the dendrites are devoid of myelin and are characterized by the presence of relatively regularly distributed tubules of varying length. This can be seen most clearly in longitudinal sections of the dendrites, when the tubules are arranged parallel to the axis of the dendrite.

As a rule, transverse sections of the dendrites show one or more mitochondria, distributed eccentrically around the axis. The fine branches of the apical and basal dendrites of the pyramidal cells in field CA_2 of the hippocampus can be seen clearly in the str. moleculare and str. lacunosum (Fig. 55). Elongated mitochondria are the most widely distributed in the dendrites.

IV. Axons are generally observed only in transverse cross-section (Figs. 54-55, A). The axoplasm contains oval or elongated mitochondria and clear areas bounded by membranes. Neurofilaments are visible in some of the pulpy cylinders. The myelin most commonly retains a periodic structure created by the alternation of light and dense (osmiophilic) lamellae every 120-160 Å.

V. The presynaptic buds contain mitochondria and synaptic vesicles (SV) having a diameter from 300 to 480 Å. The dimensions of the bud usually do not exceed 0.5-1.5 microns, although giant synaptic buds having a diameter approaching 5 microns can also be seen.

Both types of synapses isolated by Gray (641) have been seen. One of these is characterized by a dense osmiophilia of the pre- and post-synaptic membranes, and by special density of the post-synapse. The synaptic fissure is somewhat larger in this case and usually attains 300 Å. The lumen of the fissure is filled with osmiophilic extracellular material (Fig. 55, B). The /155
other type of synapse (Fig. 55, B) is characterized in our material by a punctate thickening of the membranes, which are separated by a transparent fissure having a diameter of 200 Å which is free of extracellular osmiophilic material. The thickness of the pre- and post-synaptic membranes is approximately identical.

VI. Spinous processes are also found in preparations stained by the method of Golgi. Attention should be called to the fact that their number along the trunks of the apical and basal hippocampal dendrites is decreased in animals which have been in a state of torpor for some time.

VII. As has been noted repeatedly, the distribution of the synaptic vesicles (SV) in the synaptic bud is quite variable, but two extreme variants of their synaptic localization are the most commonly observed. They either lie close

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Figure 55. a — Mitochondria in the Axoplasm of a Myelinated Axon in the Suslik Cerebral Cortex. At the Lower Right, a Type I Axodendritic Synapse. Torpor. Brain Temperature 7°C. b — Synaptic Bud of a Type II Axodendritic Synapse from a Pyramidal Cell of the Suslik Cerebral Cortex. The Presynapse Shows an Irregular Distribution of Synaptic Vesicles (SV); PRE – Presynaptic Membrane, POST – Postsynaptic Membrane, SF – Synaptic Fissure, D – Dendrite. Torpor. Brain Temperature 7°C. ×50,000.

to the pre-synaptic membrane of the junction, possibly penetrating into the subsynaptic fissure, or they are distributed relatively uniformly in the follicle.

VIII. The synaptic fissures appear either in the form of parallel and uniformly dense membranes, or as the punctate thickenings described above. In both cases, finally, variants are possible in which the fissure is filled with fine, granular, osmiophilic material, forming a bridge connecting the pre- and post-synaptic spaces (see Figs. 56-58). On the post-synaptic membrane one can see the fine elements of the subsynaptic network emerging at a depth of 150-200 Å (see Fig. 58, a). Synaptic membranes having a uniformly osmiophilic pre- and post-synaptic space are also possible. Electron micrographs in which no pre-synaptic membranes can be seen but the post-synapse is represented by a friable osmiophilic band are also common (Fig. 58, b). The synaptic vesicles generally do not exceed 500 Å in size and they are most often clear with an osmiophilic membrane, the so-called agranular vesicle. In any case, one can also see synaptic vesicles which perforate the pre-synaptic membrane and penetrate into the synaptic gap.

There is a tendency for the granular vesicles to be looked upon as the site of the catecholamines. However, both types of vesicles can be seen in the same synaptic bud. In this case, as was correctly pointed out by G.D. Smirnov, one must make a choice: either the ultrastructure of the vesicle is not necessarily related to the chemical nature of the mediator, or legitimate doubt is cast on the familiar rule of Dale concerning the unique chemical specificity of the neurons (659). /159

Proceeding to a specific description of some of these neuronal structures, we will attempt to characterize their electron-microscopic and functional organization during various stages of hibernation. The most interesting for us was naturally the ultrastructure of the synapses and the changes which could be directly related to the stages of torpor and the nature of electrical activity in the central formations. It should be emphasized that the type I and type II synapses which we found in the cerebral cortex and hippocampus of the red-cheeked suslik were in various functional states, apparently depending on the stage of hibernation in which the animal found itself when the material was taken for the electron-microscopic studies.

If, based on some of the criteria from the papers of Gray, Hamlin, De Robertis and G.D. Smirnov, various characteristics of the ultrastructure of functioning and inactive synaptic junctions are used for descriptive purposes, then the following variants can be seen in our material:

1) Synapses of both types characterized by uniformly osmiophilic membranes with clear mitochondria in the lumen of the bud and a relatively diffuse (non-oriented) distribution of synaptic vesicles (SV) (see Figs. 56, b, 57, a);

2) An oriented (polar) distribution of vesicles, either lying close to the pre-synaptic membrane or grouped in the form of rosettes along the entire diameter of the synaptic follicle (see Figs. 55, b, 56, b);

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Figure 56. Synaptic Junctions in the Stratum Lacunosum of Field CA_2 of the Suslik Hippocampus. Torpor. Brain Temperature 7°C. Along with the Dense Osmiophilic Membranes One Can See Frequent Synapses Lacking Dense Membranes (a, b). S — Synapse. ×50,000.

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Figure 57. Type I Synapse in a Suslik Cerebral Cortex. Torpor (b). Brain Temperature 7°C. The Synaptic Gap is filled with Friable Osmiophilic Material. The Subsynaptic Network and the Transverse Exhaustibility of the Fissure Can be Clearly Seen. ×50,000.

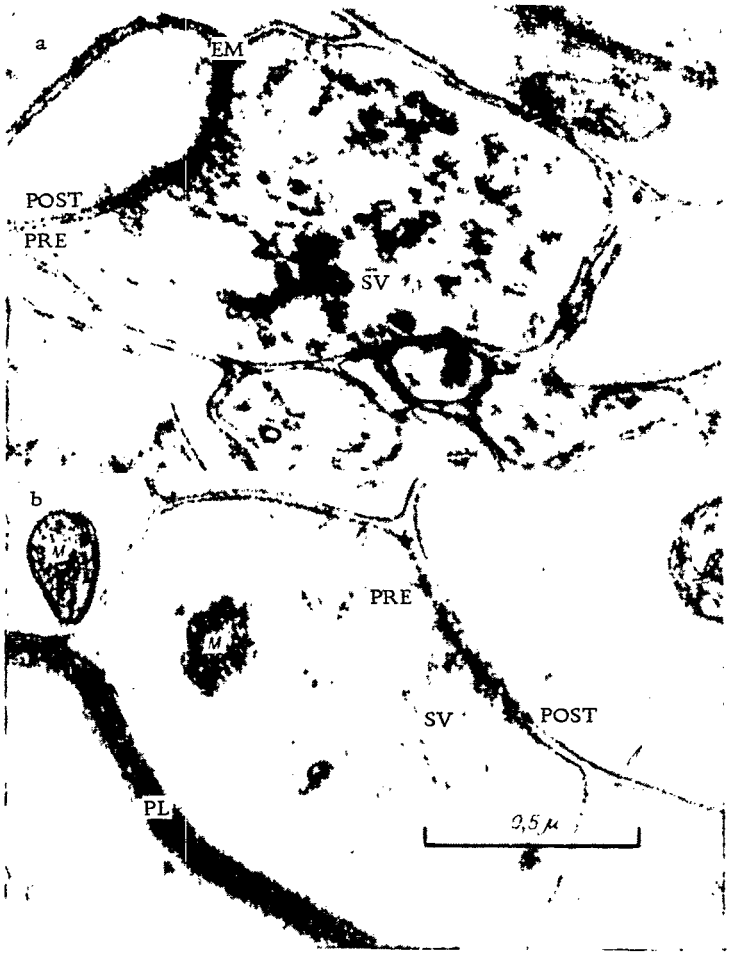

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Figure 58. Axodendritic Synapses in the (a) Hippocampal and (b) Cerebral Cortical Neurons of the Suslik; Stratum Moleculare of Field CA_2. Torpor. Brain Temperature 7°C. The Transverse Exhaustability and Extracellular Material (EM) in the Synaptic Gap (a) are Clearly Visible. ×75,000. For the Meaning of the Other Abbreviations See Figs. 54-55.

Figure 59. Light and Dark Axodendritic Synapses in the Stratum Lacunosum of Field CA_2 of the Suslik Hippocampus (a). The Synaptic Vesicles (SV) Are Seen to Lie Close to the Pre-Synaptic Membrane; Perforation of the Membrane, and Discharge of the Synaptic Vesicle into the Subsynaptic Fissure (b). Torpor. Brain Temperature 2°C. ×75,000. For the Meaning of the Other Abbreviations See Figs. 54-55.

3) Synaptic vesicles lying close to the pre-synaptic membrane, perforation of the latter by vesicles, and discharge of vesicles into the subsynaptic fissure. In this case it is very important to note the nature of the transverse exhaustability arising in connection with the distribution of granular material in the fissure, and the presence of pores in the pre-synaptic membrane (see Fig. 59, b);

4) Disappearance of the usual synaptic structure and its replacement by a uniformly osmiophilic granularity can often be observed (see Fig. 57, b). In changes in synaptic ultrastructure of the type described, a definite role is played by transformations of the mitochondria and their internal membranes — from clearing of the matrix to complete degeneration of the cristae, such as /161 has been observed in previously reported studies (650).

The principal conclusion, which we not only feel to be of theoretical importance but which may lead eventually to the formulation of new concepts relating to the fine cerebral mechanisms of hibernation, can be stated as follows. The most important finding is the presence of a large number of functioning synapses or synaptic junctions which are apparently ready to play an active role under conditions of deep torpor. These are seen primarily in the hippocampus but can also often be observed in the neocortex. This serves to confirm the comments made by Willis (633) regarding the special resistance to low temperatures of the membranes guaranteeing ion transport, cellular permeability, the transmission of the action potential and finally (and most important in the present case) the synthesis and excretion of mediators. It also seems particularly important that a large proportion of the synapses which we have found to be functional can be classified in type I of Gray. It is well known that this type is presently considered to be the excitatory type of synaptic junction (255).

The data obtained are currently being subjected to statistical analysis, which is a difficult and complex task for electron-microscopic studies. It can already be stated, however, that more than one third of the synaptic junctions in the hippocampus of hibernating animals in a state of deep torpor are characterized by the principal characteristics of functioning synapses. Low brain temperatures do not affect the ultrastructure of the principal membranes, the synaptic vesicles, the periodic elements of the myelin or the internal membranes of the mitochondria in a significant number of cellular elements in the hibernant hippocampus or cerebral cortex.

CHAPTER VII.
THE NEUROPHARMACOLOGY AND NEUROCHEMISTRY OF AROUSAL /162

Adrenergic Influences; Effects of Amphetamine and Epinephrine.
The relative "Adrenonegativity" of the Hibernant Brain under Conditions of Torpor.
Characteristics of the Cholinergic Effects.
Effects of Atropine and Galanthamine.
Potentiating Properties of Cholinotropic Preparations.
The Cholinergic and Adrenergic "Periods" of Hibernation.
Nature of the Distribution of Acetylcholinesterase and Monoaminoxidase during Various Stages of Hibernation.
Hypothesis Concerning the Neurochemical Structure of Hibernant Arousal.

It thus becomes clear that the regular sequence of the various stages of vital activity in hibernating mammals (the onset of hibernation, torpor and natural arousal) is in agreement with definite systematic interrelationships in the brains of these animals. Among the various cerebral systems which play a role in the mechanisms of hibernation, electrophysiological analysis has made possible the isolation of a few individual structural-functional complexes. One of these plays the role of a "trigger", bringing on the first stages of arousal, while others function according to the principles of cybernetic systems in order to maintain the optimal conditions for cerebral activity under low-temperature conditions. Depending on the stage of natural adaptation, one or the other of these functional systems takes a dominant role, either blocking or facilitating the activity of the subordinate neurodynamic structures. This is expressed in the form of changes in electrical activity, generalization of the synchronized forms of bioelectric activity, desynchronization of the biopotentials, and finally their suppression. In essence, within the framework of these systematic bioelectric phenomena, coordinated elements of facilitation and inhibition of the nerve impulse in specific and nonspecific conducting pathways of the network are produced which lead in turn to cyclic changes in the synaptic organization of various cerebral structures.

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It is only natural to assume that the successive and parallel (in the case of arousal) excitation of various cerebral systems must be based on

differences in their neurochemical nature. A hypothesis as to the different neurochemical characteristics underlying the various stages of mammalian hibernation should not seem particularly daring. The extensive and multi-faceted pharmacological studies of S. Ya. Arbuzov (5-8) and the later experiments carried out by Romanian physiologists (291, 292) have demonstrated convincingly that hibernants possess relatively high powers of discrimination for adrenomimetic stimuli and the hormones of the nervous system. This enabled these authors to set up a pharmacodynamic series of compounds in which the excitatory potential decreased from amphetamine via pervitin, (methamphetamine), pentetrazole, ephedrine, nikethamide, strychnine and oxedrine to epinephrine (8). These are mostly adrenotropic preparations which are able to reflect, to one degree or another, the state of the adrenergic mechanisms of the brain in hibernants.

In addition, based on the latest work of Oros et al. (517) and the experiments of Lyman and O'Brien (475, 477), it seemed necessary to study the excitatory effect of cholinotropic preparations such as atropine.

In view of the fact that the previous pharmacological studies were based mainly on an analysis of changes in animal "behavior" which could be related to the effect of the preparation, we did not feel that it was possible, on the basis of these results, to draw definite conclusions as to the state of the central nervous system in hibernants under conditions of drug-induced arousal. In connection with the necessity of analyzing the sensitivity of the adreno- and cholinoreactive zones of the brain in hibernating mammals, and as a logical extension of the experiments of S. Ya. Arbuzov, we undertook to study the effect of some adreno- and cholinotropic preparations on the course of hiberation and the bioelectric changes in the brains of hibernants.

A. Effect of amphetamine on the bioelectric changes in the brain during arousal. The intramuscular injection of amphetamine at a dose of 0.03 mg dry weight per kg body weight (studied in 8 experiments, No. 53-60) produced significant and consistent changes in electrical activity. Electrographic silence /165
typical of a state of deep torpor and low intracerebral temperatures were recorded during the course of the next 60-90 minutes (Fig. 60, a). The first signs of "spontaneous" electrical activity, which were connected with the effect of the preparation, were observed 60-90 minutes after injection when the temperature of the cortex reached 19-21°C (Fig. 60, b). During this period the EEG commonly showed synchronous groups of low-voltage, mixed, dysrhythmic waves having a broad frequency spectrum. Their amplitude usually did not exceed 20-40 microvolts. These forms of "initial" activity appeared most clearly in the cerebral cortex and the diencephalon. Here, the individual bioelectric potentials were combined with grouped activity. The statistical distribution of the EEG potentials showed considerable variability in the frequency spectrum and a rather low amplitude (histogram b', Fig. 60).

Even at the very beginning of arousal, rapid activity in the band from 20 to 30 cycles/sec. appeared in the EEG, along with slow waves on the order of 7-11 per second having an amplitude of up to 60 microvolts. Subsequently, the shift in the direction of a predominance of rapid activity became even more

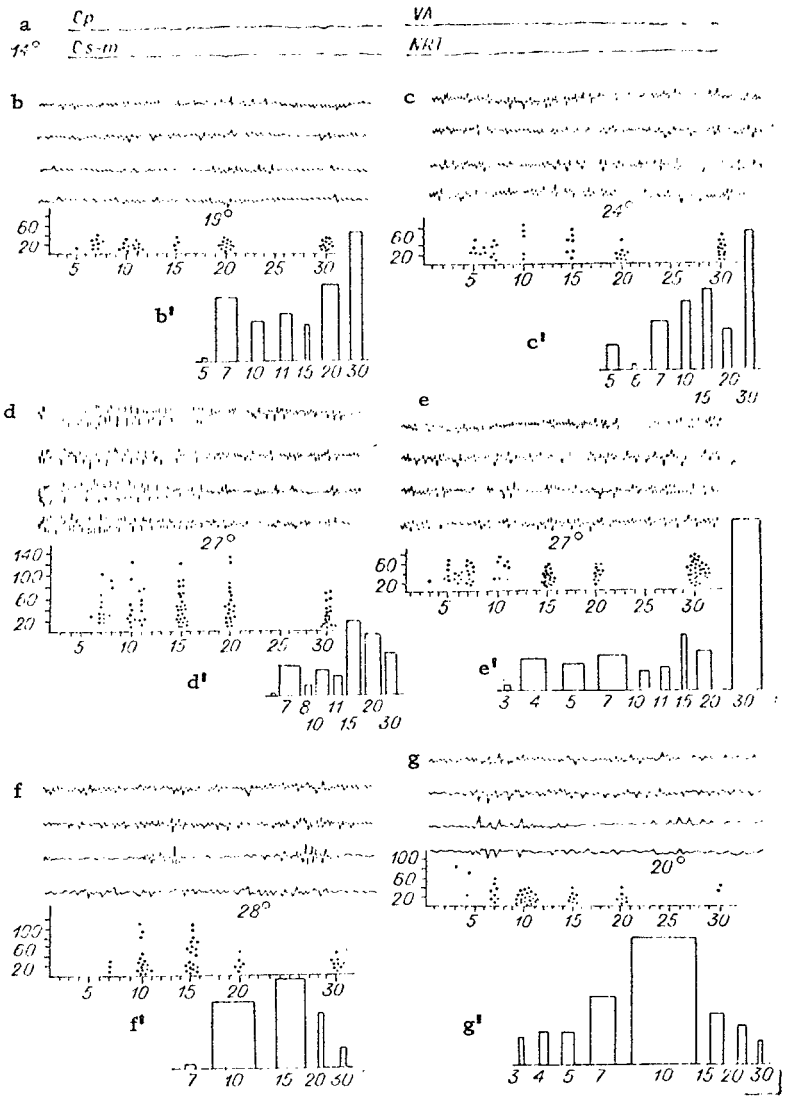

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Figure 60. Effect of Amphetamine on the Bioelectric Activity in Various Parts of the Brain of the European Hedgehog; b'-g', Histograms of the Distribution of EEG Potentials in the Cerebral Cortex (Channel I). Experiment 53, Dec. 21, 1959. The Calibration Shows 100 Microvolts and Seconds.

pronounced: desynchronized activity, the basis of which was rapid low-voltage waves having a frequency of 15-30 per second and an amplitude of 60-80 microvolts, became the dominant type of bioelectric activity in all parts of the brain, but especially in the cerebral cortex (Fig. 60, c).

The shift to the right in the frequency spectrum was accompanied by an "enrichment" of the frequency spectrum of the bioelectric activity arising in the brain of the waking animal. Along with rapid pulsation, the EEG showed significant amounts of slower activity. The phenomenon of "enrichment" of the EEG was observed mainly after arousal was well under way and the cortical temperature had risen to 24-26°C (Fig. 60, d, d'). The later stages of arousal were also characterized by the appearance, in the EEG from all parts of the brain, of groups of spike discharges mostly having a frequency of 15-30 cycles/ /167
sec. and an amplitude of up to 100-200 microvolts; these appeared spasmodically in the EEG, fading away periodically only in order to reappear 12-19 seconds after the preceding group (Fig. 60, d). Between the periods of high-voltage desynchronized activity, some specific electrical activity could be detected in the subcortical layers of the brain and in the cerebral cortex.

For us, the most interesting changes in bioelectric activity were those recorded above 24-26°C (Fig. 60, e, f). This period was characterized by a gradual increase in cortical temperature and by the appearance of grouped activity, initially in the subcortical layers and then in the cerebral cortex. Nevertheless, synchronized activity was only rarely recorded in the mesencephalic reticular formation during amphetamine arousal. Having arisen in a segment of the EEG lasting 10-15 seconds, it was quickly suppressed and altered by desynchronization (Fig. 60, e, f). During the transition from active wakefulness to incipient hibernation (in connection with the waning of the stimulatory effect of the preparation), a "burst component" appeared first in the structures of the diencephalon and the cerebral cortex (Fig. 60, f).

The amphetamine-induced changes in bioelectric activity described above usually appeared within the course of 3-4 hours after the injection. Assuming that the external temperature was maintained within ± 2°C, the activating effect began to diminish after 3-4 hours and was followed by the development of the bioelectric changes which are characteristic of the natural onset of hibernation (Fig. 60, g).

B. Effect of epinephrine on the bioelectric changes in the brain during arousal. In an animal in a state of deep torpor, the intramuscular injection of a 0.1% solution of epinephrine initially produced generalization of the autonomic rhythms, particularly the ECG complex (Fig. 61, a, b). In this series of /168
experiments (7 experiments, No. 61-67), the electroencephalographic signs of arousal appeared suddenly, almost against a background of "electrographic silence" (Fig. 61, c). This was immediately accompanied by a rapid rise in cortical temperature to 21-26°C. The bioelectric changes reached their maximal extent almost at once. The dominant activity during this period of arousal was made up of relatively slow waves having a frequency of 4-7 per second and an amplitude of 40-100 microvolts (Fig. 61, c'). The EEG also showed sharp spikes during this period. The brain temperature also increased

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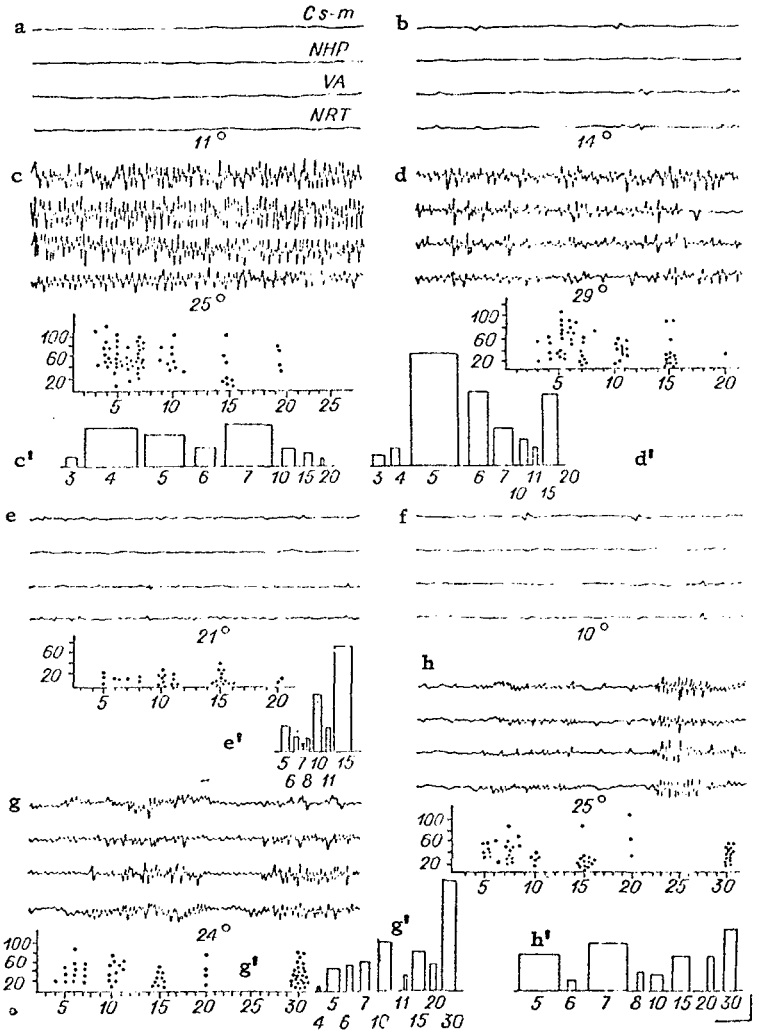

Figure 61. Effect of Epinephrine on the Bioelectric Activity in Various Parts of the Brain of the European Hedgehog. Experiment 62, April 10, 1960. Repeated induction of Hibernation (e, f) and Arousal (g, h), Possibly Connected with the Secretion of Endogenous Epinephrine; e - 120 Minutes after Injection of the Preparation, f - 240 Minutes after the Second Drop in Electrical Activity.

extremely rapidly: compared to an increase of 8-10°C during the first 60 minutes after the injection, it rose to 29-31°C during the next 15-20 minutes, a much shorter time interval. During this period of marked arousal, mixed dysrhythmic activity was recorded in the cerebral cortex and the mesencephalic reticular formation, while in the remaining subcortical areas the dominant form of activity was generally spikes having a frequency of 10-15 per second and an amplitude of 80-100 microvolts (Fig. 61, c).

As arousal progressed, the cortical temperature increased extremely slowly. Sporadic groups of slow waves and spike discharges reaching an amplitude of 90-100 microvolts (Fig. 61, d) were recorded in the EEG from all parts of the brain but especially from the cerebral cortex and the hypothalamic region (these changes were commonly distributed over all regions of the subcortex, particularly the medial portion of the optic thalamus and the mesencephalic reticular formation). The frequency spectrum was generally shifted to the left and waves having a frequency of 5-6 per second began to predominate. The increase in intensity of the epinephrine-induced bioelectric changes ceased just as suddenly as it had begun (Fig. 61, e). Signs of rhythmic activity disappeared from the cortex and were significantly reduced in the subcortical layers (Fig. 61, e). The brain temperature then fell to 20-23°C.

Against the background of renewed "electrographic silence", episodic damping waves and spike discharges were recorded showing a gradual transition to a distinct permanent activity, which appeared most consistently in the ventromedial part of the optic thalamus and the mesencephalic reticular formation (Fig. 61, g). This was the beginning of the so-called "second arousal", /169 which could be observed repeatedly in the course of a single experiment with epinephrine, most often 3-4 times during the 6-10 hours after administration of the preparation. Statistical analysis of the waves of the EEG showed that the predominant form of activity during the period of "repeated arousal" was rapid waves having a frequency on the order of 10-15-30 cycles/sec. (Fig. 61, g').

Another characteristic of the stage of "repeated arousal" was that the bioelectric changes were not as constant here as at the beginning of the experiment. The bursts of activity shown in the figures alternated with prolonged periods of electrographic silence. This phenomenon also appeared during the subsequent "spontaneous" changes in electrical activity, when the depression of activity was replaced by its transitory revival and a moderate increase in brain temperature (Fig. 61, f, h). In 4 experiments out of 7 in this series, 7-11 repeated sequences of changes in bioelectric activity were observed which were directly or indirectly related to the administration of epinephrine.

The repeated bioelectric changes resulting from arousal of the hibernant are established against a background of a general dominant activity consisting of rapid waves having a frequency of 15-30 cycles/sec. and an amplitude of 40-60 microvolts (Fig. 62, i, l). In the case of epinephrine arousal, these repeated bioelectric changes may become stabilized, accompanied by a stable state of active wakefulness (Fig. 62, k, l,). In this case, the statistical distribution of the waves in the EEG indicates "enrichment" of the dominant activity during arousal (Fig. 62); nevertheless, persistent arousal is accompanied by a reduction in the proportion of slow waves in the arousal EEG (Fig. 62).

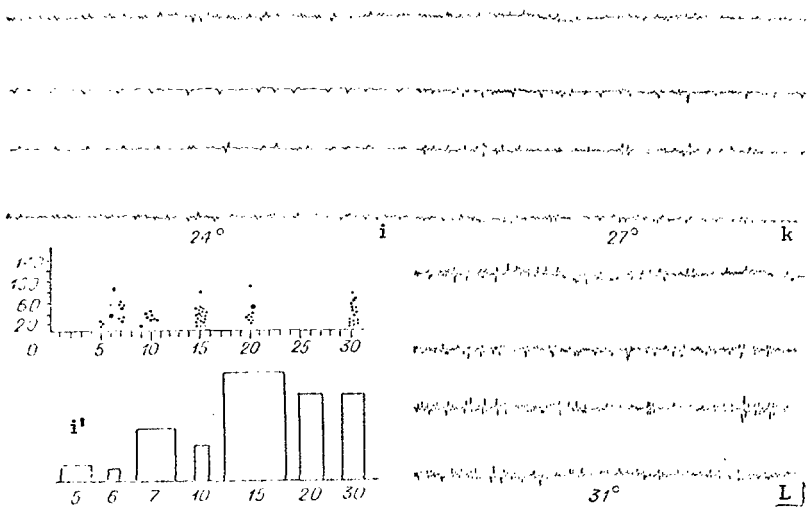

Figure 62. Continuation of Fig. 61. "Repeated Arousal", Possibly Connected with the Secretion of "Endogenous" Epinephrine. Reappearance of Electrical Activity and Increase in Cortical Temperature to 27-31°C. Stable Waking State.

C. Course of the bioelectric changes in the hibernant brain during arousal produced by intramuscular injection of atropine. Atropine produces both electrographic and "behavioral" arousal at very low cortical temperatures. Injection of atropine at a dose of 0.3-0.7 ml of 1% solution produced a marked rapid resumption of electrical activity after only 30-35 minutes, with the appearance of waves having a frequency on the order of 7-30 per second (the predominant frequencies were 7 and 25-30 cycles/sec.). These changes were generalized to a significant extent and could be seen most clearly in the cerebral cortex (Fig. 63, a, b) and the medial portions of the optic thalamus. /170
The excitatory effect in this case was quite stable; it did not disappear and hardly decreased in intensity with time. As the intracerebral temperature increased further, the phenomenon of "burst" activity appeared in the EEG (especially in the cerebral cortex and the medial portions of the optic thalamus) (Fig. 63, d). In the case of atropine arousal, signs of electrographic wakefulness could be detected for 8-10 hours after injection of the preparation. These signs were characterized by the presence of an "enriched" frequency spectrum consisting of waves having frequencies of 7-10-20-30 per second (Fig. 63, c'). In the case of atropine arousal, a distinct drop in cortical temperature did not appear earlier than 18-20 hours after injection of the preparation (Fig. 63, e, f). The excitatory effect of atropine was extremely constant and stable. Even after the cerebral cortical temperature had decreased to 19-21°C, elements of "enriched" activity consisting of waves with a frequency of 10-20-30 cycles/ sec. could be recorded quite clearly. In essence, in the case of atropine arousal,

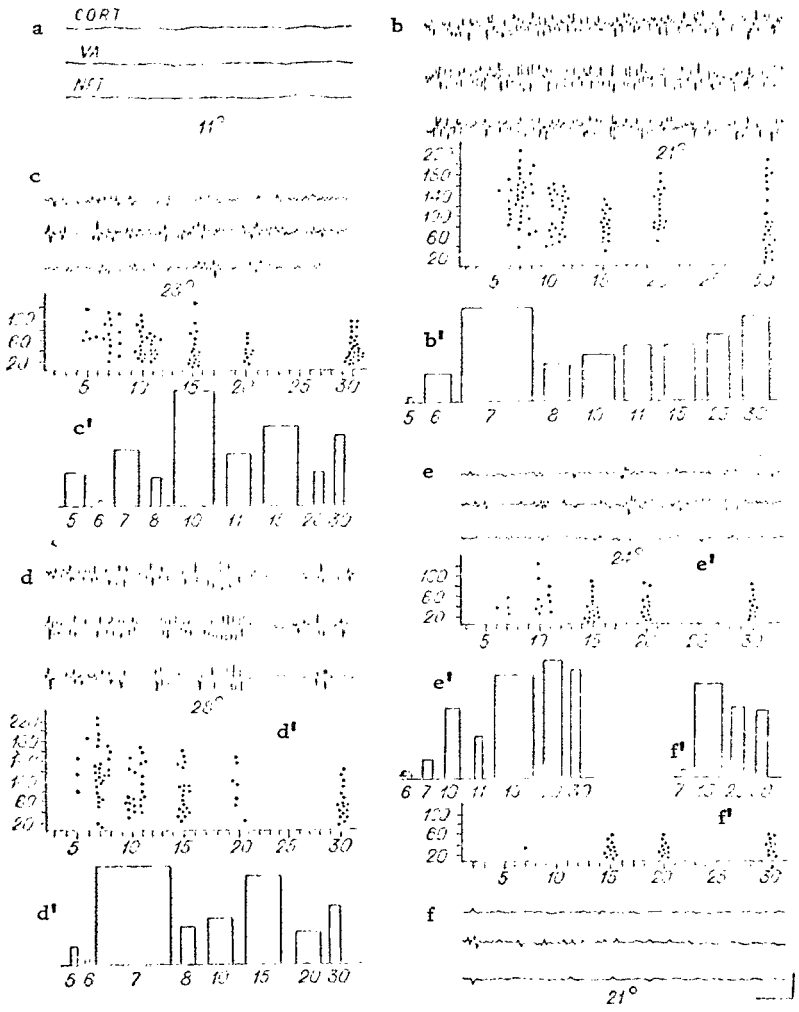

Figure 63. Effect of Atropine on Bioelectric Activity in the Brain of the European Hedgehog; b - 20 Minutes after a. Experiment 69, Oct. 10, 1960.

the low-voltage polymorphic activity seen in the EEG (Fig. 63, e, f) is a "final" form. In none of the 7 experiments devoted to the bioelectric changes developing in the hibernant brain in connection with atropine arousal did we succeed in observing either depression or a prolonged disappearance of rhythmic electrical activity in the EEG. Episodic damping waves and individual groups of slow waves could be consistently observed over the course of the following 3-4 days. /172

D. Electrophysiological characteristics of arousal induced by galanthamine. Intramuscular injection of galanthamine at a dose of 0.2-0.8 ml of 1% solution resulted in the appearance of irregular damping waves in the hippocampus, the hypothalamic region and the ventromedial nuclei of the optic thalamus. These bioelectric changes appeared against a background of generalized depression of electrical activity in all parts of the brain and a diffuse distribution of the ECG complex in the EEG (Fig. 64, a). Subsequently, in connection with the onset of arousal, the heart rate increased but the electrical activity in the cerebral structures remained essentially unchanged. The episodic waves showed a stable localization to the hippocampus (Fig. 64, b, c). The frequency spectrum in the hippocampus was enriched by the appearance of slow waves, both singly and in groups, and elements of synchronization (see the histogram and curve in Fig. 64, d'). Generalized forms of activity also appeared in the EEG 120-150 minutes after the beginning of arousal, along with spike potentials which could be observed singly and in groups during this period in all parts of the brain.

Relatively persistent desynchronization of electrical activity was recorded in the structures of the diencephalon, the ventromedial nuclei of the optic thalamus and the hypothalamus (Fig. 64, d). After the temperature had risen to 22-24°C, individual episodes of "burst activity" appeared in the cerebral cortex, the mesencephalic reticular formation and the hypothalamic region. In the neighborhood of 27-29°C the amplitude of the biopotentials decreased somewhat and the prolonged periods of desynchronized activity appeared which are so characteristic of definitive arousal. Nevertheless, the background activity consisted mainly of regular waves having a frequency of 6-7/sec., accompanied by spikes and brief periods of rapid, low-voltage activity.

After 7-8 hours (or 6-6.5 hours in most of the experiments of this series), all of the previously described types of electrical activity could be seen in the EEG: from synchronized groups of "burst activity" to spasmodic discharges appearing in the cortex and the structures of the mesencephalon and diencephalon (see Fig. 64). /174

E. Electrophysiological characteristics of arousal induced by epinephrine and "reinforced" by atropine. As was demonstrated earlier, epinephrine arousal is characteristically stepwise and discontinuous in nature. This is clearly reflected in the alternation between periods of mixed dysrhythmic activity and depression in the EEG, between generalization of the "burst spindles" and the appearance of the spikes which characterize the convulsive state. The stimulatory effect of epinephrine (or of amphetamine) fades after 120-180 minutes, followed by periods of "repeated" arousal and lapses into hibernation.

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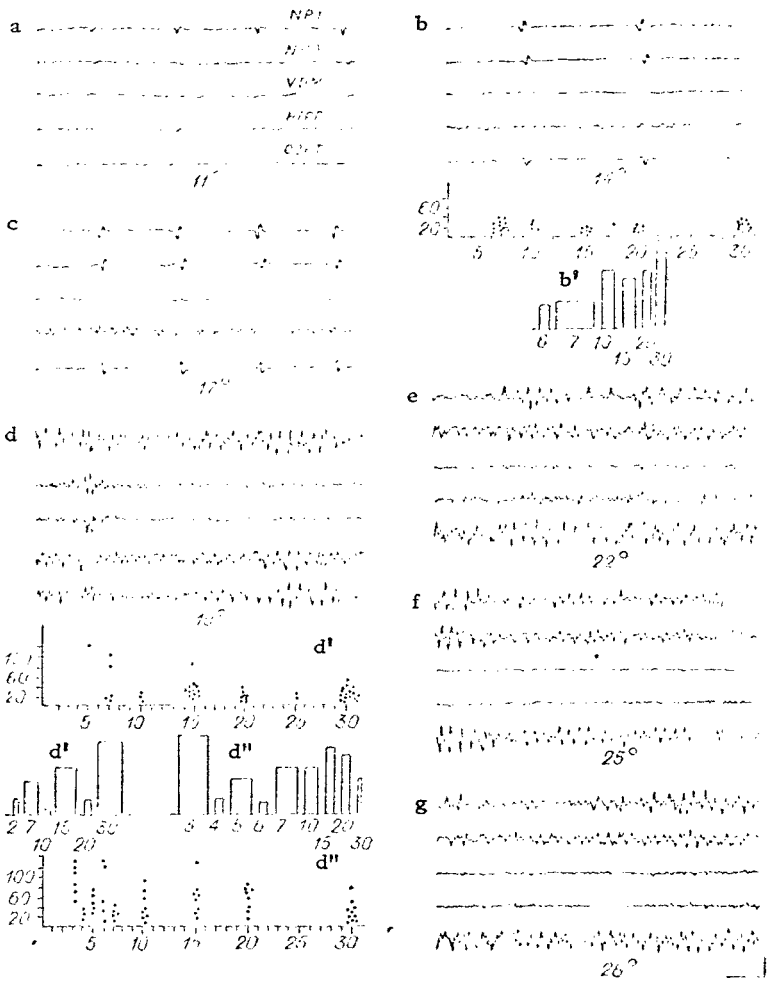

Figure 64. Effect of Galanthamine on Bioelectric Activity in the Brain of the European Hedgehog: b and c – 20 and 35 Minutes after a, respectively. Experiment 79, Dec. 26, 1960.

Atropine is characterized by instantaneous and persistent effects which appear in a state of deep torpor and in a temperature range which is significantly lower than is found under the conditions of adrenomimetic arousal.

In this connection, it seemed interesting to study the combined effects of adreno- and cholinotropic preparations under conditions when the epinephrine effect was fading away. Potentiation of adrenomimetic effects by cholinotropic substances is a phenomenon which we have seen before (9, 10).

As was already reported above, 30-50 minutes after the administration of epinephrine (within 60 minutes in all experiments) to animals in a state of deep torpor (Fig. 65, a), the initial signs of "spontaneous" activity appear in the EEG; these signs become regular and constant during the period when the cortical temperature reaches 17-20°C (Fig. 65, b). Arousal is accompanied by a significant shift to the right in the frequency spectrum, the appearance of desynchronized activity, and enrichment of the EEG (Fig. 65, b, c). From time to time, the signs of "spontaneous" bioelectric activity which appeared paroxysmally initially in connection with the effect of epinephrine, increase in strength. The stimulatory effect of epinephrine begins to fade 120-160 minutes after the beginning of the experiment. At that time, slow synchronized waves having a generalized character appear in the EEG (Fig. 65, d, e). The weakening of the stimulatory effect of epinephrine proceeds gradually and the /175 period is characterized basically by a predominance of slow polymorphic waves having a diffuse character (Fig. 65, d, e).

Administration of atropine against a background of a fading adrenomimetic effect led relatively rapidly (within 20-30 minutes) to activation of the EEG, a shift to the right of the frequency spectrum, and the predominance of rapid forms of electrical activity (Fig. 66, f-i). This was inevitably followed by an /176 increase in brain temperature and the appearance of "burst activity" in the EEG. The stimulatory effect produced by the combined and successive administration of epinephrine and atropine lasted for a relatively long period, more than 7-8 hours. In this type of experiment, the stepwise form of epinephrine arousal with prolonged periods in which activity is depressed and the brain temperature falls was not observed. Weakening of the stimulatory effect of these substances could be recorded on the day following their administration (Fig. 66, k).

An analogous sequence of events and changes in bioelectric activity was /177 observed when the stimulatory effect of amphetamine was reinforced by atropine. About 20-30 minutes after an injection of amphetamine, the rapid bioelectric activity in the hippocampus became stronger, the frequency spectrum was enriched by waves having frequencies of 10-20-30 cycles/sec., and then, after stabilization of the stimulatory effect, dominant activity on the order of 30 cycles/sec. appeared in the hippocampus (Fig. 67, a, b). At the same time, the cardiac components were generalized, although the heart rate during this period did not exceed 2-3 beats per minute (Fig. 67, b). The brain temperature at this point reached 16-20°C and the EEG was characterized by initial signs of grouped activity and the appearance of a "burst component" (Fig. 67, e, f).

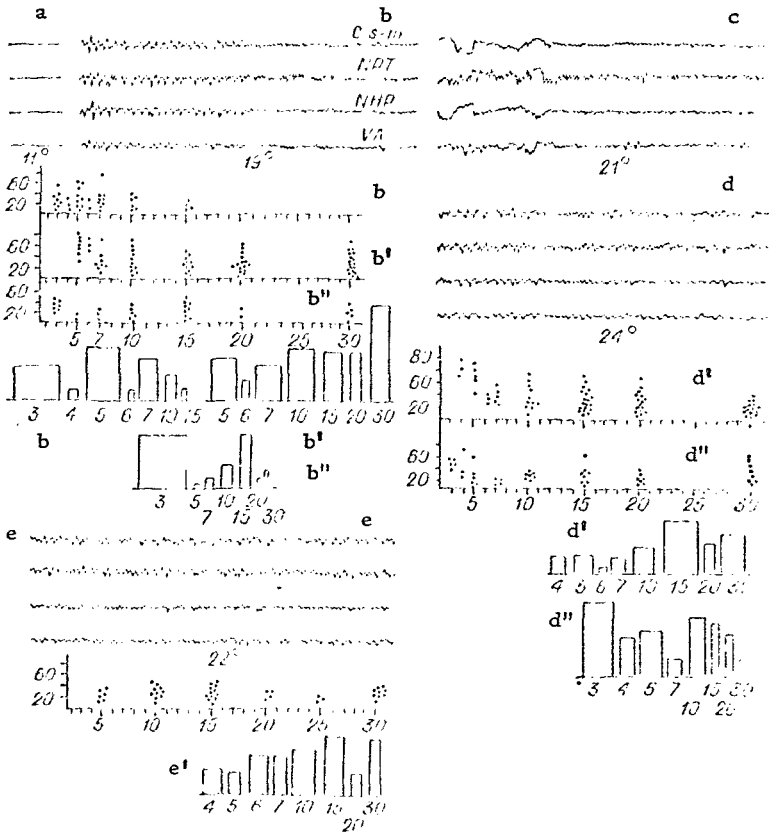

Figure 65. Effect of Epinephrine and Subsequent Administration of Atropine on the Electrical Activity of Various Parts of the Brain of the European Hedgehog. Initial Effect of the Preparation; b - 40 Minutes, c - 70 Minutes after Injection of the Preparation; a - Initial Activity in a State of Torpor; d, e - Weakening of the Stimulatory Effect of Epinephrine 120 Minutes after its Administration.

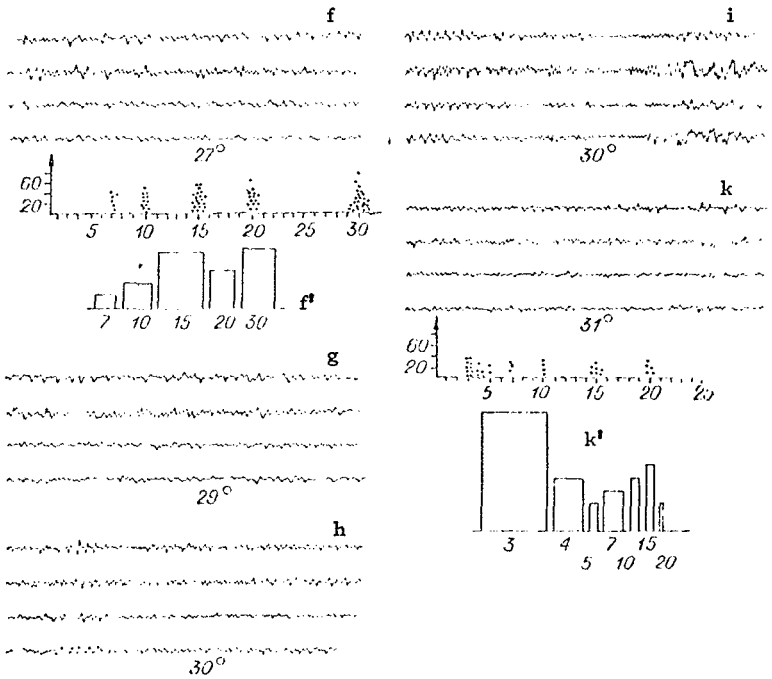

Figure 66. Continuation of Fig. 65. "Potentiation" of the Stimulatory Effect of Epinephrine by Atropine Administered at a Cortical Temperature of 24°C Against a Background of a Weakening Epinephrine Effect: h-k, Renewed Lapse into Hibernation Following Fading of the Stimulatory Effect of Epinephrine and Atropine.

At the beginning of amphetamine-induced arousal, the "burst component" was closely associated with elements of convulsive activity. By a temperature of 22-27°C, however, the "burst component" became the dominant form of electrical activity.

At this stage of the experiment, the first signs of incipient inhibition of the stimulatory effect of amphetamine appeared. The "burst component" showed stabilization in the records from the cortex and all parts of the subcortex and signs of hypersynchronization appeared with an increase in amplitude of the waves having a frequency of 7-10 cycles/sec. to 200 microvolts (Fig. 67, e-i, i'). The cortical temperature began to decrease. Even at a brain temperature of 26-27°C, the frequency spectrum already showed a definite tendency to shift to the left; this tendency became more pronounced later and is characteristic of the fading of the stimulatory effect of amphetamine. During this period of weakening amphetamine stimulation, the "burst activity" was characterized by extremely constant frequency and amplitude characteristics and by a significant resistance to external influences.

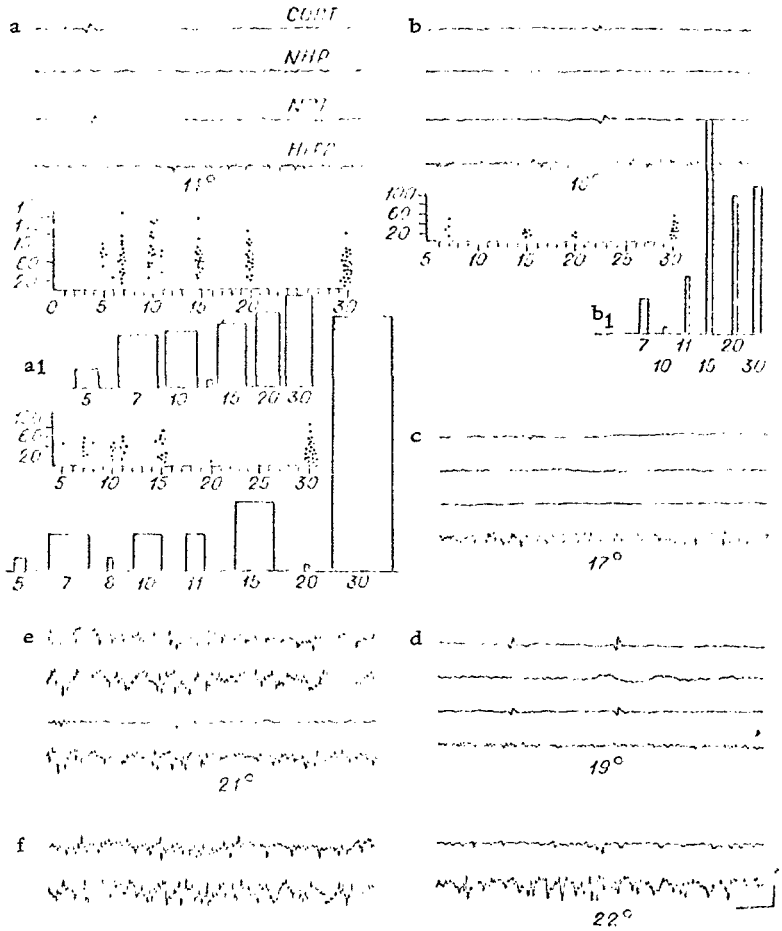

Figure 67. Effect of Amphetamine and Subsequent Administration of Atropine on Bioelectric Activity in Various Parts of the Brain of the European Hedgehog. Experiment 82, Jan. 14, 1961.

Administration of 0.5-1 ml of a 1% atropine solution during this period produced a vigorous resumption of bioelectric activity after about 20-30 minutes. Initially this activation was characterized by a reappearance of the "burst component", which had begun to fade out during this period, and then by the appearance of high-frequency discharges (Fig. 68, i-l).

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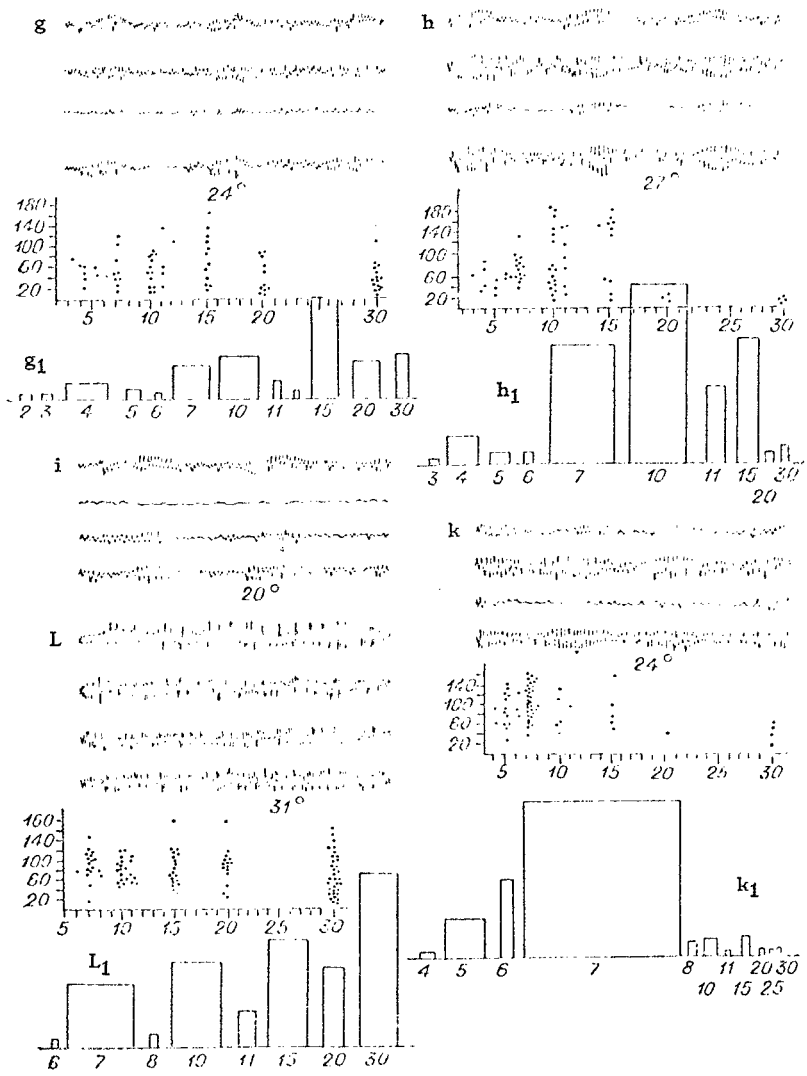

Figure 68. Continuation of Fig. 67. "Potentiation" of Adrenergic Stimulation by Atropine.

The initial changes in electrical activity produced by atropine then became generalized, with the appearance in the EEG of high-frequency peak discharges and groups of high-voltage waves. Statistical analysis of the EEG pattern showed a significant "concentration" of activity in the high-frequency ranges. /178 Activation of the EEG persists for the next 24-48 hours. Arousal thus becomes stabilized and may be accompanied by a significant increase in motor activity.

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The stimulatory adrenotropic and cholinotropic preparations thus produce a definite excitatory effect which is characterized by clear changes in electrical activity and the course of hibernation.

Adrenomimetic agents (amphetamine but especially epinephrine) produce a stepwise arousal in hibernants which are in various stages of deep torpor. This arousal, which develops paroxysmally and suddenly, usually fades away 80-120 minutes after the beginning of the active process but then reappears, often repeatedly, accompanied each time by weaker electrical effects. Arousal induced by epinephrine usually has the same characteristics as arousal induced by amphetamine, the only difference being in the latent period preceding the first signs of arousal and in the intensity and extent of the electrical effect. In both cases, arousal is inevitably reversed once it has reached its maximal level. Thus, the arousal produced by "exogenous" epinephrine and adrenomimetic agents such as amphetamine is apparently "duplicated" in connection with the repeated secretion of the endogenous mediator hormone. Repeated arousal is accompanied by the identical changes in electrical activity, but to a lesser degree. It is quite possible that we are dealing here with the persistent secretion of endogenous epinephrine during the period of torpor (619, 620).

The arousal produced by cholinotropic agents, particularly anticholinesterases and cholinolytic agents such as galanthamine and atropine, is not characterized by specific electrical changes; it is, however, characterized by a progressive course which does not show periods of fading. The electrographic and "behavioral" arousal produced by these agents develops at lower brain temperatures than the analogous effects produced by adrenomimetic substances. The first signs of arousal are observed at a temperature of 14-16°C, while epinephrine and amphetamine produce distinct electrographic arousal only above 20°C.

Statistical analysis of the data shows that the electrographic equivalent of hibernant arousal is not limited to generalization of desynchronized activity. As a rule, the change in the frequency spectrum associated with artificial arousal also involves, as an essential component, "enrichment" of the bio- /181 potential frequency band. The appearance of a "burst component" in the EEG and enrichment of the frequency spectrum during arousal are most likely interrelated processes reflecting the transition of the majority of the central neurons to an active state.

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In view of everything that has been said above, it would be logical to assume that the temperature differences noted in the effectiveness of the stimulatory agents are related to a definite and fixed temperature range for the activity of the corresponding chemical mediator (acetylcholine and norepinephrine), i.e., with a definite level of activity of the adrenergic and cholinergic processes in the brain. In both cases, we may be dealing with differences in the rates of synthesis and destruction of the mediators in the cerebral structures of hibernating animals.

Proceeding on the basis of such an hypothesis, it seemed appropriate to compare the changes in the content and distribution of acetylcholinesterase and monoaminoxidase (AChE and MAO) with the production of the corresponding substrates as described in references 618-620 and confirmed by our own electrophysiological experiments.

The histochemical localization of AChE was accomplished by the method of Ishiya and Friede (375) and that of MAO by the method of Glenner (655). The experiments were performed on 18 red-cheeked susliks and 6 European hedgehogs, in a state of either deep torpor (rectal temperature of 0.8-1°C), arousal or active wakefulness. The brains of these animals were also studied to determine the localization of MAO and AChE.

After the brain had been fixed in Lilly's fluid at 4°C for 24 hours, the skull and its contents were fastened in the head-holder of the stereotaxic device and a block having a sagittal diameter of 10 mm (F—1.0--F—7.5) was cut out with the aid of a razor blade. Frontal sections with a thickness of 60 microns were then marked off on the basis of the atlas and every other one was stained for the histochemical localization of the enzymes. Control and experimental slices (from animals during arousal and torpor, respectively) were treated on the same microscope slide.

The stained frontal sections were then subjected to digital scanning photometry (652). The area of the sequence being scanned was given by the time of the measurement and the scanning coordinates. The error caused by the nonuniform distribution of MAO and AChE in the plane of the section was minimized by using a sufficiently small probe (1-2 microns), thus eliminating the limitations connected with the shape of the spectral curve of the absorbing component, abolishing the necessity of working at maximal absorption, and making it possible to evaluate many histochemical reactions objectively. /182

Since, assuming the time is held constant, an increase or decrease in size of the specimen due to changes in scanning coordinates does not produce any change in the readings of mean optical density, this method permits the mean concentration of an absorbing substance to be calculated for a specimen larger than the scanning unit. This was particularly important in our case where we scanned large nuclei or several parts of the same nuclear formation.

The low inertia of the pulse counters made possible high-speed scanning, thus significantly decreasing the time required for a measurement. Thanks to the free decades of the PS-1000 counter, we were able to use the cumulative

principle and obtain the mean value for the amount of absorbing substance (MAO and AChE in our case) from a table. In parallel with the procedure described for scanning the sections, we utilized the switching microammeter of a valve unit to transmit the signal to an EPP-09 autorecorder; in this way, we were able to calculate the distribution of the absorbing substance in the optical section without area scanning.

The time of the measurement was given by a relay on the counter and was set at 10 seconds. Thus, within the limits of an average-size nucleus, we were able to make 10 scanning passes with an average velocity of 0.6-0.8 mm/ sec. (real scale along the section). With nuclei of average or larger than average size, this was repeated on several parallel scanning passes and the results were cumulated using the table on the counter, after which we divided by the number of passes in order to obtain the mean concentration of absorbing substance (Tables 13 and 14) *.

The chemical topography of AChE in the plane of the frontal sections from animals undergoing arousal corresponded with that described in the literature /183
for rodents and carnivores (375). In the waking animal, the gradient of enzyme concentration was represented by the following decreasing sequence: nucleus caudatus — olfactory tract — amygdalus — hippocampus — central grey matter — lateral and medial nuclei of the optic thalamus — septum.

TABLE 13. AChE IN BRAIN SLICES FROM THE RED-CHEEKED SUSLIK

Structure	Torpor			Arousal		
	M±m	$\Sigma\alpha^2$	σ	M±m	$\Sigma\alpha^2$	σ
CORT	90.4±0.22	4.40	0.69	96.2±0.19	3.60	0.63
SD	71.8±0.25	3.60	0.63	85.5±0.19	2.5	0.53
SP	80.4±0.16	2.40	0.52	92.2±0.13	1.60	0.42
AHA	95.6±0.17	2.40	0.52	92.3±0.16	2.10	0.49
AMYG	81.5±0.17	2.50	0.53	88.9±0.18	2.90	0.56
HIPP	80.4±0.17	2.40	0.52	96.0±0.26	6.00	0.82
MD	85.7±0.15	2.10	0.48	93.1±0.27	6.50	0.85
R	86.8±0.19	3.60	0.63	97.8±0.19	3.60	0.63
VM	77.2±0.19	3.60	0.63	94.1±0.22	4.50	0.70

* The author would like to thank Prof. B.B. Fuks and Engineer Yu.Ye. Morozov for putting at his disposal the digital scanning photometer of the histochemistry laboratory of the Institute of Morphology of the USSR Academy of Medical Sciences, as well as L.I. Gol'dgefter for his help in carrying out the histochemical studies.

TABLE 14. MAO IN BRAIN SLICES FROM THE RED-CHEEKED MARMOT

Structure	Torpor			Arousal		
	M ± m	$\Sigma\alpha^2$	σ	M ± m	$\Sigma\alpha^2$	σ
CORT	90.4±0.16	2.40	0.52	73.0±0.15	2.00	0.52
HIPP	87.2±0.19	3.60	0.63	56.8±0.19	3.00	0.63
VDM	88.1±0.18	2.90	0.56	63.8±0.19	3.60	0.63
PTH	80.6±0.16	2.40	0.52	55.1±0.23	4.90	0.73
MO	82.0±0.32	10.0	1	53.9±0.18	2.90	0.56
SGC	55.2±0.13	1.60	0.42	88.2±0.58	31.20	1.86
NHP	82.1±0.31	8.90	0.99	56.2±0.13	1.60	0.42
AHL	82.6±0.30	8.40	0.96	60.9±0.23	4.90	0.73
PC	96.8±0.13	1.60	0.42	59.0±0.29	8.90	0.94
AMYG	83.5±0.16	2.50	0.53	65.0±0.25	5.60	0.79
PR	83.2±0.19	3.60	0.63	53.0±0.26	6.00	0.82

The results of the quantitative evaluation of the mean AChE concentration /184
indicate clearly that the enzyme content in the cerebral cortex and subcortical muclei is increased in animals in a state of deep torpor. Arousal leads to a decrease in AChE content. This phenomenon is most clearly expressed in the nucleus caudatus, hippocampus and amygdalus and to a lesser degree in the cerebral cortex (Fig. 69, Table 13). The enzyme concentration in the hypothalamic region increases as the animal awakens from torpor.

MAO is uniformly distributed in the subcortical nuclei, elements of the neocortex and hippocampus, with a slight predominance in the nucleus caudatus, posterior hypothalamus and medial nuclei of the optic thalamus; the structures of the neo- and archicortex are characterized by a relatively low MAO content. The topography described is in complete agreement with the data obtained in rodents (669, 673, 680).

The experimental data show that the changes in MAO distribution in the brain during arousal are characterized by a tendency opposite to that of the AChE: during arousal from torpor, and especially when the brain temperature /185
reaches 18-20°C, the enzyme content increases (Fig. 70, Table 14). This effect can be seen most clearly in the cerebral cortex, hippocampus, hypothalamic nuclei and amygdalus, and is somewhat less pronounced in the nucleus caudatus and the reticular nuclei of the optic thalamus. An effect which /186
goes in the opposite direction is observed in the conducting pathways of the capsula interna (Fig. 71).

Quantitative histochemical studies have thus shown that the natural /187
arousal of hibernants is accompanied by an increase in the MAO content and a decrease in the mean AChE concentration in sections of the brain of the red-cheeked suslik. The data obtained are in general agreement with the published results of American and Finnish authors (561, 618-620) and may be used in the development of a hypothesis relating to the dual nature of natural

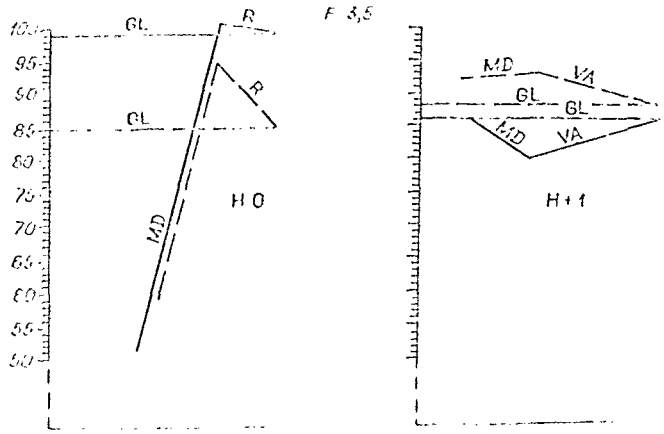

Figure 69. Graphic Presentation of the Results of Digital Scanning Photometry of Frontal Sections of Suslik Brain during Either Torpor or Arousal. The Numbers on the Abscissa Are the Same as in Fig. 70 and Represent the Length of the Nuclear Scan, while Those on the Ordinate Represent the Change in Mean AChE Concentration in the Section in Arbitrary Units; the Dashed Line Represents Arousal while the Solid Line is Torpor. H Indicates the Depth of Scanning in the Nucleus in Relation to the Horizontal Zero Reference Plane.

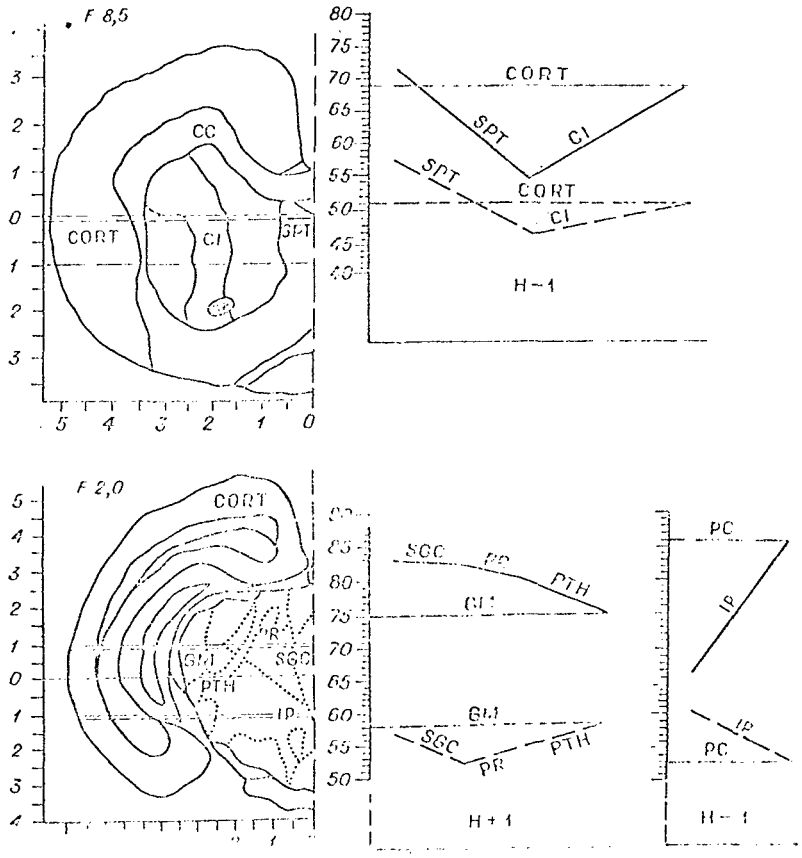

Figure 70. Graphic Presentation of the Results of Digital Scanning Photometry of Frontal Sections of Suslik Brain during Either Torpor or Arousal. Mean MAO Concentration. The Other Symbols have the Same Meaning as in Fig. 69.

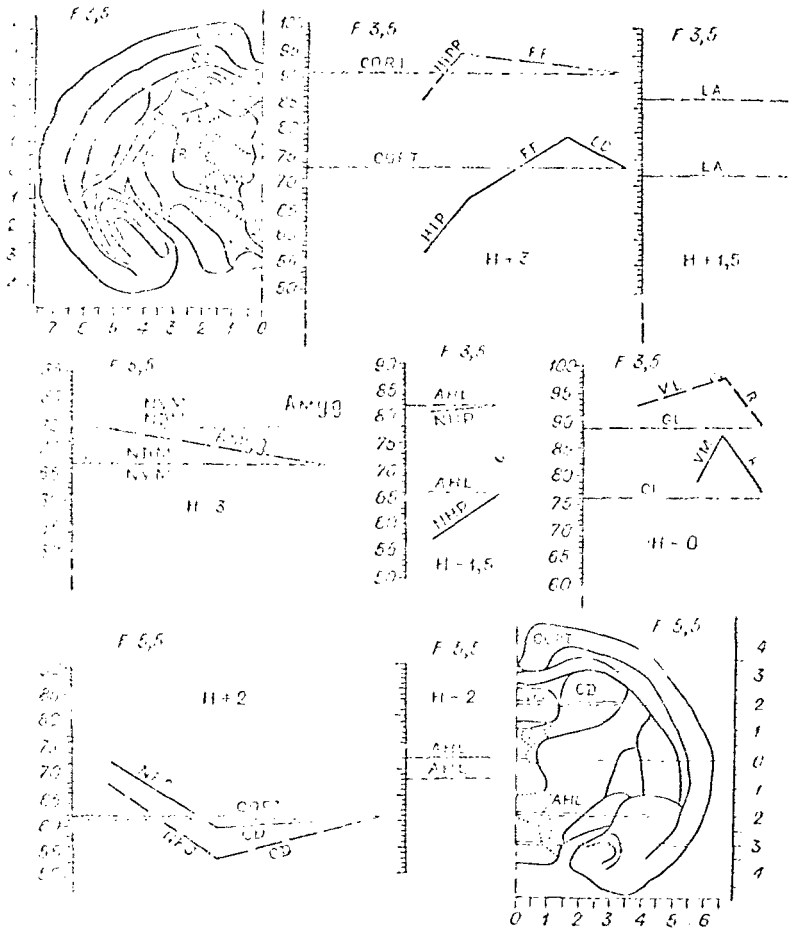

Fig. 71. Continuation of Fig. 70.

arousal and some other systemic processes taking place in the central nervous system of hibernating mammals. These subjects will be discussed in the last chapter of this book.

CHAPTER VIII.
MECHANISMS OF THE CEREBRAL ACTIVITY OF HIBERNATING MAMMALS AND SOME PROBLEMS OF NEUROPHYSIOLOGY /188

Neurophysiological (Electrophysiological) Phenomenology of Mammalian Hibernation.
Cortico-Subcortical Relationships in Hibernants.
Role of the Limbic Structures during Various Stages of Natural Adaptation.
General Neurophysiological Principles of Hibernation.
The Neurochemistry of Hibernation and Arousal.
Hypothesis as to the Dual Nature of Arousal.
Physiological and Morphochemical Prerequisites for Sequential Processes (Memory) in Hibernants.
Possible Structural Mechanisms Underlying the Consolidation of Memory in Hibernating Mammals.

This analysis of the experimental results will consist, in a general way, of an evaluation of the factual material obtained in our laboratory followed by some hypothetical postulates in which we will attempt to evaluate the mechanisms of cerebral activity in hibernants and to relate the facts pertaining to this basic problem with some borderline aspects of neurophysiology. We hope to show that some of the questions discussed during the past few years and connected with the problem of systematic cerebral activity may acquire a new interpretation on the basis of the experimental findings concerning the cerebral mechanisms of natural adaptation in hibernating mammals. In this connection, we will attempt to shed light on the following questions:

A. The neurophysiological (electrophysiological) phenomenology of hibernation;

B. The structural and functional organization of the cerebral systems which /189
guarantee the homeostatic equilibrium and the interaction of the hibernating organism with its environment; the neurochemical basis of the activity of these systems during the various stages of natural adaptation;

C. Physiological and morphochemical prerequisites for memory in hibernants.

Such an analysis of the basic aspects of the problem will naturally result both in conclusions based completely on the factual material and in hypotheses related to a definite type of extrapolations and not directly dependent on the experimental results. The longevity of these hypotheses will depend purely on the results of future experimentation.

A. Electrophysiological phenomena of hibernation. The experimental results show that onset of hibernation, torpor and arousal are accompanied by regular and to a certain degree mutually interdependent changes in the transmission of stimuli along the specific and nonspecific pathways of the brain. As shown by our studies of the induced potentials, transmission of the signal is limited in the first place to the polysynaptic nonspecific systems, particularly the polysynaptic connections in the reticular formation of the brainstem. The changes in secondary bioelectric responses can serve as a final proof of this conclusion. The gradual changes in and suppression of these potentials can best be observed in the temperature range 22-26°C.

To this general conclusion we can add that an analysis of the induced potentials in the hibernant cortex also demonstrates some characteristics of these phenomena which are found, as far as we know, only in hibernants. The first of these is that a secondary response, the parameters of which are identical to those described for homoiothermic animals (186, 372, 407), can be obtained in unanesthetized hibernants approaching a state of torpor. As the depth of hibernation increases, however, actual torpor is inevitably accompanied by the disappearance of the secondary response, which is in precise agreement with the temperature range for the block of transmission of excitation in the polysynaptic pathways of the brainstem reticular formation and the diencephalon.

This leads to at least two conclusions. The first is that the secondary response is not exclusively a result of barbiturate narcosis, but can be looked upon in our experiments as an indication of a transitional functional state of the hibernant brain between the waking state and torpor under conditions of /190 natural adaptation. The second conclusion can be formulated as follows: within the framework of an analysis of the mechanisms of hibernant cerebral activity, the biological significance of the secondary electrical response is probably as an indication of a new constricted range of homeostatic reactions connected with the gradual exclusion of the structures of the brainstem from active participation. This hypothesis will shortly be subjected to an analysis based on some experimental facts.

Suppression of the secondary bioelectric responses during this stage of hibernation takes place without any significant changes in the primary complex. The first signs of a block of transmission in the specific conducting pathways begin to appear at a brain temperature of 18-20°C; the duration of the latent period increases and reaches 200-300% of the original value at 14-16°C, there is a drop in amplitude of the negative component of the primary response and an increase in its duration. These phenomena are still quite clearly visible after significant depression has set in the secondary responses. The primary positive wave disappears below 8-11°C. Thus, the pyramidal neurons of the third

and fourth layers, which are responsible for the generation of the positive wave of the primary response, are capable of local depolarization during this stage of hibernation but are unable to generate a stimulus which will be distributed in the upper layers of the cerebral cortex.

The course of the induced cortical responses during arousal and the return of the cerebral temperature to its original level is characterized by the reverse sequence of phenomena: the primary positive waves appear first of all, followed by the negative component of the primary response and finally by the secondary waves and trace waves of a generalized type. The onset of hibernation and incipient torpor are thus characterized primarily by a block in transmission of excitation along the nonspecific pathways. Within this temperature range, the specific pathways do not show any significant change in their physiological properties, so that transmission of the afferent impulses is only inhibited in deep torpor. The first signs of arousal and an increase in brain temperature are accompanied by the restoration of transmission along the thalamo-cortical pathways. The negative component of the primary response and "facilitation" of /191 the formation of the nonspecific secondary response appear only after the brain temperature has reached 18-20°C.

Similar definite and consecutive changes take place in the post-synaptic potentials of the apical dendrites of the cerebral cortex. Attention should be called first of all to the significant resistance of these dendrites to low temperatures, which distinguishes these dendrites from the same structures in the cortical neurons of homoiotherms, in which the post-synaptic potentials of the apical dendrites are suppressed at a brain temperature of 19-22°C (288, 312).

The absence of a pronounced positive component in the dendritic potentials under conditions of deep torpor (with persistence of the negative wave) may indicate that the body of the pyramidal neurons, the local excitation of which results in the superficial positive wave of the dendritic potentials, becomes refractory to stimulation at temperatures at which the apical dendrites are still capable of local depolarization as a result of electrical stimulation of the superficial cortex.

This may indicate, first of all, the presence of active and probably inhibitory properties, in relation to the pyramidal neurons, in the dendrites of the upper layers of the hibernant cortex. This hypothesis is in agreement, to a certain extent, with the ideas of I.S. Beritov and A.I. Roytbak concerning the nature of cortical inhibition (19). In any case, this hypothesis seems quite adequate within the framework of an analysis of hibernant cerebral activity.

It would be valuable to compare the special properties of the post-synaptic potentials of the apical dendrites of the pyramidal cells, which we have described above, with the data obtained by means of histochemical and electron-microscopic studies. An analysis of the facts concerning the temperature resistance of the post-synaptic potentials of the dendrites is facilitated by the previous literature indicating a special role for oxidative metabolism in the dendrites of the cortical neurons (163, 676), as well as by our own data on the preservation of high succinic dehydrogenase activity in the dendrites of the pyramidal neurons.

In connection with this question, the electron-microscopic data on the significant percentage of "active" axodendritic synapses in the neocortex of hibernants in a state of deep torpor acquire theoretical significance. The significant accumulations of synaptic vesicles oriented towards the presynaptic membrane, the high contrast of the pre- and post-synapse, and the preservation of the internal mitochondrial membranes in the synaptic buds all suggest that the synthesis of chemical mediators and thus the capacity for trans-synaptic transmission continue without interruption under conditions of deep torpor. /192

The facts relating to the morphochemical and physiological organization of the dendrites of the cortical neurons in hibernants thus indicate that these neuronal structures may play an exclusive role in the cerebral mechanisms of mammalian hibernation.

* * *

We began this evaluation of the problem as a whole with an analysis of the electrocortical responses primarily because it is only in the cerebral cortex that it has been possible to follow both the "individual" behavior of the functional unit, the neuron, and the activity of their associations during the complex work of the hibernant brain in the various stages of natural adaptation. These changes are naturally determined, to a great extent, by the ascending stimuli from the subcortical systems and formations of the archicortex; we will now proceed to an analysis of the activity of these formations.

It follows from the experimental data that the "disconnection" of the subcortical formations and the cerebral cortex as the depth of hibernation increases spreads from structure to structure in the following order: cerebral cortex — reticular formation of the brainstem — medial thalamus — lateral thalamus — hypothalamus — hippocampus and some other structures of the limbic system (amygdalus). The restoration of electrical activity follows the reverse sequence. Within the framework of these general laws, it might be useful to consider the following questions: a) the interaction of specific and nonspecific systems in the process of hibernation, and b) characteristics of the systemic bioelectric changes of the generalized type during the various stages of torpor.

The histographic analysis of the EEG shows quite clearly that the "systemic" bioelectric changes of the diffuse type correspond to a definite temperature range and stage of hibernation. Desynchronized electrical activity in the cerebral cortex and the "desynchronization reaction" produced by external stimulation or the administration of chemical stimulants are the least resistant to a decrease in temperature. The possibility of desynchronization of electrical activity is preserved only in the temperature range from 34 down to 26°C (we are speaking here of electrical activity in the cortex). /193

The predominance of synchronized forms of biopotentials in the EEG and particularly of the "burst component" remains possible in the temperature range from 28 down to 22°C. Episodic slow waves as well as individual spike discharges and sporadic, paroxysmal, group discharges which tend to fade out as the brain temperature drops are seen in the range from 17 down to 11°C.

Finally, depression of activity usually sets in at a cortical temperature in the neighborhood of 7-10°C.

A phenomenological description of the diffuse bioelectric changes — desynchronization, synchronization and depression — thus demonstrates a connection between the bioelectric activity of the brain and a certain temperature range. This very important finding can serve as the basis for certain conclusions. In the first place, it indicates that the activity of the cerebral systems which take part in the organization of these types of bioelectric activity is limited to a definite temperature range.

It is important to note, in connection with this conclusion, that both an analysis of the electrophysiological data obtained abroad (267, 317, 320, 417, 437, 589-592) and the results of our own studies (231, 232, 236, 241, 244) indicate that the temperature range for the systemic bioelectric changes described above is approximately the same for all representatives of the hibernants. Consequently, for all representatives of the hibernants, the bioelectric changes reflecting definite interconnected functional states of the central neurons are fixed within a definite temperature range and, more important, a definite period of adaptation.

This conclusion makes it possible for us to disagree with the rather widely held opinion, expressed in the papers of non-Soviet investigators of the hibernant brain (320, 417), that there are so-called species differences in the relationship between electrical activity and brain temperature. Statistical analysis of the data shows that the temperature range of generalized bioelectric changes, which is reflected quite accurately in the dynamics of electrogenesis, is a criterion of the essential similarity, rather than of the differences, in the cerebral activity of the various representatives of the hibernants. In other words, we are dealing with general principles of the activity of cerebral systems in hibernating mammals under conditions of natural adaptation, in any case to the /194
extent that this is reflected in the EEG. This basic position has been confirmed by further evaluation of the problem of the "systemic" organization of cerebral activity in the hibernant.

* * *

As was shown above, the period of suppression of bioelectric activity in the nonspecific structures of the brainstem is preceded by a stage characterized by the predominance of generalized desynchronized activity, especially in the formations of the brainstem. This same period is also characterized by both an increase and a "spontaneous" decrease in amplitude of the negative component of the primary response.

In susliks and hedgehogs, the background bioelectric activity in the mesencephalic reticular formation and especially in the medial segments of the optic thalamus begins to fade out below 26°C. The initial period of natural adaptation is characterized by activation of the nonspecific cerebral systems and "facilitation" of stimulus transmission in the upper layers of the cerebral

cortex. Inhibition of the afferent signals in the reticular formation of the brainstem results in suppression of the activated forms of electrical activity; this coincides with the initial drop in amplitude of the negative component of the primary response and the secondary discharges.

The decrease in "spontaneous" activity in the mesencephalic reticular formation in hibernants is accompanied by "facilitation" of the activity of the nonspecific thalamic nuclei, expressed in the EEG by generalization of the "burst component" which is quite similar in origin to the alpha rhythm of man. This type of activity arises primarily in the structures of the diencephalon and cerebral cortex. In the medial nuclei of the optic thalamus and the hypothalamus, such "burst" spindles may be followed by cortical bursts having a duration of 50-60 milliseconds, which are apparently a consequence of circular excitation within the limits of the thalamo-cortical circular connections. As the depth of hibernation increases, the "burst activity" becomes generalized in the structures of the mesencephalon and archicortex. The onset of hibernation and arousal are consistently accompanied by the appearance of this type of electrical activity.

The onset of hibernation is thus associated with the successive inhibition of the functional activity of the reticular activating system and facilitation of the activity of the nonspecific thalamic structures. /195

Experiments have shown that the disappearance of "burst activity" from the EEG of the cerebral cortex and subcortical structures may be indicative of the final onset of hibernation. This period is also characterized by preservation of background activity in the specific thalamic nuclei, the hypothalamic region and the structures of the limbic complex. The ability of these parts of the brain to generate regular forms of bioelectric activity is preserved even after the brain temperature has fallen to 9-17°C. Consequently, it is these parts of the brain which are least sensitive to low temperatures; at these temperatures, the activity of these structures can be maintained in the presence of minimal energy reserves. In connection with these facts, the question naturally arises of the role of the structures of the functional limbic complex in the maintenance of cerebral homeostatic equilibrium and in the cortico-subcortical interactions.

The presence of a relatively constant rhythmic activity in the hippocampus, spetum and olfactroy lobe of the Digger ground squirrel was confirmed by the work of Strumwasser (589). In this work, however, the author did not go beyond establishing the fact that electrical activity is preserved in these cerebral formations at low temperatures and during torpor. Besides, these studies did not constitute the type of systematic analysis of the different stages of adaptation which is the basic feature of our own experiments.

It follows from the experimental findings that torpor is preceded by a series of so-called "tentative" lapses into hibernation and periods of arousal. These are always characterized by the appearance of increasing bioelectric activity in the hippocampus followed by generalization of the bioelectric changes to the various parts of the brain. This process, which begins with the appearance of regular activity in the hippocampus, continues for a considerable period of time and usually leads to complete arousal of the animal, followed in turn by renewed

torpor. The definitive onset of hibernation and torpor may be preceded by a number of such "attempts". The so-called tentative hibernation and arousal of hibernants is thus accomplished primarily by the intervention of the hippocampus, which plays the role of a "trigger" in this process.

The hippocampus and some other structures forming a part of the functional limbic complex are still able to generate normal and regular types of electrical activity at a brain temperature of 9-11°C. Impulse activity of the individual neurons can still be recorded in the cellular elements of the hippocampus at a brain temperature of 6-8°C. All of the waves of the EEG under these conditions are strictly synchronous in character and are usually generalized to the limbic cortex, the hypothalamus and the amygdaloid nuclei. Besides these forms of generalization there are also spasmodic equivalents. Synchronization of activity in certain parts of the archicortex is thus an outward expression of irradiation of hippocampal influences to adjacent regions and may be produced by quite limited expenditures of energy. /196

The formation and generalization of paroxysmal activity which take place with the involvement of the hippocampus and other formations of the limbic complex may almost certainly be dependent on other factors besides the "metabolic" characteristics of the neurons of these parts of the brain. It is quite possible that the cybernetic functions of the hippocampus are realized not only with the aid of the usual trans-synaptic excitation but also by way of extra-synaptic neuronal stimulation.

In connection with an analysis of the role of limbic structures in the stages of hibernation, definite interest attaches to the results of micro-electrode and electron-microscopic studies which we carried out on the hippocampal neurons of hibernants which were either subjected to physical cooling or in a state of natural torpor.

One finding in particular is worthy of being emphasized: the presence of action potentials in the hippocampal neurons at quite low brain temperatures (6-8°C) and the preservation of "active" synaptic contacts or synapses which are structurally "ready" to be excited under conditions of natural torpor.

Finally, the well-known existence of two "trigger" zones in the neurons of the hippocampus which are capable of generating excitatory stimuli independently of one another may also help to clarify the special properties of the cellular elements in this part of the brain during torpor.

During various stages of natural adaptation in hibernating mammals, the neurophysiological mechanisms of hibernation are thus based on the regular sequential uncoupling (demonstrated in our experiments) of the structures responsible for the maintenance of cerebral homeostasis. The homeostatic optimum depends entirely on strict adherence to this sequence. The simultaneous /197 or parallel excitation or inhibition of several functional systems, which may take place under conditions of extraneous arousal, is merely a particular case of these universal mechanisms operating in the brain of hibernating animals.

The neurophysiological concept of mammalian hibernation which we have developed on the basis of the experimental results consists of the consecutive, "programmed" excitation and uncoupling of four functional levels: the brainstem, thalamus, archicortex and neocortex. The smooth onset of hibernation and the physiological arousal of the hibernant organism from a state of deep torpor is made possible precisely by the differentiation of the functions of these systems within the context of natural adaptation and the preservation of the ultrastructure of the pathways of synaptic transmission. At each stage of hibernation, a dominant role is played by a particular structural and functional system (Fig. 72).

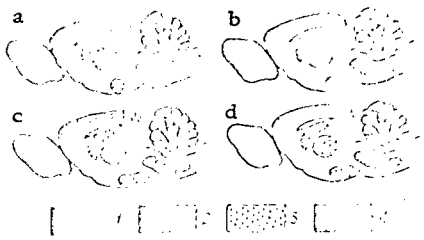

Figure 72. Schematic Representation of the Sequence of Functional States in the Various Parts of the Brain of Hibernating Mammals during Various Stages of Natural Adaptation: a - Onset of Hibernation, b - Torpor, c - Incipient Arousal, d - Definitive Arousal from Torpor; 1 - Incipient Inhibition, 2 - Inhibition, 3 - Incipient Excitation, 4 - Excitation.

We have no intention of "tying up" our ideas concerning the cerebral mechanisms of mammalian hibernation into an integrated neurophysiological concept of natural adaptation since, as pointed out by I.M. Sechenov (188), despite all the distinctiveness and internal perfection of the functional systems of the animal organism, their appropriate activity remains to a great extent dependent on the active participation of external ecological factors in the environment.

B. The neurochemistry of hibernation and arousal. The results of our neuropharmacological studies have shown that the arousal effect of chemical stimuli on the EEG is limited to a definite temperature range. Using adrenomimetic preparations such as epinephrine /198
and amphetamine, arousal can be achieved in the range from 19 to 26°C. Regardless of the original functional state and the dominant form of electrical activity, the adrenomimetics produce a statistically significant shift in the frequency spectrum in the direction of the appearance of rapid bioelectric potentials of low amplitude. The stimulatory effect of adrenomimetic agents is thus "localized" to the temperature range between 19 and 26°C.

Atropine and galanthamine may result in the appearance of the electrographic signs of arousal at lower temperatures and in states of deep torpor. Once started at 14-16°C, arousal is accelerated after the brain temperature reaches 19-20°C. The cholinotropic preparations which have an arousal effect on the EEG may also potentiate the activating effect of the adrenomimetics, which tends to fade rapidly. With some caution, our experimental results permit us to hypothesize that the stimulatory effect of galanthamine is realized via the limbic system with the subsequent involvement of the activating system of the brainstem.

It is possible to demonstrate a definite level of acetylcholine-synthesizing processes in the neurons of the archi- and neocortex even in the deepest stages of torpor. Based on electron-microscopic findings and some of the data in the literature (561), it can be concluded that the synaptic vesicles (fine transparent vacuoles) in the buds of the axodendritic synapses of the neo- and archicortex are mostly acetylcholine-containing, although some of the synaptic buds also contain large, dense, osmiophilic vesicles which may contain norepinephrine (674). In connection with these facts, it is possible to define the following stages as being directly or indirectly related to arousal: acetylcholine synthesis, which continues even in a state of deep torpor; the activation of cholinergic processes resulting in an exponential drop in acetylcholinesterase content (561) and changes in the ultrastructure of the synapses of the archi- and neocortex; incipient arousal (generalization of the electrical activity of the limbic structures); an increase in MAO in the hippocampus, hypothalamic nuclei, the septal region and the medial cortex; activation of cortical electrogenesis; and definite arousal of the animal.

Histochemical data on the distribution of AChE during hibernant arousal also tend to support the hypotheses arrived at previously on the basis of the results of electron microscopy. Actually, the increase in the number of active synapses (expressed as an increase in the number of synaptic vesicles containing mediator and lying close to the pre-synaptic membrane), the loosening of the synaptic fissure and the appearance of pores therein, and the increase in contrast of the pre- and post-synapses may be looked upon as signs of activation of cholinergic processes in the hibernant brain during the very early stages of arousal. During this period the animal shows no outward signs that it is emerging from hibernation. Electrographically, however, one can record local activity in the limbic structures and especially in the hippocampus and amygdalus. /199

In view of all that has been said, it is possible to formulate an hypothesis as to the dual nature of arousal in hibernating animals, which may therefore consist of two stages - a cholinergic stage and an adrenergic. Adrenergic arousal sets in quite rapidly but is heterogeneous by its very nature and incorporates two periods which are connected with the effects of endogenous and exogenous mediator hormone. The second stage (which arises due to the secretion of endogenous epinephrine) leads to definitive arousal, but the first is usually short-lived and may terminate in a lapse back into hibernation. This leads to the conclusion that a persistent activating effect can be achieved only by mobilization of endogenous epinephrine.

This hypothesis is strengthened considerably by certain data which have been published abroad only very recently. We are referring first of all to the work carried out in the laboratory of P. Suomalainen (581, 618-620) in which it was shown that mammalian hibernation (the European hedgehog) is accompanied by a significant decrease in the content of the mediator hormone in the brain (20-30% compared to the active state). Besides, the content of dopamine, a precursor of norepinephrine, was found to rise during hibernation, particularly during periods of torpor close to the arousal of the hibernant. These facts may serve as further clarification for the stepwise course of adrenergic arousal: the persistent stimulatory effect is absolutely dependent on intense hormone

synthesis leading to the establishment of the "optimal" hormone concentration in the brain and blood which is sufficient for the stabilization of definitive arousal.

It seems to us that these considerations are also supported by the histochemical localization of MAO in the brain. In all the subcortical formations and the structures of the brainstem, as well as in the cerebral cortex, the mean concentration of this enzyme rises during arousal from torpor. This is indicative of a significant activation of adrenergic processes in the cerebral structures, especially in the hypothalamus, hippocampus, medial nuclei of the optic thalamus and the nucleus caudatus. /200

Cholinergic arousal is connected in some way with the initial neutralization of the blocking effect of AChE in the cerebral structures. This point of view is supported by the studies with galanthamine. Persistent arousal is guaranteed by the interaction of central cholinotropic substances with the receptor fields in the limbic complex, where "spontaneous" electrical activity can be detected, and by the maintenance of the synaptic ultrastructure during this stage of hibernation.

The rapidity with which cholinotropic arousal is accomplished is due first of all to the nature and properties of the mediator and is responsible for the function of these cerebral systems as a sort of "trigger". The adrenergic systems of the hypothalamus and the reticular formation of the brainstem are responsible for establishing an optimal level of cerebral activity and for coordinating the vital functions of the waking animal.

It is quite interesting that the pharmacological activity of the stimulators can also be related to the content of some other substances in the brain. We are thinking of the extremely interesting data (especially considering the relationship between the tocopherols and acetylcholine synthesis) obtained by N. I. Kalabukhov et al. (25, 97, 99-101) with reference to the intracerebral localization and concentration of vitamin E in hibernating rodents.

This then is a possible outline, from our point of view, of the neurochemical nature of natural hibernant arousal which is more or less in agreement with the latest experimental data. This fits in a definite way into the neurophysiological concept of hibernation which we have postulated and points to the possible chemical basis for the sequential "switching on and off" of hibernant cerebral systems guaranteeing the appropriate cortico-subcortical interaction during the emergence of the animal from a state of torpor.

C. Physiological and morphochemical prerequisites for memory in hibernating animals. What might be the role of the neurophysiological and neurochemical relationships described above in the maintenance of the principal forms of complex behavior in hibernants emerging from torpor ? We will attempt to consider this question from the point of view of an analysis of the mechanisms of memory in hibernants — animals which remain for long periods under hypothermic conditions but are able to retain acquired habit patterns. /201

The effects of prior and subsequent hypothermia described in the literature enable us to classify physical cooling in the same group with other physical agents, such as electroshock and hyperthermia, which "erase" the memory: it is usually effective for from one to four hours after conditioning and successfully blocks the consolidation of memory in homoiothermic animals (359-361, 596-598). In hibernants, cold seems to be considerably less effective (510, 512 and others). It might be suggested that the physiological mechanisms of short-term memory and early consolidation, particularly the reverberation processes in the central neurons, the phenomenon of post-activational facilitation and, finally, the synthesis of mediator in the synaptic bud and its transport into the sub-synaptic fissure are more resistant to the effects of cold. What facts have we uncovered and can we glean from the non-Soviet literature which might support these hypotheses?

The first of these is the persistent and regular "spontaneous" electrogenesis which can be detected in some parts of the hibernant brain at cerebral temperatures close to 0°C (137, 230-246, 317, 320, 588-593). This phenomenon is seen most clearly in the limbic and hippocampal structures, the amygdalus and the limbic cortex. During some stages of hibernation (we are thinking particularly of the "tentative" onset of hibernation and arousal which we have described above), the electrical activity of these parts of the brain is characterized by cyclic changes in structure. A comparison of these facts with some of the data found in scattered other publications (317, 320, 588-590) provides a basis for concluding that we may be dealing, in this case, with a special form of temporary memory mediated via the basic homeostatic mechanism of thermoregulation in hibernants. This is especially attractive since the possibility that the limbic structures, especially the hippocampus, may play a role in the sense of time has been discussed in the literature (639, 661, 677).

Prolonged observations of the amplitude-modulated electrical activity in the hippocampus and recordings of the action potentials of individual neurons in this same area at temperatures of 6-8°C have indicated that the pyramidal cells of the hippocampus may be resistant to low brain temperatures during torpor.

It is difficult to specify at the moment at what level (pre- or post-synaptic) /202
the endogenous and induced activity of the cerebral structures (the neurons of the hippocampus and cortex, the apical dendrites of the pyramidal cells) is blocked during torpor. It seems logical to suppose that this probably takes place via a presynaptic mechanism, although this supposition is contradicted by some of the results obtained in our own electron-microscopic studies (although the presence of a sufficient number of synaptic vesicles in a presynaptic terminus still does not prove the continued effectiveness of the synaptic mechanism as a whole).

Long-term cyclic changes in electrical activity, the persistent excitability of the post-synaptic membrane of the cortical dendrites, and the continued synthesis of mediator in the synaptic buds thus provide a basis for relating the phenomena which we detected to the mechanisms guaranteeing the preservation of memory in hibernants during the prolonged periods of extremely low brain temperature associated with torpor.

From our point of view, among the morphological criteria of changes in the central neurons of hibernants which might be used in an analysis of trace phenomena (memory), attention should be called to the following three: the "structural modifications" reported by Dr. and Mrs. Scheibel (cited in (660)) in the spinous processes of the axodendritic synaptic fields, the presence of "active" presynaptic termini in animals which have been in hibernation for prolonged periods (just analyzed above), and finally the histochemical localization of AChE in the hibernant brain.

A. and M. Scheibel were able to show that the number of spinous processes in the dendrites of the cortical neurons in hibernants is sharply reduced during torpor. As might be expected, these authors also reported that the number of axodendritic synapses is restored to normal during arousal from hibernation. It is tempting to suggest, as was done by G. D. Smirnov (660), that this structural phenomenon may be related to the morphological reconstruction of the cortical synaptic mechanism and may be looked upon as a possible guarantee of the preservation of the engram. It should be point out that with the onset of torpor the spinous processes are "retracted" into the body of the dendrite, thus significantly interfering with the transmission of excitation from the presynapse to the post-synaptic membrane. The wide fissures in the axodendritic synapses which we detected during electron-microscopic studies can also be interpreted as supporting the likelihood of the facts described by the Scheibels. A similar mechanism of "synaptic modifications" may serve as a flexible guarantee of the maintenance of long-term memory in hibernating animals (660, 671, 672).

The fact that there are still "active" synapses in the neocortex and hippocampus of hibernating hibernants can also be related to the increased AChE content in the brain of hibernants during torpor. This phenomenon is interpreted /203 in the literature as proof of an increase in the number of synapses (659, 667). Correlations between the formation of new synapses and the AChE content are often observed during post-natal ontogenesis.

There is some reason to believe that the structural modifications in the area of the synaptic junctions about which we spoke above must be brought about by the genetic apparatus of the neurons, the activity of which varies significantly during the vital activity of hibernating mammals. There are still very few facts about the role of the genetic apparatus of the central neurons in hibernants. It has been shown that some parts of the brain, particularly the limbic structures, are characterized by the most intense and dynamic RNA synthesis during all stages of hibernation and especially during torpor. It is possible that this should not be looked upon as an exception which can be observed only in hibernants. It has been shown experimentally (243) that the hippocampus in general is characterized by the highest levels of RNA and protein synthesis.

Quite recently, Japanese cytologists showed (570) that orotic acid is incorporated into RNA in the brains of hibernating animals even during deep torpor. Some studies carried out in the laboratory of A. V. Palladin (17, 151) also point indirectly in the same direction.

Finally, the last indication that the biosynthetic apparatus of the neuron plays a role in the regulation of periods of activity and hibernation can be arrived at only by extrapolation. We refer here to the extremely interesting experiments of Strumwasser dealing with the mechanisms of the circadian rhythms in vertebrates and invertebrates during the onset of hibernation and torpor (594, 595). He was able to show that administration of actinomycin, an inhibitor of DNA-dependent RNA synthesis, completely disrupts the regulation of circadian rhythms in mollusks, erasing their genetically determined temporal memory. On the basis of these facts, it can be hypothesized that the /204 genetic apparatus of the central neurons is also characterized by periodic activity during the various stages of the vital activity of hibernating mammals, including the state of deep torpor (570). This may also guarantee a flexible reserve for the structural changes in the hibernant brain and the specific transformations of the macromolecules (RNA and protein) playing a role in the consolidation of memory.

CONCLUSION

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In the wonderful book by Margaret and Lorissa Milne entitled "The Senses of Animals", one finds the passage: ". . . until such a time that astronauts will be able to fall into a state of hibernation in a special cabin with automatic equipment which will guarantee their return to a normal temperature, the cost of launching astronauts while in a cooled state would significiantly exceed any benefits to be gained from such a launch."

Rather similar thoughts arise in connection with an analysis of the experimental material pertaining to the activity of the hibernant brain. These animals can remain in a state of deep torpor for several weeks and months while maintaining a thermodynamic equilibrium with the external environment. Their brain does not lose the ability to generate electrical activity and to establish a special but effective functional relationship between its parts even at a temperature close to 0°C. The spike-generating properties of the neuronal membranes, active synaptic junctions, the synthesis of mediator, post-synaptic excitability, and the cyclic course of the process of excitation are all preserved.

In such a state, interruption of the oxygen supply to the hibernant brain for period of several hours does not produce symptoms of menacing hypoxia or disruptive structural changes in the cellular elements of the brain; arousal and normothermia, the restoration of unconditioned reflex activity, memory and complex forms of behavior are not prevented. The ability of the cerebral systems of hibernating animals to regenerate information fixed before the onset of hibernation is particularly striking. It is precisely this area of the problem which seems to us to be extremely important and which is receiving the most attention at the present time. The studies are unsystematic, preliminary and in my opinion, largely intuitive in nature. Since the information which is presently available on /206 the current activity of the hibernant brain is extremely limited and uncoordinated, we are completely helpless when the question arises of the mechanisms for the preservation and restoration of this activity during emergence from torpor. Acknowledgement of this situation, about which we feel there can be no doubt, must be the first step in solving the problem of the neurophysiology and neurochemistry of arousal and the early recuperative period which follows immediately after the emergence of the animal from hibernation.

In connection with what has been said above, the study of the mechanisms of the preservation of memory and complex forms of behavior in hibernating mammals which have been in deep torpor for prolonged periods occupies the most important place in the scientific analysis of the high resistance of the neural elements to low temperatures. The possibility of controlling this resistance, of simulating its basic principles and mechanisms and using them under conditions of homoiothermia and during the prolonged stay of man and animals under extreme conditions, represents the basic theoretical and experimental direction of the studies which must be devoted to the problem of mammalian hibernation in the interests of practical biology and medicine.

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