ACTA BIOLOGIAE EXPERIMENTALIS

Jan Dembowski
1889—1963

IN MEMORY OF JAN DEMBOWSKI

On September 22, 1963, Professor J a n D e m b o w s k i, an eminent Polish biologist, died in Warsaw. His life and scientific activities were closely connected with the history of the Nencki Institute of Experimental Biology. During half a century of his scientific career, Jan D e m b o w s k i worked in this Institute over a period of 30 years and it was precisely in its laboratories that most of his experimental investigations were carried out.

He was working at the Nencki Institute since 1918, that is, from its very foundation, successively holding the posts of an assistant, department chief, and, afterwards, also a director of basic research of the Institute. As a director (1947—1960), he rendered great services in the reconstruction of the Institute, completely destroyed during World War II and, subsequently, in its development.

Professor J. D e m b o w s k i was also one of the founders of the ACTA BIOLOGIAE EXPERIMENTALIS (vol. 1 appeared in 1928) and, until his last days, a member of the Editorial Board of this journal.

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Jan D e m b o w s k i, a son of Kazimierz Dembowski, an engineer-technologist, and Józefa Dembowska née Mazurkiewicz, was born in Petrograd, Russia, on December 26, 1889. It was also in the then capital of Russia that, after completing his zoological studies in 1912, he started his scientific career under the guidance of V. A. D o g e l at the Department of the Invertebrates Zoology, Petrograd University. His two publications, one, dealing with the eye anatomy of the crab, *Ocypoda ceratophthalma*, the other, dedicated to the interpretation of the motion mechanism of gregarines come from that period. In 1914, D e m b o w s k i, sent by the Petrograd University, continued his specialization at the Biologische Versuchsanstalt in Vienna. Upon the outbreak of the World War I, D e m b o w s k i, then a Russian citizen, was committed to the internment camp where he spent two years. After his release, he under-

took his work anew and, together with H. P r z i b r a m, investigated the bonds between chemical properties of the tyrosinase and animals' adaptive coloration, as well as a relationship of the coloration of young salamanders with the coloration of the background. It was also in Vienna that D e m b o w s k i's first monograph was elaborated (Das Kontinuitätsprinzip und seine Bedeutung in der Biologie, 1919), which constituted a criticism of some concepts in the field of the evolutionism, genetics and embryology, then accepted.

In 1918, D e m b o w s k i arrived in Warsaw and undertook scientific work at the Nencki Institute which was then founded. The same year, he married W i k t o r i a   S t a n i s ł a w a   D e m b o w s k a née Swiniarska. Professor W. S. Dembowska (1891—1962), a well-known and appreciated (particularly for her classical studies on the regeneration of the *Stylonychia metilus* and maritime *Hypotricha*) biologist, was throughout her life her husband's nearest friend and closest scientific associate.

During a period of the first two years of his work at the Nencki Institute, two fundamental trends in his experimental interests were developed, to which he kept faith for the rest of his scientific research work, that is, (1) animal psychology (ethology) and (2) physiology of protozoa.

In the former trend, the ciliate *Paramaecium caudatum*, the crabs *Dromia vulgaris* and *Uca pugilator*, as well as the larva of the caddis fly *Molanna angustata* and in the latter — exclusively *Paramaecium caudatum* were his experimental objects.

In 1920, Dembowski obtained his doctor's degree at the Warsaw University and, in 1922, for his investigations on the *Paramaecium caudatum*'s food preference, he became a Docent. Professor Konstanty J a n i c k i, an eminent zoologist and parasitologist, was his supervisor. Over 1924 and 1925, Dembowski, as a scholarship holder of the Rockefeller Foundation worked, together with his wife, at the maritime stations Villefranche, France, and, Woods Hole, Mass., USA, where he experimentally investigated the behavior of the crabs *Dromia vulgaris* and *Uca pugilator*. Up to 1927, Dembowski was employed as a senior research associate at the Department of General Biology. Between 1927 and 1934, he was in charge of the Experimental Morphology Department and, over the period of 1933—1934, he also hold the office of the President of the Nencki Institute. His studies on the geotaxis of the *P. caudatum* and on the plasticity of the instinct of the *Molanna angustata* larva come from that period.

In 1934, Dembowski was appointed a professor at the Department of Biology, Stefan Batory University in Wilno and occupied this post up to the World War II. In Wilno, he organized a very well equipped labora-

tory where he employed his numerous disciples and wrote a book, „Animal Psychology" which was about to be ready for press in 1939. Most of those achievements were destroyed after the outbreak of World War II. Some of his associates perished, the remaining ones were scattered all over the world. D e m b o w s k i survived the Nazi occupation in Wilno where he made his living by selling books and writing, for other people, applications and petitions.

After Wilno was liberated in 1944, he went to Moscow where, up to 1947, he held the office of a scientific attaché of the Polish Embassy and, at the same time, worked scientifically at the Medical Academy Institute of Experimental Biology. During his stay in Moscow, D e m b o w s k i revised and submitted for publication in Poland his book, „Animal Psychology", and worked on another book, „Psychology of Apes".

„Animal Psychology" (published by the „Czytelnik" Press in 1946) was a profound and, in Polish literature, quite a new elaboration of this extensive branch of knowledge in the development of which the author and his associates took active part. „Psychology of Apes" (published by the „Książka" Press in 1946), constituting the continuation and conclusion of the former work, presents a comprehensive experimental and theoretical material.

In 1947, after his return from Moscow, D e m b o w s k i was in charge of the Nencki Institute, temporarily reëstablished in Łódź, (Lodz), and, at the same time, he managed in person its Department of General Biology. In addition, he was the head of the Experimental Biology Chair at the Univeristy of Łódź (1947—1952).

In 1952, he took the sponsorship over the construction of a new building of the Nencki Institute in Warsaw to which finally all its departments were transferred from Łódź in 1955.

Professor Dembowski was a scientist with a versatile biological education and extensive horizons of knowledge and interests. Besides the psychology of animals and physiology of protozoa, the sphere of his interests covered such problems as, morphogenesis, regeneration, genetics and evolutionism, as well as methodology and organization of the science. He published several reports and theoretical articles in these fields.

D e m b o w s k i did not restrict himself to the specialistic scientific work only. He published many popular science books and brochures, among others, a classical work in Polish literature of this subject, entitled, „The Natural History of a Protozoan". In general, Jan D e m b o w s k i's bibliography consists of over 120 titles, in this number 28 experimental papers and 9 books some of which were published in five editions and translated into foreign languages. Thus, for instance, „Animal Psy-

chology" appeared in two Polish editions (in 1946 and 1950), one German (in 1955) and one Russian (1959). „Psychology of Apes" was twice issued in Polish (1946, 1951) and once in Italian (1950), German (1956) and Russian (1963).

Many times, Professor D e m b o w s k i revised and extended these two books. It was still at the beginning of 1963 that new paragraphs were added by him to the Russian translation of the „Psychology of Apes".

Besides his own scientific creative work, Professor D e m b o w s k i's attention was paid to a considerable extent to the pedagogical activity.

Before the last war, D e m b o w s k i's disciples published over 30 experimental papers of which 6 were doctor dissertations. When he returned to Poland, his department was organized anew and his associates were mostly students who graduated only about 1952. During a period of recent 11 years, D e m b o w s k i's disciples published over a hundred experimental papers and 11 of them made the doctor's degrees.

Jan D e m b o w s k i's contribution to the organization of the scientific activities in Poland was of a particularly creative character. He prepared and presided over the First Congress of Polish Science (1951). On the basis of this Congress' resolution, the Polish Academy of Sciences was founded and Dembowski was elected its first President (1952—1957).

During that period, Jan Dembowski held high state offices. He was the President of the Polish Parliament (Sejm) and the Vice-president of the State Council (1952—1957).

Jan D e m b o w s k i's scientific and civic activities were highly appreciated by Polish society and by the scientific world. He was an ordinary member of the Polish Academy of Sciences, honorary fellow of the Academy of Sciences of the USSR, foreign member of the Hungarian Academy of Sciences and of the National Academy of Science in New York. Two times, he was awarded the State Prize of the 1 st degree (in 1949 and 1955). He was also an owner of highest Polish distinctions.

The work was the main activity of Professor D e m b o w s k i's life. Its intensivity was not diminished either by his retirement (in 1960), or the loneliness after the death of his wife, Professor W. S. D e m b o w s k a (1962), or even by an ever more intensified illness. Until the last months of his life, Professor D e m b o w s k i patronized the doctor and docent dissertations prepared by his pupils, supplemented the texts of new editions of his books and presided over the Scientific Council of the Nencki Institute. He also was never tired with experimenting and with ever new investigations, particularly those, dedicated to the animal psychology.

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D e m b o w s k i's experimental investigations on the animal psychology were always focused on the behavior of *Paramaecium caudatum* and on animals' instinctive actions. As to the latter problem, the larva of the caddis fly, *Molanna angustata,* as well as the crabs *Uca pugilator* and *Dromia vulgaris* were his fundamental experimental objects.

The behavior of ciliates was first investigated by D e m b o w s k i when he studied food preference in *P. caudatum* (1922a, 1922b, 1922c). By the application of an original method of diluting the food suspensions, he showed that food intake is two-phasic and that it consists of (1) the automatic and rhythmic actions of swallowing and (2) the ingestion of suspended particles. Furthermore, he found that a considerable concentration of dyes, contained in a fluid, can occur in the course of its intake. These facts threw a new light on the physiology of food intake in Protozoa and served as a starting point for subsequent investigations, conducted by other protozoologists.

It was also shown by D e m b o w s k i that, despite the fact that ciliates have the capability of the food preference, the refusal to ingest some suspensions by *P. caudatum,* even after a few days of staying in them, cannot be considered a symptom of memory — as believed by M e t a l n i k o v — but only the result of a non-specific injury of the ciliate cell body. Likewise, no conditioned response to the boundary of light and shadow could be established in *P. caudatum* (1950). At the same time, on the basis of ingeneous experiments, D e m b o w s k i succeeded in showing the nature of the error, committed by authors who, in such cases, obtained positive results.

Another range of problems of the ciliates' behavior was dealt with by papers concerned with the regularities of motion and geotaxis in *P. caudatum.* A new law of the ciliate motion, formulated by D e m b o w s k i, consisted in the fixity of the angle of reflection (about 70°) from a solid obstacle, which allows one to envisage the path of the ciliate as it moves in a vessel of any shape. The changes in the motion response were explained by D e m b o w s k i from the standpoint of normal living conditions of the ciliate and supported by special experiments.

On the basis of a series of experiments, D e m b o w s k i showed (1929a, 1929b, 1931) that the theory of the statocyst is unconvincing. At the same time, by means of accurate methods, he found that the center of gravity of *P. caudatum's* body is shifted posteriorly and that the posterior, heavier half of the ciliate's body constitutes a subtle means of its orientation, replacing the sense of equilibrium. Consequently, D e m b o w s k i developed a theory of geotaxis, according to which the taxis

is not a compulsory response but a response of a ciliate to external stimuli and a response in which a considerable degree of plasticity is manifested.

Simultaneously with the investigations on the behavior and physiology of ciliates, D e m b o w s k i analyzed the invertebrates' instinctive actions. In fact, he was one of the first zoopsychologists who started experimental research in this field. A series of investigations in this respect was initiated by his work (1923), devoted to the behavior of the larva of the caddis fly, *Molanna angustata*. The same caddis fly served as an object of further investigation (1933a, 1933b, 1937, 1950b) which showed an unusual plasticity in the behavior of an animal which manages to adapt its actions to the changes in the environment and responds purposely even under many circumstances with which it never had anything to do before. This work allowed D e m b o w s k i to carry out a keen and profound analysis of the instinct and provided much new valuable experimental material in the field of the ciliates' behavior. Similarly to *Molanna angustata,* a conspicuous plasticity in the behavior was discovered by D e m b o w s k i in the crabs *Dromia vulgaris* and *Uca pugilator* (1925a, 1925b, 1925c, 1925d, 1926).

The problem of the instinct and its plasticity constituted a subject of one of the last articles (1960) by Professor D e m b o w s k i which was written on the basis of his lecture, delivered at the Congress of Polish Anatomists and Zoologists in Kraków (Cracow) in September, 1959. At the beginning of this article, D e m b o w s k i's fundamental theses are precisely and unequivocally formulated.

"In the present lecture, I shall defend my thesis that the order of animal's movements, aimed at some adaptive effect, is always controlled by its system which examines given situations and correlates its behavior with the results of these examinations. This is a typical example of a reciprocal coupling so characteristic of the cybernetics. This modern way of the presentation of animal's behavior becomes an important ally of an ethologist. A common objection that, doing so, we examine the behavior under artificial conditions, often considerably departing from ethological standards, seems to be immaterial because we never act at will but we always try to follow the direction, pointed out by animal's organism. If we face field problems, resulting from the behavior, we investigate it under field conditions. At the same time, we remember that any more profound analysis of factors, responsible for an animal's behavior, is possible only at the laboratory where appropriate conditions are available. Let us consider, from the standpoint, indicated above, some instances of an animal's instinctive action, focusing our attention on the motoric aspect of the phenomenon."

The instances selected came from Professor D e m b o w s k i's and his disciples' own investigations.

"When the caddis fly *Molanna angustata* larva constructs its case, it applies a definite succession of movements. Broken and sieved eggshells were mostly used as building materials for this purpose. (1) The larva quickly sorts particular grains

with its legs before it is ready to seize one of them. Not all the grains are fit to be used for construction and, therefore, the material must be carefully checked; (2) The larva seizes a grain and holds it in its fore extremities; (3) The larva turns it over and, at the same time, palpates its edges with mandibles; (4) The larva tries the grain, applying it in various positions to the edges of other grains, already stuck in, thus manifesting a clear intention to find such a position in which the grain can be best and most tightly attached to them; (5) Now, there begins the operation of "sewing". The larva tightly presses the edge of the new grain to the edges of the grains, previously stuck in, connecting them firmly by means of a sticky thread which fastens the edge of the new grain to the edge of an adjoining grain or grains. This connecting thread forms a zig-zag line which we call " a seam".

"The operations described form a single, consistent and purposeful process, caused by an animal's need to hide its body inside a tube and, at the same time, depending on the construction technique. I have described here, however, only the most commonplace, routine and repeated movements. The problem becomes mare complex owing to the fact that the case of our caddis fly is specific. Thus, for instance, the tube of a natural case is dorsally arched, hence the necessity of adding each new grain by applying it at a definite angle to the previous ones. Since it is a tube that is to be built, the grains must be accurately orientated. Next, the grains, forming the dorsal part of the case, its roof and side wings, are much larger than those in the ventral part and, therefore, the acceptance or rejection of each grain depends on the degree of the progress of the entire structure or, on the place which a particular grain will take in the future structure. Once more, we have to do with a reciprocal coupling, with a control of each movement and it is only this important operation that allows to build a case with a morphologically typical shape.

"This problem must be, however, more accurately examined. In a natural case, we observe a regular anteroposterior lessening of the size of grains. The idea is that with the growth of the larva and lengthening of its body the case tube becomes too tight. The larva manages this situation by gradual forward extension of the tube and by cutting off and rejecting its posterior parts. In other words, the size of grains is correlated with the larva's dimensions. In a case, built at the laboratory we can observe grains of, roughly speaking, two sizes: (a) larger in the dorsal part and in the side wings together with the roof and (b) much finer in the ventral part. The grains in the ventral wall of the tube are, however, of a uniform size. In the laboratory, the case was built in a few hours and, therefore, the factor of the larva's growth does not occur at all.

"A characteristic feature of an instinctive action was revealed by the control experiment. We supplied the larva with a building material in the form of discs and triangles, punched out of a celluloid film. Discs were 1 mm in diameter, triangles were equal in area with discs. There were equal numbers of both kinds of grains. This experiment was carried out by Rasza S z l e p. Individual larvae built their cases in fairly different manners. There were specimens, however, with very distinct preference considerably exceeding all statistical criteria. Thus, in a few instances the entire case, consisting of several scores of grains, individually stuck in, contained only discs.

"This experiment can be also considered from another point of view. As we have already mentioned above, the larva turns many times particular grains, trying to fit it at several points of the case edge. It might be presumed that, doing so, it wants to find such a place where this new grain can tightly adhere to the

ones previously stuck in. Let us, however, check it by a control experiment. We supply the larva with a building material consisting exclusively of discs 1 mm in diameter. The larva palpates the edge of each grain "examined" as described above, turns it over and over and looks for the best position for it in which it should be placed in the entire structure. This time, discs are identical and could be stuck in without any examination. The animal takes, however, certain movements, purposeful and adaptive under usual conditions that is, in the case of a mixed material, and thus it proceeds in a routine manner under artificial conditions which do not require the selection of grains.

"The substitution of the larva's extremities in case of various kinds of amputations belongs to the same category of phenomena. These problems were dealt with in this country by G r ę b e c k i, K u ź n i c k i and K i n a s t o w s k i. After the removal of a certain number of extremities, the larva was given its building material. In most instances, larvae were able to build quite normally looking cases. It was only when the larva was compelled to build by means of one leg only that very irregular cases were made. It is possible that in such events, mechanical reasons are decisive of the results. It should be born in mind that the third pair of legs does not take part in building operations and, therefore, our experiments must deal with only the anterior and middle legs. Under normal conditions, an uninjured larva can hold any grain in a rigid position and to stuck it in at any necessary angle. The amputation of one of the four legs does not change these conditions. Operating with three legs in any arrangement, the larva builds its case that does not differ from a normal one. If a larva has two extremities only, it can still keep the grain in a motionless position and at proper angle to the other ones. This time, it is also kept at three points which are not situated along a straight line: the grain touches the edge of the tube in construction and is held by two legs. But, when a larva has one leg only, it can make use of two immobile points only, that is, the edge of a tube and this single sound leg. These two points form an axis around which the grain revolves as on a hinge which makes difficult any kind of a rigid coupling. This is the reason why the case of a onelegged larva is irregular and has only tube in which the larva can hide but it is deprived of the side wings and the roof. Such a case, without its specific structure, retains only minimum properties, allowing larva for hiding in it when attacked. Instead of closely fitting grains, the walls of such a case contain grains, irregularly stuck in, the surface of the tube is uneven and some grains are stuck in on end. In short, the entire tube is structure, hurriedly stuck together, meeting only the most rudimentary needs of its dweller and nothing more.

"The description of the behavior of young garden spiders (*Aranea diadema*) when building their nets is another example that I would like to discuss. The garden spider's cocoons, collected in autumn, were put in an unheated room which allowed us to keep them for several months. When such a cocoon is moved to another, heated room, the development process, stopped before, is resumed and — — about January — young spiders are hatched from the eggs. After they consume all their supply of the embryonal yolk, which takes place approximately after two weeks, they are ready to be experimented on. Such spiders we put by ones on vertical, square wire frames with mesh size 10×10 cm. The order of the movement of a spider, which spins the net for the first time in its life, is as follows: (1) Not satisfied with the net prepared, the spider builds its own net, made of the silk; (2) the spider draws the beam of a future net which takes place in a definite and settled manner; (3) in the center of the net formed a small platform

is built by the spider of a dense silk on which it rests motionlessly after completing its work; (4) by means of a few threads of the spiral, situated very near to each other, the spider strengthens the center of the net; (5) it draws the auxiliary spiral, serving as a sort of a scaffolding and which will be broken during the subsequent period of its work; (6) it builds a final „hunting" spiral, consisting of sticky threads.

"The work of such a spider is a very typical example of a behavior which we tend to interpret as a chain reaction. It is a very important fact, first discovered in our Department by Eliza P e t r u s e w i c z, that the order of the spider's movements specified can be subject to considerable changes which do not affect the final structure of the net built. In other words, the normal circular net can be formed with its typical shape despite the existence of considerable differences in the quality and order of particular movements.

„This can be proven by the following facts. Single spiders were placed on vertical frames. At first, such a spider crept "aimlessly" all over the frame with a "safety thread", always dragged behind. After some time, almost the entire opening of the wire net was spun around with a dense, dry and entangled silk. Instead of speculations upon sudden changes in the manner of the animal's work, it is much simpler to assume that during this initial period, the spider does not build the net at all and that its entangled silk has nothing to do with a proper hunting net. But after passing through one or two exuviae, the spider begins, directly and without any preliminaries, to build its hunting net. The formation of such net was described many times and, therefore, there is no need to do it once more. A young spider supplies us, however with important indications. It needs about 20 minutes to complete its building work. It may sit still on the frame for hours and hours, but once its work is started, the spider does not interrupt it until it is completed. The following experiment was carried out by Eliza P e t r u s e w i c z. Ten wire frames were placed vertically next to each other. At 2-minute intervals, individual spiders were placed on single nets. After 20 minutes, there were several nets in various stages of completeness, from those barely started to complete nets. Now, spiders were transferred from one net to another in various combinations. For instance, a spider which just started its work, was transplanted into the frame with almost finished net and the producer of it took the former's place or vice-versa, that is, a spider of phase 4 is taken to phase 1 and that from phase 1 — to phase 4. There can be many combinations but the experiments always consisted in transferring spiders to nets of a different phase of finishing than their own nets. These investigations, made by Eliza P e t r u s e w i c z and, much later, confirmed by Rasza S z l e p, did not yield uniform results. It can be, however, stated in a quite general form that a spider can adapt itself to new circumstances and conditions it finds in a new place, that it can skip certain phases of its work, repeat the work already done or, even take at once the place in the center (where it "lies in wait") of a net, fully constructed by some other insect.

"The things, described above, cannot be in conformity with the hypothesis of chain reflexes. Although it is only the behavior of spiders that we have described which cannot be accepted as a rule for other animals, the possibility of knocking the bottom out of the principle itself of the chain reflexes establishes a precedent which we can always refer to".

In the next part of his lecture, D e m b o w s k i presents a critical review of both older and modern literature, dealing with the instinctive

behavior, with a particular consideration of such authors as, L o r e n z, T i n b e r g e n, T h o r p e and P r o m p t o v. An estimate of the contemporary state of research in this respect, as well as investigation trends, promising a further progress, are thus discussed in the conclusion of his lecture:

"To recapitulate our considerations of the instinct, it should be emphasized that many valuable and interesting facts, pertaining mostly to the behavior of birds have been discovered over the recent decades. The theoretical aspect of these facts, however, has admittedly been insufficiently made use of. Indeed, we lack a satisfactory theory of instinct.

"In the light of this statement, many an argument and view become irrelevant, in particular a debate whether the instinct is or is not a form of a reflex. Mostly, it is a matter of convention and it will remain so until exact definitions of both the reflex and the instinct are formulated. The controversy over the instinct being inborn or acquired is also purely verbal. It is an indisputable fact that, without exception, each character of a living organism must be derived from something to which it does bear no resemblance and it appears as a result of a very complex process of individual development during which it is impossible to distinguish the inborn from acquired elements. From this point of view we can hardly agree with either L o r e n z or P r o m p t o v who point out the possibility of distinguishing the inborn element in the ontogenesis but they fail to indicate how it is to be done in practice.

"Eventually, biology must turn to more modern ways of reasoning in which it will be effectively helped by the already powerful cybernetics".

Leszek Kuźnicki,

on behalf of the Department of General Biology,
The Nencki Institute of Experimental Biology,
Warsaw, Poland

AN INCOMPLETE LIST OF D E M B O W S K I'S ORIGINAL PUBLICATIONS ON ANIMAL BEHAVIOUR

I. ORIGINAL PAPERS BOTH EXPERIMENTAL AND THEORETICAL

1922a — Über die Nahrungswahl und die sog. Gedächtniserscheinungen bei Paramaecium caudatum (in Polish with German summary). Trav. Laborat. Biol. Génér. L'Inst. Nencki, *1* (*1*) 1.

1922b — Weitere Studien über Nahrungswahl bei Paramaecium caudatum (in Polish with German summary). Trav. Laborat. Biol. Génér. L'Inst. Nencki *1* (*2*), 1.

1922c — Über den Einfluss der Suspensionskonzentration auf die Anzahl der gebildeten Nahrungsvakuolen bei Paramaecium caudatum (in Polish with German summary). Trav. Laborat. Biol. Génér. L'Inst. Nencki, *1* (*5*), 1.

1923 — Experimentell-biologische Studien über die Larve der Köcherfliege Molanna (in Polish with German summary). Trav. L'Inst. Nencki, *2* (*31*), 1.

1924 — Über die Bewegungen von Paramaecium caudatum. Arch. f. Protistenk., *47*, 25.

1925a — Experimentelle Untersuchungen über das Verhalten von Dromia vulgaris M. Edw. I. Das Abstreifen der Schlinge (in Polish with German summary). Trav. L'Inst. Nencki *3* (*40*), 1.

1925b — Experimentelle Untersuchungen über das Verhalten von Dromia vulgaris M. Edw. II. Versuch einer Deutung der Bewegungen eines gefesselten Krebses (in Polish with German summary). Trav. L'Inst. Nencki *3* (*42*), 1.

1925c — Experimentelle Untersuchungen über das Verhalten von Dromia vulgaris M. E. III. Über die Reaktion des Umdrehens (in Polish with German summary). Trav. L'Inst. Nencki, *3*, (*45*), 1.

1925d — On the "Speech" of the Fiddler Crab, Uca Pugilator. Trav. L'Inst. Nencki, *3* (*48*), 1.

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1929b — Die Vertikalbewegungen von Paramaecium caudatum. II, Einfluss einiger Aussenfaktoren. Arch. f. Protistenk., *68*, 215.

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1933b — Über die Plastizität der tierischen Handlungen. Beobachtungen und Versuche an Molanna-Larven. Zool. Jahrb., *53*, 261.

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1950b — (together with Sulimata, Staropolska). An attempt of analysing the variability in the bahavior of the caddisfly larva Molanna angustata. Acta Biol. Exp. (Warsaw), *15*, 37.

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1957 — On the compensation of locomotory function in invertebrata (in Russian with English summary). Trudy Leningrad. Obsc. Estestvoisp., *73*, 218.

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II. BOOKS

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MYELOARCHITECTONICS OF THE PARIETAL CORTEX IN DOG

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The present paper is devoted to a myeloarchitectonic study of the parietal cortex of the dog. It is the continuation of my previous papers on the myeloarchitectonics of the dog brain and based on the same material as used before (K r e i n e r 1961, 1963, 1964). This material consisted of 12 brains of dogs sectioned in the three cardinal planes and stained by the methods of Weigert-Wolters, Nissl, and Klüver-Barrera. Emphasis was laid on the Weigert preparations and the study of myeloarchitectonics. In the myeloarchitectonic analysis I followed my own scheme, explained in details in my previous papers (K r e i n e r 1961).

The term "parietal cortex" will be applied here for the portion bounded medially by the splenial fissure and laterally by the suprasylvian fissure. The line drawn from the point of bifurcation of the lateral fissure and the fissura ansata marks its oral boundary, whereas the caudal boundary is arbitrary. It begins at the ascending branch of the splenial fissure (ramus ascendens fissurae splenialis, E l l e n b e r g e r 1891) and runs along its extension laterated across all the fissures and gyri.

The parietal cortex of the dog covers several parasagittal gyri and fissures (Fig. 1a, b). The most conspicuous of the gyri is the suprasplenial gyrus (E l l e n b e r g e r 1891), called also the marginal gyrus (A d r i a-n o v 1959). It lies along the medial edge of the hemisphere. It is bounded laterally by the fissura ansata and the entolateral fissure, medially by the suprasplenial fissure. The presplenial gyrus appears on the medial aspect of the hemisphere and it is bordered ventrally by the splenial fissure and caudally by the ascending branch of the splenial fissure

(Ellenberger 1891). The oral portion of the splenial gyrus is situated beyond this groove. An occasionally present sulcus may divide the presplenial gyrus into two parts.

On the dorsal aspect of the hemisphere (Fig. 1b) the parietal cortex is divided by the deep lateral fissure into two parts. The medial portion consists of the suprasplenial (marginal) gyrus mentioned above and the
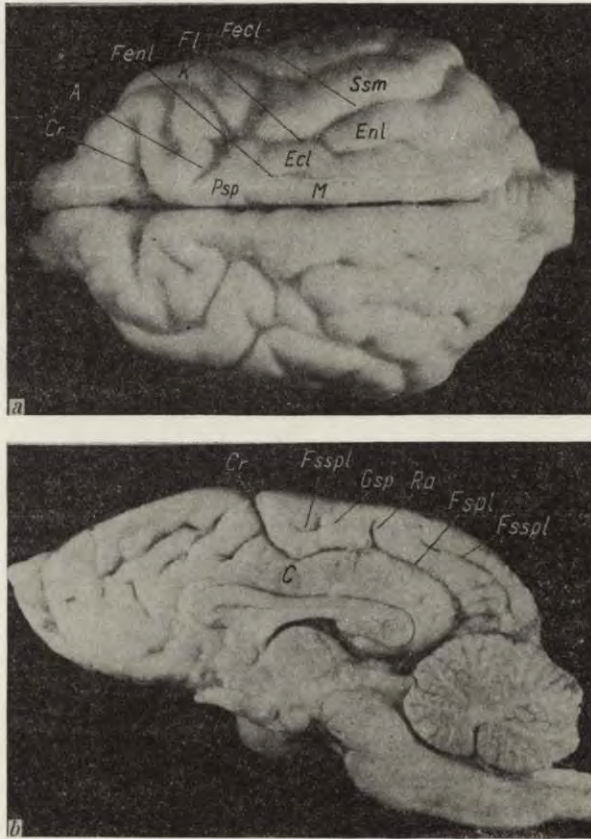

Fig. 1. The parietal region of the dog. a — medial aspect; b — dorsal aspect
A — fissura ansata; C — gyrus cinguli; Cr — fissura cruciata; Ecl — gyrus ectolateralis; Enl — gyrus entolateralis; Fecl — fissura ectolateralis; Fenl — fissura entolateralis; Fl — fissura lateralis; Fspl — fissura splenialis; Fsspl — fissura suprasplenialis; Gsp — gyrus presplenialis; K — gyrus coronalis; M — gyrus suprasplenialis (marginalis); Psp — gyrus presplenialis; Ra — ramus ascendens fissurae splenialis; Ssm — gyrus suprasylvius medius

entolateral gyrus bounded by the lateral and entolateral fissures. In many specimens, the latter fissure may be lacking and then the two gyri are delimited only by a cryptosulcus, or they fuse. The ectolateral gyrus, lying between the lateral and ectolateral fissures, and the suprasylvian

gyrus between the ectolateral and suprasylvian fissures can be distinguished in the lateral portion of cortex. The oral extension of the suprasylvian gyrus is the coronal gyrus.

OBSERVATIONS

Suprasplenial (marginal) gyrus

This long parasagittal gyrus comprises four myeloarchitectonic areas: the area marginalis anterior (MA), the area marginalis lateralis (ML), the area fissurae ansatae (FA), and the area fissurae suprasplenialis (FN).

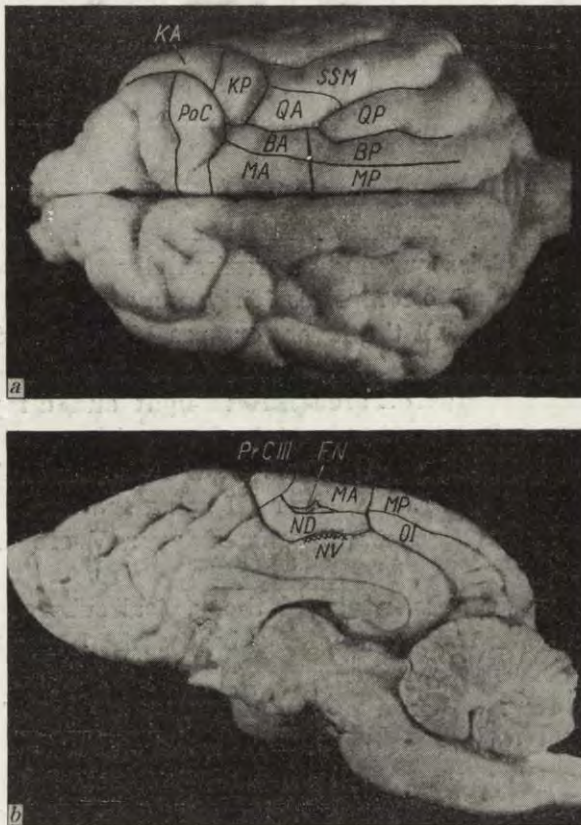*The anterior marginal area* (MA) occupies the anterior portion of the

Fig. 2. Myeloarchitectonical areas of the parietal cortex of the dog. a — medial aspect; b — dorsal aspect

BA — area entolateralis anterior; BP — area entolateralis posterior; FN — area fissurae presplenialis; KA — area coronalis anterior; KP — area coronalis posterior; MA — area marginalis anterior; MP — area marginalis posterior, ND — area presplenialis dorsalis; NV — area presplenialis ventralis; O I — area splenialis I; FoC — area postcentralis; PrC III — area precentralis III; QA — area ectolateralis anterior; QP — area ectolateralis posterior; SSM — area suprasylvia media

gyrus. Orally, it touches area precentralis III (PrC III; K r e i n e r 1964) and caudally passes into the area marginalis posterior (MP). The boundary lies a little orally to the extension of the ascending branch of the splenial fissure. The lateral border of area MA is marked by the fissura ansata and the entolateral fissure, while the suprasplenial fissure is the medial border. Where this groove is lacking, area MA is in direct contact with the area presplenialis dorsalis (ND) (Figs. 2a, b, 3a—d).

Area MA is a large well-myelinated field about 392 mm² *. Its radial fibers are fine, medium-sized, and thick, grouped in fascicles 15—25 μ thick and about 25—40 μ apart. The fine fibers reach up to 5/6 of the thickness of the cortex, the medium-sized and thick ones up to 3/4 of this thickness. Proximally the radial fibers can be traced a long way lateroventrad within the white matter.

The tangential fibers are abundant and include many very fine "grundfasern" as well as a large number of thick, mediumsized, and fine fibers running in various directions. The bundle Mα consists of fine fibers passing towards the lower layers of the area fissurae ansatae. The bundle Mβ is composed of fibers differing in size and connecting various portions of the cortex within area MA only. Numerous thick and medium-sized fibers running approximatelly orocaudally form the bundle Mγ. The bundle Mδ comprises fairly numerous, mostly fine fibers extending ventrad from all the layers or area MA to the deep layers of area FN (cf. below).

The superficial plexus of area MA is light and composed of fine parallel fibers running in the frontal plane with some mediumsized fibers among them. Nissl preparations show layer I about 240 μ thick. Layers II—IV are cf small cells, layer III being sparser than the others. The small well-stained pyramidal cells of layer V are arranged more loosely.

In area MA we can distinguished the typical subarea MA a at the edge of the gyrus and less myelinated subareas MA b and MA c situated medially and laterally respectively.

Moreover, we can observe a gradual quantitative differentiation in the density of myelination: the fibers of the oral portions are more densely arranged than those of the caudal portions. Nevertheless, there being no qualitative differences, they cannot be delimited or divided into any more areas.

The area marginalis lateralis (ML) is a fairly densely myelinated paragyral field, about 29 mm² in area, in the lateral wall of the fissura ansata (Fig. 3b).

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* All data on the areas of the field were obtained on only one specimen and should be considered as but a rough approximation.
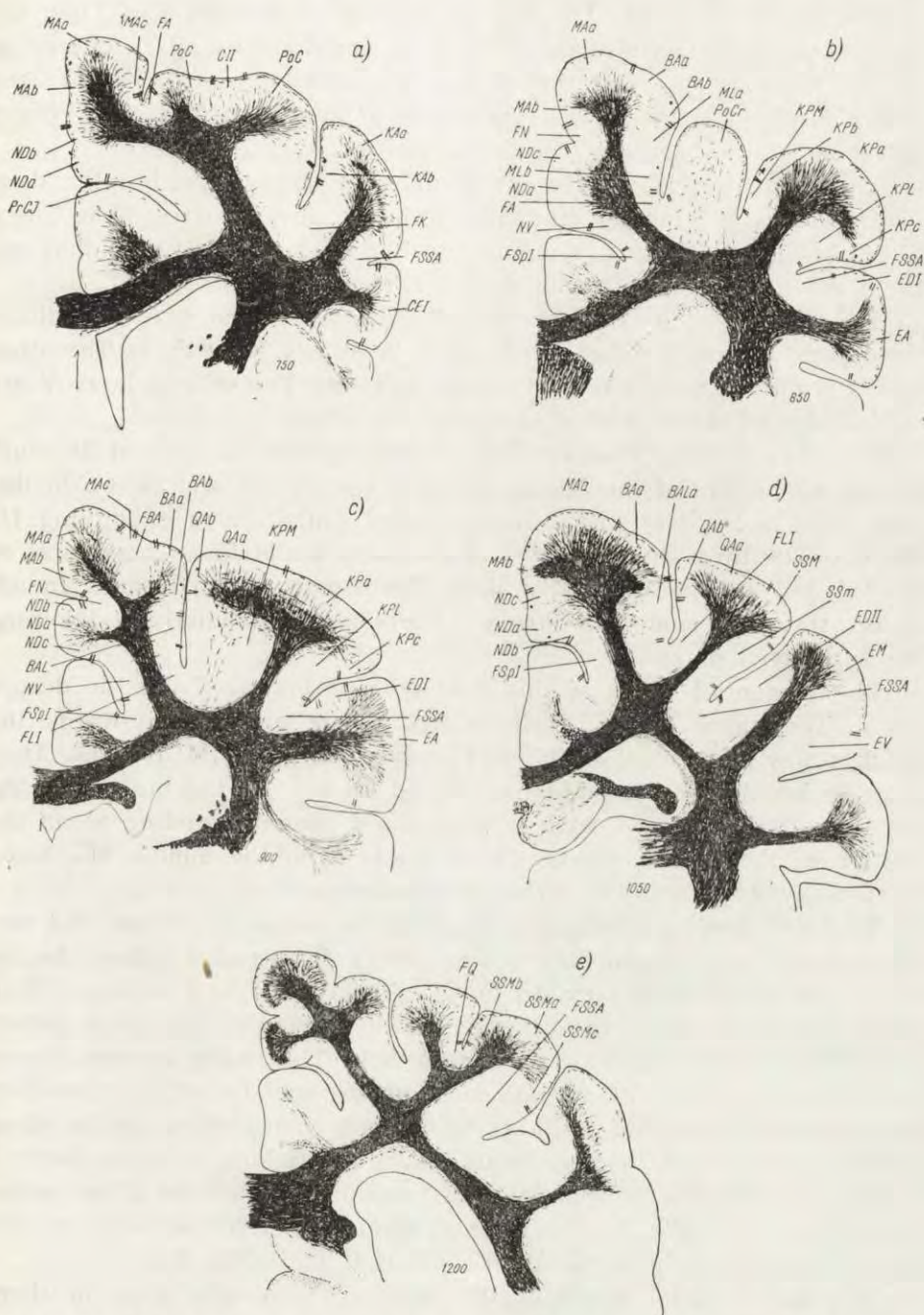
Fig. 3. A series of transverse sections showing the myeloarchitectonical areation of the cortex. Semischematic, Weigert-Wolters stain. Numbers refer to preparations, 100 preparations = 5 mm. Labellings explained in text

The radial fibers of area ML are fine or of medium size. They are grouped in small fascicles about 7 μ thick and 30—50 μ apart, extending 2/3 of the way through the cortex. Proximally, most medium-sized fibers turn ventrad and join the fibers of area MA. The other fibers disappear at the border of the white matter.

The tangential fibers are rather densely arranged within the deep layers. They are mostly fine and directed towards area MA. These fibers belong to the above-mentioned bundle Mα. The fibers that are their extensions run towards the area fissurae ansatae (FA).

The superficial plexus consists of some fibers and a few medium-sized ones. In cresyl-violet preparations layer I is 300 μ thick. The other layers are made up of small round perikaryons. The cells of layer V are a little larger, better stained and more dispersed.

The area fissurae ansatae (FA), extending over an area of 28 mm², lies in the bottom of the fissura ansata (Figs. 1b, 2b, and 3a, b). In this area there are a few single medium-sized radial fibers extending 1/3 the distance through the cortex. Proximally these fibers bend oral or caudad and run parasagittally along the course of the fissure. In addition, there are some fine straight fibers running radially and joining the superficial plexus.

The tangential fibers of this area are present mostly in the deeper layer. They are fine or medium-sized fibers and tend towards the neighboring postcentral (PoC) and lateral marginal (ML) areas. They may be labelled Mε and Mα (mentioned above). At the margin of the white matter there are some medium-sized fibers extending along the bottom of the fissura ansata. These fibers from the bundle Mζ. Some of them bend towards the cortex and become radial.

The well-developed superficial plexus is composed of fine and medium-sized fibers. Some of its fine fibers turn radially towards the deeper layers of the cortex. In the Nissl picture layer I is about 360 μ thick, the thickness of the other layers being 750 μ. The latter layers are built of small round or pyramidal cells. The limits between layers are indistinct. Layer VI appears more diffuse and its cells are smaller. A few larger triangular cells are to be seen among them and in silver preparations they show very large dendrites reaching as far as layer I.

The area fissurae presplenialis (FN) occupies the bottom of the presplenial fissure parallel to the splenial fissure. The surface area of this fissural field of slight myelination is about 46 mm² (Fig. 3b-c).

The scarce radial fibers of this area are fine-only some of them being of medium-size — and grouped in fascicles about 120 μ aparat. They extend 1/3 of the way through the cortex. Some of the very fine fibers reach as far as the superficial plexus. Proximally the radial fibers

disappear at the border of the white matter. There they probably bend
to sink into the tangential fiber systems.

In this area there are many fine and very fine fibers running from
both the neighboring areas MA and ND (cf. below). Part of them run
from the upper layers of these areas to the deeper layers of area FN.
They form the bundles Mδ (mentioned above) and Mη connecting area
FN with area ND. At the margin of the white matter there appear nu-
merous medium-sized fibers probably belonging to the bundle Hγ. Fine
„grundfasern" are also present.

The superficial plexus of area FN is light and shows some con-
nections with very fine radial fibers. In Nissl preparations layer I is
about 280 μ thick. Layers II—IV are made up of small round cells. The
cells of layers V and VI are triangular or elongated and more diffusely
arranged.

Presplenial gyrus

This gyrus lies dorsally to the anterior part of the splenial fissure
(Fig. 1 a) between the cruciate fissure and the ascending ramus of the
splenial fissure (Ellenberger 1891). Dorsally, it is delimited from
the suprasplenial (marginal) gyrus by the suprasplenial fissure. The
cortex of the presplenial gyrus can be divided into three myeloarchite-
ctonic areas. In some specimens the occasional sulcus presplenialis (mihi)
is present in the middle of this gyrus. Sometimes it may occur in one
hemisphere only.

The area presplenialis dorsalis (ND) occupies the dorsal portion of
the presplenial gyrus neighboring upon the area fissurae presplenialis
or, if this groove is absent, touching area MA immediately (Figs. 2a,
3a-d). In the last case the limit is marked by a cryptosulcus. Area ND
is a moderately myelinated field. Its area is about 220 mm².

This area contains fine and medium-sized radial fibers. They run
single or in loose thin fascicles disposed at intervals of about 30—50 μ.
Most fibers extend only 1/3 the way through the cortex, the remaining
ones 3/4 of its thickness. Proximally they enter the white matter and
join the fibers coming from area MA.

The tangential fibers are moderately dense. A diffuse bundle of fine
fibers (Mη) runs from all the layers of this area towards the lower
layers of the area fissurae presplenialis (FN). The ventral extension of
bundle Mγ, composed of thick orocaudal fibers, occurs at the margin of
the white matter. Some straight fine fibres connect this area with the
area fissurae splenialis (FSp). They are denoted by MΘ.

The superficial plexus of area ND is poorly developed. It consists

of fine fibers, most of which run obliquely dorsocaudally. In Nissl preparations, layer I is about 200 μ thick. Layers II—IV consist of small round cells, of which those within layer IV are arranged more loosely. Layer V contains a little larger cells, while the cells of layer VI are arranged in columns and some of them are dispersed within the white matter.

Area ND can be divided into typically developed subarea ND a and less myelinated subareas NB b and ND c. This area is very variable and in different specimens shows considerable differences in development and position.

The area presplenialis ventralis (NV) is situated in the ventral portion of the presplenial gyrus (Fig. 3b, c). There it occupies a cryptogyrus and is often hidden in the splenial fissure. In some dogs it is separated from area ND by an occasional shallow sulcus or by a cryptosulcus. The arrangement of this area is very variable. In my specimen its area is about 58 mm².

The radial fibers of area NV are fine and rather abundant. They extend about 1/2 or 1/3 of the thickness of the cortex. They run parallel showing but little tendency to gather into fascicles. Proximally the radial fibers turn ventrad and can be seen as a superficial layer of the white matter as far as the bottom of the splenial fissure.

The tangential fibers are fairly dense. A diffuse bundle of fine fibers (Mι) runs towards the area fissurae splenialis. There are also fibers running to area ND; they form bundle MΘ and a part of Mδ.

The superficial plexus is poor, composed of fine diffuse fibers. In cresyl-violet preparations layer I is 250 μ thick. Layer II is dense and layers III and IV thin and more diffuse. Layer V contains round cells somewhat larger than those in the other layers, while layer VI is wide and many of its cells are dispersed within the white matter.

Area NV may be divided into typical subarea NV a and subarea NV b, surrounding it and far more poorly developed.

The area fissurae splenialis I (FSp I) extends over the bottom of the named fissure. It is a fissural field of very poor myelination with an area of about 168 mm² (Fig. 3b-d).

The radial fibers of area FSp I are represented only by a few single short fibers, which bend proximally so as to mingle with the sagittal fibers. The tangential fibers are fine except for a few ones which are of medium size. They belong to the systems MΘ and Mι, connecting area FSp I with areas ND and Nv. Another system of tangential fibers (Mϰ) originates from the limbic cortex, from the area limbica anterior lateralis (LAL, K r e i n e r 1963) and the area limbica anterior dorsalis II (LAD II).

The superficial plexus is poor and consists of a small number of very fine fibers. In Nissl preparations, layer I appears to be 600 μ thick, the other layers being 720 μ. Layers II and III harbor densely arranged small cells, while in layer IV the cells are arranged more loosely. The round perikaryons of layer V are somewhat larger; layer VI is thin.

Entolateral Gyrus

The entolateral gyrus (figs. 1b, 2b) lies along the medial lip of the lateral fissure. Its medial border is marked by the entolateral fissure. Orally this fissure is usually lacking, and then a cryptosulcus, being its extension, delimits the entolateral gyrus from the suprasplenial (marginal). The entolateral gyrus contains four myeloarchitectonic areas.

The most conspicuous of them, *the area entolateralis anterior* (BA), is situated in the anterior portion of the entolateral gyrus (Figs. 2b, 3b-d). It is a pendant to areas MA of the suprasplenial gyrus. Both these areas either touch immediately or, in other specimens, are separated by the entolateral sulcus. Many U-fibers connect area BA with area MA. The surface area of BA is about 140 mm².

The area entolateralis anterior (BA) is a strongly myelinated field. Its radial fibers are thick, medium-sized, or fine with a few very thick ones. They form very loose fascicles grouped around the thick fibers. The fascicles are about 7—10 μ thick and 25—35 μ apart. They extend about 4/5 the way through the cortex, while a lot of fine radial fibers end at 1/3 of its thickness. Proximally, the radial fibers run ventrad and somewhat caudad.

The tangential fibers are scarce. A bundle (Mλ), composed of fine and medium-sized fibers, runs to the entolateral fissure (area FBA) and another similar bundle (Mμ) tends to area BAL and to the cortex of lateral fissure (FL). There are also some fine „grundfasern".

The superficial plexus is built of fairly dense parallel fine and medium-sized fibers. In cytoarchitectonic sections layer I is 300 μ thick and layers II—IV contain undifferentiated small round cells. Layer V, with some loosely arranged pyramidal cells, is well visible and layer VI, made up of small cells, is wide.

Within area BA we can distinguish a central typical subarea (BA a) and, around it, two other subareas (BA b and BA c) showing less typical development.

The area entolateralis anterior lateralis (BAL) is hidden in the medial wall of the lateral fissure (Fig. 3c-d). Its area is about 53 mm². It is a slightly myelinated field joining area BA.

The radial fibers of area BAL are of medium and small sizes. They

run singly, extending halfway through the cortex. Proximally they turn orad or caudad at the border of the white matter and thus become tangential. A few of them join the fibers of area BA.

The tangential fibers are seen mostly in the deeper layers of this area. They are fine, only some of them being of medium size. The bundle Mμ runs to area BA and another conspicuous bundle (Mν) connects area BAL with the area fissurae lateralis (FL) and with the cortex of the ectolateral gyrus. Some mediumsized fibers extend parasagittally at the margin of the white matter.

The superficial plexus contains fairly dense fine fibers most of which run ventroorad. Nissl preparations show layer I to be 350 μ thick. Layers II—IV are built of densely arranged small round cells, while layer V is more diffuse and its cells are somewhat larger. The cells of layer VI are denser and small.

The area fissurae entolateralis pars anterior (FBA) occupies the bottom of the fissure of the same name. In my specimen its area is about 17 mm². It may vary very considerably with the stage of development of the fissure from dog to dog. This area is myelinated very poorly (Fig. 3c).

The radial fibers of area FBA are scarce and extend singly 1/4 the way through the cortex. They disappear at the border of the white matter. The tangential fibers are abundant. The bundle Mλ comprises fibers coming from all the layers of area BA, whereas the bundle Mσ is built of a large number of fibers running from area MA to BA.

The superficial plexus is rather dense, composed of fine fibers running in various directions. In cresyl-violet preparations layer I is 300 μ thick and layers II—IV are built of palely stained small round cells. Layer V contains some triangular cells.

The area fissurae lateralis (FL) is a very slightly myelinated large fissural field (Fig. 3c-d), about 105 mm² in area, occupying the bottom and partly the walls of the lateral fissure.

The radial fibers of this area are practically lacking. The few ones present are short and bend to join the tangential fibers. The tangential fibers are fairly dense. They run towards the neighboring areas BA, BP, BAL, and BPL (bundles Mμ and Mν) as well as to the areas of the ectolateral gyrus. Part of them are probably U-fibers connecting the entolateral and ectolateral cortex; they form the bundle Mζ. A peculiarity of this area is the small number of the orocaudal tangential fibers, which are common in the other fissural fields.

The superficial plexus of area FL is composed of fine and very fine fibers running in various directions. Some of them descend radially into the cortex. In Nissl preparations layer I is about 250—300 μ thick.

Layers II—IV consist of pale small cells and layer V harbors some larger triangular perikaryons. Layer VI is thin and its cells are small. Silver preparations show the triangular cells provided with long ramifying dendrites.

Ectolateral gyrus

The ectolateral gyrus lies along the lateral lip of the lateral fissure. Laterally it is bordered by the ectolateral fissure. The parietal cortex covers only anterior portion of this long gyrus. Three myeloarchitectonic areas can be distinguished in it (Figs. 1b, 2b).

The area ectolateralis anterior (QA) is a well-myelinated gyral area limited laterally by a branch of the suprasylvian or by its extension, medially by the lateral fissure and orally by the coronal fissure. Its area is about 150 mm² (Figs. 2b, 3c-d).

The radial fibers of area QA differ in size, they are mostly thick or of medium size, and form very poorly defined fascicles. The fine fibers extend 4/5 the distance through the cortex and the thick ones 2/3 of this distance. Proximally these fibers run ventrad, then turn slightly mediad and penetrate as far as the tapetum.

The tangential fibers are abundant. At the border of the white matter there is a rich bundle of medium-sized and thick fibers running laterocaudad towards the suprasylvian gyrus. I label it Mπ. The fine and medium-sized fibers seen in the middle of the cortex take a similar course and form a conspicuous bundle, abelled Mρ. Some thick fibers running in the frontal plane are intermingled with it. The bundle Mσ consists of fine fibers and a few medium-sized ones connecting subarea QA a with subarea QA b, situated in the wall of the lateral fissure, and with area FL. A similar bundle (Mτ) runs from the lateral portion of subarea QA a to subarea QA c in the wall of the ectolateral fissure (along area QA there are no paragyral fields similar to KPL or KPM but large marginal subareas).

The superficial plexus consists of fine and medium-sized fibers running in the frontal plane with a slight caudolateral inclination. In Nissl preparations layer I is about 200 μ thick. Layer II is composed of small round cells and layers III—V of small round cells mixed with small pyramidal perikaryons. Layer VI shows small cells varying in shape.

Area QA can be divided into a large well-myelinated subarea (QA a) on the ridge of the gyrus and two worse developed subareas in the wall of the neighboring fissures. They show rather a small number of radial and tangential fibers, which are more dispersed and shorter. In Nissl

sections layer I is over 250 μ thick, the development of all the other layers is uniform.

The area ectolateralis medialis (GM) is a small field (38 mm²) situated in the lateral wall of the lateral fissure. Its radial fibers are mostly fine with some medium-sized ones. They join to form very loose fascicles extending 2/3 of the way through the cortex. Proximally they enter the white matter and disappear in it.

The tangential fibers are fine. Most of them belong to the bundle Mυ running to this area from the area ectolateralis anterior (QA). The bundle Mυ made up of fine fibers extends through the deep cortical layers ventrad to the area fissurae lateralis (FL) and to area BAL. Some of these fibers turn radially within the area ectolateralis medialis and then run on as radial fibers.

The well-developed superficial plexus is composed of fine fibers running parallel dorsocaudad. In Nissl preparations layer I is about 270 μ thick; the other layers show no distinct lamination.

The area fissurae ectolateralis (FQ) lies at the bottom of the ectolateral fissure. This field of rather poor myelination is about 36 mm² in area (Fig. 3e).

The radial fibers of area FG are scarce, fine, and dispersed. Most of them turn to join the tangential fibers, some disappear within the white matter. Great part of the fibers are connected with the superficial plexus. The tangential fibers are abundant. The bundle Mχ connecting the neighboring areas (QM, QA and BAL) appears here on the background of a dense net of fine „grundfasern". Moreover, there are some thick fibers originating from the white matter and running through the peripheral portions of area FQ to area QA.

The superficial plexus is dense. It is composed of very fine fine, and a few medium-sized fibers extending in various directions. Many of these fibers continue their course in the radial direction. In cresylviolet preparations layer I is 250 μ thick. Layer II is built of densely arranged small round cells. The deeper layers, showing no distinct lamination contain small pyramidal or triangular cells.

Coronal gyrus

The parietal cortex extends only over the caudal portion of the coronal gyrus, its oral portion being occupied by the sensorimotor cortex. There are four myeloarchitectonic areas in the caudal portion (Figs. 1b, 2b-c).

The area coronalis posterior (KP) is the most conspicuous and well-myelinated gyral fields (Figs. 2b, 3). Dorsally it touches the area ecto-

lateralis anterior (QA), caudally the area suprasylvia media (SSM) and orally the area coronalis anterior (KA). The suprasylvian fissure separates it ventrally from the area ectosylvia anterior (EA, cf. Kreiner 1964). Its surface area is approximately 228 mm². The cortex of this area is thinner than in other regions and in the middle it has a stripe of intenser myelination seen with the naked eye.

The radial fibers of the area coronalis posterior are fine, thick or of medium size, grouped into fascicles 10—12 μ wide and about 25—50 μ apart. The fascicles extend 4/5 of the distance through the cortex and show a varying structure. Their lower portion is composed mostly of medium-sized, thick, and only a few fine fibers. The upper portion is built of fine fibers. It is so because fine fibers connecting the upper and lower layers of the cortex join the fascicles and prolong them. Their occurrence is responsible for the presence of the white stripe in the middle of the cortex.

The tangential fibers are abundant and run in various directions. They appear particularly dense in two places. One of them contains chiefly fibers running in the frontal plane and can be seen approximately in the middle of the cortex. It can be seen with the naked eye, since it coincides with the occurrence of the additional radial fibers described above. The other aggregation, for the most part of orocaudal fibers, is localized at the border of the white matter.

The great density of the tangential fibers makes the distinction of any definite bundle very difficult. However, I have distinguished a bundle (Mχα) composed of thick and medium-sized fibers running orocaudally and aggregating at the margin of the white matter. The bundle Mψ consists of fine fibers extending more or less in the frontal plane, crowded in the mentioned stria situated halfway through the cortex. These fibers probably serve as an intraareal association pathway. A similar function my be ascribed to the bundle Mω composed mostly of medium-sized and thick fibers running in various directions. These fibers are present chiefly in the lower layers of the cortex. Many of them run obliquely from the deep layers to the superficial ones. A relatively small number of fibers go beyond the limits of area KP. The bundle Mαα, made up of fine fibers with an addition of some medium-sized ones, passes from area KP to area KPL, and not very numerous fibers of the bundle Mαβ tend towards area KPM.

The superficial plexus of area KP consits of very fine and single thick fibers. They run in various directions joining the net of „grundfasern" repeatedly. Just under the surface of the cortex there occurs a slight density of the plexus.

In cresyl-violet sections layer I is 200 μ thick, layer II contains pale

small round cells and layers III—IV small pyramidal cells. Layer V is light and has some small elongate pyramidal cells, while layer VI consists of small polymorphous cells.

Area KP can be divided into a large typical subarea (KP a) and a narrow subarea (KP b) bordering it.

The area coronalis posterior lateralis (KPL) is found in the medial wall of the suprasylvian fissure bordering area KP laterally. The area of this slightly myelinated field is about 62 mm^2 (Figs. 3b-c).

The radial fibers are here curled and short. They run singly or in very loose fascicles extending 1/3 and, some of them, 1/2 of the way through the cortex. Proximally they disappear among the parasagittal fibers at the border of the white matter. Some of them join the fibers of area KP. The medium-sized or fine tangential fibers belong to the bundle Mαα. There are also many „grundfasern". At the margin of the white matter there are numerous parasagittal fibers, which are the extension of the bundle Mχα. Some of these fibers probably turn to run radially and become radial fibers. There is also a bundle connecting this area with the area fissurae suprasylviae (FSS). This bundle is labelled Mχγ. The superficial plexus is diffuse and made up of fine fibers running in various directions.

The Nissl preparations show layer I to be 300 μ thick, the limits of the other layers being indistinct. The cells of layer II are pale and round, those of layers III—IV small and triangular or pyramidal. There are some larger rounded cells in layer V, while layer VI is thin and composed of small cells.

The area fissurae suprasylviae anterior (FSSA) is a moderately densely myelinated area at the bottom of the suprasylvian fissure (Fig. 3a-e). There are some fine or medium-sized pale radial fibers in it. Proximally part of these fibers penetrate mediad rather deep into the white matter and join the fibers coming from the area ectosylvia anterior (EA).

The tangential fibers, which are numerous, run through the deep layers and connect area KPL with the area paraectosylvia dorsalis (ED) situated on the opposite side of the suprasylvian fissure. These fibers form the bundle M mentioned above. In this bundle the largest number of fibers occurs just at the bottom of the fissure. It may be assumed that it contains also the fibers passing from area KPL to area FSS and those from area FSS to ED. There are also fine fibres of the bundle Mαδ connecting area FSS with the upper layers of the area paraectosylvia dorsalis (ED). Many „grundfasern" are present, too.

The superficial plexus is poor, represented only by a few fine fibers.

In cresyl-violet preparations layer I is over 360 μ thick. Layers II—VI are made up of small round and triangular perikaryons. Some larger triangular cells appear among them. Fissural cells with large dendrites are observed in silver preparations.

The *area coronalis posterior medialis* (KPM) is a small (about 14 mm²) fairly well-myelinated field in the lateral wall of the coronal and lateral fissures; it is the counterpart of area KPL (Fig. 3b). Its radial fibers, fine or of medium size, run singly about halfway through the cortex. Their number is smaller than that observed in area KPL. Proximally the fine fibers disappear at the border of the white matter, whereas the medium-sized ones bend ventrad and join the projection fibers of area KP.

The tangential fibers are fairly dense. The bundle Mαε connects area KPM with area KP, and is composed of fine fibers, while the fine fibers of the bundle Mαζ run towards the bottom of the coronal fissure. The superficial plexus is poor and consists of a small number of fine fibers running in the frontal plane. In cytoarchitectonic sections layer I is about 250 μ thick. The other layers can hardly be distinguished. They are built of small pyramids mixed up with a small number of round cells. The pyramids which are situated deeper are larger. In the deepest layer the cells are polymorphic.

Suprasylvian gyrus

The suprasylvian gyrus is a long gyrus situated between the suprasylvian fissure and the ectolateral (Figs. 1b, 2b). Orally, it continues into the coronal gyrus. The parietal cortex covers only its portion known as the medial suprasylvian gyrus. From the myeloarchitectonical standpoint the suprasylvian gyrus can be divided into two areas, suprasylvia media (SSM) and suprasylvia accessoria (SSm).

The area suprasylvia media (SSM) is a heavily myelinated field occupying the medial suprasylvian gyrus. Its area is about 360 mm² (Fig. 3d-e).

Area SSM contains medium-sized and fine radial fibers grouped in fascicles about 12 μ thick and 10—20 μ apart. The fibers extend 4/5 of the distance through the cortex. In the upper course the fascicles are accompanied by fine short fibers connecting the different levels of the cortex radially and giving this field its striped appearance. Proximally the radial fibers run medioventrad and disappear among the fibers of the semioval center.

The tangential fibers of the area suprasylvia media (SSM) are dense. They can be found in all layers but form two particularly dense aggre-

gations: one at the border of the white matter and the other approxima-
tely halfway through the cortex. The lower aggregation consists of fibers
running in all directions. Most of them form a system composed of me-
dium-sized and a few thick fibers. The fibers of this bundle (Mαη) arch
from the ectolateral fissure (FQ) to the area fissurae suprasylviae (PSS).
The bundle MαΘ is built of fine and medium-sized fibers passing from
all the layers of the cortex to the area fissurae ectolateralis (FQ), whe-
reas the bundle Mαι connects all the layers of area SSM with the cortex
of the suprasylvian fissure (FSS). The upper aggregation of the tan-
gential fibers consists of fine and medium-sized fibers. This bundle,
labelled Mαϰ, connects various regions of area SSM.

The superficial plexus is moderately well developed and shows
a distinct density of fibers just below the surface. In Nissl preparations
layer I of area SSM is about 180 μ thick. Layer II is fairly wide and
harbors densely arranged small round cells. Pyramidal cells appear in
layer III, while the other layers are hard to distinguish and contain
small and diffuse cells.

The area suprasylvia media (SSM) can be divided into a typical
subarea, SSM a, and two accompanying subareas, SSM b and SSM c.
In the latter subareas the myelinated elements are worse developed
and the cytoarchitectonics less distinct.

The area suprasylvia accessoria (SSm) lies in the wall of the supra-
sylvian fissure along area SSM. Its surface area varies from one spe-
cimen to another. It is an area of moderate myelination (Fig. 3d).

The radial fibers of area SSm are fine, grouped in thin fascicles
comprising a few fibers each, or running singly. The fibers reach as far
as 2/3 of the cortex. Proximally, they disappear in the white matter.
The tangential fibers form a bundle (Mαι) connecting area SSM with
the area fissurae suprasylviae. Part of these fibers probably end in
area SSm.

The superficial plexus of area SSm is poor, composed of fine parallel
fibers running in the frontal plane. Cresyl-violet preparations show
layer I to be about 200 μ thick. Layers II—IV are made up of small,
mostly round cells without any visible lamination. Layer V is distin-
guished by a few larger cells and layer VI by a more diffuse arrange-
ment of perikaryons.

DISCUSSION

The observations on which the present paper is based cover the
cortical region extending between the region of the sensori - motor
orally and the occipital cortex condally. This region does not coincide

with the textbook notion of the occipital lobe, which reaches anteriorly
as far as the central sulcus. Instead, it corresponds to the region intro-
duced and called „regio occipitalis" by B r o d m a n n (1909), including
cytoarchitectonic areas 5, 7, and 52.

I have discussed the boundaries of the parietal cortex in the dog
according to my conception in the descriptive part of this paper. Here
I wish to attract attention again to the caudal boundary of this region.
I have defined it as arbitrary. There is, really, no sulcus or any other
formation there to mark it macroscopically. According to the literature,
this boundary does not exist in other brains either, the human brain
not excepted. Only in some of the dogs a vertical branch of the splenial
fissure (ramus ascendens fissurae splenialis Ellenberger) is visible on
the medial aspect of the hemisphere, and the prolongation of this
branch constitutes the boundary in question. It crosses several fissures
and gyri at right angles. Nevertheless, it is clear-cut between areas MA
and MP, QA and QP, and BA and BP, forming a transverse line deli-
miting the occipital cortex from the parietal cortex. Only part of area
SSM passes caudally to this line.

In this paper I present a myeloarchitectonic map of the parietal
cortex of the dog based on my own scheme of the myeloarchitectonics
and my concept of the myeloarchitectonic area. A comparison of this
map with the available maps of C a m p b e l l (1904), B r o d m a n n
(1909), K l e m p i n (1921), G u r e w i t s c h - B y c h o w s k y (1928), and
A d r i a n o v (1959) will show great differences. In the map of C a m p-
b e l l there is no region outlined that might be considered comparable
with my concept of the parietal cortex. In the map of K l e m p i n
and in that of G u r e w i t s c h - B y c h o w s k y the parietal region is
represented by fields labelled 5 and 7, situated one behind the other.
Their limits, however, differ considerably. In the map of G u r e-
w i t s c h - B y c h o w s k y there is no division into parasagitally exten-
ding areas, which division is essential to my map. In the map of K l e m-
p i n such a division is shown only in the lateral portion of area 7.
In the map of A d r i a n o v the parietal region is occupied by areas PC,
P, and T_4. The limits of these areas also differ considerably from those
marked out according to my concept, except area T_4, which may be
compared with my area suprasylvia media (SSM).

The map of B r o d m a n n (1909) does not concern the dog but Cer-
coleptes caudivolvulus. None the less their brains are to some extent
similar. Here the parietal cortex includes areas 5, 7, and 52. Area 52
seems to be homologous to my area suprasylvia media (SSM), while the
other limits much differ from each other.

SUMMARY

The paper presents a myeloarchitectonis analysis of the parietal region of the dog's cerebral cortex. The region is limited orally by the fissura ansata and a line drawn medially from the point of bifurcation of this fissure, then by the splenial fissure. The lateral boundary is marked by the suprasylvian fissure, while the caudal one is arbitrary. Basing on his own simplified scheme of the myeloarchitectonics and his own concept of the cortical area the author has distinguished 20 areas in this region. Six of them are well-myelinated gyral areas (ND, MA, BA, QA, KP, and SSM). Seven areas (ML, NV, BAL, KPL, KPM, QM and SSm) are moderately well myelinated paragyral fields. The remaining ones are poorly myelinated fissural areas.

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EXTINCTION OF FOOD-REINFORCED RESPONSES AFTER MEDIAL OR LATERAL HYPOTHALAMIC LESIONS

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It has previously been demonstrated that lesions of the medial hypothalamus in rabbits induce hyperphagia together with a preference for oats when the rabbits are trained to work for and make a choice between carrots, purèe-type potatoes and grains of oats (B a l i ń s k a 1963a). It has also been demonstrated (B a l i ń s k a et al. 1961, B a - l i ń s k a and B r u t k o w s k i 1963) that rabbits with medial lesions but still in the dynamic phase of hyperphagia show an impairment in suppression of the Type II conditioned reflex (instrumental CR) response on inhibitory trials in a ‚go-no go' differentiation task (‚errors of disinhibition').

Damage to the rabbit's lateral hypothalamus, on the other hand, results in aphagia, which can be stopped by simple subcutaneous injection of Ringer's solution and glucose. When complete aphagia occurs CR Type II activity immediately drops to zero. As a result of the hydration procedure, animals with lateral hypothalamic lesions begin to accept food, and, eventually, return to the preoperative level of feeding behavior and may even become hyperphagic. They will also eventually begin to perform an instrumental response in order to obtain a food reinforcement (B a l i ń s k a 1963b). Moreover, like dynamic hyperphagic animals, they then show difficulty in withholding the CR response on inhibitory trials in a differentiation task (B a l i ń s k a and B r u t k o w - s k i 1963). It is of interest that, in a free-choice situation, the first response which is recovered after the operation is that reinforced by carrots (B a l i ń s k a 1963b).

It was considered important to compare the CR performance of rabbits with either medial or lateral hypothalamic lesions during sessions of extinction. The present investigation is concerned with this question.

METHODS

Details of the procedure have been previously described (B a l i ń s k a 1963a, b). The present experimental series consisted of 12 adult rabbits. The animals were trained in a free-choice situation to select either of two kinds of food reinforcements with one of two CR Type II responses. During regular conditioning sessions the animals ate *ad libitum*, that is, as long as they performed the CR's (ca. 1 hr.). While not in the testing cage, the animals were maintained on a standard diet different from that used in the experimental situation. In all animals, the forelimb CR was trained in accordance with our standard procedure (B a l i ń s k a 1963a) used primarily by K o n o r s k i and M i l l e r (1933) in the dog. This response consisted of the mere placing by the animal of its right forelimb on a board attached to the food tray No. 1 which was situated on the left side of the testing cage. Each of these forelimb responses was immediately reinforced with grains of oats for some animals, or with a ration of cooked puree-type potatoes for the other animals. The second instrumental CR was trained by the M a l i n o v s k i technique (1952), and consisted of grasping and pulling with the teeth a bakelite ring attached to the right side of the experimental cage next to food tray No. 2. This response was followed by a carrot reinforcement.

After the two CR's were well established an axtinction session was performed for both responses stimultaneously. This extinction session followed a 5-min. conditioning session, and was terminated when no response occurred for 5 min. Then, ,gratis' food reinforcements were given, thereby recovering the performance. In each animal, there was one extinction preoperatively.

Upon completing the preoperative testing, in 6 animals the medial hypothalamus was damaged (group M), in 6 others, lesions were made in the lateral hypothalamus (group L). The regions were coagulated by passing a 3 mA D.C. for 15 sec. The details of operative procedures have been previously described (B a l i ń s k a 1963a, b). One animal from group M died after operation.

Testing the animals of group M was resumed from 3 to 5 days following operation, whereas that of group L was started a few days later (after the recovery of voluntary eating). The lateral hypothalamic rabbits were kept alive by forced hydration. This was accomplished by daily subcutaneous injection of 100 cc. of 40 per cent glucose with isotonic salt solution. In isolated instances, aphagic animals which were rapidly losing weight were given an additional injection of 10 cc of 20 per cent glucose, intravenously. Forced hydration was discontinued when voluntary acceptance of food recovered.

In group M, the extinction session was carried out at early postoperative stages. On the other hand, group L was retested on extinction after a complete recovery of both CR's. This occurred a few weeks after surgery. Postoperative conditioning and extinction sessions were performed in exactly the same manner as the preoperative sessions. From 3 to 4 months after the operations the animals were sacrificed, and the placements of lesions verified histologically.

RESULTS AND CONCLUSIONS

Preoperatively, the animals extinguished slower the response associated with carrot presentation than that associated with either potatoes or oats. There were no signs of excitement nor any other emotional manifestations throughout the extinction period.

After lesions of the *medial hypothalamus,* the animals in the dynamic phase of hyperphagia showed a drastic decrease in response rate during extinction of the CR associated with carrot reinforcement. They also decreased or maintained the preoperative level of responding of the CR associated with potatoes. Conversely, these animals significantly increased responsiveness while extinguishing the CR reinforced with oats (Tables I and II). With regard to the general aspects of behavior, the animals with madial lesions performed all responses much more vigorously than before operation, and they were also easy to irritate.

Table I

Extinction of CR's associated with the presentations of carrots or oats in rabbits before (pre-op) and after (post-op) lesions of the medial hypothalamus

Animal No.	Extinction time in min.		Number of responses during extinction session			
			carrots		oats	
	pre-op	post-op	pre-op	post-op	pre-op	post-op
11	10.5	7.3	26	5	11	15
12	8.2	7.8	18	4	4	13
13	4.0	3.4	23	8	4	9

Table II

Extinction of CR's associated with the presentations of carrots or potatoes in rabbits before (pre-op) and after (post-op) lesions of the medial hypothalamus

Animal No.	Extinction time in min.		Number of responses during extinction session			
			carrots		potatoes	
	pre-op	post-op	pre-op	post-op	pre-op	post-op
16	5.6	5.0	16	7	8	7
17	6.2	4.9	19	7	12	2

The animals with lesions of the *lateral hypothalamus* following recovery of CR performance greatly increased responsiveness while extinguishing the CR's reinforced with all types of food used. The slowest extinction rate in these animals was that under conditions of reinforcement with carrot (Tables III and IV). In gross behavior, the lateral hypothalamic lesioned animals were surprisingly tame and placid. Their increase in the response rate in extinction seems to be a reflection of a somatoperseverative type of behavior.

The finding that animals with lateral hypothalamic lesions after recovery of eating behavior extinguish a particular food-reinforced

response much slower than animals with medial hypothalamic lesions still in the dynamic phase of hyperphagia adds support to the argument (B a i l l e and M o r r i s o n 1963, L e w i ń s k a 1964, M i l l e r, B a i l e y and S t e v e n s o n 1950, T e i t e l b a u m 1962) that the relations of the hypothalamic ,feeding' and ,satiaty' systems are more complex than it was thought before, and the motivation for food in animals with medial and lateral lesions is still difficult to assess. We therefore consider it is worth reporting these results, even though we lack an explanation.

Table III

Extinction of CR's associated with the presentations of carrots or oats in rabbits before (pre-op) and after (post-op) lesions of the lateral hypothalamus

Animal No.	Extinction time in min.		Number of responses during extinction session			
			carrots		oats	
	pre-op	post-op	pre-op	post-op	pre-op	post-op
19	7.8	24.6	28	93	5	17
20	20.0	30.0	34	105	13	13
21	6.3	14.4	15	58	5	12

Table IV

Extinction of CR's associated with the presentations of carrots or potatoes in rabbits before (pre-op) and after (post-op) lesions of the lateral hypothalamus

Animal No.	Extinction time in min.		Number of responses during extinction session			
			carrots		potatoes	
	pre-op	post-op	pre-op	post-op	pre-op	post-op
24	2.5	12.3	12	43	0	23
25	5.3	13.5	9	40	11	15
26	9.0	25.7	27	75	7	25

A pilot study conducted by us demonstrates that (a) rabbits with medial hypothalamic lesions prefer to take grains of oats or cooked potatoes due to a small quantity of water in these two types of diet, on the other hand, (b) rabbits with lateral hypothalamic lesions accept more carrots because they show a preference for food containing more water. This demonstration helps to understand the slow extinction rate for carrots in laterally lesioned animals.

SUMMARY

Extinction of a food-reinforced response is much slower in lateral hypothalamic lesioned rabbits who have recovered eating behavior than in medial hypothalamic lesioned rabbits still in the dynamic phase of hyperphagia. When offerred free choice between carrots, purée-type potatoes, or grains of oats, the animals with medial lesions show the slowest extinction rate while working for oats, whereas the animals with lateral lesions show the slowest rate while working for carrots.

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THE EFFECT OF FOOD DEPRIVATION
ON BLOOD SUGAR LEVEL, FOOD INTAKE AND CONDITIONING IN RABBITS WITH MEDIAL HYPOTHALAMIC LESIONS

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Since the discovery that lesions, located in the hypothalamic "satiety center", result in overeating and obesity (H e t h e r i n g t o n 1942a, 1943, A n a n d and B r o b e c k 1951), many attempts have been made to clarify the problem of the hunger drive and the effort to satisfy it in animals with lesions of the ventromedial hypothalamus.

A fact that some animals with ventromedial lesions eat more ravenously than the normal ones (B r o b e c k e t a l. 1943, and others) and greatly increase their food intake is an indication of their similarity to unoperated hungry animals. It has, however, been revealed by recent studies with the use of the motivation tests for measuring the efficacy of the drive (M i l l e r e t a l. 1950, M i l l e r 1955, 1957, A n l i k e r and M a y e r 1956, 1957, T e i t e l b a u m 1957, 1958) that these problems are more complex than believed so far and the behavior of ventromedial animals is still difficult to estimate.

The views on the mechanism of activation and inhibition of feeding centers in hunger and satiety stages are also under discussion. A glucostatic (M a y e r 1952, 1953a, b), lipostatic (B r u c e and K e n n e d y 1951, K e n n e d y 1953) and thermostatic (B r o b e c k 1946, 1948, S t r o m i n g e r and B r o b e c k 1953) theories, as well as a theory, relating hunger phenomena with the amino acid content of the blood serum (M e l l i n k o f f e t a l. 1955) were developed over the last decade. Continuing the subject, taken up by J a n o w i t z and G r o s s m a n n (1951), S h a r m a e t a l. (1961) proved that the satiety center responds to the stomach extension. Attention was drawn by many authors, and recently by A n d e r s s o n and L a r s s o n (1961), to the correlation, existing between the food intake control centers and the water intake control centers.

An effect of lesions of the medial, particularly the ventromedial regions of the hypothalamus on the feeding behavior and the blood sugar level in rabbits in various stages of food deprivation is dealt with by the studies, described in the present paper. Our previous experiments indicated (L e w i ń s k a 1963) that the blood sugar level and food intake of food deprived rabbits are subject to rhythmic changes, depending on the previous feeding once in 24 hrs. A negative correlation was shown between the changes in the blood sugar level and the changes in the food intake. The present paper refers to those results which seem to confirm the assumptions advanced by the glucostatic theory. The participation of the ventromedial nucleus and primarily of the paraventricular nucleus in the control of the carbohydrate metabolism and of the blood sugar level has been pointed out by many authors for a long time. There were, however, few studies in which the blood sugar level in the hypothalamic hyperphagia was determined in addition to the food intake. M a y e r (1952, 1953b) maintains that the blood sugar level in rats was lowered in their dynamic phase of the hypothalamic obesity. A tendency was observed by H e i n b e c k e r et a l. (1944) to the blood sugar level drop in hyperphagic dogs with lesions placed caudally to the paraventricular nucleus. On the other hand, in mice with the hyperphagia, produced either by the electrocoagulation, or by giving the goldthioglucose, the blood sugar level remained unlowered (M a y e r et a l. 1955).

In view of these divergences, we attempted to investigate the postoperative correlation between the blood sugar level and food intake in rabbits, both under usual conditions and after various periods of food deprivation. The food CR Type II has been trained in these rabbits. The observation of the CR activity and of animals' gross behavior before surgery was aimed at a comparison with corresponding measures during the period of the hyperphagia, produced by hypothalamic lesions. In addition, we were interested whether starving rabbits in the dynamic hyperphagic stage will or will not increase their food drive and food intake.

MATERIAL AND METHOD

Experiments were carried out on 16 male and female rabbits about 2 years old. This number comprised 3 groups of 5 rabbits each, that is, groups A, B and C, as well as a rabbit, denoted as. No. 10 and used for the preliminary control tests. The average body weight amounted to 2.57 ± 0.25 kg. During the CR experiments. the group A animals were fed carrots, group B — potatoes (purèe) and, group C — grains of oats. This basic diet was supplied *ad libitum* only during experiments which took place once in 24 hrs. After experiments, the rabbits of group A were

given 50 g of potatoes, oats and hay each, those of group B — 100 g of carrots and 50 g of oats and hay and, those of group C — 100 g of carrots and 100 g potatoes and 50 g of hay. Water was not given at all.

The CR training. Experiments were carried out on an empty stomach at fixed morning hours. A food CR Type II (K o n o r s k i and M i l l e r 1933), manifested by placing the paw on a platform situated near the food tray, was trained in rabbits in a feeding cage (B a l i ń s k a et al. 1961). In response to a correct CR performance, a bowl with food was presented to an animal. The amount of food, necessary as a single reinforcement of the CR Type II was always the same. Rabbits of group A and the rabbit No. 10 were fed a piece of carrot, weighing about 3 g, rabbits of group B — 10 g of cooked potatoes and rabbits of group C — 1 g of oats. With so selected values of particular doses, the level of the CR rate, measured by the number of CR performances per minute in all rabbits was very stable.

Recording. The CR responses were recorded on a kymograph by means of a device, consisting of two Marey drums, one of them placed under the plate, the other — connected to the recorder. The number of the CR responses per minute served as an indicator of the rate of eating under experimental conditions. Since, satisfying their hunger, rabbits ate at an ever slower rate and since, towards the end of the experiments, intervals occurred sometimes in the food intake, the number of movements per unit of time was calculated on the basis of the initial minutes of the experiment. In groups A and C in which experiments lasted for about fifteen to thirty minutes, the mean number of movements was computed from the first ten minutes of the experiment. In group B, rabbits' satiety was reached in about 10 minutes and so, the number of movement responses per minute was calculated on the basis of the first five minutes.

Food deprivation. The adaptation of animals to the conditions, described above, lasted for 3 months (from October to December). Over the next three months (January, February, March), the animals were subject to food deprivation every few days. The length of the deprivation period was conventionally determined by the number of hours by which 24-hour intervals between the food intake experiments were extended. Each rabbit was subjected to the series of such deprivations 6-, 12-, 24-, 36-, 48- and 60-hour long. The intervals between particular deprivations were irregular because they depended on a condition of individual animals and amounted from 5 to 12 days. Food deprivation was continued only when an animal recovered its previous body weight. In doing so, the CR behavior, the amount of the food ingested during regularly made experiments and the body weight, were taken into account.

Weighing. Prior to the food intake experiments, weighing took place once a week. During food deprivation experiments, rabbits were weighed on a day when deprivation was started and after the end of it but prior to their being fed once more.

The blood sugar level was determined by the Hagedorn and Jensen method. The blood was taken from the ear marginal vein. The determination was effected in the morning, on an empty stomach and directly prior to the food intake experiments. The blood sugar level was determined in each rabbit several times over a 1-month period, preceding the food deprivation. During the period of the deprivation experiments, initial determinations were carried out, as usually, in the morning, then, food deprivation took place and, after its termination, the blood sugar level was determined once more. In the case of longer food deprivation

periods, the determination was carried out every 12 hours. In the rabbit No. 10, the blood sugar level was not determined.

Surgery. After completing a series of deprivation experiments, when an animal has recovered its body weight, rabbits were operated upon in the Horsley--Clark stereotactic apparatus with the head holder, designed by Sawyer et al. (1954). A lesion was made by means of double stainless steel electrodes 0.5 mm in diameter. The electrodes were fully insulated, except for their 1 mm long tips. A distance between electrodes amounted to 1.5 mm. Rabbits were operated under the Nembutal narcosis with the observance of aseptic conditions. The Nembutal dose applied amounted to 40 to 42 mg/kg. According to the atlas by Sawyer et al. (1954), the ventromedial nuclei are situated near ventricle III, 14 to 16 mm beneath the brain surface. They stretch anteroposteriorly from plane A1 to plane P1 over a length of over 2 mm. The width of the nuclei in the frontal APO plane equals about 1 mm. The points of the electrode insertion were selected in the lateral direction 0.8 mm from the sagittal suture and, in the rostrocaudal direction, the first electrode was implanted on the boundary of the bregma and the second, 1.5 mm behind it. Electrodes were inserted into the brain to a depth of 15 mm. Lesions were produced by passing a direct current of 3 mA for 15 secs. This operation was made bilaterally.

Postoperative treatment. After surgery, rabbits were dealt with identically as during the preoperative period. On the first days, food was given only in home cages. The CR experiments were resumed 3 to 4 days after the operation. Afterwards, food deprivation experiments were started. The CR experiments were completed about 3 months after the surgery. Over the next 3 weeks, animals were *ad libitum* fed carrots, potatoes, oats and haẏ.

Histology. About 4 months after operation, rabbits were killed and their brains were histologically verified. Sections, 20 microns in thickness, were stained by the Nissl and Klüver methods. Every tenth section was analyzed.

Statistics. Prior to statistical comparisons, it was ascertained by means of the Mosteller modified test "A$_1$" that the results obtained come from the same general population of rabbits. The results were compared either by test "A$_1$" or by the use of the so-called "zero hypothesis" according to the following formula:

$$U = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\sigma_1^2 n_2 + \sigma_2^2 n_1}} \sqrt{n_1 n_2}$$

RESULTS

Before surgery

Food intake. In most cases, the amount of the food ingested by animals during the CR experiments was not subject to any major fluctuations. As shown in Table I, rabbits' average food intake was: in group A — 87.3 g of carrots, in group B — 76.8 g of purée type potatoes and, in group C — 28.8 g of grains of oats. The rabbit No. 10 ate 131 g of carrots. A standard deviation in groups A and B averaged several, and in group C — a few grams.

Table I

Mean food intake during CR experiments before
medial hypothalamic lesions in rabbits

	No.	Mean food intake		
		$\bar{X}$ gms	σ gms	V %
Group A (carrot)	1A	85.5	15.0	17.5
	2A	102.6	18.4	17.9
	3A	81.0	13.6	16·9
	4A	70.0	7.7	11.0
	5A	97.6	10.6	10.8
	Mean	87.3		
Group B (potatoes)	1B	84.1	20.6	24.4
	2B	91.1	19.2	21.0
	3B	96.0	22.2	23.1
	4B	41.3	14.8	35.8
	5B	71.5	14.1	19.7
	Mean	76.8		
Group C (oats)	1C	24.5	4.5	18.4
	2C	30.3	4.7	15.5
	3C	29.3	7.2	24.6
	4C	28.3	6.9	24.4
	5C	32.3	7.1	22.0
	Mean	28.8		

X — mean value, σ — standard deviation, V — coefficient of variation

Food intake after food deprivation intervals. After increased periods
of food deprivation, the food intake showed characteristic fluctuations.
In group A, the consumption of carrots, as compared with that during
experiments which preceded the food deprivation, was increased after
each deprivation period. It has been shown, however, by a statistical
comparison that a mean food intake in a given series of measurements
in relation to the mean, obtained from previous measurements, was
increasing after 12, 36 and 60 hours, while it was decreasing after 24
and 48 hrs (in all cases $p < 0.05$). It was shown by test "U" that a signi-
ficant increase in the food intake occurred in group B (purée type po-
tatoes), after 12-, 36- and 60-hour starvation, while, in group C (grains
of oats) — after 6-, 12-, 36- and 60-hour starvation ($p < 0.01$).

During experiments, shifted in time by 24 and 48 hrs, the figure,
denoting the consumption of potatoes and oats, did not differ to any
significant degree from the mean value, obtained from experiments,
which took place once in a 24-hour period. The maximum carrot and
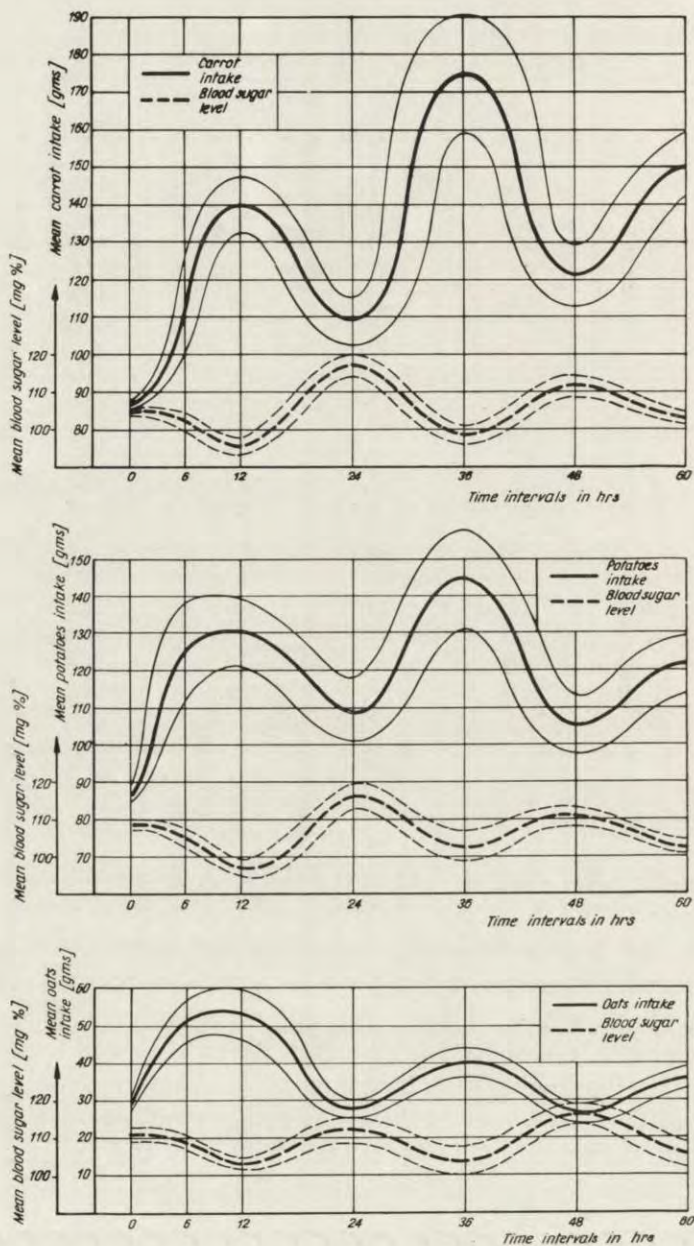
Fig. 1. The effect of food deprivation periods on blood sugar level and food intake in rabbits before lesions of the medial hypothalamus. Mean deviation from mean value $\frac{\sigma}{\sqrt{n}}$ is depicted with a light line
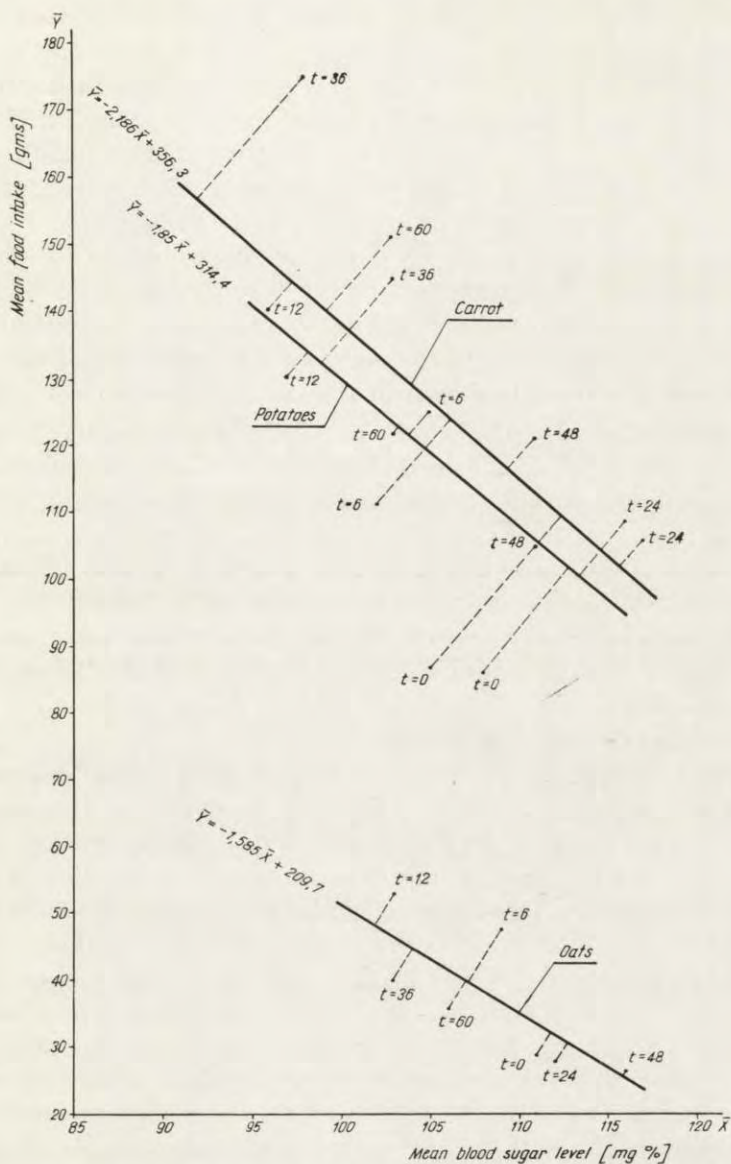
Fig. 2. The correlation between food intake and blood sugar level

potato consumption was recorded after a 36-hour food deprivation, while the maximum ingestion of oats — after a 12-hour deprivation (Fig. 1).

Blood sugar level. On an empty stomach, it showed a considerable stability and, in the rabbits investigated was contained within limits of 98 and 122 mg⁰/o. In individual cases, mean deviation was ca. 4.2 mg⁰/o.

Blood sugar level after different periods of food deprivation. During food deprivation, changes in the blood sugar level were of the order of a few to several or so mg⁰/o (Fig. 1). After successive deprivation periods, two series of measurement results were compared with other two series. A preceding series was always treated as a distinguished one. A significant decrease in the blood sugar level occurred after 12, 36 and 60 hours of deprivation (in all cases, $p < 0.05$).

The drop in the blood sugar level was accompanied by the increase in the food intake and, the increase in the blood sugar level — by the decrease in the food intake (Fig. 1). The course of the regression equations, determining the theoretical correlation between the food intake and the blood sugar level is shown in Fig. 2.

The food CR Type II. In general, the CR reaction occurred without any disturbances, being performed at a progressively decreasing rate with the experiment duration. Rabbits 1B and 4B were the only exceptions. The decrease in the rate of the food CR often occurred, in their case, under the influence of certain slight noises. In rabbit 4B, the response was limited sometimes to a few movements. Except for rabbit 4B, the number of CRs per minute was, in each rabbit, a characteristic and considerably stable magnitude. Mean numbers of CRs per min. in individual rabbits were contained within limits of 1.8 to 3.4 and of mean deviations — of. 0.14 to 0.36.

The food CR Type II after different periods of food deprivation. The degree of the intensification of the CRs depended on the length of the starvation period and it fluctuated rhythmically parallel to the changes in the food intake. Shifting experiments by 6, 12, 36 and 60 hrs was followed by a considerable increase in the general mobility of the animals. When put into the feeding cages, rabbits wriggled, tried to break away and quickly jumped to the food trays. The movements of their paws were violent and, in some animals, stronger which was depicted by the rise of the amplitude in the kymograms. In some cases, repeated movements or scratchings were recorded, in others — prolonged keeping of their paws on the food tray. Of the symptoms, mentioned above, changes in the rate of eating were common for all rabbits of a given group and simultaneous during the same experiment. Comparisons showed that the statistically significant increase in the number of CRs per minute occurred, in rabbits of group A, after shifting experiments by 12, 36 and 60 hrs, in rabbits of group B — by 36 and 60 hrs and, in rabbits of group C, by 6, 12 and 36 hrs (in all cases, $p < 0.01$). After the 24- and 48-hour food deprivation, despite some animation, occurring now and again, no statistical differences were recorded in the rate of eating (Fig. 5).

After surgery

Anatomy. Lesions, stretching mostly bilaterally along ventricle III are indicated in Fig. 3 which presents a diagram of the cross section of the rabbit brain in a frontal APO plane. These lesions covered more or less the ventromedial nuclei and, in some cases, encroached on the dorsomedial nuclei. The paraventricular nuclei were damaged in rabbits 2A, 1B and 4B. Unilateral lesions of the hypothalamus were obtained in rabbits 3B and 10. Paraventricular and dorsomedial nuclei, as well as a lateral area, situated at the same level and dorsolaterally covering the fornix were partially injured in rabbit 3B. An area, comprising the paraventricular and dorsomedial nuclei, as well as the fornix was partially destroyed in the rabbit No. 10.

Table II

Mean food intake during CR experiments after medial hypothalamic lesions in rabbits

|	No.	Mean food intake			Food intake in % % as compared to the pre-op level
		$\overline{X}$ gms	σ gms	V %	
Group A (carrot)	1A	156.5	20.0	12.7	181
	2A	235.1	20.4	8.7	230
	3A	135.3	10.9	8.1	167
	4A	137.0	12.7	9.3	196
	5A	202.0	19.3	9.6	207
	Mean	173.2			196
Group B (potatoes)	1B	157.6	23.7	15.0	187
	2B	180.0	12.4	6.9	198
	3B	105.5	24.8	23.4	110
	4B	126.1	21.2	16.8	305
	5B	146.0	26.2	18.0	204
	Mean	152.8*			227*
Group C (oats)	1C	47.6	11.3	23.7	194
	2C	55.6	4.8	8.6	183
	3C	55.5	8.6	15.5	189
	4C	60.6	11.5	18.9	215
	5C	60.3	14.6	24.3	187
	Mean	55.9			194

* Rabbit No. 3B is not included in means due to the failure of hyperphagia.

$\overline{X}$ — mean value, σ — standard deviation, V — coefficient of variation.
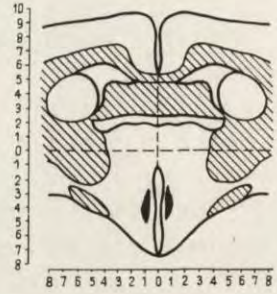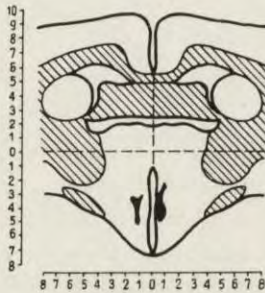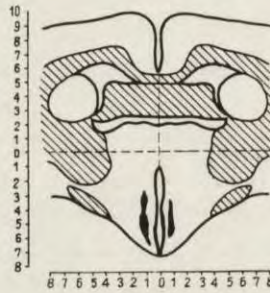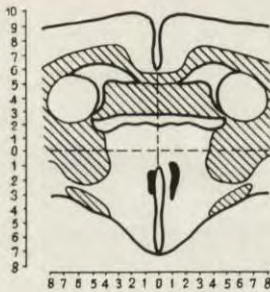
Group A
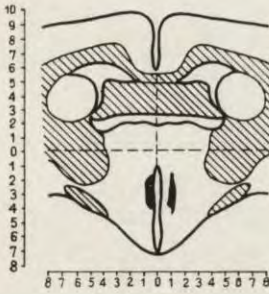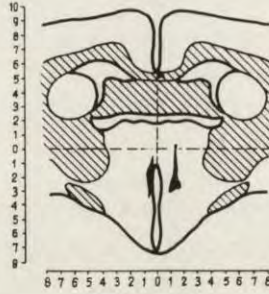
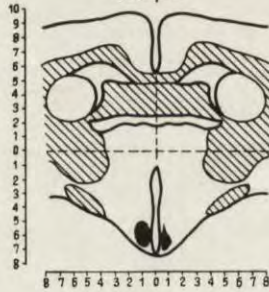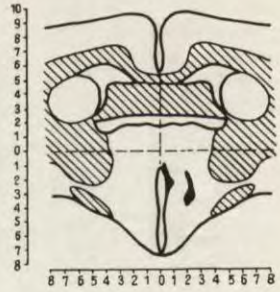Rabb. No 1A   Rabb. No 2A   Rabb. No 3A

Rabb. No 4A   Rabb. No 5A
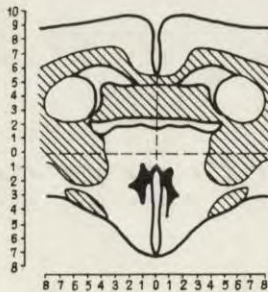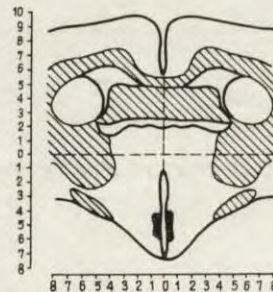
Group B

Rabb. No 1B   Rabb. No 2B   Rabb. No 3B
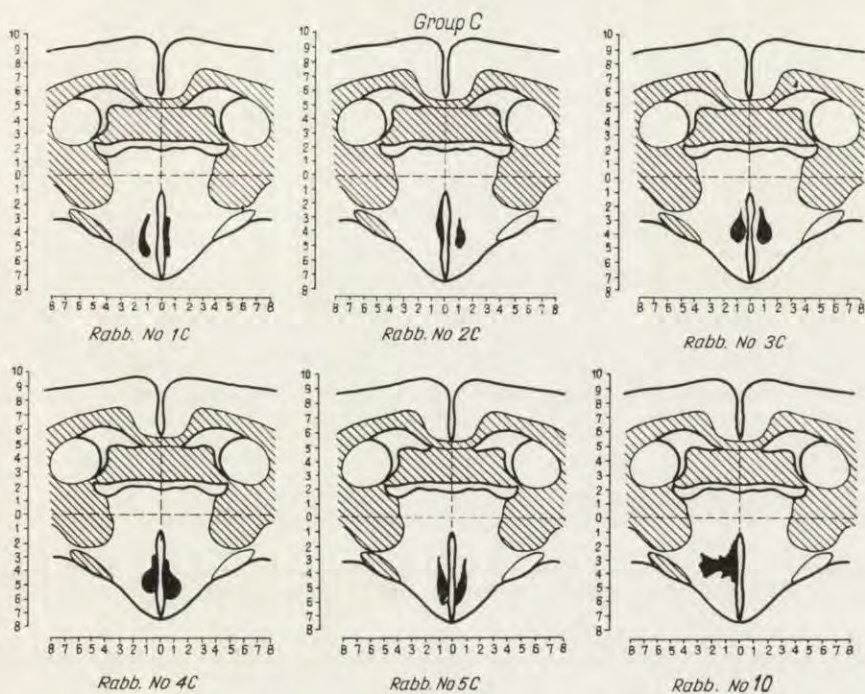
Rabb. No 4B   Rabb. No 5B

Fig. 3. Reconstructions of lesions of the medial hypothalamus in rabbits at APO frontal plane

Dorsoventrally, the lesions covered an area about 2 to 3 mm long laterally and about 2.5 mm — rostrocaudally. In most cases, lesions, stretching rostrocaudally, were contained between planes A1 and P1.

Food intake. It was already immediately after the surgery that the hyperphagia occurred in most rabbits. In some of them (Nos. 3A, 4A, 1B and 1C) it was observed after a few days. In rabbit 2B, the food intake increase started only after the 8th postoperative day. Prior to that moment, the rabbit was distinctly sunk in apathy. Its fur was wet near the ears. These disturbances disappeared after 2 weeks. There-after, hyperphagia occurred and was maintained up to the end of the experimental period, that is, for about 3.5 months. The mean increase in the food intake was about 2 times as much as at first and was con-tained within limits of 167 and 230 per cent. (The case of the triple food intake, recorded in rabbit 4B is disregarded since its small food intake during the preoperative CR experiments was caused by a neurosis). Rabbit 3B was an exception. Immediately after the operation, it showed a very uneven extent of hunger, sometimes ingesting considerably increased amounts of food. After a number of days, the food intake in
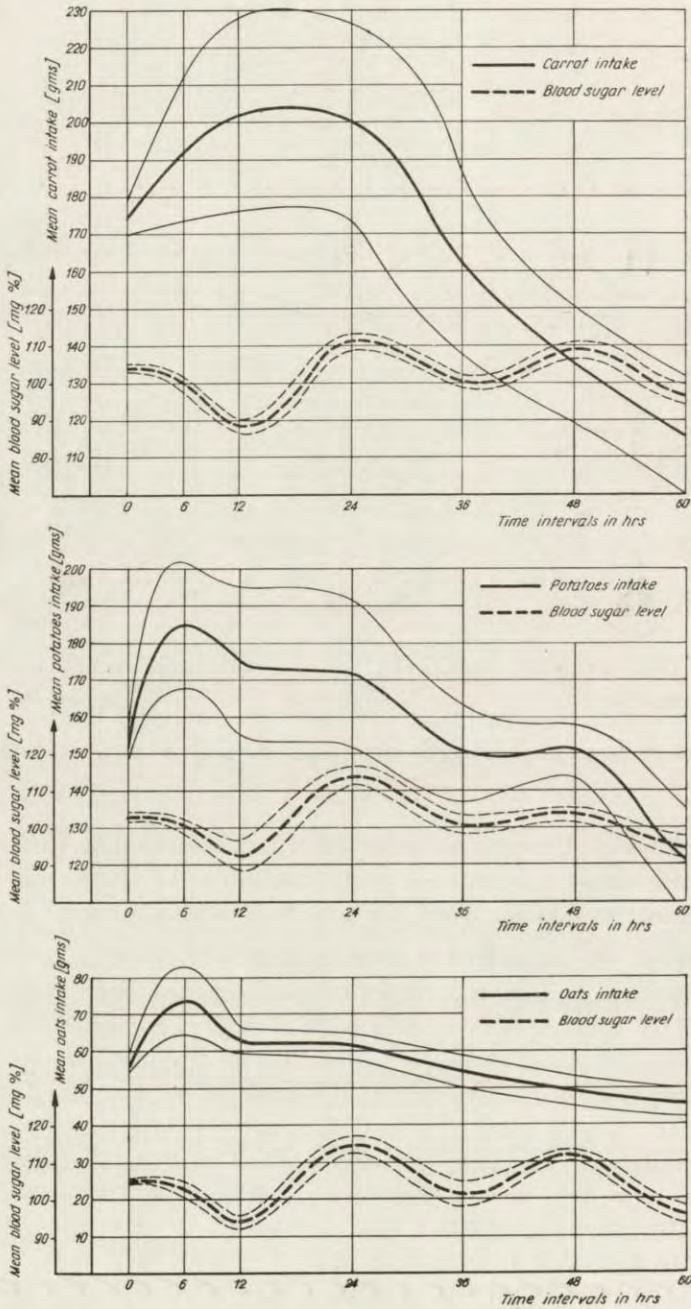
Fig. 4. The effect of food deprivation periods on blood sugar level and food intake in rabbits after lesions of the medial hypothalamus. Mean deviation from mean value $\frac{\sigma}{\sqrt{n}}$ is depicted with a light line. Rabbit No. 3 is not included in means due to the failure of hyperphagia

this rabbit recovered it preoperative level (Table II). In the control rabbit reached that recorded preoperatively. In the remaining animals,

Food intake after food deprivation intervals. It is clear from Fig. 4 that, against the background of hyperphagia, caused by the medial lesions of the hypothalamus, an increase in the food intake, provoked by deprivation was very slight and, as shown by statistical computations, insignificant. An essential decrease in the ingestion of carrots and grains of oats ($p < 0.01$) was caused by a 60-hour food deprivation period.

As regards absolute values, the amount of food, eaten by operated animals after 6, 12 and 24 hours of deprivation, exceeded the amount of food, ingested after indentical periods of the preoperative deprivation. Longer periods of food deprivation, primarily those of 60 hours, caused — like before the operation — a food intake of approximately identical amounts.

Blood sugar level. The blood sugar level was first determined on the fourth postoperative day. It was found that, in most rabbits, the blood sugar level was not changed to any considerable extent. It was only in rabbit 3B that the hyperglycemia was recorded, amounting to 240 mg⁰/o. After 2 weeks of fluctuations, the level of the blood sugar in this rabbit reached that recorded preoperatively. In the remaining animals, over the entire 3-month postoperative period, the blood sugar level showed a relative stability and was contained within limits of 98 to 111 mg⁰/o. Similarly to the preoperative stage, mean deviations did not exceed a few mg⁰/o. A statistically significant drop in the blood sugar level was recorded in rabbits 1B, 2B, 4B, 1C and 5C. It was not, however, very large, except in rabbit 1C (about 20 mg⁰/o).

Blood sugar level after different food deprivation periods. Similarly to the preoperative stage, a statistically significant drop in the blood sugar level occurred after 12, 36 and 60 hrs of the food deprivation and an increase — after 24 and 48 hrs (except for the results, obtained after 48 hrs in group B where the increase was not, however, significant). In all the remaining cases, $p < 0.05$.

After the operation, the changes in the blood sugar level did not show any correlation with the changes in the amounts of the food consumed (Fig. 4).

Food CRs Type II. Changes which occurred in the CRs and in gross behavior of animals were not uniform. They were connected with the rate of eating, character of movements, as well as with general emotionality and they occurred in different combinations. Due to the fact that, prior to the operation, a considerable increase in food intake was accompanied by the increase in the rate of eating, particular attention

was paid to make sure whether this symptom occurred or not in the rabbits operated.

The results obtained, were divided into three groups.

Group 1. An increase in the number of CRs per minute (p < 0.01) was obtained in rabbits 2A, 1B and 10. Rabbit 4B, in which the changes in the CR reaction were not statistically compared because of a small and non-uniform preoperative reaction, was also assigned to this group. After the operation, a timid and slug-gish behavior of the rabbit was replaced by a lively and sure movements. The postoperative kymograms were characterized by the uniform continuity. Some-times, double movements were observed. An unteased rabbit behaved quite nor-mally, but at the sight of a hand, stretched out towards it, reacted aggressively. The behavior of rabbits 2A and 1B considerably changed after the operation. At the sight of a person, these rabbits took an aggressive attitude which was never observed in them when they were deprived of food before the operation. They dashed at the hand, tried to bite it and squealed. They attacked the food tray with their teeth and claws and, afterwards, they began to eat hurriedly, some-times clumsily, spilling the food around. In their cages, they often did not finish eating the portions offered and — by scratching — demanded new portions. Otherwise, their paws were correctly placed on the food tray. The biting reflexes were pronouncedly increased in rabbit 2A which gnashed at all objects in its reach, mostly the floor of the cage.

Lesions in the rabbits, mentioned above, were bilateral, located high at the level of the paraventricular nuclei or, at least partially, involving these regions.

A considerable increase in the CR performance, expressed either by the rise of the amplitude, or by keeping the paw on the food tray, or an increased number of the food CRs per minute, occurred in the rabbit No. 10. The lesion of the hy-pothalamus in this rabbit was unilateral at the level of the dorsomedial nucleus and of the fornix. A slightly similar lesion was found in rabbit 3B in which scratching, gnashing the food tray and increase in the aggressiveness of behavior were recorded. These changes persisted up to the end of the experimental period despite the fact that the hyperphagia was never observed in this animal.

Group 2. A decrease in the number of the CR performance per minute (p < < 0.01) was recorded in rabbits 2B, 1A and 5A. Rabbit 5A manifested an aggres-sive-defensive behavior. When taken out of its living cage, it resisted, wriggled, tried to escape and bite and, when placed in the experimental cage, attacked the food tray. In this animal, the CRs were disturbed. Over a 3-week postoperative period, this rabbit performed the CRs with its left or right or even both fore limbs simultaneously. These movements, when not reinforced, resulted in scratching, in other types of incorrect responses or in intervals during which the rabbit alter-nately raised its fore limbs but refrained from final placing them on the food tray board. Its movements were violent and, at the same time, disorderly and clumsy as compared to the precision with which it performed them prior to the operation. Mostly, the animal kept both its fore limbs on the food tray board. The amplitude of the kymograms rose. After a few weeks, the right limb movements were resto-red.

In rabbit 1A, the manner of the CRs was not subject to any distinct changes. The rabbit, put in the testing cage, after a few movements, fell into a drowsy torpor. So, the duration of experiments was sometimes considerably extended. The degree of the intensity of this state was subject to fluctuations. Nevertheless,

the drowsiness and reluctance to react persisted to the end of the investigations, that is, for about 3 months. After transferring it to the living cage, the rabbit usually brisked up and started eating the food, prepared for it.

Rabbit 2B, refused reacting for a week after the operation. Put into the experimental cage, it turned its back upon the food tray. Taken in hand, it tried to break free. After 10 days, the drowsiness disappeared and the CR performance returned. Its responses were not, however, uniform. The periods of considerable activity alternated those of drowsiness.

The lesions in rabbits, mentioned above, were bilateral but they differed in their location. In rabbit 5A, they occurred at the level of the ventromedial and dorsomedial nuclei. On one side they were situated near ventricle III, on another — slightly more laterally. In rabbit 1A, the lesion was stretched from the area of the dorsomedial nucleus in the P1 plane up to the ventromedial nucleus in the A1 plane. In rabbit 2B, the lesions covered the lower area of the hypothalamus and ventromedial nucleus, stretching from the A1 through the APO and reaching the P2 planes.

Group 3. The number of the CR performances per minute was not changed in rabbits 3A, 4A, 5B and 1—5C. A fundamental difference in the rabbits' feeding behavior was not made up by the increase in the eating rate but by the increase in the experiment duration. On the first postoperative days, certain disturbances also occurred in some rabbits of this group, consisting mostly in a prolonged keeping of their fore limbs on the food tray.

In most rabbits of this group, lesions were symmetrically located at the level of the ventromedial nucleus and fairly near ventricle III. In rabbit 4A, lesions stretched in a sagittal direction from the ventromedial nucleus to the A2 plane. After the operation, an aggressiveness of a medium intensity was recorded in this rabbit.

An aggressive behavior, observed in a few rabbits did not disappear under the influence of satiety.

The food CRs Type II after different starvation periods. A clearly observable activation in the hyperphagic animals were caused by shorter food deprivation periods. This was primarily reflected in a rapid eating rate. The number of CRs per minute increased in a statistically significant manner in group A, after 6, 12, 24 and 36 hrs of starvation, in group B, after 6, 12 and 24 hrs and, in group C, after 6 and 12 hrs. In all cases, $p < 0.01$. It is clear from Fig. 5 that while the mean food intake in operated rabbits increased about 2 times, the mean number of CRs per minute remained unchanged. On the other hand, after short food deprivation periods, the mean number of CRs per minute increased significantly, while the food intake did not show any significant differences. Periodical fluctuations in the number of the CR responses per minute disappeared.

The effect of food deprivation and hypothalamic lesions on animals' body weight. Losses in body weight, caused in animals deprived of food were rapidly compensated after normal feeding was resumed. In the case of the greatest body weight loss which amounted to about 0.25 kg, the compensation of the loss took a few days. The losses in body weight
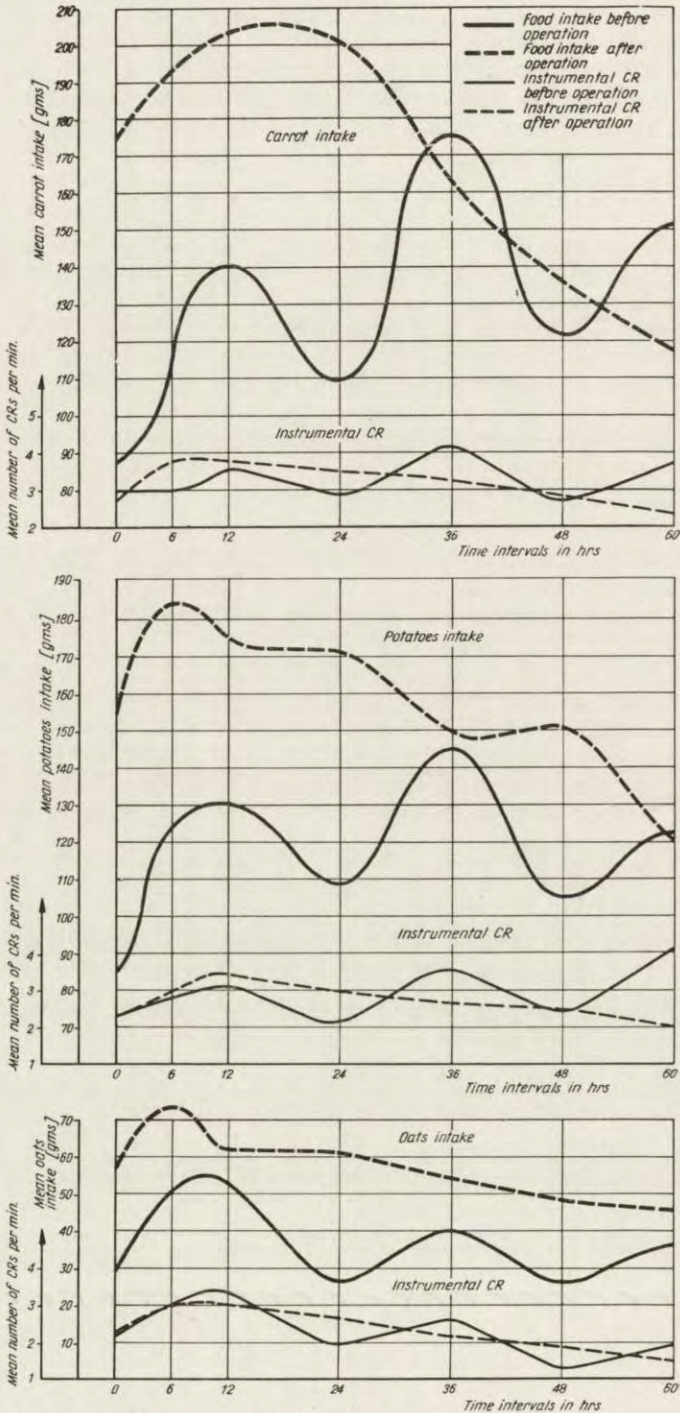
Fig. 5. Food intake and the number of CRs per min. before and after medial hypothalamic lesions at various deprivation periods. Rabbit Nos. 3B and 4B are not included. Explanation in text
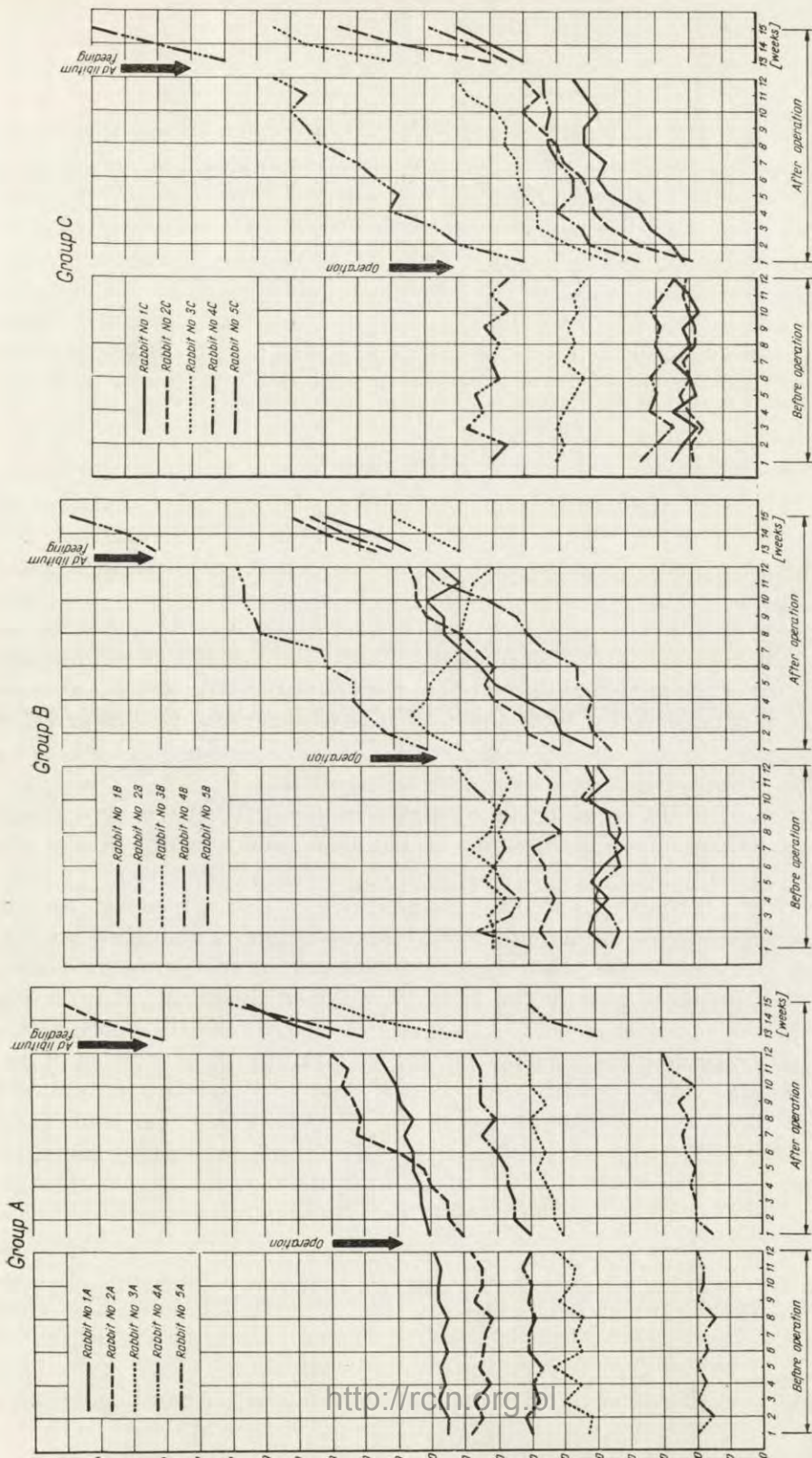
Fig. 6. Body weight before and after medial hypothalamic lesions

caused by both the pre- and postoperative deprivations were of the same
order. The effect of the hyperphagia on the rabbits' body weight is
illustrated by diagrams (Fig. 6). A conspicuous increase in the body
weight occurred in the animals which, during experiments, were *ad
libitum* fed grains of oats or the purée potatoes. After a 3-month hyper-
phagia, the increase in the body weigth in various rabbits amounted to
0.25 to 0.57 kg. After the termination of the CR experiments, rabbits
were fed ad libitum (carrots, potatoes, oats and hay) for 3 weeks. Their
mean body weight increase over that period amounted to about 0.5 kg.

DISCUSSION

The above data show that the lesions of the medial areas of the
hypothalamus change the food intake and the CR behavior not only
under usual conditions, but also during the food deprivation. In addition,
they cancel a negative correlation between the changes in the blood
sugar level and the food intake which was previously established on the
basis of determinations, performed in starvated rabbits, in the morning
and in the evening. Rhythmic phenomena, related with the blood sugar
level and the magnitude of the food intake under the food deprivation
conditions, have been discussed in the previous paper (L e w i ń s k a,
1963).

In our results, particularly striking was the fact that the hyperphagia
was obtained after the lesions of both the ventromedial nuclei and
regions of the paraventricular nuclei. According to H e t h e r i n g t o n's
(1941) and K e n n e d y's (1950) pioneer investigations, made on rats, an
area, occupied by the hypothalamic satiety center is identified with an
area, occupied by the ventromedial nuclei and an area, situated ventro-
laterally to these nuclei. The hyperphagia was also obtained in rats as
a result of the lesions of the fibers stretching between the ventromedial
and lateral nuclei (A n a n d and B r o b e c k, 1951), as well as of sec-
tioning the fibers which run ventrocaudally from the ventromedial
nuclei towards the mammillary bodies (H e t h e r i n g t o n and R a n-
s o n 1942a, B r o b e c k 1946). On the other hand, an obesity which not
always was accompanied by the increase in the food intake was observed
by H e i n b e c k e r et al. (1944) in dogs with lesions of the paraven-
tricular nuclei and areas, caudally situated to this nucleus. The expe-
riments of P o i r i e r et al. (1962) made on monkeys, should be men-
tioned among recent investigations which confirm these results. These
authors believe that, „lesions which produced obesity in the absence of
absolute hyperphagia were located somewhat dorsally and rostrally in
relation to the ventromedial hypothalamic nuclei". According to these

authors, certain metabolic and endocrinological disturbances, occurring simutaneously with these lesions, prove the break of the neuroendocrine balance.

In our operated rabbits, the tendency to the obesity was always accompanied by higher or lower increase in the food intake. The rabbits in which lesions were unilaterally located in the APO plane in the region of the fornix constituted the only exception. These rabbits showed neither the permanent hyperphagia nor the obesity. A less intensive hyperphagia seemed also to be produced by lesions, located at the level of the dorsomedial nuclei and in the near vicinity of ventricle III. On the other hand, the increase in the body weight always occurred primarily in the hyperphags which were fed grains of oats and the purée potatoes as their basic diet (groups B and C). In rabbits, *ad libitum* fed on carrots (group A), the body weight was not subject to any significant changes. Rabbit 2A, showing a clear tendency to putting on weight was an exception in this group. The lesions in this animal, similarly to rabbit 4B, also distinguished by an increased inclination to corpulence, were located in the region of the paraventricular nuclei. Maybe, this type of lesions introduced, beside hyperphagia, some additional metabolic disturbances, manifested by the tendency to putting on weight.

Cn the average, the increase in the food intake we observed after operations, was about twofold and, therefore, not so excessive as mentioned by some authors. It should be, however, emphasized that we estimated the hyperphagia once in 24 hrs on the basis of a definite food intake up to the satiety point. On the other hand, this approximately double increase in the food intake reached the upper limit of an amount of food which animals were able to ingest at one sitting because the food deprivation intensified this effect only slightly. Irrespective of the lesion location, after 3 months, all hyperphagic rabbits, given the *ad libitum* diet, were able to ingest larger amounts of food daily and all of them showed a similar tendency to put on weight. This fact confirms a conclusion that hyperphagia was the main cause of obesity in the cases we observed.

In some animals, the increase in the food intake was accompanied by considerable emotional changes.

Such definitions of the hypothalamic hyperphagia as, „voracious tigerish appetite" or, „high degree of voracity" (K e n n e d y 1950), etc. can be met with in the literature. In many cases, animals were characterized by the irritation, the increase of the sensibility to the touch, the aggressiveness and violent movements. An increase in the food CRs and a partial disinhibition of the food inhibitory reactions were observed by B a l i ń s k a e t a l. (1961) and by B a l i ń s k a (1963) in rabbits with

medial hypothalamic lesions. On the basis of experiments, carried out on rats, a hypothesis was put forward by K e n n e d y (1950) that the hypothalamic hyperphagia seems to represent the increase in the primitive food drive which might be defined as hunger. On the other hand, experiments performed by M i l l e r e t a l. (1950), M i l l e r (1955, 1957), A n l i k e r and M a y e r (1956, 1957), as well as T e i t e l b a u m (1957, 1958) indicated that the „hypothalamic" animals do not show an increased hunger, if the capability of accomplishing tasks, connected with the food intake, the rate at which the food is secured and ingested and the consumption of a food with unpleasant taste are accepted as a measure of hunger. L a r s s o n and S t r ö m (1957) also expressed a view that there are no sufficient reasons to believe that, as a result of the destruction of the satiety center, the increase can take place in the activity of the food center in an uninjured lateral part of the hypothalamus. These authors can be charged with drawing their conclusions from experiments, made, in most cases, on animals fed *ad libitum* and, therefore, being in the stage of a fairly far-reaching obesity. This was precisely the reason why in our studies, we fed animals once in 24 hrs. In estimating the degree of the food drive in hyperphagic animals, we compared their behavior to that, recorded in various stages of the preoperative deprivation. During the preoperative period, in the correlation with the duration of the food deprivation, we observed the increase in the food intake, animal's agility and rate of eating, as well as the slowing down, dullness and lower food intake capability. In no case, however, regardless of the length of the deprivation period, an aggressiveness was manifested by the rabbits similar to that, observed in a few of them after the operation. The increased food intake, accompanied by the aggressive behavior gave indeed an impression of a high degree of voracity. On the other hand, however, the increase in the aggressiveness of its behavior was manifested, after the operation, by rabbit 3B in which the hyperphagia did not occur. And contrariwise, a part of hyperphagic rabbits was not aggressive at all. Besides, in animals, aggressive as a result of the operation, their aggressive behavior was only insignificantly mitigated by the satiety which indicates that this reaction occurred regardless of the state of hunger or of satiety. A fact that the region of the ventromedial nuclei was related with the aggressive reactions, was known from W h e a t l e y's paper (1944). Our observations, aimed at the localization of the areas the electrocoagulation of which causes aggressiveness are in conformity with R o m a n i u k's (1962, 1963) results, obtained from the experiments with rabbits and cats. It was also in our experiments that the aggressiveness occurred mostly after lesions of areas, located in the dorsal part of the medial hypothalamus.

It seems very likely that a close correlation exists between the food centers and areas, related with the defense and attack reactions but, as a result of lesions, these reactions might occur quite idepedently.

During the preoperative period, the increase in the food drive was primarily manifested in rabbits by the increase in the rate of eating and in the number of CRs per minute. After the operation, changes of this type occurred only in some rabbits and, despite the fact of their being caused by lesions located above the ventromedial nuclei, it was not in all cases that they were accompanied by the aggressiveness and even the hyperphagia. In the rabbit No. 10 with lesions located unilaterally in the APO plane at the level of the dorsomedial nuclei and the fornix, the hyperphagia was quite insignificant, while a considerable increase was recorded in its CR performance. On the other hand, in the cases in which lesions were located lower, mostly in the region of the ventromedial nuclei, animals' behavior, although undoubtedly hyperphagous, was free of any symptoms of voracity. In these rabbits, changes were primarily manifested by an increased food intake and, consequently, by a considerable increase in the duration of the food experiments. Slowing down, the languor in performing motor CRs, as well as the drop in the number of the food CRs per minute were even recorded in some rabbits. Rabbit 5A showed disturbances in the performance of motor CRs even a few weeks after the operation. The course of changes in the CR reaction up to the moment of its full reproduction approached that which occurred during the period of the original formation of the reflex. Perhaps, the disturbances, observed in this rabbit, were caused by the damage to certain elements of the medial forebrain bundle disposed in the medial area of the hypothalamus. This bundle, running in the APO plane through the lateral hypothalamus, is believed to play an important role in reactions motivated by food reward (Miller 1957, 1960 Morrison et al. 1957, Olds 1958, Brodie et al. 1960, Morgane 1961). After a few weeks, however, these disturbances were compensated. No disorders of this type have been recorded in the remaining rabbits. The CR Type II in rabbits 1A and 2B was quite correct. The drowsiness, observed in rabbit 1A, could be caused by damage done to structures, related with the sleep control. In this rabbit, lesions were reaching the P2 plane. According to Johnson and Kaelber (1961), the center of wakefulness is supposed to be located in this region. The lowered agility of rabbit 2B with lesions, involving the ventromedial nucleus and adjacent regions, situated anteriorly and beneath this nucleus, was also of a different character. On the first postoperative days, this rabbit did not show an increased food drive, refused to perform the CR and showed disturbances in the water balan-

ce. It has recently been found by Wyrwicka (1964) that the stimulation of an area, located anteriorly to the ventromedial nucleus in cats, causes the food intake. The effect we produced in rabbit 2B, is probably related with a partial lesion in this region.

Considering the problems of the intensity of the CR performances after ventromedial lesions of the hypothalamus, we cannot omit the results indicating the drop in the general locomotor activity of animals with these lesions (Hetherington and Ranson 1942b, Brooks 1946, Mayer et al. 1955, Brobeck 1955, Gladfelter and Brobeck 1962, and others). A view is expressed by many authors that the lowered activity is independent of other symptoms accompanying these lesions. Nevertheless, it seems very likely that the hypoactivity may be reflected in the CR performance of hyperphagous animals.

It is clear from these data that a behavior which is or is not voracious in character depends — in the hypothalamic hyperphagia — on the location of a lesion. In this light, it is not altogether a surprise that controversies as to the mechanism of the satiety center can be met with in the literature. It seems doubtful, therefore, if, in view of such variety of effects, produced by lesions even of a not very extensive area, the rate of eating and magnitude of the CR, applied before the operation as indexes of the excitation of the feeding center can continue to be considered of full value.

However, regardless of this view, it should be emphasized that, in animals with hypothalamic lesions after a 6, 12 and 24 hrs. of food deprivation, the mean number of CRs per minute considerably increased, but it did not exceed the preoperative mean.

On the one hand, this fact is a proof that the connections between the „food center" and the „CR response centers" were not surgically injured to any considerable extent and, on the other, that the stimulation of the food center can be changed independently of the satiety center. However, in contradistinction to the preoperative period, the food deprivation did not increase the food intake. If, therefore, the „initial" food drive, influenced by hunger, could be subject to certain fluctuations, the quantitative control of the food intake did not depend on these fluctuations to such an extent as prior to the operation. The amount of food which could be ingested by animals at a single sitting did not exceed a certain upper limit and it could be only significantly decreased as a result of a prolonged deprivation. This fact seems to indicate that a control, related with reaching the stage of satiety was disturbed as a result of the electrocoagulation of the medial regions of the hypothalamus. There can be no doubt that this control, despite the change in its limits and in its lability, still exists in a more primitive form. It is

not clear which factors in the hypothalamic hyperphags are decisive of reaching the point of satiety. On the basis of S h a r m a's et a l. (1961) experiments, a supposition seems to be permissible that the stimuli, resulting from the stomach extension, caused by the food ingested, were, at least partially, removed from this control. A conclusion occurs primarily from our experiments that the glucostatic control was subject to a certain disorder.

The glucostatic theory both in its original version (a correlation between the appetite and the blood sugar level) and its secondary version (the appetite need not necessarily accompany a lowered blood sugar level, but it must accompany a definite value of a difference in the A–V content of the blood sugar) was criticized by some authors (H a n s o n and G r o s s m a n 1948, J a n o w i t z and G r o s s m a n 1949a, b, G r o s s m a n 1955, Q u a a d e 1962, Q u a a d e and J u h l 1962) but confirmed by others (S t u n k a r d and W o l f f 1954, 1956, A n a n d et a l. 1961, A n a n d et a l. 1962).

In our experiments, a definite value of the blood sugar level was not related with a definite stage of hunger, if the food intake and the CR performance are used as a measure of hunger. With the prolonged starvation, the blood sugar level showed rising tendency and, despite this fact, we continued to observe the symptoms of hunger. A negative correlation, we recorded before the operation, was not expressed in any absolute values, but it concerned a tendency to the changes in the blood sugar content and in the food intake. In the cases in which the blood sugar level showed relative decrease, the quantity of the food consumed and the number of CRs in rabbits rose and vice versa. Except for a few cases, after the operation, lesions did not cause a permanent and significant lowering of the blood sugar level in rabbits, examined on an empty stomach. The hyperphagia did not, therefore, show any correlation with changes of this type. Moreover, the blood sugar level continued to be correlated with the time of day in which experiments were carried out, but the correlation between these changes and the food intake was abolished. The abolition of natural rhythms in the food intake in the hyperphagic hypothalamic mice was also reported by A n l i k e r and M a y e r (1956). In their experiments, the blood sugar level was not determined and no closer analysis of this phenomenon was ever published. Our results could be explained, from the standpoint of the glucostatic theory, by the fact that the satiety center with glucoreceptors, inhibits the food center in response to the rise in the blood sugar level. Hence the preoperative excitation of the feeding centers was larger when the blood sugar level dropped within certain limits than in the case when the blood sugar level rose. The injury of the satiety center

disturbed this correlation and hence, the correlation between the blood sugar level and the quantity of the food consumed was abolished. The fact that the lesions of both the ventromedial nucleus and the region of the paraventricular nucleus produced similar effects as to the disturbances in the glucostatic control, could be explained by the inhibition channels from the ventromedial nucleus to the food center being directed for the most part through the region of the paraventricular nucleus. Due to a small number of animals with lesions, located in the region of the paraventricular nucleus, this problem requires continued studies.

Changes in the food intake, caused after the operation, by 6 to 60 hrs of the food deprivation did not show characteristic daily fluctuations. Moreover, they showed a trend opposite to that prior to the operation. With ever longer deprivation, the food intake gradually decreased and after the lapse of 60 hrs it reached a level which, in normal animals, was obtained after the identical food deprivation period. It seems likely that the prolonged food deprivation disturbs a quantitative consumption control in hyperphags in a way similar to that in normal animals. T e p p e r m a n (1943) concluded that the respiration coefficient in hyperphagic animals can be higher than 1.0 which shows that most carbohydrates in these animals are transformed into fats. On the other hand, the starved hyperphagic rats, similarly to the control rats, were characterized by a low respiration coefficient (0.7 to 0.76), typical of the oxidation of fatty acids. Switching over metabolic processes to the exclusive use of own stores, a general exhaustion as a factor taking the edge off the appetite, a dehydration and many other changes, occurring in an internal environment during a prolonged food deprivation could make the behavior and food control of normal animals similar to that of hyperphags.

SUMMARY

(1) In rabbits who had previously been deprived of food the food intake, the number of the food CRs per minute and the blood sugar level alternately increased or decreased, showing rhythmical changes, caused by earlier feeding once a day. The CR performance was changed parallel to the changes in food intake. Changes in the blood sugar level and food intake showed a negative correlation.

(2) Bilateral lesions of the medial and ventromedial regions of the hypothalamus caused a double increase in food intake in rabbits. On the other hand, only in 5 rabbits of 15, the blood sugar level showed a slight drop.

(3) Lesions, located above the ventromedial nucleus caused the agres-

siveness, voracity and increase in the number of the food CRs per min-ute. These reactions occurred also apart from each other and independ-ently from hyperphagia and were not observed after lesions of the ven-tromedial regions.

(4) Rhythmic changes, occurring during starvation, were retained in hyperphags only in the blood sugar level. The abolition of the corre-lation between the blood sugar level and the food intake suggests a pos-sible disturbance in the glucoreceptive mechanism, acting through the satiety center.

(5) The increase in the number of the food CRs after short food deprivation periods indicates that the connections between the food center and the CR reaction centers were preserved and that the excita-tion of the food center can be changed after the lesions of the satiety center.

(6) In food deprived hyperphags, the food intake did not increase to any significant degree and it gradually dropped with ever longer deprivation periods. After a 60-hour food deprivation, both before and after the operation, the state of satiety was reached by rabbits after ingesting approximately identical amounts of food.

(7) An absence of a visible increase in food intake, despite an in-creased motivation for food after short starvation periods, indicates that food intake in the hypothalamic hyperphags cannot be a measure of the „hunger" to an identical extent with that before the operation.

The results obtained confirm an assumption that disturbances, caused by the lesions of the satiety center, concern not so much the mechanism, responsible for the initiation of the act of eating, as the mechanism, res-ponsible for stopping the act of eating.

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ISOLATED CEREBRUM

OF MIDPONTINE PRETRIGEMINAL PREPARATION: A REVIEW

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I. Introduction

Midpontine pretrigeminal preparation (MPP) in the cat was first decribed by B a t i n i, M o r u z z i, P a l e s t i n i, R o s s i and Z a n-c h e t t i in 1958. MPP results from a sectioning of the brain stem just in front of the trigeminal roots and is composed of two neurally independent parts, the rostral and the caudal. The rostral part is also referred to as "isolated cerebrum".

B a t i n i, M o r u z z i et al. (1958) observed eye movements and pupillary dilation in response to visual stimuli, i.e. the oculomotor reflexes typical for an awake animal, in the rostral part of the MPP. Later on, it was shown by several authors that, i) these ocular reflexes can become habituated and may be considered as components of an orientation reflex (A f f a n i, M a r c h i a f a v a and Ż e r n i c k i 1962a, Ż e r-n i c k i and D r e h e r 1963, 1965, E l u l and M a r c h i a f a v a 1964, D r e h e r and Ż e r n i c k i, unpubl.), and, ii) positive and inhibitory CRs may be established in the MPP (A f f a n i, M a r c h i a f a v a and Ż e r-n i c k i 1962b, Ż e r n i c k i and O s e t o w s k a 1963). These data indicate that higher nervous activity is retained in the isolated cerebrum of the MPP.

It seems, therefore, that the MPP has the properties of both a "preparation" and a normal animal offering new experimental posibilities for investigating cerebral functions. The aim of the present article is to describe the MPP in detail, and to outline its potentialities for such investigations.

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II. Anatomy

The rostral part of the MPP consists of the forebrain, the midbrain and the rostral part of the pons (cf. Fig. 1), the four rostral pairs of the cerebral nerves and the structures innervated by them, the eyes and the olfactory mucous membrame. In consequence, the isolated cerebrum receives sensory impulses from the optic and olfactory nerves. Possibly, it may also receive some proprioceptive impulses from the extraocular muscles. However, an existence of the extraocular muscle afferents in their motor nerves is still not settled (cf. Whitteridge 1960). It is
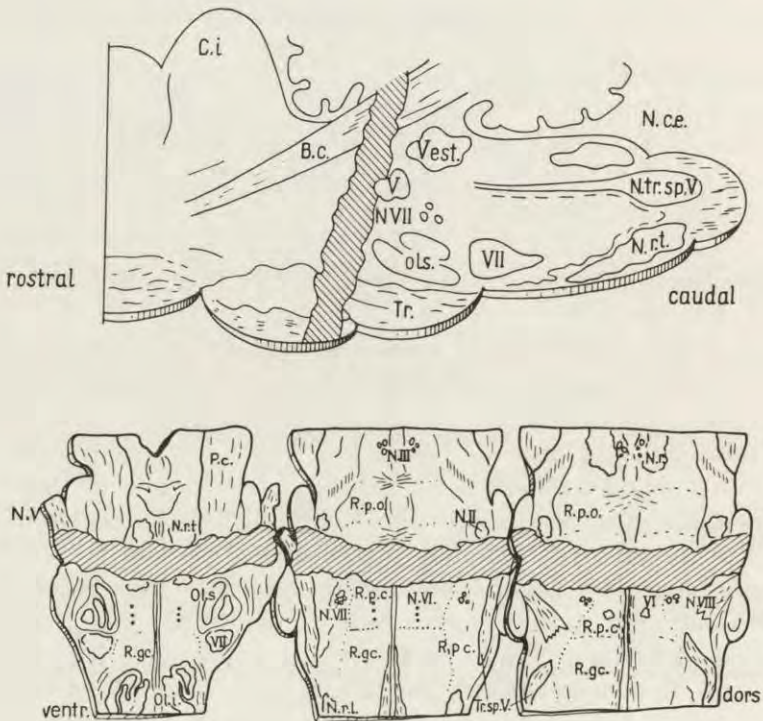

Fig. 1. Midpontine pretrigeminal section

Drawings of the saggital section (above) and horizontal sections (below) of the brain stem of cat. Cross hatched areas indicate level and extent of brain stem lesion. Anatomical abbreviations: B.c. brachium conjunctivum; C.i.: colliculus inferior; N.c.e.: nucleus cuneatus externus; N.r.: nucleus ruber; N.r. l.: nucleus reticularis lateralis; N.r.t.: nucleus reticularis tegmenti pontis; N.tr.sp.V: nucleus tractis spinalis nervi trigemini; N. III, V, VI, VII, VIII: root fibers of cranial nerves; Ol.i.: nucleus olivairs inferior; Ol.s.: nucleus olivaris superior; P.c.: pes pedunculi cerebri; R.gc.: nucleus reticularis gigantocellularis of Meessen and Olszewski; R.pc.: nucleus reticularis parvocellularis of Meessen and Olszewski; R.p.c.: nucleus reticularis pontis caudalis of Meessen and Olszewski; R.p.o.: nucleus reticularis pontis oralis of Meessen and Olszewski; Tr.: corpus trapozeideum; Tr. sp.V: tractus spinalis nervi trigemini; V, VII: motor nuclei of cranial nerves. (Figure composite from Batini, Moruzzi et al. 1959, and Batini, Magni et al. 1959, by courtesy of Arch. ital. Biol.

known that all, or, at least, a majority of them run in the trigeminal nerves and that in this way they enter caudal part of the MPP. The isolated cerebrum sends efferent impulses to the extraocular muscles excluding the lateral recti, to the pupillary sphincters, the ciliary muscles and the levators of the superior eyelids.

During the transection no large vessels are cut and thus the blood supply of the isolated cerebrum is not seriously affected.

III. Discussion on methods used

1. *Animal.* Until now the MPP has been performed only on cats. Because the actual level of the brain stem transection is critical, the use of cats that are either larger or smaller than average should be avoided. It is very important that the animal used for the experiment is in an excellent state of health.

2. *Type of preparation.* Either the acute or the chronic MPP may be used for experiments. An acute MPP may be kept alive for one to four days, whereas a chronic animal may be kept alive for some weeks or longer, if necessary (cf. Ż e r n i c k i and O s e t o w s k a 1963).

3. *Anaesthesia.* During the transection for the acute MPP ether anaesthesia is used. In order to obtain the chronic MPP, barbiturate narcosis instead of ether anaesthesia may be employed. In the latter case, bleeding during the operation is reduced and consequently the lesion of the brain stem due to the hemorrhage is less extensive. On the other hand, barbiturate narcosis often causes respiratory difficulties which increase the postoperative mortality of the animals.

4. *Surgical procedures.* There are some differences in the mode of operation depending on the type of preparation. In the acute MPP a tracheotomy may be performed and both the nictitating membranes and the upper eyelids removed to allow better observation of the eyes. The chronic MPP should be carried out under aseptic conditions.

The brain stem is transected either electrolytically or with a spatula while the animal is in a stereotaxic apparatus. In order to avoid the bony tentorium the plane of the section is inclined from the frontal plane and goes through the cerebellum (Fig. 2). The inclination is usually 30° but may be also 45° (cf. G l ä s s e r and M a n t e g a z z i n i 1960). The plane of the section is calibrated 1 mm anteriorly to AP = 0. However, if it is difficult to avoid the tentorium during the operation, the plane may be moved back 0.5 — 1.0 mm.

The electrolytic method of sectioning by means of a monopolar thin electrode which is completely insulated except at the tip was used originally by B a t i n i, M o r u z z i et al. (1959). After its insertion into

the brain stem, the electrode is pushed downwards in 1 mm steps, the tissue of each position being subjected to a constant current, 3 ma for about 20 sec is usually quite sufficient. The electrode is then withdrawn,
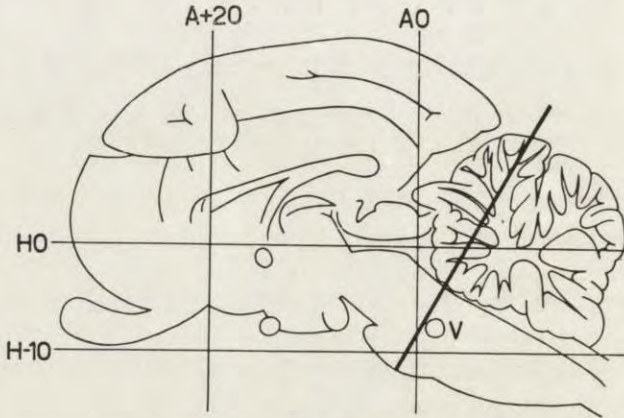
Fig. 2. Saggital section of the brain of cat

Horsley-Clarke planes of references and midpontine pretrigeminal section are designated. (Figure from M e u l d e r s 1962, slightly altered)

displaced laterally by 1.5 mm and the whole procedure is repeated. The ether narcosis may be discontinued after the first penetrations.

Recently, the transection has been often made more quickly with a thin spatula (Fig. 3). This procedure is more simple because the spatula is usually not fixed in the stereotaxic holder but can be slid by hand along the stereotaxically fixed plate. The first penetration is made in the

Fig. 3. Spatula for brain stem transection

midline. When the base of the skull is reached (any strong pressure here should be avoided), the spatula is raised back about 15 mm, turned laterally, and the second penetration is made. If the diameter of the tip of the spatula is about 7 mm, three penetrations, one in the middle and two laterally, are sufficient.

Immediately after the transection, done either with an electrode or with a spatula, the middle part of the cerebellum is removed by suction and the fourth ventricle opened to prevent edema.

It may be added that during the transection made with a spatula some of the most rostral fibers of the brachia pontis may be sometimes spared. However, this anatomical connection between rostral and caudal parts of the MPP seems to have no physiological meaning because the brachia coniunctiva are cut and the dentate nucleus is also affected during the suction of the cerebellum.

A r d u i n i and H i r a o (1959) have described a completely different method for obtaining MPP. They introduced a thin spatula into the mouth and carried out the transection through the base of the skull. The advantage of this method is that the size of the lesion of the brain is reduced, and in fact, the cerebellum needs neither to be cut nor to be removed by suction. Moreover the cerebellar peduncles can be spared if necessary. On the other hand, this method is more difficult surgically, and cannot be used for obtaining chronic MPP because of injury to the mouth.

5. *Criterion of a good preparation.* Just after the transection, the rostral part of the MPP remains "behaviourally inefficient" for some time, easily seen by the absence of the orientation reaction to visual stimuli *. This "behavioural inefficiency", which may be partially due to the ether anaesthesia, may last for some minutes to some hours in the various preparations, and usually lasts about 30 minutes.

However, some preparations (about 15%) remain permanently behaviourally inefficient. Sometimes, in these preparations clearly synchronized EEG patterns can be observed, and later anatomical verification usually reveals that either the brain stem transection had been too far forward or the reticular formation anterior to the section was damaged by hemorrhage. In consequence, instead of MPP a rostropontine pretrigeminal preparation had been obtained (cf. B a t i n i , M o r u z z i e t a l. 1959).

However, in some preparations the behavioural inefficiency may be accompanied by desynchronized EEG patterns. It may be reminded that a similar phenomenon has also been observed in cats with a lesion

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* To my knowledge, "behavioural efficiency" was first introduced by L i n d s l e y (1952) as a term denoting a capacity for response typical for an awake animal.

in the mesencephalic reticular formation (B a c h, K e l l y and S t a u b 1955), in human beings as a result of hemorrhage in the central portion of the pons (L o e b and P o g g i o 1953), and also under ether narcosis. Its mechanism is not yet understood. In any case the experiments of B a t i n i, M a g n i et a l. (1959) have shown that in MPP the desynchronization of EEG patterns is not due to either an increase of the adrenaline discharge or to high concentration arterial carbon dioxide. The possibility that this may be a permanent paradoxical stage of sleep should also be considered. The data of J o u v e t (1962), who found that the centre responsible for this stage of sleep is located in the nucleus reticularis pontis caudalis lying in the caudal part of the MPP, seem to exclude such a possibility. However, recently R o s s i, M i n o b e and C a n d i a (1963) claim that such a centre is located rather at the level of the caudal part of the nucleus reticularis pontis oralis. In this case, it could remain partially within the rostral part of the MPP. But the absence of the typical bursts of rapid eye movements also argues against the presence of a paradoxical stage of sleep in the isolated cerebrum of the MPP.

In any case, it is quite understandable that there is a fundamental difference between a behaviourally efficient and inefficient rostral part of the MPP. The importance of this difference is apparent for all who are interested in its higher nervous activity. However, there is little doubt that during the behavioural coma the more simple cerebral functions are also affected to a greater or lesser extent. Therefore, it seems justifiable to consider behavioural efficiency of the MPP as an essential property of it. In consequence, a behaviourally inefficient MPP must be considered a completely unsatisfactory preparation and should be rejected. Furthermore, during the experiment the behavioural state of the MPP should be controlled as well as its temperature and respiration. It is important to remember that behavioural efficiency may be easily lost after cerebral ablation or simple extensive craniotomy. If behavioural efficiency disappears, the experiment should be suspended and the cause sought. Without such precautions, which, unfortunately, by some authors were neglected until now, equivocal results can easily be obtained.

6. *Caudal part of the MPP.* In most cases, the general state of caudal part of MPP is good so that conditions are satifactory also for the isolated cerebrum. Just after the brain stem transection breathing usually stops, but, as a rule, it resumes after a few minutes of manual artificial respiration and is usually regular, averaging about 20 breaths per minute. Arterial pressure, measured through canulation of the femoral artery, ranges from 120 to 140 mm Hg. Decerebrate rigidity and phasic reflexes

are present. The temperature of the preparation fluctuates with the temperature of the environment. At a room temperature of about 26°, the rectal temperature of MPP is usually kept at about 36° to 38°. Defecation is usually spontaneous. Vomiting occurs in some preparations.

7. *Nursing care.* In an acute preparation the nursing care is limited to controling the body temperature, administering 5⁰/₀ glucose injections, and the protecting the eyes from drying. When the MPP is alive for more than one day, antibiotic therapy is adminstered and the bladder manually evacuated.

The chronic animals are housed in cages in a thermoregulated room. Antibiotic therapy is given during the first three postoperative days and also later if necessary. The preparations are fed by a stomach tube twice daily. However, in the first two postoperative days the food should be given rectally to avoid vomiting. Urine is evacuated manually twice daily and an enema given when necessary. As the lid reflex is disturbed the eyes are kept closed with bands attached to the lids by a glue and the corneal rinsed twice daily with aseptic solution.

8. *Experimental chamber.* Investigations are performed in a small chamber which isolates the animal optically from the experimenter (Fig. 4 A). An acute MPP may remain in the chamber for the whole survival period being fixed permanently in a stereotaxic apparatus while chronic animals are placed there in a holder only for the duration of the experimental session. The chamber is illuminated continuously with a light sufficiently strong to produce a moderate myosis of the pupils in the animal. With the pupils in such a condition the pupillary dilation produced by „indifferent" or conditioned stimuli may be easily observed while the intensity of the light is usually sufficient for taking photographs. The visual stimuli are placed on the sagittal plane, on the wall of the chamber about 10 cm in front of the animal's eyes (Fig. 4B). Small lamps giving either a constant or intermittent light, at a rate about 2/sec, and small objects rotating or oscillating at a rate of about 1 cycle/sec, are usually applied.

9. *Classical conditioning.* The "indifferent" visual stimuli mentioned above may be used as CSs (A f f a n i et al. 1962b, Ż e r n i c k i and O s e t o w s k a 1963). The utilization of olfactory stimuli is technically difficult in MPP just as it is in an intact animal. However, the cerebrum may be stimulated directly.

It has been shown in normal animals that electrical stimulation of the thalamus (B e c k 1963, I o r d a n i s 1964), the medial geniculate body (N i e d e r and N e f f 1961), the lateral geniculate body (R o u g e u l and B u s e r, personal communication), the auditory radiations (N i e d e r and Ne f f 1961), the cerebral cortex (L o u c k s 1935 and 1938, D o t y, R u t-

ledge and Larsen 1956, Doty and Rutledge 1959, Doty and Giurgea 1961, Mogenson 1959, Iordanis 1964), the nucleus caudatus (Lagutina and Rozhanskii 1949, Nielson, Doty, Rutledge 1958, Sadowski 1960), the hippocampus (Iordanis 1964), septum (Iordanis 1964), and reticular formation (Tuge, Kanayama and Yuch 1962) can be safisfactorily used as the CS.

Indifferent visual stimuli are unsatisfactory as the USs because the orientation reflexes produced by them are relatively easily habituated. The only response to the visual stimuli, which is resistant to habituation, is the pupillary reflex to the change of illumination. However, many authors have demonstrated in human begins that the pupillary constriction to light is either unconditionable (Wedell, Taylor and Skolnick 1940, Young 1954, Crasilneck and McCranie 1956), or
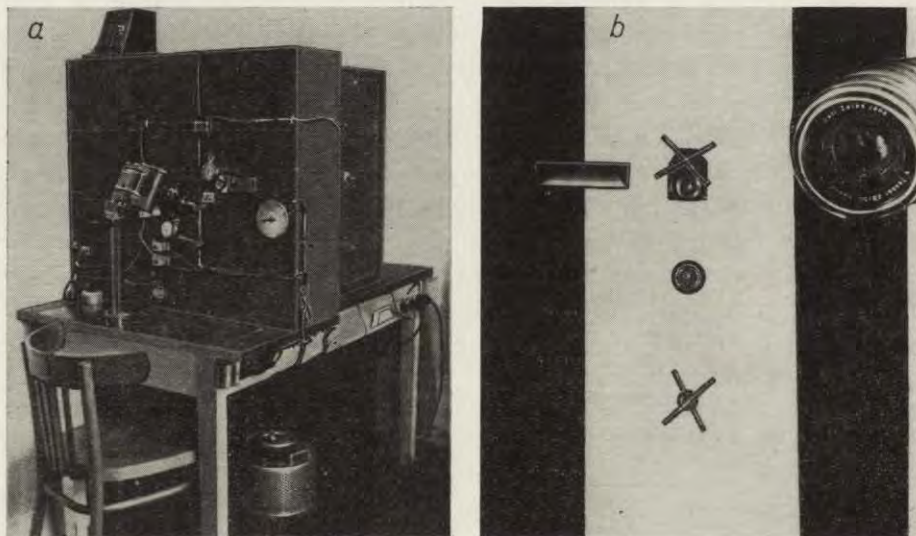
Fig. 4. Experimental chamber

a — External view. b — The screen with visual stimuli (neon lamps and "rotors"). The stimuli are located within the horizontal through the nodal point of the eye and about 30° above or below it. On the left above, the aperture for direct observations

that the CR obtained is very poor and unstable (Steckle and Renshaw 1934, Steckle 1936, Hilgard, Dutton and Helmick 1949). Also pupillary dilation produced by light offset may not be conditioned either in the human being (Hilgard, Miller and Ohlson 1941, Gerall, Sampson and Boslov 1957) or in the cat (Gerall and Obrist 1962). In conclusion, in MPP direct stimulation of the cerebrum should be used as the US.

A cerebral stimulation producing a clear ocular and EEG effects seems to be preferred. Stimulation of the perifornical area, producing in

the MPP a strong pupillary dilation and EEG arousal (S a n t i b a-
n e z - H., T a r n e c k i and Ż e r n i c k i 1960), was satisfactorily used as
the US in this preparation by A f f a n i e t a l. (1962b) and Ż e r n i c k i
and O s e t o w s k a (1963). Stimulation of the reticular formation whose
EEG and behavioural effects were recently conditioned in a normal ani-
mal by S e g u n d o, R o i g and S o m m e r - S m i t h (1959), and which
produces in the MPP pupillary dilation, EEG arousal and an upward
movement of the eyes (Ż e r n i c k i and D r e h e r, 1965), may possibly
also be used. It is also worth mentioning that recently C l e m e n t e,
S t e r m a n and W y r w i c k a (1963) have shown that EEG synchroni-
zation produced by stimulation of the preoptic basal forebrain zone or
the intralaminar thalamic nuclei, may also be satisfactorily conditioned
in the normal animal. On the other hand, B a n and S h i n o d a (1956)
have found that pupillary constriction produced by stimulation of the
lateral hypothalamic nucleus is unconditionable.

It would be tempting to see whether a cerebral stimulation, which
does not produce an efficient ocular and EEG effect could also be used
as the US. In a normal animal stimulation of the motor cortex (N i k o-
l a e v a 1957, D o t y and G i u r g e a 1961, T c h i l i n g a r y a n 1963)
and of the cerebellum (B r o g d e n and G a n t t 1942) was successfully
used as the US. If such a reinforcement were used in the isolated cere-
brum of the MPP then the electrical activity of the cerebral efferent
motor paths (e.g. cerebral peduncles) should be recorded instead of the
movements of the limbs.

A general procedure of conditioning is in the MPP similar to that
applied in the intact animal (cf. A f f a n i e t a l. 1962b, Ż e r n i c k i
and O s e t o w s k a 1963). However, due to the limitation of time the
CR sessions are more often conducted; in chronic MPP twice daily, and
in acute cats from 2 to 5 daily. If the CR becomes irregular this means
that the training is too rapid. One CR sesion usually consists of about
20 trials. The intertrial intervals depend on the duration of the uncon-
ditioned effect of the preceding trial and usually vary from 1 to 2 min.
At the very beginning of the CR training the CS should be reinforced
almost immediately by the US. Later on, when the CR is established,
the CS-US interval may be gradually increased to several seconds.

10. *Instrumental conditioning.* At present we have no experimental
data concerning elaboration of instrumental CRs in MPP, therefore we
can only offer some theoretical considerations. As far as the reinforce-
ment for the instrumental CR is concerned, it has been shown by many
authors on normal animals, especially with the self-stimulation technique,
that stimulation of many cerebral points may be utilized as the positive
or negative US. A vertical eye movement may be used as an instrumental

reaction. The electrical response in cerebral efferent motor paths may also be used as a substitution for the motor activity of a normal animal. If the piramidal tracts were protected during the performance of the operation the observation of the responses would be easier. Such a „pyramidal" cat has been described in 1953 by Whitlock, Arduini and Moruzzi and this technique has been used by several authors in acute and chronic preparations (Preston and Whitlock 1960, Batsel 1963, Agnew, Preston and Whitlock 1963).

It is worth noting that in the MPP the ways in which the motor response may be evoked are greatly limited compared to that of a normal animal. Initial provocation of the eye movement could be effected by either the fixation reflex to a properly localized visual stimulus or the stimulation of the reticular formation. The provocation of the eye movement by stimulation of colliculus superior, which produces in MPP a strong usually upward eye movement and relatively weak pupillary dilation (Żernicki and Dreher, unpublished experiments), seems to be an ineffective way of producing conditioning due to the more efferent character of this reaction. The initial provocation of electrical activity in the efferent motor paths can be produced by stimulation of the sensori-motor cortex. This method has been used for the elaboration of the instrumental CR in an intact animal (Loucks 1935, Konorski and Lubińska 1939, Tarnecki 1962).

The absence or paucity of proprioceptive feedback from the ocular muscles in the MPP must also be considered. However, there is evidence (Jankowska 1959, Górska and Jankowska 1963, Knapp, Taub and Berman 1963), that the instrumental CR may be elaborated in a deafferentated limb. The pilot study of Tarnecki (see Konorski 1962), who elaborated an instrumental CR in the deafferentated limb of a cat using stimulation of the motor cortex for its initial provocation, is especially interesting in this connection.

11. *Recording*. Due to the immobilization of the caudal part of the MPP, recording the electrical activity and photographing the ocular activity is relatively easy. The eye movements may be filmed and recorded (EOG). Changes in pupillary diameter can be filmed and the activity of the short ciliary nerves may be recorded (cf. Bonvallet and Zbrożyna 1963, Zbrożyna and Bonvallet 1963). However, a precise analysis of the oculomotor activity is sometimes difficult because of an incomplete data regarding the topographic anatomy of the cat's orbit and the eyeball. Therefore, the recent paper of Bishop, Kozak and Vakkur (1962), Vakkur, Bishop and Kozak (1963) and Vakkur and Bishop (1963), partially filling this gap, seem to be very important.

IV. Behavioural and electrophysiological properties of the MPP

1. Ocular behaviour and EEG paterns in absence of intentional stimulation. The eyes of MPP look forward. The pupils are more constricted than in the intact animal. Three factors may be responsible for this phenomenon: i) the elimination of bulbar tonic inhibitory influence on the Edinger-Westphal nucleus (cf. Z b r o ż y n a and B o n v a l l e t 1963), ii) the extensive deafferentation of the isolated cerebrum, and, iii) the absence of tonic sympathetic cerebral influences. As in the normal animal the pupils are not still and carefull observation detects constant very small changes of their diameter. Occasionally, spontaneous ocular activity, consisting of rapid clear pupillary dilations and eye movements, may also be observed.

B a t i n i, M o r u z z i et al. (1959) have shown that the desynchronized EEG patterns are on the average more persistent in the MPP than in the intact animal. This observation was soon confirmed by several authors (A r d u i n i and H i r a o 1958, M a n c i a, M e u l d e r s and S a n t i b a n e z 1959bc, G l ä s s e r and M a n t e g a z z i n i 1960) and C o r d e a u and M a n c i a (1959) have also found that in the cats with midpontine pretrigeminal hemisection the EEG activity of the homolateral hemisphere is more desynchronized than of the heterolateral one. That finding was unexspected because we know that: i) extensive deafferentation of a dog or a cat may produce a strong tendency to behavioural sleep (G a l k i n 1933, A b u l a d z e 1936, K r a v c h i n s k i 1945, A i r a p e t i a n c 1955, K l o s o v s k i and K o s m a r s k a i a 1957, L o b a n o v a 1957, S e r g i e v s k i and I v a n o v 1962) and synchronization of the EEG patterns (S e r g i e v s k i and I n v a n o v 1962), ii) after the cerebelloctomy the EEG patterns become more synchronized (D o w, F e r n à n d e z - G u a r d i o l a and M a n n i 1962, A n g a u t, M a s s i o n and L e l o r d 1962, G r i g o r y a n 1963), iii) bulbar and pontine reticular formation is a constituent part of the activating reticular system (cf. M o r u z z i and M a g o u n 1949), and, iv) following bilateral trigeminal deafferentation the EEG synchronized patterns prevail in the „encéphale isolé" cat (R o g e r, R o s s i and Z i r o n d o l i 1956, B a t i n i, M a g n i et al 1959). Moreover, B a t i n i, P a l e s t i n i et al. (1959) have found that after olfactory and visual deafferentation the isolated cerebrum of the MPP shows only temporary strong synchronization of EEG activity (cf. also A r d u i n i and H i r a o 1959), the desynchronized EEG patterns reappearing after some days. Recently, a considerable evidence has been accumulated that this desynchroniza-

tion of the EEG patterns in the isolated cerebrum of the MPP is probably due to an elimination of the synchronizing influances of the lower brain stem, chiefly of the bulb, upon the cerebrum (C o r d e a u and M a n c i a 1959, M a g n i et a l. 1959, B o n v a l l e t and B l o c h 1961, F a v a l e et a l. 1961, M a g n e s, M o r u z z i and P o m p e i a n o 1961, C a s- p e r s 1962, D e m e t r e s c u and D e m e t r e s c u 1962, B o n v a l l e t and A l l e n 1963, cf. also M o r u z z i 1960, 1963, and 1964).

M e u l d e r s and collaborators (M e u l d e r s, M a s s i o n and C o l l e 1961, M e u l d e r s 1962) found that various cortical regions of the MPP may show different EEG patterns. According to the obser- vations of those, the EEG activity within the middle suprasylvian gyrus and the anterior sygmoideus gyrus is clearly synchronized.

D r e h e r and Ż e r n i c k i (unpubl.) have also studied the sponta- neous EEG activity in the MPP. However, these authors have always in- vestigated cats in the conditioned-reflex chamber which isolated the animals from the accidental visual stimuli, and they selected behavioural- ly efficient animals for their experiments (cf. Section III.5). D r e h e r and Ż e r n i c k i found that there are strong individual differences be- tween the EEG patterns of different MPP's (cf. Fig. 5). In some MPP's, the EEG activity was clearly desynchronized, whereas in the majority of them the EEG patterns were more or less synchronized and spindle bursts could be often observed. They also found that the EEG patterns recorded from the occipito-parietal areas were always more desynchronized than those recorded from the fronto-parietal ones, and that there was no clear tendency for rhythmical changes of the EEG activity corresponding the sleep-wakefulness rhythm of an intact animal.

The considerable individual differences in the EEG activity of the MPP's are not surprising if we consider that the isolated cerebrun of every MPP is a little different anatomically because of the individual differences of the brain stem dimensions and differences in the extent of haemorrhage produced by the transection. Moreover, it is probable that the configuration of the temporarily depressed cerebral structures is also different in each MPP. The existence of these temporarily depressed structures may explain why some drowsy chronic MPPs become alert (Ż e r n i c k i, unpublished observation), and why the EEG patterns in the chronic cerveau isolé become, with time, less synchronized approa- ching the spectrum of the normal animal (B a t s e l 1960, 1963 and V i l l a b l a n c a 1962).

The fact that the EEG activity of the occipital lobes, receiving sensory inflow from the eyes, is more desynchronized needs no comment. The lack of a diurnal rhythm in the EEG patterns of the isolated cerebrum
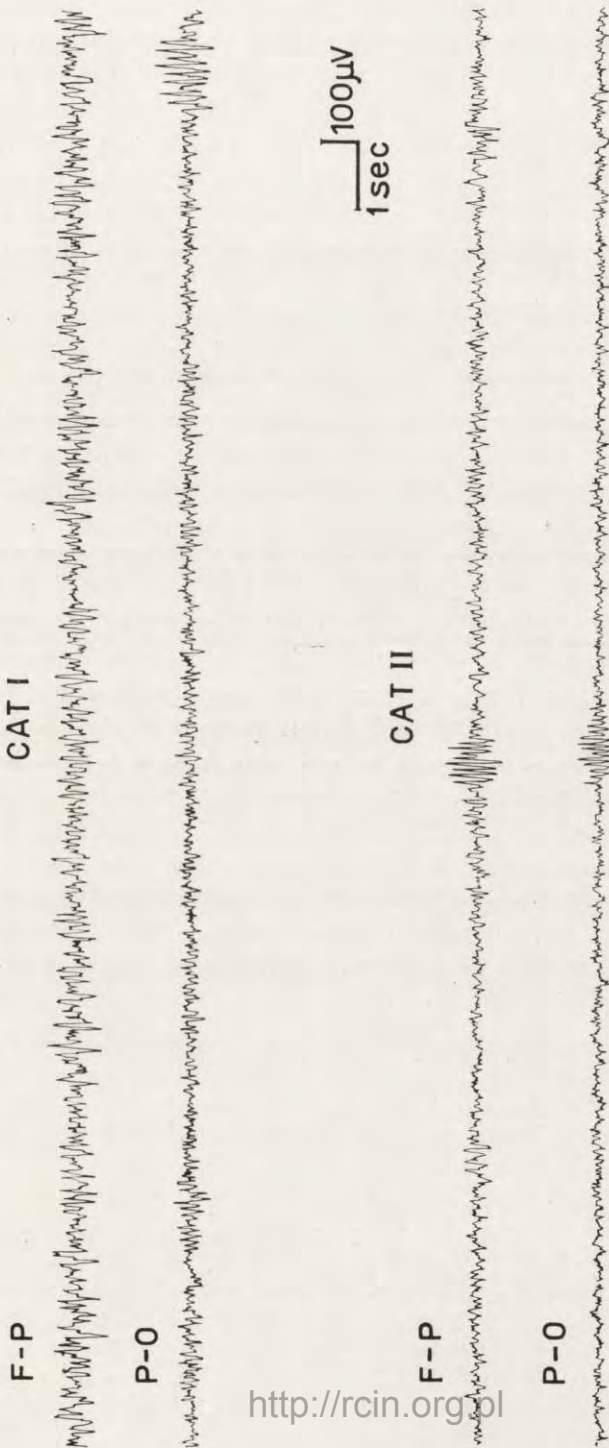
Fig. 5. Typical spontaneous EEG patterns in the midpontine pretrigeminal cats

EEG recordings from left fronto-parietal (F-P) and parieto-occipital (P-O) leads. The cats remained in the optically isolated conditioned-reflex chamber. The interior of the chamber was illuminated. Both cats were behaviourally efficient

of the MPP may be at least partially due to its isolation from the intero-
ceptors. However, this feature of the MPP needs further investigation.

2. *Light reflex*. An increase of illumination produces in the MPP
a clear constriction of the pupils. If one eye is only illuminated, the con-
sensual light reflex is also present. That reflex is not accompanied by
any visible change in the EEG pattern (D r e h e r and Ż e r n i c k i
unpubl.). However, A r d u i n i and H i r a o (1960b) have found that
a continuous illumination of the eyeballs from above, with an inclination
of 45° to 90° to the horizontal plane, produced in the MPP a clear syn-
chronization of EEG activity. This interesting finding needs a further
investigation.

3. *Orientation reflex*. The rostral part of the MPP usually responses
to the first presentation of the „indifferent" visual stimulus with a clear
reaction consisting of: i) dilation of the pupils, ii) conjugate vertical eye
saccadic movement towards the stimulus and maintenance of the eyes
upon it for some time (fixation reflex), iii) accomodation reflex of eyes,
and, iv) EEG arousal (Fig. 6), A f f a n i et a l. 1962a, Ż e r n i c k i and
D r e h e r 1963, 1965, E l u l and M a r c h i a f a v a 1964). If the visual
stimulus is presented only to one eye, the consensual reaction is also
present. There is much evidence that that reaction may be considered
an orientation reflex. First, it is a constituent part of the orientation
reflex of an intact animal. Second, it has properties similar to those of
the orientation reflex in a normal animal and especially it can be habitua-
ted (see Section IV, 5).

It is well known that both in the human being and in the normal
animal the first time an indifferent visual stimulus is presented it produ-
ces an EEG arousal, fixation reflex and accomodation of the eyes. Ż e r-
n i c k i and D r e h e r (unpubl.) have also observed a clear pupillary
dilation to visual stimuli in a normal cat under a constant illumination
similar to that used in the experiments with pretrigeminal pre-
parations*.

As in a normal animal the strenght of the orientation reflex depends
on the efficiency of the specimen (see Section IV, 4) and the physical
properties of the stimulus. Moving objects produce a stronger reaction

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* In human beings although the indifferent stimuli from other analyzers always
produce pupillary dilation also, the visual stimulus usually produces pupillary con-
striction (S h a c h n o v i c h 1956, L i b e r m a n 1958). One of the possible expla-
nation of this discrepancy may lay in the fact that some visual stimuli may produce
in addition to the orientation reflex the light reflex and in the conditions of the
moderate background of illumination the light reflex may overhelm the orientation
one. It is also necessary to remember that in the cat the constriction of the pupils
to a near object is less pronounced than in the human being.

than light*. The fixation and accomodation component of the orientation reflex depends on the position of the stimulus. Under certain experimental conditions, some components of the orientation reflex may be absent in the MPP at all. In dim light, when the pupils are relatively dilated, the orientation reflex will not include pupillary dilation. Whereas, when a light is used as a visual stimulus, the antagonistic photic reflex may even over helm the orientation reaction and pupillary constriction is ob-
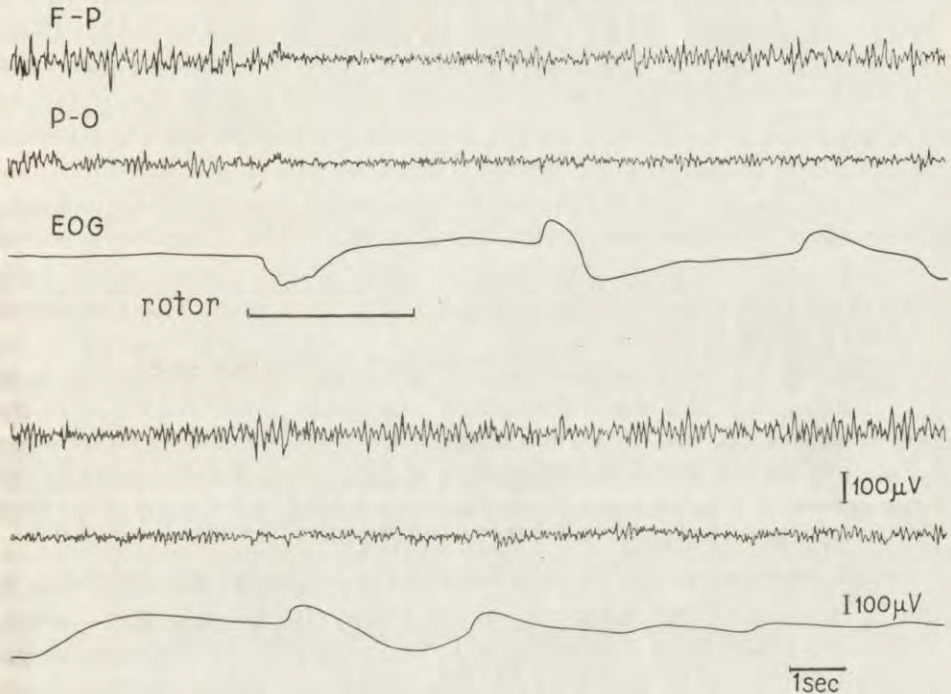

Fig. 6. Fixation and EEG components of orientation reflex to the "rotor inferior"
The rotor is located 30° under the visual axis of the animal. Note that EEG arousal is more long-lasting in the parieto-occipital (P-O) than in the fronto-parietal (F-O) area. EOG is recorded with an a-c amplifier (time constant = 0.7 sec). The cat looked at the rotor during its rotation and about two seconds after it has stopped. Some after-movements were present

tained. When the source of the stimulus lies on the visual axis of the cat's eye, the fixation reflex is absent. Finally with a background of strong EEG desynchronization the EEG arousal may be undetectable.

As in a normal animal the strong indifferent stimulus produces a long-lasting effect. The pupillary dilation may still increase after the stimulus

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* This agrees with recent observation in anaesthetized animals by H u b e l and W i e s e l (1959), A r d e n (1963) and K o z a k, R o d i e c k and B i s h o p (in preparation).

is off and the maintenance of fixation and new vertical eyes movements can be observed. Some residual pupillary dilation, EEG arousal, and slight raising or lowering of the eyes may be detected some minutes later.

Let us discribe some further properties of the individual components of the orientation reflex in MPP. The pupillary dilation to the indifferent stimulus may occasionally be very strong in spite of the fact that its mechanism in MPP is strongly affected. We know that after sympathectomy pupillary dilation may be obtained in the cat to emotional (S e y-b o l d and M o o r e 1940) or acoustic stimuli (S h a c h n o v i c h 1958). However, in this case it is produced by both neural (parasympathic) and humoral factors (cf. L o e w e n f e l d 1958). In the MPP the pupillary dilation to an indifferent stimulus is produced only through the inhibition of the tonus of Edinger-Westphal nucleus.

The eye-balls of the MPP have one degree of freedom; they can only rotate about a transverse horizontal axis while the lateral eye movements (conjugate as well as disjunctive) are absent. Consequently only the vertical component of the fixation reflex is present. The absence of horizontal movements in the MPP suggests that the nervous centre for them is located in the caudal part of the MPP. Such supposition is in agreement with some other observations that this centre is located in the abducens nucleus (cf. H o l m e s 1921, C r o s b y 1953). On the other hand, it is known that a corresponding neural centre for the vertical eye movements is located in the superior colliculus (cf. C r o s b y 1953, D r e h e r, M a r c h i a f a v a and Ż e r n i c k i 1965).

The amplitude of the fixation response is greater in the MPP than in the intact animal under normal conditions of life because the synergic movement of the head is eliminated in the preparation. However the general character of the fixation reflex in the MPP is similar to that observed in an intact cat (Ż e r n i c k i and D r e h e r 1965) and in an intact infant chimpanzee (B e r k s o n and F i t z - G e r a l d 1963).

The course of accomodation reflex to a near object in the MPP is similar to that observed in an intact cat. The total range of accomodation amounts to about 4 to 5 diopters. The accomodation reflex is not accompanied by a covergence of the eyes and by constriction of the pupils. However, these components of the accomodation response to a near object are also little pronounced in an intact cat.

The EEG arousal is always longer-lasting when recorded from the occipito-parietal area than from the fronto-parietal.

Finally, should be added that the orientation reflex may be also obtained to olfactory stimuli (D r e h e r and Ż e r n i c k i unpubl.). An odour of butyric acid or collidine introduced into the nostrils may produce

a slight pupillary dilation, a short-lasting EEG arousal and sometimes even a slight vertical movement of the eyes. According to our observations a similar but less pronounced effect may be obtained by an introduction of a stream of room air. On the other hand, A r d u i n i and H i r a o (1960b) were able to obtain the long-lasting EEG arousal using the latter method.

4. Behavioural and EEG efficiency*. Preliminary observations on the ocular behaviour of the MPP (B a t i n i, M o r u z z i et a l. 1959) seemed to indicate that on the whole its behavioural efficiency was more persistent than that of an intact animal, but its level was lowered i.e. the reactions were weaker. However, a careful analysis of the ocular components of orientation reflex shows that the level of behavioural efficiency of an average active MPP seem to be similar to that observed in a normal animal. Furthermore, the presence of the EEG component of the orientation reflex shows that MPP is also electroencephalografically efficient. It is important that as in a normal animal both behavioural and EEG efficiency appear to be closely related.

There are stronger individual differences in the level of behavioural and EEG efficiency in the MPP than in the intact animal; we may even speak about drowsy and alert MPPs. These differences, as those in the spontaneus EEG activity, probably depend on the anatomical and functional level of the transection.

However, there is no close relation between the level of behavioural and EEG efficiency and the level of synchronization of spontaneous EEG activity. In various MPPs similar efficiency may sometimes coexist with different EEG patterns, and similar EEG activity with different efficiencies.

In the MPP as in a normal animal the behavioural and EEG efficiency depends strongly on the presentation of the external (visual) stimuli. An increase of efficiency is then always accompanied by an increase of pupillary diameter and some desynchronization of EEG patterns (cf. A f f a n i et a l. 1962a). During the residual ocular and EEG reaction after the presentation of the strong visual stimulus, the orientation reflex to another visual stimulus is increased and its sensory threshold lowered (Fig. 7). If the stimuli are given freguently, the pupils of the MPP remain continuously relatively dilated the EEG relatively remain desynchronized, and the efficiency high. On the other hand, if an MPP remains

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* We introduce the term „electroencephalographical efficiency" (analogous to the term behavioural efficiency) for denoting a capacity for EEG response typical for an awake animal.

for some time in the experimental chamber without presentation of
visual stimuli, its pupils become more and more constricted, its EEG
more synchronized and its efficiency lowered.

It is interesting that the rostral part of the MPP may be capable of
full behavioural and EEG efficiency in spite of the extensive deafferen-
tation which, as it has been already mentioned, should produce a strong
tendency to behavioural sleep (see Section IV, 1). Therefore, it may be
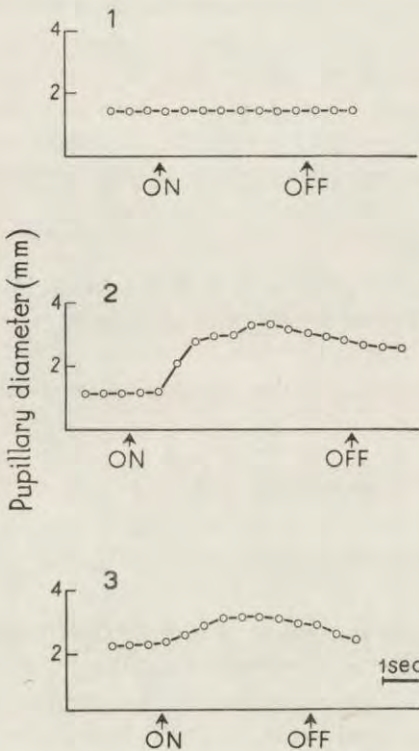concluded that the midpontine pretrigeminal section eliminates some

Fig. 7. Increase of efficiency by the
application of a strong visual sti-
mulus

Reading from top to bottom, pupillary
orientation reactions in three successive
trials. There is no reaction to the weak
visual stimulus (a train of weak flashes)
in the first trial but the strong visual
stimulus (rotor) produces a clear reac-
tion two minutes later with the same
pupillary condition (the second trial).
The same train of flashes one minute
later (third trial) produces a reaction,
also. This time the diameter of the pupils
was greater than in the first trial as an
after-effect of the reaction to rotor.
(From A f f a n i et a l. 1962a, by cour-
tesy of Arch. ital. Biol.)

tonic inhibition influencing the normal behavioural and EEG responses.
As we have already mentioned, the structures synchronizing the EEG
activity in a normal animal appear to be located in the lower brain stem.
The usual relationship between EEG patterns and the efficiency could
indicate that the structures inhibiting behavioural and EEG efficiency
are situated in the same place. This hypothesis is supported by the obser-
vations of B ü r g i and M o n n i e r (1943) and F a v a l e et a l.
(1961) who demonstrated that stimulation of the bulbar and pontine
reticular formation produces behavioural changes suggesting those of

sleep and also by the studies of J o u v e t (1962), who observed behavioural activation after the destruction of nucleus reticularis pontis caudalis.

5. *Habituation of orientation reflex.* When an indifferent visual stimulus is repeated at short intervals (from 15 sec. to 2 min) the orientation reflex thus produced becomes gradually habituated; the decrement of reaction is especially strong during the initial few trials (Fig. 8 and 9). The speed of habituation depends on several factors, i) the strength of the orientation reflex, ii) the length of the intervals between stimuli, and iii) the individual features of the preparations.
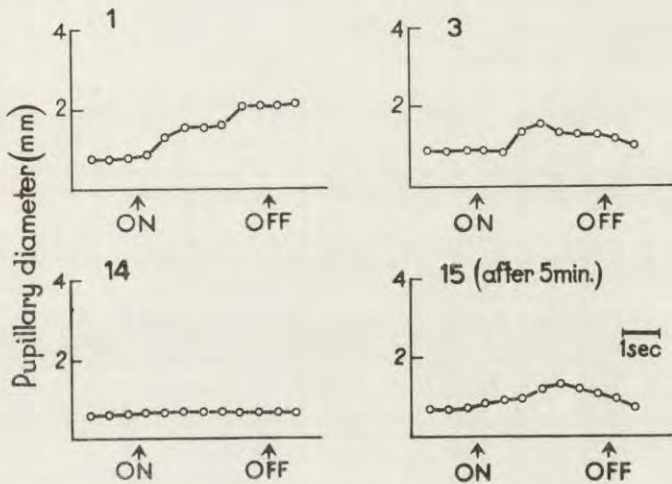

Fig. 8. Habituation and spontaneous recovery of orientation pupillary dilation to the rotor

Rotor stimuli given with intervals of one minute. The first rotor application produced a clear pupillary dilation. In the third trial the reaction was much weaker, and became absent in 14 th trial. After 5 min of rest, the rotor again produced some reaction (the 15 th trial). (From A f f a n i et a l. 1962a, by courtesy of Arch. ital. Biol.)

When the fixation reflex is absent from the very beginning (see conditions specified in Section IV, 3), the pupillary and EEG components of the orientation reaction habituate rapidly. Even in the second or third presentation of the stimulus the responses are greatly reduced, but sometimes after 20 trials the stimulus still produces a small effect. The fixation component of the orientation reflex is often relatively resistant to habituation and it usually disappears after 5 to 50 trials. As long as the fixation reflex is present the EEG arousal may be usually also detectable.
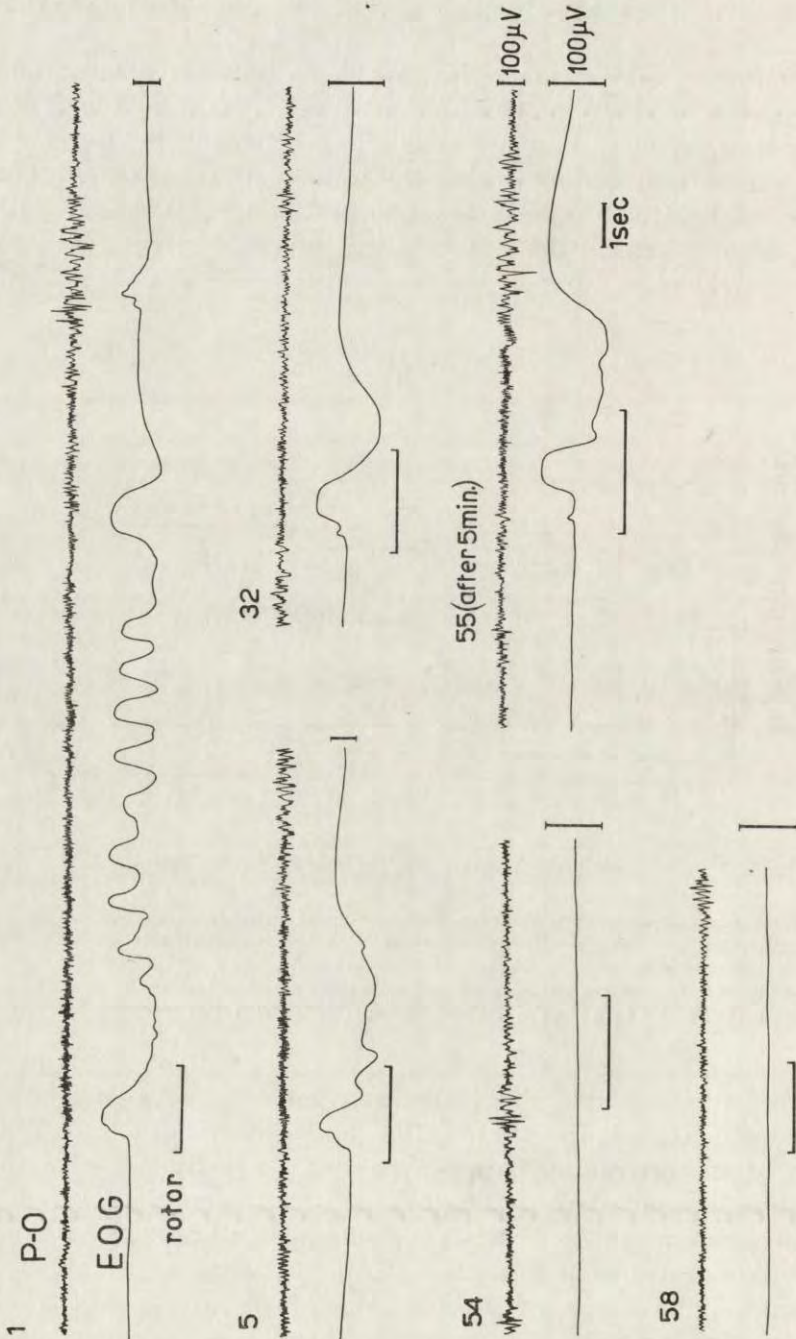
Fig. 9. Habituation, spontaneous recovery and rehabituation of orientation reaction to rotor superior
In the first trial the rotor produces a strong reaction. The reaction is much weaker in the fifth trial. In the 32-nd trial the reaction is quite weak and is absent in the 54th trial. Spontaneous recovery of the reaction appears after 5 minutes of rest (55th trial) but again there is no reaction after three trials (quick rehabituation). In the very beginning the intertrial intervals were about 2 minutes and later were gradually shortened to 15 seconds. Note that in the first and fifth trials the amplification of EOG was 2 × weaker

After complete habituation has taken place, the orientation reflex may be partially dishabituated by a 5 to 15 min. break in stimulation (Fig. 8 and 9) or by the application of another visual stimulus. The dishabituated reflex can be easily rehabituated. When given stimulus has been habituated during several experimental session, it produces non or only a slight effect even after an interval of many hours (chronic habituation).

It would be interesting to compare the development of habituation in the MPP with that observed in an intact organism. Habituation of the EEG arousal to visual stimuli has been investigated by several authors on human beings (M u s h k i n a 1956, N o v i k o v a and S o k o l o v 1957, R o g e r, V o r o n i n and S o k o l o v 1958, M i k h a l e v s k a j a 1958, V o r o n i n and S o k o l o v 1960, J u s and J u s 1960). Their individual results are not quite consistent but it may he concluded that the EEG arousal to visual stimuli habituates rapidly except for its „phasic" component. This type of habituation of the EEG arousal is also observed in the isolated cerebrum of the MPP. The fixation reflex in human being habituates rapidly according to the observations of V o r o n i n and S o k o l o v (1960). On the other hand, Ż e r n i c k i and D r e h e r (1965) found that in a normal cat the fixation reflex is as resistant to habituation as in the MPP. We have no information on the habituation of the pupillary dilation to visual stimuli in the normal animal except for preliminary observations of D r e h e r (unpublished), who found that such habituation is rapid in the cat. However, the habituation of pupillary dilation to different modalities of stimuli in a normal cat (L i b e r m a n and S t r e l t s o v a 1952, G l e z e r 1952, S h a c h n o v i c h 1956, L i b e r m a n 1958, S o k o l o v 1959) resembles closely that to the visual stimulus in the MPP. In conclusion, it may be stated that the course of the habituation of the orientation reflex in the MPP seems to be similar to that in an intact animal. The same may be concluded about the dishabituation and rehabituation of the orientation reflex.

The orientation reflex to olfactory stimulus (pupillary dilation and EEG arousal) habituates completely within a few trials (D r e h e r and Ż e r n i c k i unpubl.).

6. *Ocular following reflex.* The rostral part of the MPP as a normal animal can follow with vertical pursuit eye movements any object moving up and down within its visual field (Fig. 10), (B a t i n i, M o r u z z i et al. 1959, K i n g and M a r c h i a f a v a 1963, Ż e r n i c k i and D r e h e r, in preparation). This response is generally refferred to as a „following reflex" and it may be considered as a kind of fixation reflex, in which during the phase of maintenance of fixation (cf. Section IV, 3) the
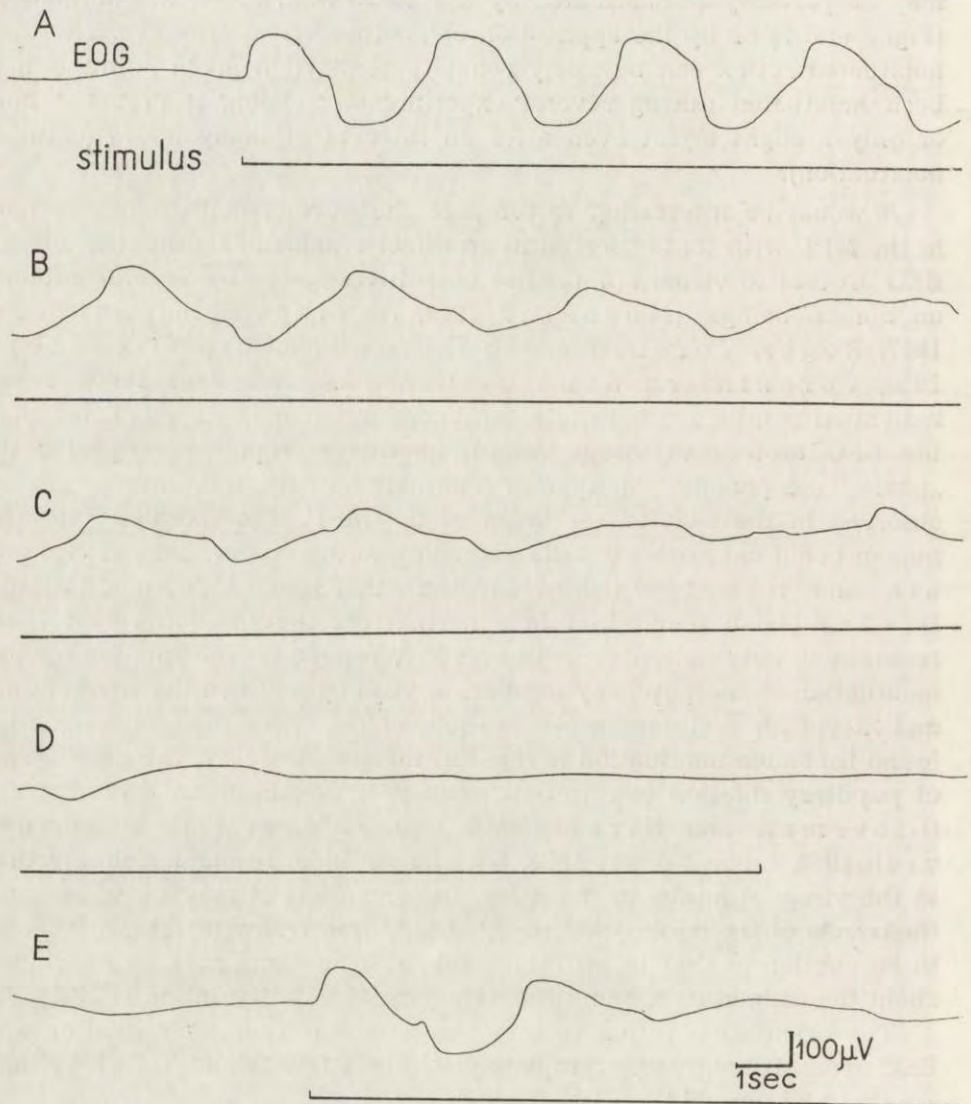
A

EOG

stimulus

B

C

D

E

100μV
1sec

Fig. 10. Habituation, spontaneous recovery and rehabituation of the following reflex to an object moving up and down within the visual field of the midpontine pretrigeminal cat

In the beginning the speed of the object was about 0,3 cycle/sec and later had to be slightly decreased due to the slower course of following response. A, Strong reactions in the beginning of stimulation. B, Smaller reactions after 1 min of stimulation. C, Slight reactions after the next 0,5 min of stimulation. D, Habituation after the next 0,5 min stimulation. E, Dishabituation after 15 sec of rest and quick rehabituation

eyes follow the object. If the moving object stops the eyes may either stop also or may still perform a few movements. A similar tendency „to look up and down" is also observed in the ocular behaviour of an intact animal.

The following reflex is very resistant to habituation. If it is evoked at rate of 0.3/sec, it, usually, habituates completely after 1 to 5 min. of stimulation (Fig. 10). One can also observe a spontaneus recovery of the following reflex after an interval of some minutes, its relatively easily obtained rehabituation, and chronic habituation. When the frequency of the moving object is increased the decerement of the following reflex is more rapid (cf. K i n g and M a r c h i a f a v a 1963), but then the effect oh fatigue should also be considered. It is worth noting that in a normal animal a similar course of habituation of the following reflex can be observed (Ż e r n i c k i and D r e h e r, unpubl.).

Due to the easeness of the evocation of the following reflex and its relative resistance to habituation, this reflex can be utilized in practice as a test for the behavioural efficiency of the isolated cerebrum of an MPP.

It is worth noting that the optokinetic reflex (optokinetic nystagmus) is absent in the MPP (K i n g and M a r c h i a f a v a 1963). There are some indications (see M o r u z z i 1962) that this is due to the interruption of ascending vestibular paths passing along the medial longitudinal fasciculus. The investigations of K i n g and M a r c h i a f a v a performed on the intact animal have shown that there are further differences between the following and optokinetic reflexes: i) optokinetic reflex does not show habituation, ii) the optokinetic reflex is present after visual decortication, and, iii) the optokinetic reflex occurs to the moving contrasting figures in the absence of a reference background while the following reflex does not. All these observations show that the mechanism of the following and optokinectic reflex are different.

7. Conditioned reflex. As has been previously mentioned (Section IV, 5), the pupillary dilation and the EEG arousal produced by a visual stimulus can be easily habituated in the MPP. However, if the visual stimulus is reinforced by hypothalamic stimulation producing a qualitatively similar but stronger reaction which does not habituate, the pupillary dilation and EEG arousal evoked by the visual stimulus does not only disappear but often becomes stronger after several CR sessions (A f f a n i et al. 1962b, Ż e r n i c k i and O s e t o w s k a 1963). If the orientation reaction to the visual stimulus is small or undetectable (this often happens as a light is used as the CS, cf. Section IV, 3) a gradual elaboration of the CR may be observed. Then a clear pupillary dilation and an EEG arousal to the CS may appear during the second experimental session and, occasionally, even during the first session. The

constant CR is usually established after about five experimental sessions (cf. Fig. 11). There is a large body of evidence which shows that this reaction may not be explained as a preservation or the appearance of an orientation reflex on the background of frequent hypothalamic stimulation (pseudoconditioning) but that is due to a formation of a real condi-
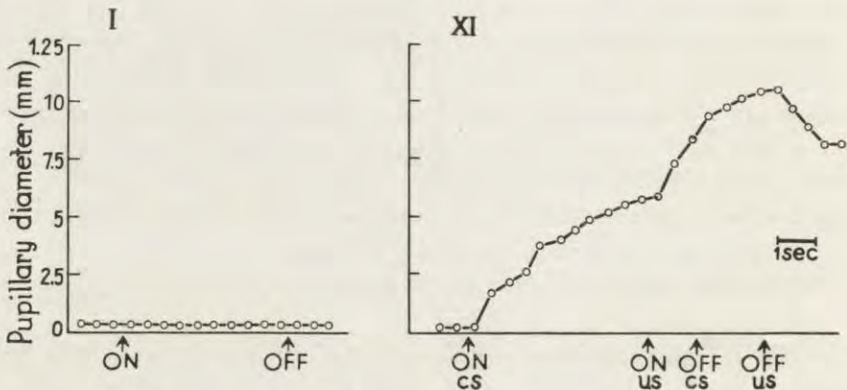

Fig. 11. Elaboration of conditioned pupillary dilation to visual stimulus (train of weak flashes)

On the left, no pupillary dilation to the train of flashes (weak stimulus) applied during the first experimental session before conditioned training. On the right, strong pupillary dilation to the same train of flashes in the first trial of the XIth experimental session. (From A f f a n i e t a l. 1962b, by courtesy of Arch. ital. Biol.)

tioned connection. First of all, the aforementioned gradual increase of the pupillary and EEG reaction may often be observed. Furthermore, this reaction is also present to the CS on the first trial of each session (i.e. before the first application of the hypothalamic stimulation). The crucial evidence, however, lies in the fact that the orientation reaction to another visual stimulus applied without reinforcement but on the same background of hypothalamic stimulation gradually disappears either completely or almost completely.

In experiments carried out on chronic MPPs in collaboration with O s e t o w s k a (1963) we used two small mobile objects as CSs which were located very close together so that they could be regarded as similar for the animal. After elaboration of pupillary conditioned dilation to one of the stimuli, the second visual stimulus was introduced and applied among the excitatory trials but without the hypothalamic reinforcement. When this stimulus was first given it produced, as a rule, a considerable pupillary dilation which could be regarded not only as an orientation

reaction to the new stimulus but also as a generalization of the positive CS. However, this reaction diminished rapidly with successive presentations of the stimulus and after several CR sessions it became fully differentiated in the majority of the cats (Fig. 12). In other words, inhibitory CRs could be elaborated in the rostral part of the MPP.

Fig. 12. Differentation of pupillary dilation to two similar visual stimuli
From left to right, conditioned reactions in three successive trials of a session. Pupillary dilation is present to the positive CS given in the 7th and 9th trials but is not present to the differentiated CS given in the 8th trial. Because the stimuli were located a little above the visual axis of the cat, the positive CS produced also a slight fixation reflex. (A part of Figure from Ž e r n i c k i and O s e t o w s k a, 1963)

The process of conditioning in the MPP is apparently similar to that observed in a normal organism. Such similarity is especially obvious if we analyze the development of pupillary conditioned dilation (H a r l o w and S t a g n e r 1933, H a r l o w 1940, G i r d e n 1942, B a n and S h i n o d a 1956, G e r a l l, S a m p s o n and B o s l o v 1957, S e g u n d o, R o i g and S o m m e r - S m i t h 1959, G e r a l l and O b r i s t 1962) or of the conditioned EEG arousal (S e g u n d o, R o i g and S o m m e r - S m i t h 1959, Y o s h i i et a l. 1960, S a k h i u l i n a 1960, L i v a n o v 1960) in a normal animal or human being. As far as the speed of CR elaboration is concerned it may be recalled that many authors have claimed that conditioned pupillary dilation develops rapidly in the normal organism (a peripheral noxious stimulation or a stimulation of hypothalamus or reticular formation was employed as the US). A similar rapid development of conditioned pupillary dilation may be also obseved in the MPP.

8. Photically evoked cortical and subcortical responses. A r m e n g o l, L i f s c h i t z and P a l e s t i n i (1961) compared the primary and associative cortical evoked potentials recorded in the intact cats with those obtained in the same animals after midpontine pretrigeminal transection. Both kinds of evoked potentials showed a clear potentiation after the midpontine section. However, similar potentiation was also obtained by these autors after rostropontine transection. On the other hand, M e u l-

d e r s and collaborators (M e u l d e r s and M a s s i o n 1961, M e u l-
d e r s, M a s s i o n and C o l l e 1961, M e u l d e r s 1962) claimed that
associative cortical evoked responses and evoked potentials in the median
centre were clearly stronger in the cerveau isolé preparation than in
the MPP.

A r d u i n i and collaborators (A r d u i n i and H i r a o 1960a,
A r d u i n i and G o l d s t e i n 1961) found in the MPP that the primary
cortical evoked potentials to shocks delivered to lateral geniculate body
were enhanced during either steady retinal illumination or deafferenta-
tion. According to these authors, this potentiation seems to result from
a decrease in the neural traffic in the optic tracts in such experimental
conditions.

P a l e s t i n i et a l. 1959, A r m e n g o l et a l. 1961 and P a l e s-
t i n i and L i f s c h i t z 1961 have claimed that the primary and asso-
ciative evoked potential are much more resistant to habituation in the
MPP than in an intact animal. Similar results were also obtained by
M a n c i a, M e u l d e r s and S a n t i b a n e z - H (1959b) who could
obtain habituation of evoked responses in the lateral geniculate nuclei
and in the primary visual cortex only after many hours of repetitive
visual stimulation.

The problem of habituation of photically evoked responses is worth
an extensive discussion. Only a few important points will be made here.
The term "habituation" is used in physiology for two phenomena: i) the
rapid decrement of the behavioural or electrical component of an orien-
tation reflex, and ii) decrement of an evoked response which usually takes
place only after many hours of repetitive stimulation. There is a little
doubt that completely different physiological mechanisms are respon-
sible for these two phenomena.

There is a large body of evidence demonstrating that habituation of
an orientation reflex is like extinction of CR due to the process of
"internal inhibition". On the other hand, the recent data suggest that
habituation of photically evoked responses in the cerveau isolé (A f f a-
n i, M a n c i a and M a r c h i a f a v a 1962), encéphale isolé (F e r n á n-
d e z - G u a r d i o l a et al. 1961) and in an intact animal (F e r n á n-
d e z - G u a r d i o l a e t a l. 1961, G a l l a r d o e t a l. 1962, B e c k and
G a s t a u t, in preparation, P a l e s t i n i e t a l., in preparation) is due to
a very gradual constriction of the pupils which decreases the strenght
of the visual stimulus. It was also shown in MPP (M a n c i a e t a l.
1959c) that habituation of evoked potentials is accompanied by EEG syn-
chronization and pupillary constriction.

It is very probable that EEG synchronization and pupillary con-
striction following longlasting repetitive visual stimulation, are "sui

generis" effects and not only due to the isolation of the animal from varied external stimuli which causes the EEG synchronization and pupillary constriction both in an intact animal as well as in an MPP after only a few minutes (A f f a n i e t a l . 1962a). On the other hand, the "habituation" of evoked potentials does not seem to be a process related to learning because its development is completely different from that of learning and it appears in cerveau isolé preparation even more quickly than in either an MPP or a normal animal (cf. M a n c i a e t a l. 1959ab). It may also be added that there is no crucial evidence for dishabituation of evoked potentials, because the partial reappearance of the reaction after an application of a different external stimulus or after stimulation of reticular formation may be due to an increase of the strength of the visual stimulus caused by an increase of the pupillary diameter.

9. *Conclusion.* As it was shown in this Chapter, the activity of the isolated cerebrum of the MPP is basically the same as that of the cerebrum of an intact animal. We see that the strong limitation of the exteroceptive input, the probable complete absence of the interoceptive one (cf. Chapter II), the strong isolation from the effectors and finally the isolation from the rest of the CNS, which all the things take place in the cerebrum of the MPP, may only reduce strongly a number of the reflexes avalaible but do not change definitely the activity of the cerebrum as a whole. This finding seems to be interesting from both physiological and psychological point of view.

V. The MPP as an instrument for future cerebral investigations

1. *An intact animal versus a preparation.* Cerebral functions can be investigated either in an intact animal or in any of the existing preparations (nembutalized or chloralosed preparations, cerveau isolé, etc.). Obviously, the experimental procedure is easier with a preparation and also the investigated problem can often be simplified. On the other hand, the cerebral activity of a preparation is different from that characteristic of an intact animal. Therefore, a preparation can be successfully used only either the investigated cerebral function is relatively "simple" (and so only slightly changed by anaesthesia), or when such abnormality may be neglected for some reason. However, because of the great recent interest of physiologists in complicated cerebral functions, which are absent in preparations, the number of investigations carried out in the intact animal is continually incresing.

The MPP, which shows higher nervous activity, may be considered

as both an animal and a preparation. Due to this peculiarity of the MPP the problem of which indications should be considered for choosing it as an experimental animal arises. In order to answer this question, the differences between the MPP, an intact animal and an anaesthetized preparation will be summarized here.

2. *The MPP versus an intact animal.* The MPP as an instrument for studying cerebral functions has the following advantages over an intact animal: i) due to an analgesia it can be fixed in a stereotaxic apparatus, and thus the recording of the electrical and oculomotor activity of the cerebrum and the changing of the position of electrodes in easier, ii) any surgical procedure may be undertaken without anaesthesia, and, iii) the cerebrum is effectively isolated from most possible accidental stimuli.

On the other hand, several disadvantages of the MPP may be enumerated: i) in chronic preparations the postoperative care is time consuming and unpleasant for the experimenter, ii) behavioural activity is limited to oculomotor activity, iii) the utilization of external stimuli is strongly reduced, iv) mortality is greater, and, v) the general state of the animal is less stable.

3. *The MPP versus anaesthetized preparation.* The MPP has only one, but essential, advantage over an anaesthetized preparation, viz. its cerebrum is behaviourally and electroencephalographically efficient and shows higher nervous activity. On the other hand, all disadvantages mentioned in the comparison with the intact animal are valid here.

4. *An acute MPP versus a chronic MPP.* It will be also useful for further considerations to compare the acute MPP with the chronic one. In the chronic MPP the CR training is easier (more time is available) and consequently more complicated CRs may be elaborated. However, in addition to the rather tedious nursing care required (in acute MPP the care is similar as in other preparations) the experimenter's freedom during an experiment in the chronic preparation is more limited because the electrodes are fixed with cement and the surgical procedure needs aseptic conditions.

5. *Types of investigations proposed.* Taking into account all the above mentioned properties of the MPP we can see that although this preparation cannot replace an intact animal or an anaesthetized preparation it may be very useful for some types of investigations.

Since the first description of the MPP in 1958 by Batini Moruzzi et al about 30 papers have been done either totally or basically on this preparation *. Some of the papers were chiefly devoted to the analysis of

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* C o r d e a u and M a n c i a 1958 and 1959, A r d u i n i and H i r a o 1959, 1960ab, A r d u i n i and G o l d s t e i n 1960, A r d u i n i and P i n n e o 1962, 1963,

the properties of the MPP while other papers were concerned with investigating definite physiological problems. As was already shown the majority of the investigations was devoted to studying the photically evoked potentials, EEG activity and orientation reactions in this preparation.

In the authors opinion, in the acute MPP the following things may be particulary succesfully investigated: i) the process of learning (habituation and elaboration of the CRs), ii) the imidiate effect of lesions, iii) topographic interrelations, and, iv) ocular activity itself.

i) Two types of investigations of higher nervous activity may be distinguished: a) investigations of the process of learning and b) investigations of the ready-made CRs (cf. K o n o r s k i 1948). Experiments of the first type which are usually shorter, may often be performed more easily on an isolated cerebrum of the MPP than on an intact animal. One reason for this is that such investigations need the careful recording of permanently variated responses and the recording is easier in the MPP. Moreover, "recent" CRs and orientation reflexes in the course of habituation are very senstive to accidental stimuli which are strongly reduced in the isolated cerebrum of the MPP.

ii) It is well known that in a normal animal the effect of cerebral lesions may usually be tested no earlier than 3—7 days after the operation. In the MPP it may be investigated even during the operation.

iii) The location of the electrodes in the isolated cerebrum of the MPP may be easily changed during the experiment. In consequence, many neuronographic experiments performed earlier on various types of preparations may be successfully repeated in the MPP.

VI. Conclusions

1. Midpontine pretrigeminal preparation (MPP) of the cat is obtained by sectioning the brain stem just in front of the trigeminal roots, and it is composed of a neurally isolated rostral part (isolated cerebrum) and a caudal part which acts as its nourisher.

2. Chronic MPP may be kept alive for several weeks.

Batini, Magni et al. 1959, Batini, Moruzzi et al. 1959, Batini, Palestini et al. 1959, Mancia et al. 1959bc, Armengol et al. 1960, Glässer and Mantegazzini 1960, Santibanez et al. 1960, Palestini and Lifschitz 1961, Affani et al. 1962ab, Meulders 1962, Meulders and Massion 1961, Meulders, Massion and Colle 1961, King and Marchiafava 1963, King et al. 1963, Żernicki and Osetowska 1963, Elul and Marchiafava 1964, Dreher, Marchiafava and Żernicki 1965, Żernicki and Dreher 1965.

3. The activity of the isolated cerebrum of the MPP is basically the same as that of the cerebrum of the intact animal.

4. The pupils of the MPP are more narrow than in an intact animal. Spontaneous EEG activity shows considerable individual differences; in some MPPs the EEG patterns are strongly desynchronized while in other specimens they are more or less synchronized and spindle bursts may be often observed.

5. A behavioural and EEG efficiency, i.e. the capacity for the behavioural and EEG responses to external stimuli typical for an awake animal, is essential feature of the rostral part of the MPP.

In the MPP there are stronger individual differences in the level of efficiency than in the intact animal. In a given specimen the level of efficiency depends on the presentation of visual stimuli and is closely related to the pupillary diameter and the EEG patterns.

6. The MPP shows an orientation reflex to visual stimuli consisting of a dilation of pupils, a conjugate vertical eye movement towards the stimulus, an accomodation reflex and EEG arousal response. The reactions are much longer than duration of the stimulus. The orientation reflex can be habituated. Olfactory stimuli may also produce an orientation reflex.

7. The rostral part of the MPP reacts to any object moving up and down within its visual field with the following reflex. This reflex may be considered as a kind of fixation reflex. It can be also habituated.

8. The conditioned pupillary and EEG reflex may be easily obtained to a visual stimulus reinforced by hypothalamic stimulation producing strong pupillary dilation and EEG arousal. In chronic MPP inhibitory CRs may be obtained.

9. The MPP displays several advantages over a normal animal as an instrument for studies of cerebral functions; i) it can be fixed in the stereotaxic apparatus which facilitates the recording of electrical and oculomotor activity of the cat and the changing of the position of the electrodes, ii) any surgical procedure may be done without anaesthesia, and, iii) the cerebrum is effectively isolated from most possible accidental stimuli. On the other hand, there are several disadvantages of the MPP: i) in chronic preparations the postoperative care is tedious, ii) behavioural activity and utilization of external stimuli is strongly reduced iii) the mortality is increased, and iv) the general state of the animal is, sometimes, unstable.

10. The MPP seems to be convenient instrument for the following types of cerebral investigations: i) the study of habituation and elabo-

ration of CRs, ii) the study of the immediate effect of cerebral lesions, iii) neuronographic studies, and, iv) investigations of ocular activity itself.

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