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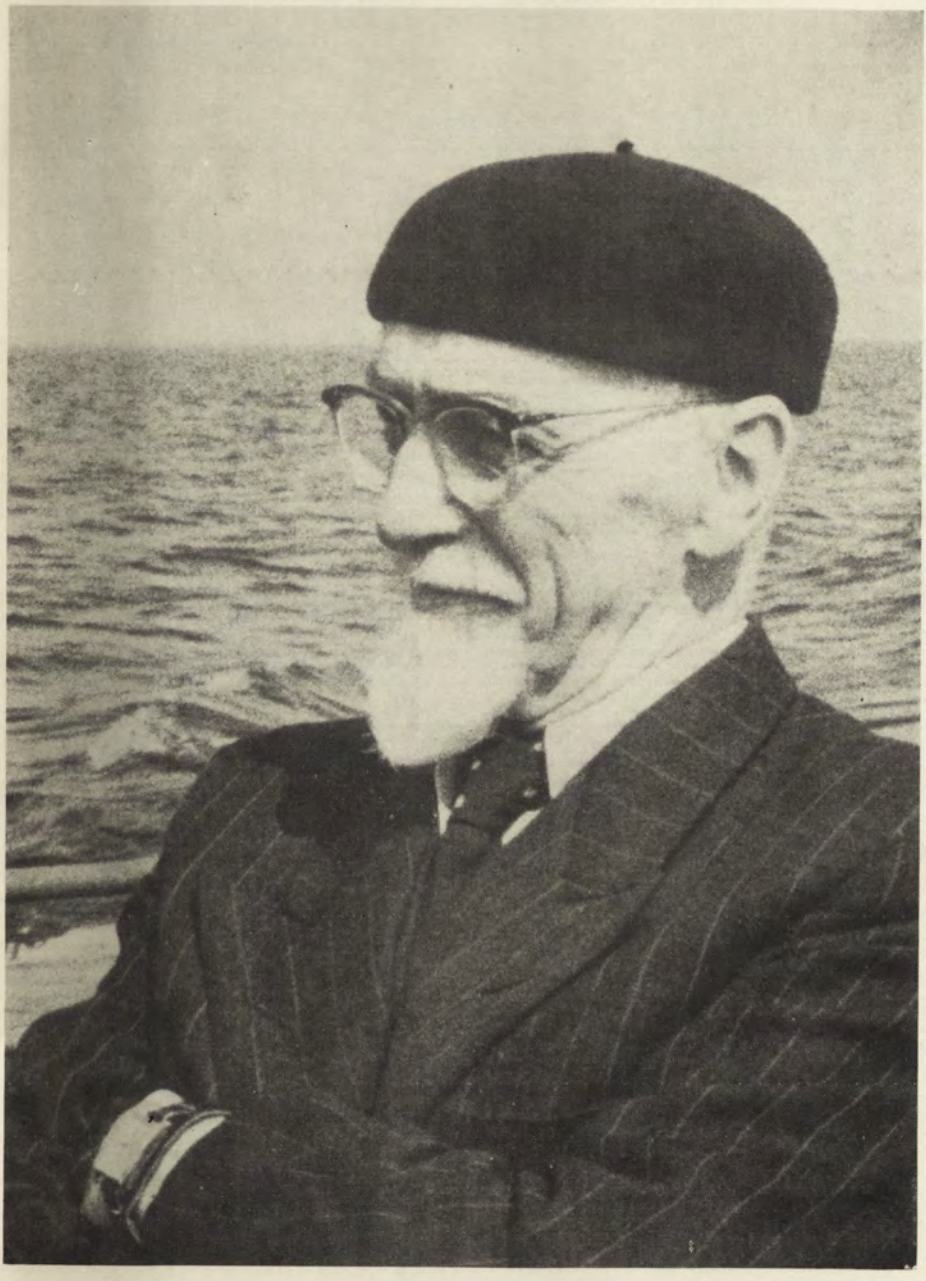

Mieczysław Bogucki, 1884—1965

Mieczysław Bogucki,
1884—1965

In the death of Professor Mieczysław B o g u c k i, which occurred on February 8, 1965, the Polish hydrobiology and physiology lost a distinguished scholar. For a period of many years, Professor B o g u c k i held several posts at the Nencki Institute of Experimental Biology. From 1953 till his death, he was the Editor of the Polskie Archiwum Hydrobiologii, and, in the years 1956—1961, he was the Editor of the Acta Biologiae Experimentalis.

B o g u c k i was born in 1884 at Łódź where he went to school. His education at the local gymnasium was interrupted shortly before his matriculation because he was charged for being active in the preparation of a school strike in the opposition to the then Poland ruling tsar regime. After graduation at a Moscow gymnasium he, in 1905—1907, studied at the Jagiellonian University in Cracow which then was under Austrian domination. In 1907, B o g u c k i went to Warsaw and joint the Polish Socialistic Party (PPS). For half a year he was imprisoned. Following his release from prison and exile from Kongresówka, the part of Poland under Russian domination, B o g u c k i studied in Paris. He was graduated from the Faculty of Science in 1912. On his return home he was offered the assistantship at the Chair of Embryology at the Jagiellonian University in Cracow. At the outbreak of World War I in 1914, B o g u c k i volunteered for the Polish Legion which as a part of the Austrian Army fought against Russia. In 1916, he returned to Cracow and received his doctor's degree. In the years 1917—1919, B o g u c k i served as associate in the Laboratory of Histology and Embryology at the Warsaw University, and later in the Laboratory of Physiology at the Nencki Institute. In 1920, B o g u c k i's work had been interrupted again by his service in the Polish-Soviet war, but he resumed his research afterwards. In 1928, he became Docent at the Warsaw University. After passing through the intermediate grades of professorship he was appointed Professor in 1959. During 1934—1939 he was Research Director of the Nencki Institute of Experimental Biology.

In 1932, B o g u c k i organized a maritime research station in Gdynia, a station which was very dear to his heart. He remained with the station

until the outbreak of World War II in 1939. On the end of the hostilities in 1945, B o g u c k i took up again his activities at the station, which he terminated in 1951. During the war years, that is in dark times of Hitler regime in Poland, he was teaching at the illegal Warsaw University School of Medicine which was officially a Nursing School.

Since 1951 B o g u c k i was domiciled in Warsaw. He was invited to work at the Nencki Institute and stayed in its Department of Biochemistry until his death. In 1963, he was elected the chairman of the Scientific Board of the Institute. His research activities continued ofter his official retirement in 1961.

Professor B o g u c k i was responsible for much important work in the field of embryology an experimental ecology. He particularly centered his attention on osmoregulatory mechanisms in the polychaete *Nereis diversicolor*. For a period of more than 50 years he worked indefatigably and his contributions to the biological science remain as example of well-conducted experiment. He was active scientifically to the very end of his long life. The enthusiasm and vigor of Professor B o g u c k i always reminded one of a young man.

Professor B o g u c k i was a member of many zoological, physiological and hydrobiological organizations, and numerous honors were bestowed upon him by both scientific societies and the Polish Government.

Professor B o g u c k i will be missed by all who really knew him and worked with him.

Stefan Brutkowski

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RECONSTRUCTION OF NEOCORTICAL LESIONS WITHIN THE DOG'S BRAIN: INSTRUCTIONS[1]

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This paper is dedicated to the physiologists, who, making the lesions within the dog's brain, face the necessity of an accurate reconstruction of such lesions or of the electrode placement and a determination of the degeneration range. Its aim is to make their task easier by giving several practical indications with regard to the brain preservation and section, as well as the analysis of mounted serial sections. To a certain extent, it also complements my previous papers on the myeloarchitectonics of the neocortex (Kreiner 1961, 1962, 1964a, b, c).

The lesion reconstruction is an indispensable consequence of any surgical intervention in the brain or of inserting electrodes in it. The term reconstruction involves a description of a lesion and of the general state of the brain, made by means of macro- and microanatomical analyses. Such a description should be as accurate as possible, based on appropriate schemes of the anatomical structure and on the exact anatomical terminology, determining particular component parts of the brain in an unequivocal and satisfactory manner. A reconstruction which determines either the lesion or the implantation of the electrodes in the form of generalities, referring only to superficial, textbook descriptions (mostly, only gyri and fissures) is worthless and, moreover, it diminishes the value of the results of physiological investigations.

The animals, destined for reconstruction are usually killed by administering lethal doses of narcotics. All manners of killing which involve bleeding should be avoided as disadvantageous for staining the sections.

[1] This research was partially supported by Foreign Research Agreement No. 287 707 of the Us Department of Health, Education and Welfare, under PL 480.

After taking out of the cranium, the brain is fixed in 10 per cent formalin (1 part of commercial formol to 3 parts of water or saline). While fixing the brain, attention should be paid so that the quantity of the fixing fluid was several times larger than the volume of the brain. After the lapse of one day, the fluid should be exchanged. The bottom of the vessel in which the brain is fixed should be covered with a few layers of filter paper or cotton. Thus, we prevent the brain from flattening, otherwise caused by its own weight (this is particularly important with large brains) and assure the access of the fixing fluid to the bottom surface of the brain which should not directly touch the glass. The perfusion of the brain with formalin accelerates fixing but, on the other hand, it also may cause the extension of vessels. The fixation of a dog brain, about 70 to 110g in weight, lasts for about 7 to 10 days. The advance in this process may be recognized by the change in the color of the blood and by the peculiar elasticity the brain acquires. A brain with red vessels is not yet fixed. Other fixing fluids such as, alcohol, mercuric chloride, chromium salts, etc., are used for special methods of staining and, to the reconstruction, are not of any greater importance. The fixed brain may be preserved for several years but, with time, it becomes less stainable.

In exceptional cases, when we are in a hurry, the brain may be fixed together with the skull, provided that the skin and muscles are removed from the skull and the brain will be fixed as soon as possible. The brain, fixed in formalin, is subject to swelling.

Macroscopic analysis. For the macroscopic examination of the brain, the meninges should be first removed. It is made by means of a delicate (ophtalmologic) pincette and a sharp scalpel. The preparation should be made very carefully so as not to damage the brain tissue. Particular attention should be paid to scars in the places of lesions where — together with dura — fragments of cortex, sometimes fairly large, may be removed involuntarily and this in turn may result in an increased lesion.

In the description of the fixed brain, attention should be paid to the range of lesion, as well as to any visible external damage and to general changes such as those caused by parasites, blood congestions, abscesses, etc. It is advisable to take a photograph or to draw a picture of the brain. In taking a photograph, a slip of paper with a distinct denotation of a dog should be placed close to the brain which will prevent from any future mistakes. The fixed brain may, even in this state, without a histological analysis, undergo a macroscopic examination. Such a reconstruction is, however, very superfical. In many cases it does not comprise such very important details as, demyelinizations, glial degenerations, accurate determination of the lesion depth and secondary alterations of adjacent regions. On the other hand, it has the advantage of being quick and simple. However, a considerable damage done to the experimental material and the limitation of the possibilities of an additional microscopic analysis make up the disadvantages of this method. For the macroscopic autopsy, the brain is dissected into 2 to 4 mm thick slices. Thinner slices are too fragile and thicker slices do not allow the experimenter to investigate the structure. In each case, however, they should be as uniform in thickness as possible. This applies to both the thickness of a particular slice and to the thickness of all slices of a given brain. The brain should be never be cut with scalpel freehand into uneven slices. Much valuable material has thus been irretrievably destroyed.

To dissect the brain into slices, a macrotome is used which should be an item of a basic equipment at every neurological laboratory. Where unavailable, it may be subs-

tituted by two bars, parallel to each other, fastened perpendicularly to a piece of board and making up a support for the knife. The brain, dissected with a large knife, is, after each slice, shifted exactly the same distance.

The base of the brain is not an even surface and this affords great difficulties in obtaining slices uniform in thickness. To overcome this difficulty, the brain should be embedded in gelatin. For this purpose a 25 per cent solution of a common gelatin is prepared with a small addition of the carbolic acid and at a temperature of about 40°C. To remove formalin, the brain is thoroughly washed in running water for at least 1 day. Hereafter, the gelatin solution is poured to a rectangular, cardbeard box so as to form a 2 to 3 cm high layer at its bottom and the brain is put in this layer when it is still liquid. The brain should be slightly immersed in the gelatin and placed in the box with its longer axis parallel to the length of the box. When the gelatin solidifies (for this purpose it may be put to refrigerator), its excess should be cut off and the entire preparation is dissected into slices by means of the macrotome. Thus prepared slices are kept in a 4 per cent formalin where they may be separated from each other by slips of blotting paper.

Prior to investigations, the slices of brain should be macroscopically stained so as the white and gray matter might be better visible. For this purpose, the oil red solution (its synonyms, oil scarlet, fast oil orange II, fat ponceau, orange RR) is used in the ratio of 2g of oil red to 120ml of benzene. With the use of a soft brush, the surface of each slice is carefully covered with this solution and, after a few minutes, the excess of this dye is washed off with running water. As a result of this operation, the white matter should acquire a red color and the gray matter — a pale-pink. This staining is not very durable but it may be repeated. The stained slices of brain are compared with the figures, showing cross sections and then, the lesion is determined in the identical manner with that in microscopic sections.

Microscopic analysis. A proper preparation of the brain to the reconstruction consists in making a series of microscopic sections. For this purpose, the entire brain or its section is embedded in paraffin and stained alternately by the Klüver-Barrera or Woelke and by the Nissl method. At our laboratory, it has been shown in practice that the most appropriate thickness of these sections amounts to 20μ. Dissecting the brain, we keep every 5th section which, of course, should be stuck on an object glass of adequate size. Of this supply, every 4th section is stained alternately by the Nissl and Klüver-Barrera method. The remaining sections are kept in store for additional staining which may be done, for instance, by the Landau silver stain or by the van Gieson method. They also may be used to replace the sections destroyed during staining. Sometimes, they may be of use to condense a series in some part of the brain, particularly interesting to the experimenter.

Reconstruction. The reconstruction of the range of lesion or of the implantation of the electrodes in the brain of an experimental animal consists in comparing the sections of this brain with the sections of a normal brain. The atlas of sections, enclosed with this paper, and the schemes of systems of fissures and gyri, as well as myeloarchitectonic fields, based on these sections, presented in the lateral, dorsal, ventral, medial and also lateral aspect but after the gyrus compositus anterior is removed to reveal the fields, hidden in the wall of the fissura presylvia, serve for the purposes of such comparisons. In using this atlas, a section as similar to the investigated section of the experimental brain as possible should first be found in the series of figures. Looking for a proper figure, we should take into account the outline of the cortex and of the white matter, as well as the details of the brain stem structure. It should be born in mind that the dog brains often display

a considerable degree of the individual variation and may, for instance, have additional fissures or gyri, as well as, vice-versa, some fissures may be in a state of disappearance. In comparing with the figures in the atlas, a certain difficulty may be afforded by the differences in the positions of section planes. When small parts of a section are examined, this does not make itself felt. It may happen, however, that, following a slightly oblique position of the section plane, a difference arises between the ventral and dorsal part of the hemisphere as compared with figures. In such cases, each half of the section should be determined separately.

After proper figures have been selected for the sections of the series examined, the most difficult part of the work is done. What remains now, is the projection of the boundaries of the lesion on the diagram of the cortex surface which should be done on the basis of diagrams A to E of the present paper. They represent a pattern of fissures and gyri with the determination of the myeloarchitectonic areas. For the readers guidance, the position of all cross sections, presented in Figures I to XXIII is shown in the plates. The pattern of fissures together with cross sections allow one to easily plot the boundaries of a lesion on, preferably, traced over small diagrams, prepared separately for each specimen investigated. The extent of the brain tissue removed within each myeloarchitectonic area is judged by eye. In practice, it turned out that it is best to determine the destructions according to the following scale: total (t), that is, about 90 to 100 per cent, nearly total (nt) — 90 to 75 per cent, partial (p) — 25 to 75 per cent and slightly dameged (sd) — 10 to 25 per cent. Here, we must not forget the areas which, invisible on the surface, are hidden in the walls of the fissures! The list of destroyed areas and the extent of lesions are read from the diagram shown on the scheme.

Preparing graphical documents in the form of figures, representing lesions, is the last stage of the work. The traced over patterns of areas and gyri on which the square of a lesion may be marked by any graphic symbol (dots, dashes, colored spots, etc.) will serve for this purpose. Introducing a gradation of these symbols (for instance, more or less densely distributed dots), we may also show the depth of the damage. Schematized drawings of cross sections supplement these documents. They are drawn either by means of a slide projector directly from selected cross sections, or the use may be made of the schemes of sections, traced over from the present paper with the boundaries of the lesion marked by means of any symbol. The outline of the lesion, plotted on the scheme makes up a sufficient reconstruction of the lesion. However, in each case it should be supplemented by the description of the general state of the brain. In such a description, the following elements should be considered: the inflammation foci, parasites, state of vessels and brain ventricles, as well as alterations, resulting from surgery, as, demyelinization, abscesses and proliferation of glial nuclei which, in many cases, may cause changes, obscuring the intended lesion.

ABBREVIATIONS

BA, area entolateralis anterior (XI—XV),
BAL, area entolateralis anterior lateralis (XIII—XV),
BP, area entolateralis posterior (XVI—XXIII),
BPL, area entolateralis posterior lateralis (XVI—XX),
CA, area composita anterior (IV—VI),
CE I, area composita ectosylvia I (XIII—X),

CE II, area composita ectosylvia II (VIII—X),
CJ I, area composita interna I (V—VIII),
CJ II, area composita interna II (VI—VII),
CJ III, area composita interna III V—IX),
CPL I, area composita posterior lateralis I (XVI—XVII),
CPL II, area composita posterior lateralis II (XVIII),
CPL III, area composita posterior lateralis III (XIX—XX),
CPM I, area composita posterior medialis I (XV—XVII),
CPM II, area composita posterior medialis II (XVII—XVIII),
CPM III area composita posterior medialis III (XIX—XX),
CS, area composita sigmoidea (V—VIII),
CSL, area composita sigmoidea lateralis (VI—VIII),
CX, area composita precruciata (V—VII),
C I, area sulci centralis I (IX),
C II, area sulci centralis II (X),
EA, area ectosylvia anterior (XI—XIV),
EAc I, area ectosylvia accessoria I (XIV),
EAc II, area ectosylvia accessoria II (XVII—XX),
ED I, area paraectosylvia dorsalis I (XI—XII),
ED II, area paraectosylvia dorsalis II (XIII—XVII),
EM, area ectosylvia media (XV—XIX),
EM′, area ectosylvia media prim (XVI—XIX),
EP I, area ectosylvia posterior I (XVII—XVIII),
EP II, area ectosylvia posterior II (XV—XVI),
EV, area paraectosylvia ventralis (XI—XVII),
FA, area fissurae ansatae (X—XI),
FBA, area fissurae entolateralis anterioris (XII—XIV),
FBP, area fissurae entolateralis posterioris (XIX),
FCM, area fissurae callosomarginalis (VIII—XVIII),
FCP, area sulci intracompositi posterioris (XIX—XX),
FE, area fissurae ectosylviae (X—XVIII),
FG, area fissurae genualis (V—IX),
FK, area fissurae coronalis (V—XI),
FL, area fissurae lateralis (XII—XXIII),
FN, area fissurae presplenialis (XI—XVI),
FO, area fissurae suprasplenialis (XVIII—XXIII),
FORB, area fissurae intraorbitalis (V—VI),
FPG, area fissurae pregenualis (II—IV),
FPRc, area fissurae pararecurrentis (XX—XXII),
FpS, area fissurae presylviae (VI—XI),
FQ, area fissurae ectolateralis anterioris (XIII),
FQP, area fissurae ectolateralis posterioris (XVI—XXII),
FR, area fissurae retrosplenialis (XX—XXI),
FRc, area fissurae recurrentis (XX—XXI),
FRE, area fissurae retrosplenialis externae (XXI—XXIII),
FRh, area fissurae rhinalis anterioris (I—XIII),
FRhP, area fissurae rhinalis posterioris (XIV—XIX),
FS, area fissurae sylviae (XIV—XVI),
FSp, area fissurae splenialis (VIII—XX),
FSS, area fissurae suprasylviae (VIII—XX),
FSSJ, area fissurae suprasylviae inferioris (XV—XXI),
FZP, area fissurae postrecurrentis (XXI—XXIII),
G I, area genualis I (VI—VIII),

G II, area genualis II (IX),
H, hippocampus precommissuralis (VIII—IX),
KA, area coronalis anterior (V—X),
KM, area coronalis medialis (VI—X),
KP, area coronalis posterior (XI—XIII),
KPL, area coronalis posterior lateralis (XI—XIII),
KPM, area coronalis posterior medialis (XI—XII),
LAD I, area limbica anterior dorsalis I (—)
LAD II, area limbica anterior dorsalis II (XI),
LAL, area limbica anterior lateralis (XI),
LAV, area limbica anterior ventralis (X),
LM, area limbica media (XI—XVI),
LPD I, area limbica posterior dorsalis I (XIII—XV),
LPD II, area limbica posterior dorsalis II (XVI—XX),
LPL, area limbica posterior lateralis (XIV—XIX),
LPV I, area limbica posterior ventralis I (XIII—XVI),
LPV II, area limbica posterior ventralis II (XVII—XVIII),
MA, area marginalis anterior (XI—XV),
MP, area marginalis posterior (XVI—XXIII),
ML, area marginalis lateralis (XI),
ND, area presplenialis dorsalis (XI—XVI),
NV, area presplenialis ventralis (XI—XIII),
O I, area splenialis I (XVII—XXIII),
O II, area splenialis II (XVII—XXII),
OAc, area splenialis accessoria (XVII—XIX),
ORB I′, area orbitalis I′ (I—IV),
ORB I″, area orbitalis I″ (II—V),
ORB II, area orbitalis II (IV—VII),
ORB III, area orbitalis III (VIII—X),
OVL, area recurrens lateralis (XXI—XXII),
OVM, area recurrens medialis (XXI—XXII),
PG I, area pregenualis I (II—III),
PG II, area pregenualis II (II—IV),
PG III, area pregenualis III (IV),
PoC, area postcentralis (X—XI),
POL, area polaris (I),
PORD′, area paraorbitalis dorsalis prima (V—VII),
PORD″, area paraorbitalis dorsalis sec. (VI—VII),
PORV, area paraorbitalis ventralis (II—VI),
PR, area prorea (I—V),
PrC I, area precentralis I (VIII—IX),
PrC II, area precentralis II (VIII—IX),
PrC III, area precentralis III (VIII—X),
PrCJ, area precentralis interna (VIII—X),
PrCL, area precentralis lateralis (IX),
PRL I, area prorea lateralis I (II—IV),
PRL II, area prorea lateralis II (III—V),
QA, area ectolateralis anterior (XII—XV),
QL, area ectolateralis lateralis (XIII),
QM, area ectolateralis medialis (XIII),
QP, area ectolateralis posterior XVI—XXIII),
QPM, area ectolateralis posterior medialis (XXI),
R I, area retrosplenialis I (XXI),
R II, area retrosplenialis II (XXI—XXII),
R III, area retrosplenialis III (XXI—XXII),
R IV, area retrosplenialis IV (XX—XXII),
RJ, area retrosplenialis interna (XXI),
RV, area retrosplenialis ventralis (XXI),
S, area sylvia (XI—XVII),

SC I, area subcallosa I (VIII—IX),
SC II, area subcallosa II (VIII—IX),
SD, area parasylvia dorsalis (XI—XVI),
SG, area subgenualis (V—VII),
SJ, area sylvia insularis (X—XII),
SPR I, area subprorea I (I—VI),
SPR II, area subprorea II (VI—VI),
SPRL I, area subprorea lateralis I (II—III),
SPRL II, area subprorea lateralis II (IV—V),
SSD, area suprasylvia dorsalis (XIII),
SSM, area suprasylvia media (XIV—XXI),
SSm, area suprasylvia accessoria (XIV—XVII),
SSP, area suprasylvia posterior (XXI),
SSV, area suprasylvia ventralis (XX—XXI),
XC, area precruciata centralis (VI—VII),
XL, area precruciata lateralis (VIII—X),
XM I, area precruciata medialis I (V—VII),
XM II. area precruciata medialis II (V—IX),
XP I, area precruciata posterior I (VIII),
XP II, area precruciata posterior II (IX—X),
ZA, area pararecurrens anterior (XXI),
ZL, area pararecurrens lateralis (XXII—XXIII),
ZM, area pararecurrens medialis (XXII—XXIII).

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EXPLANATION FOR FIGURES

I—XXIII. A series of cross sections through the right hemisphere of the dog with the denotation of myleoarchitectonic areas. Stained according to Weigert--Wolters. Semischematic.

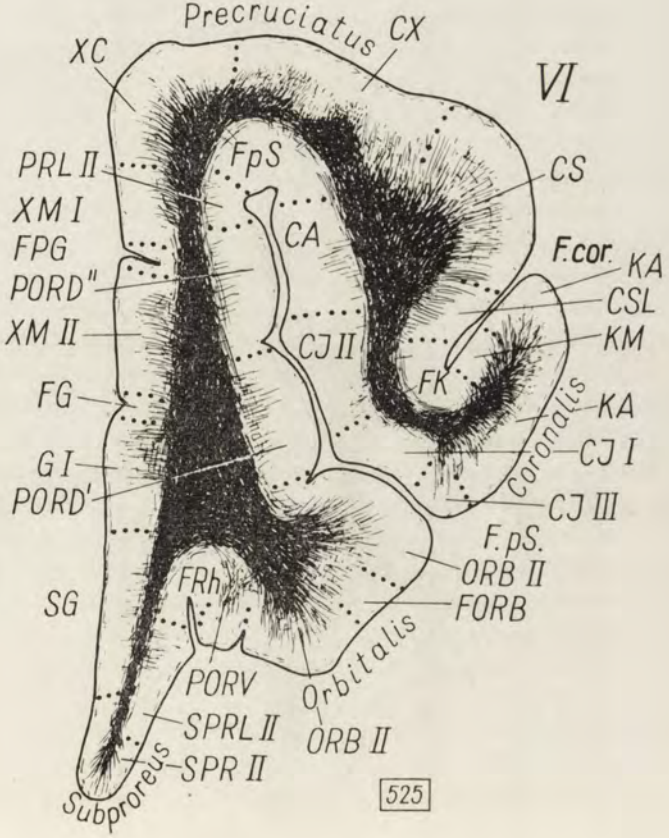
Fig. A. Lateral view of the right hemisphere of the dog with the myeloarchitectonic areas. Planes of sections I—XXIII marked.

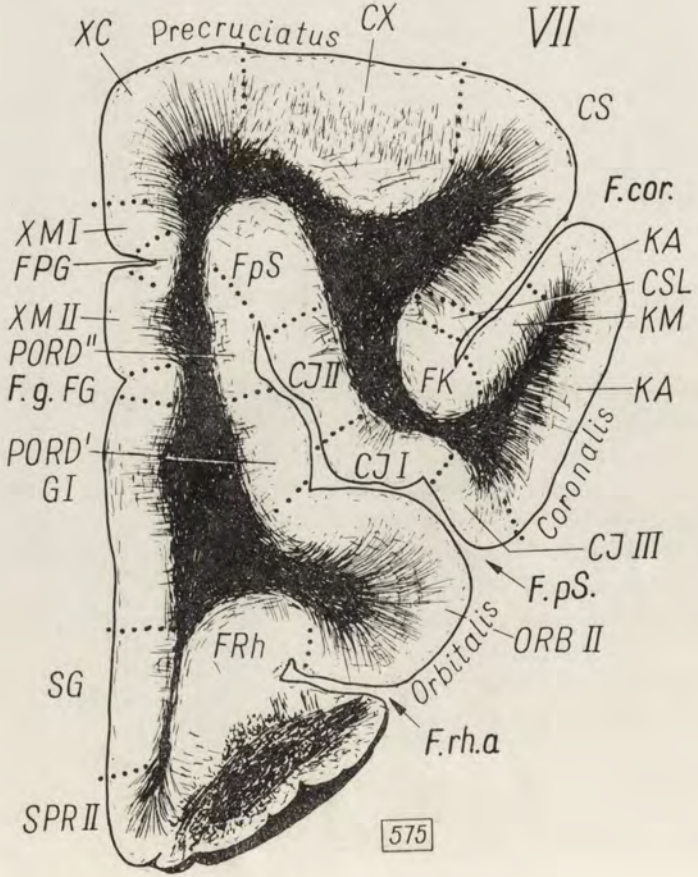
Fig. B. Lateral view of the frontal lobe of the hemisphere of the dog with the denotation myeloarchitectonic areas. Planes of sections I—X marked. Zigzag line, outline of the removed gyrus compositus anterior.

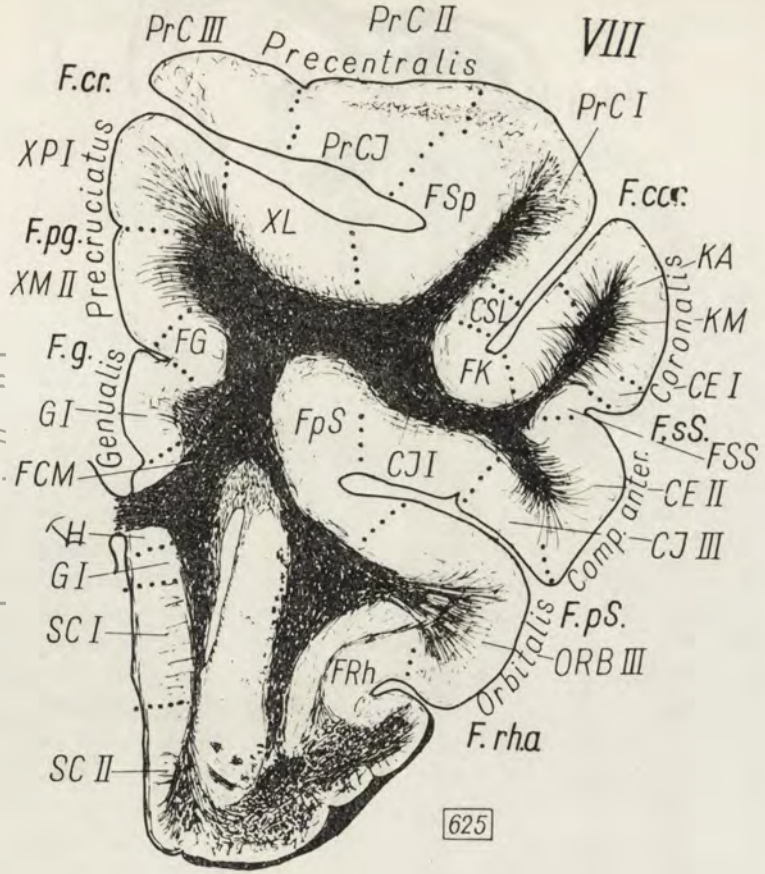
Fig. C. Medial view of the right hemisphere of the dog with the denotation of the myeloarchitectonic areas. Planes of sections I—XXIII marked.

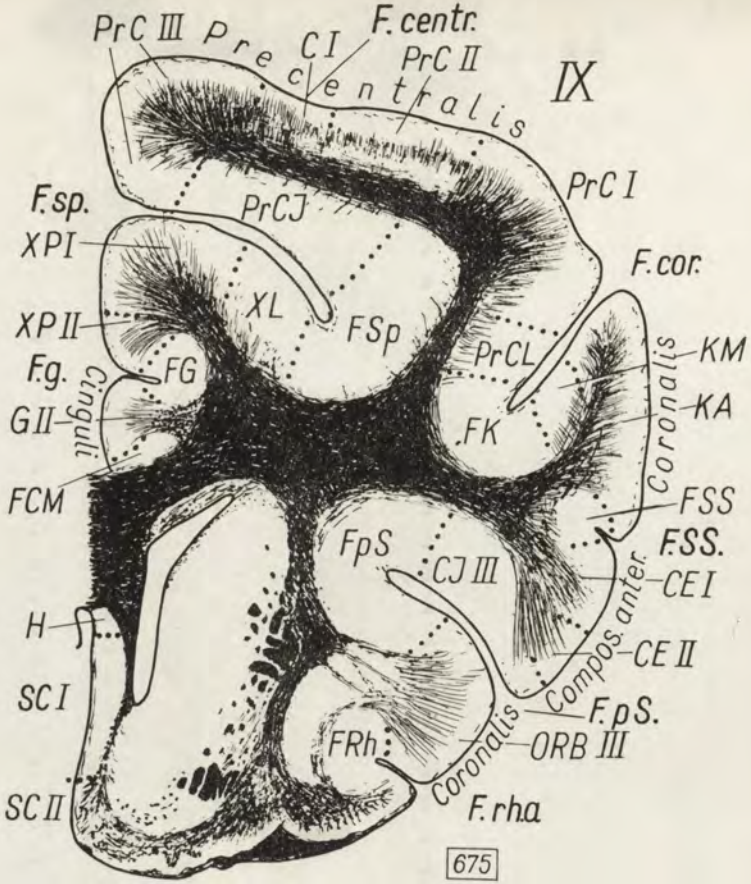
Dig. D. Dorsal view of the right hemisphere of the dog with the determination of the myeloarchitectonic areas. Planes of sections I—XXIII marked.

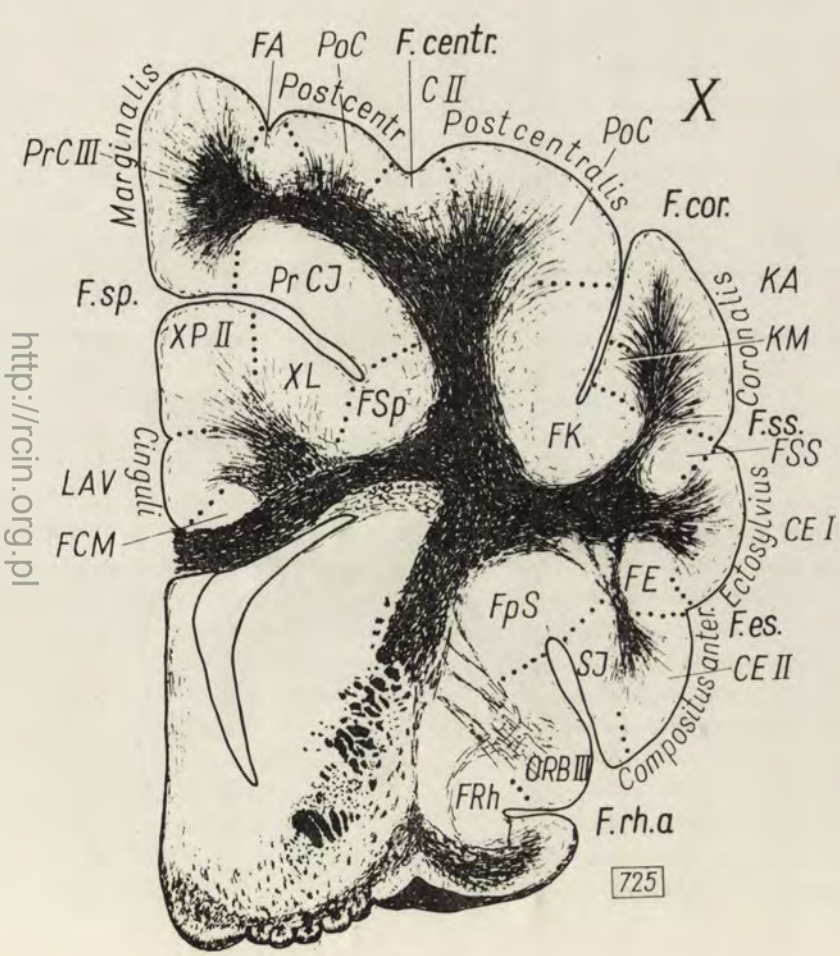
Fig. E. Ventral view of the right hemisphere of the dog with the denotation of the myeloarchitectonic areas. Planes of sections I—XXIII marked.

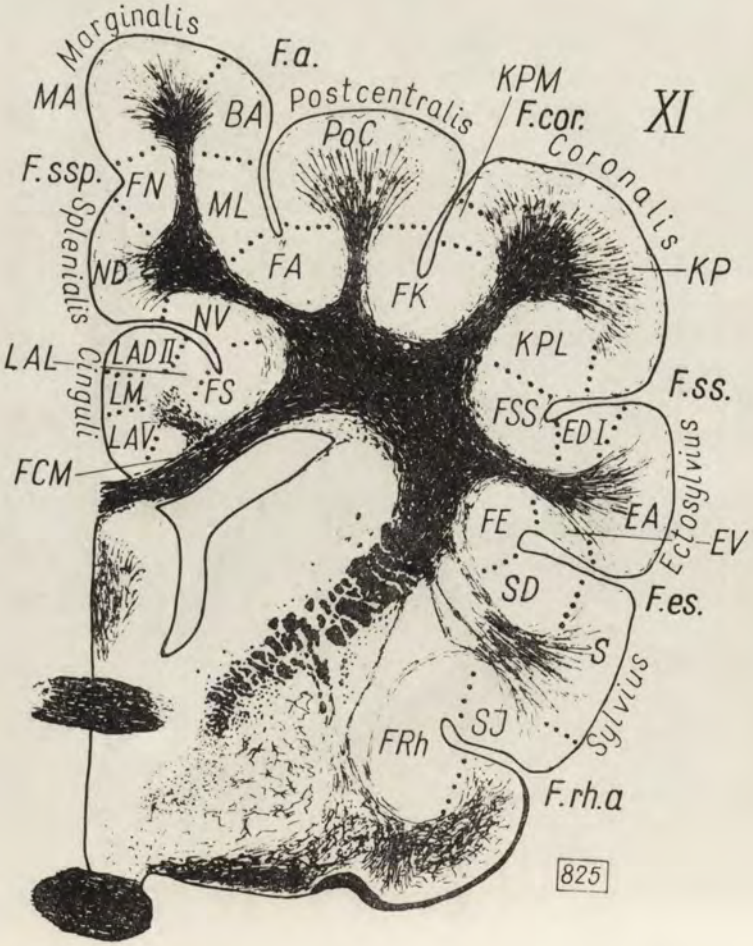


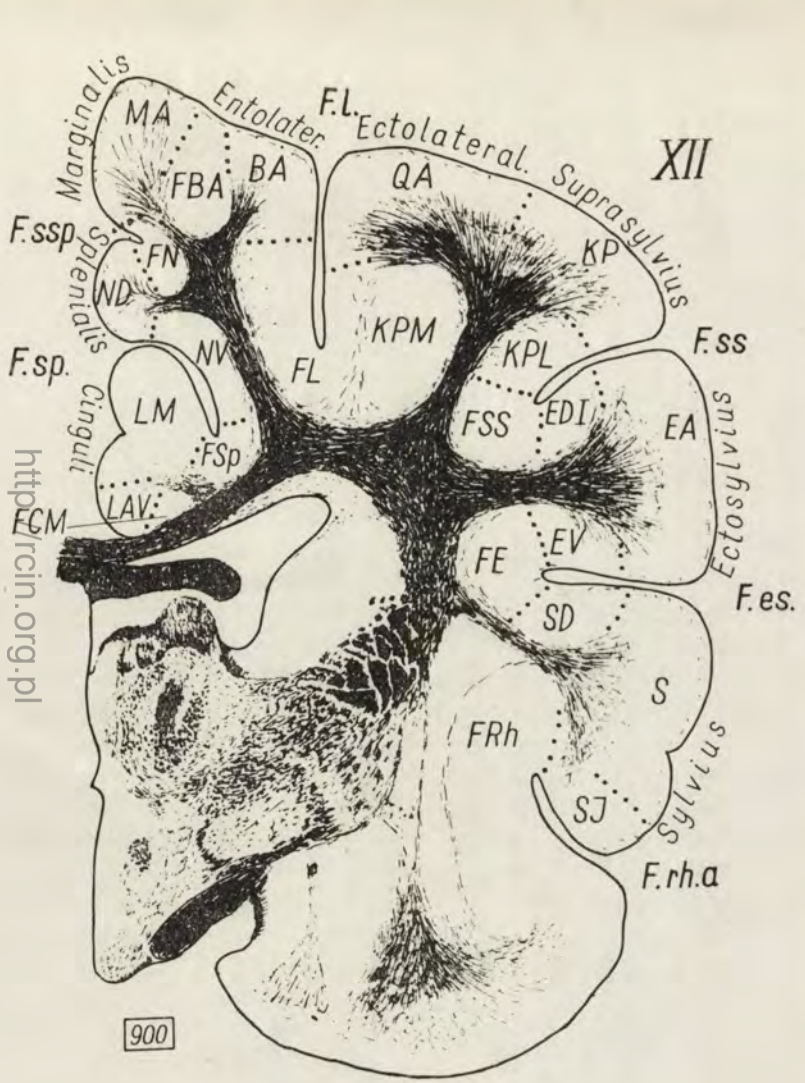


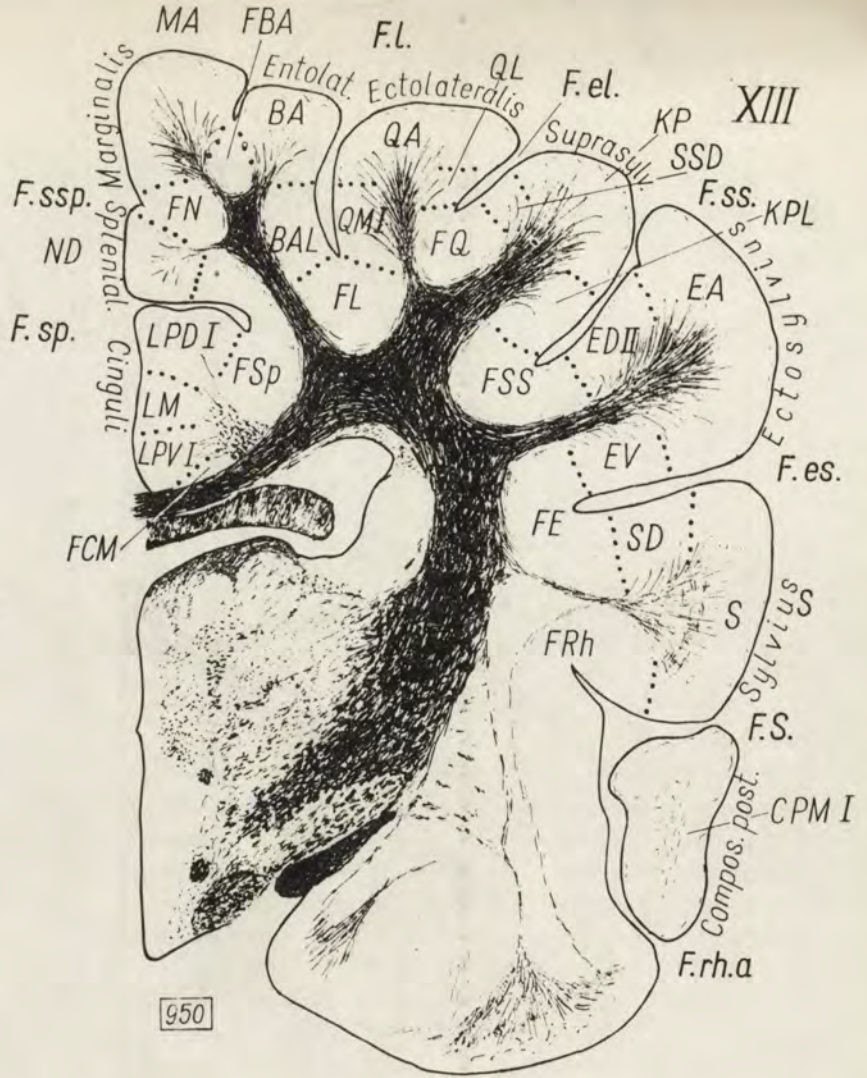


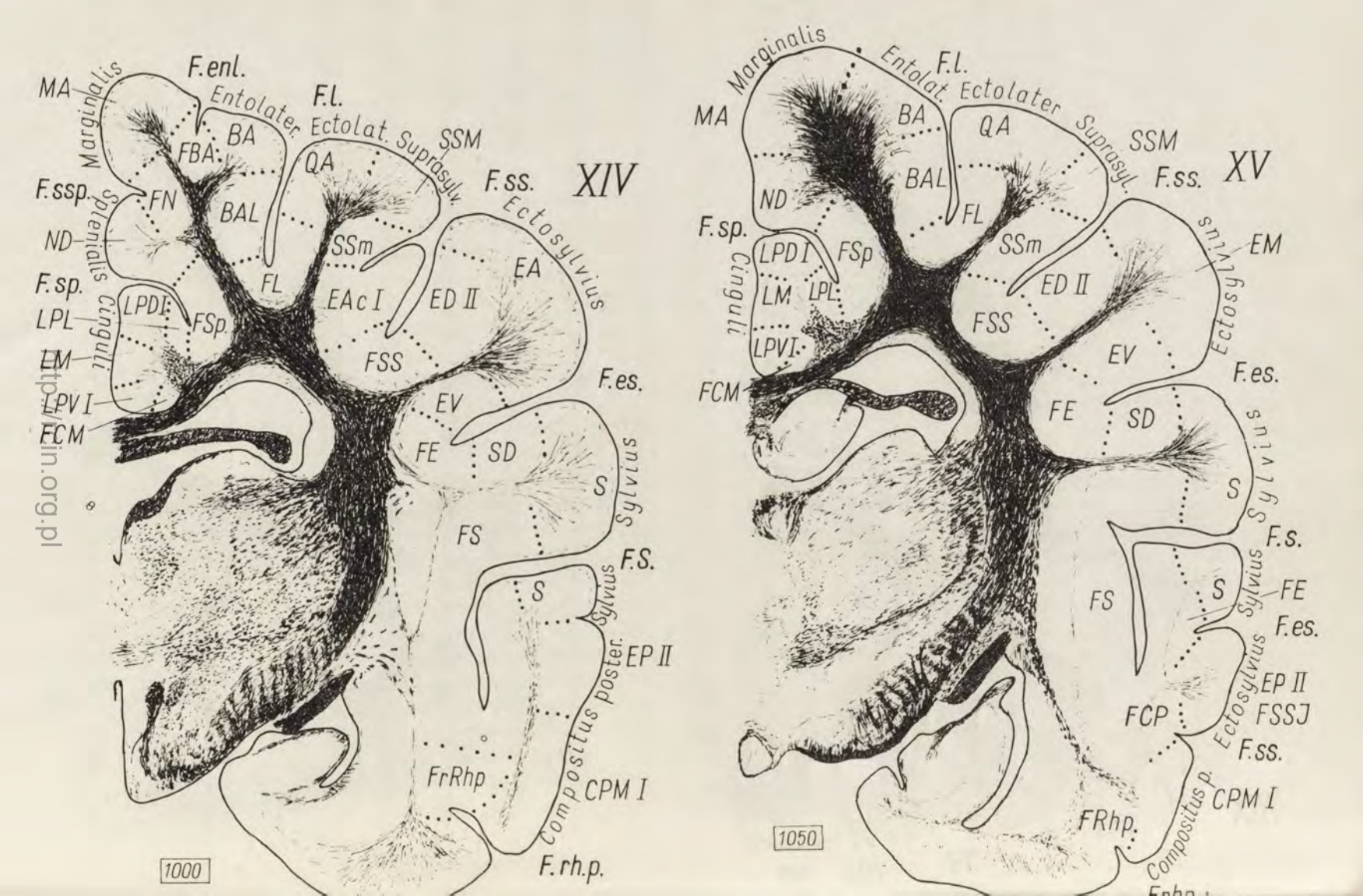


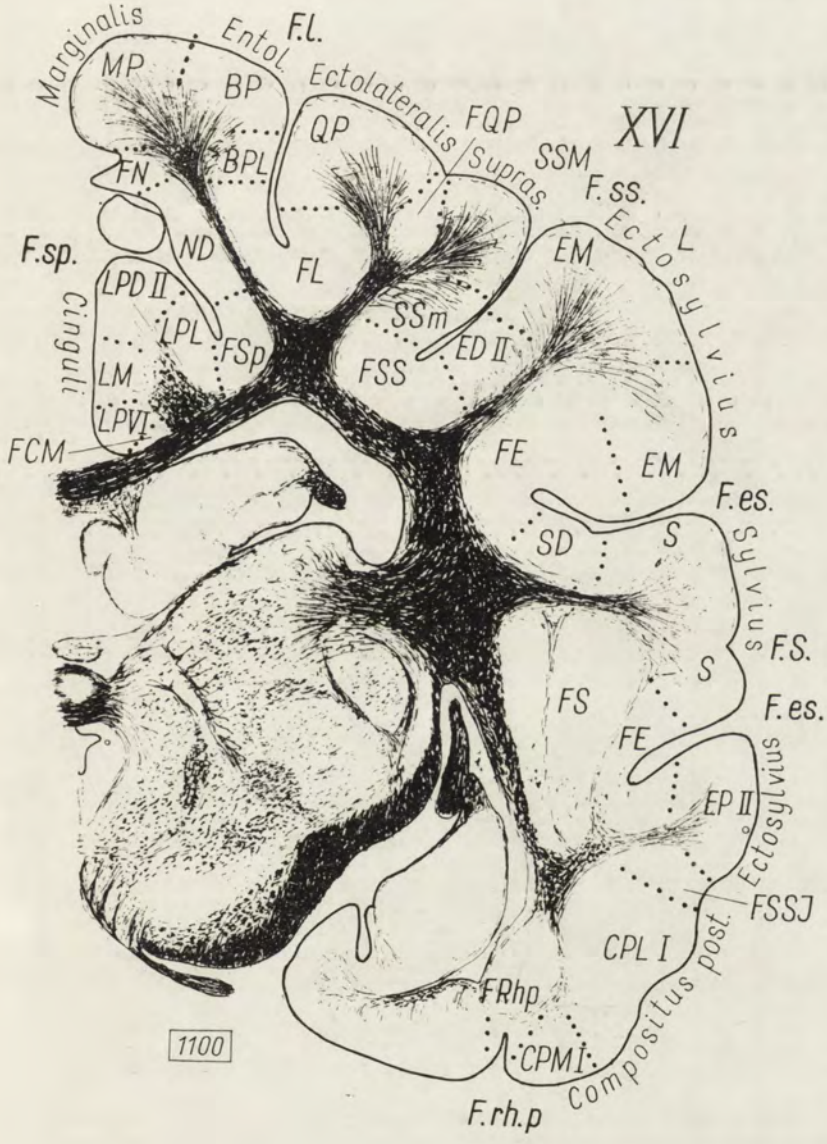


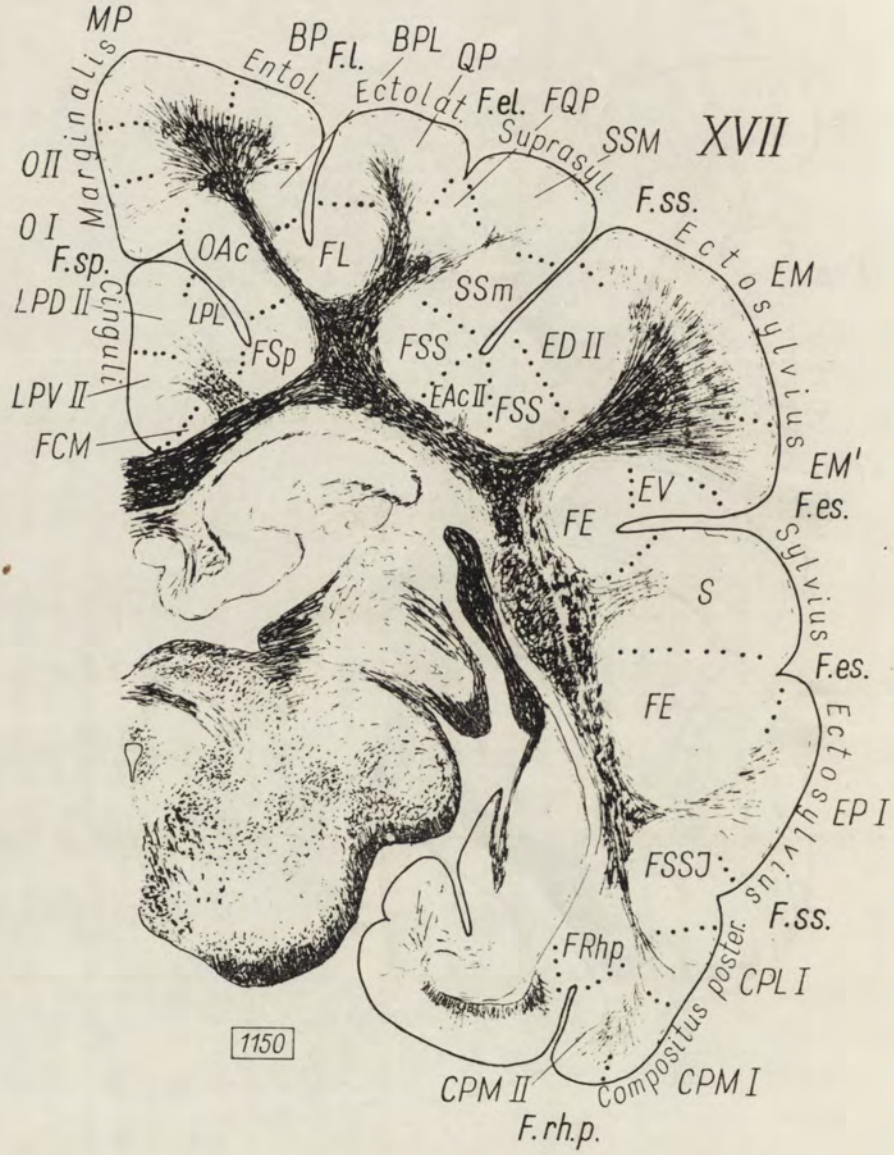


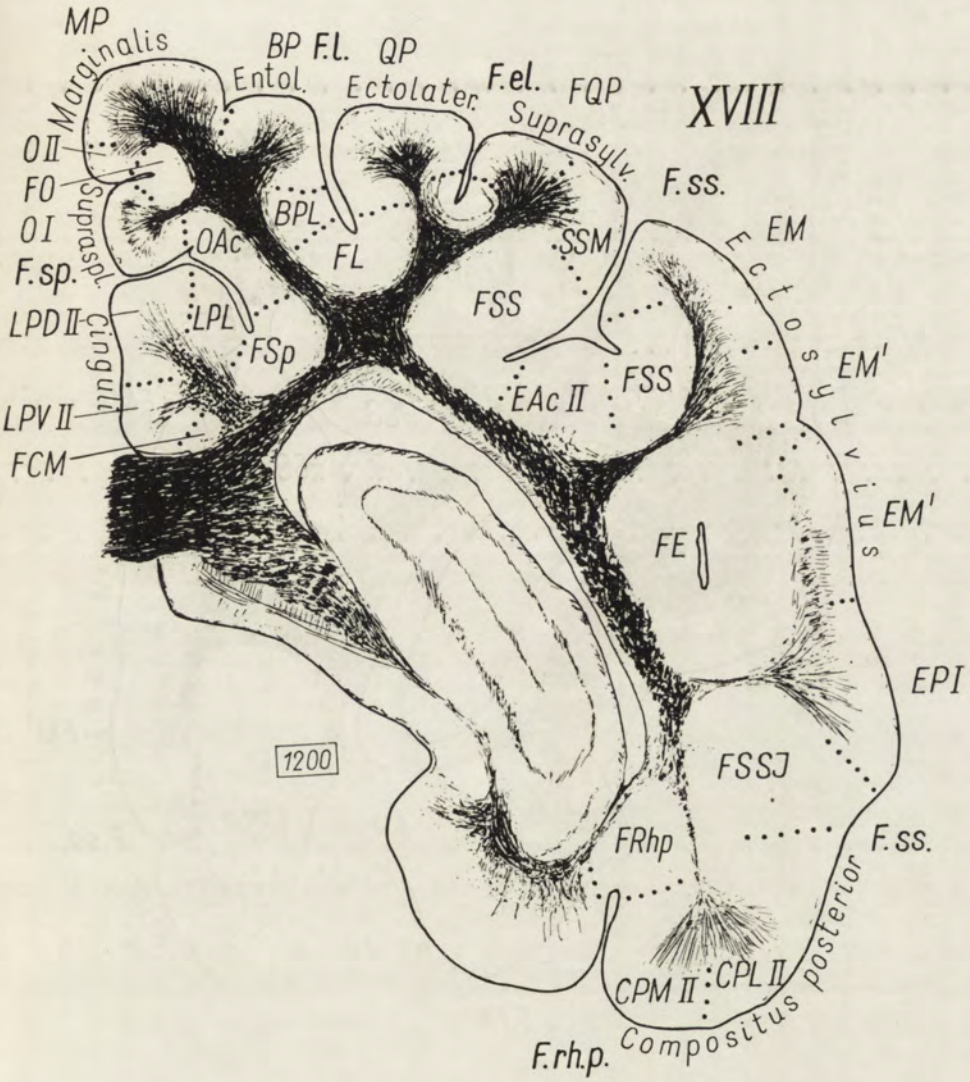


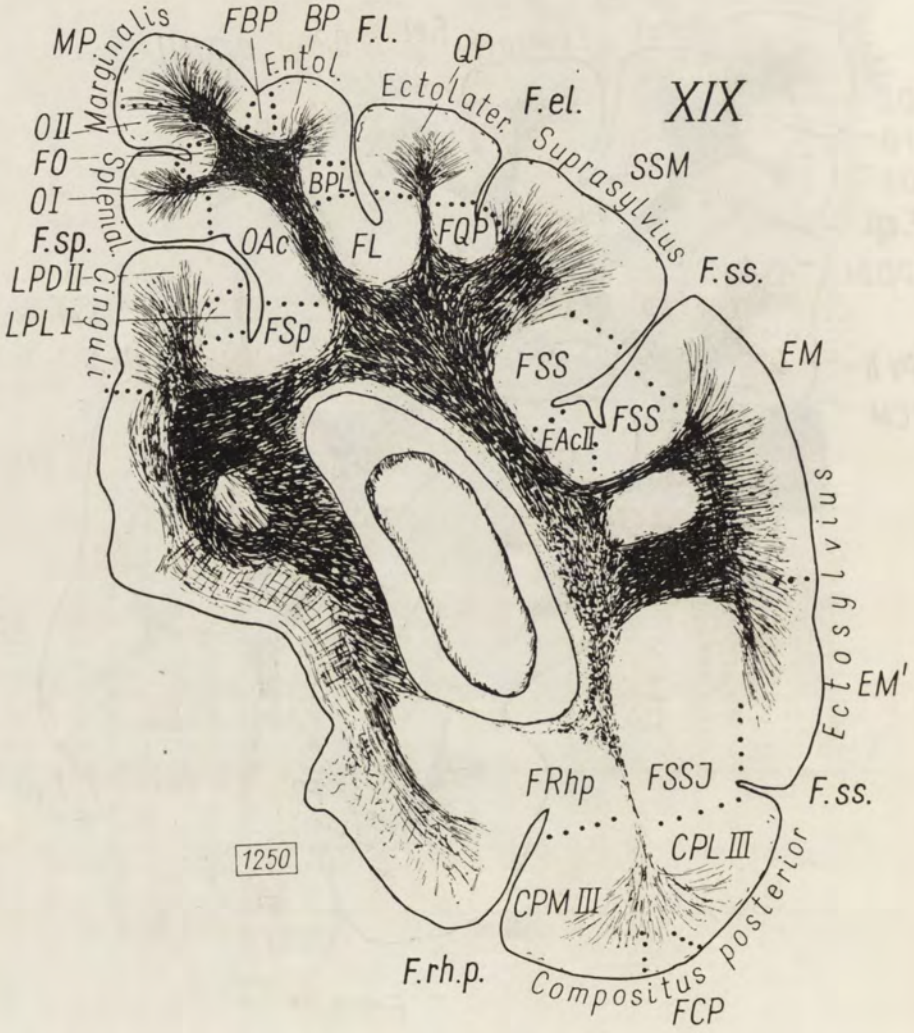


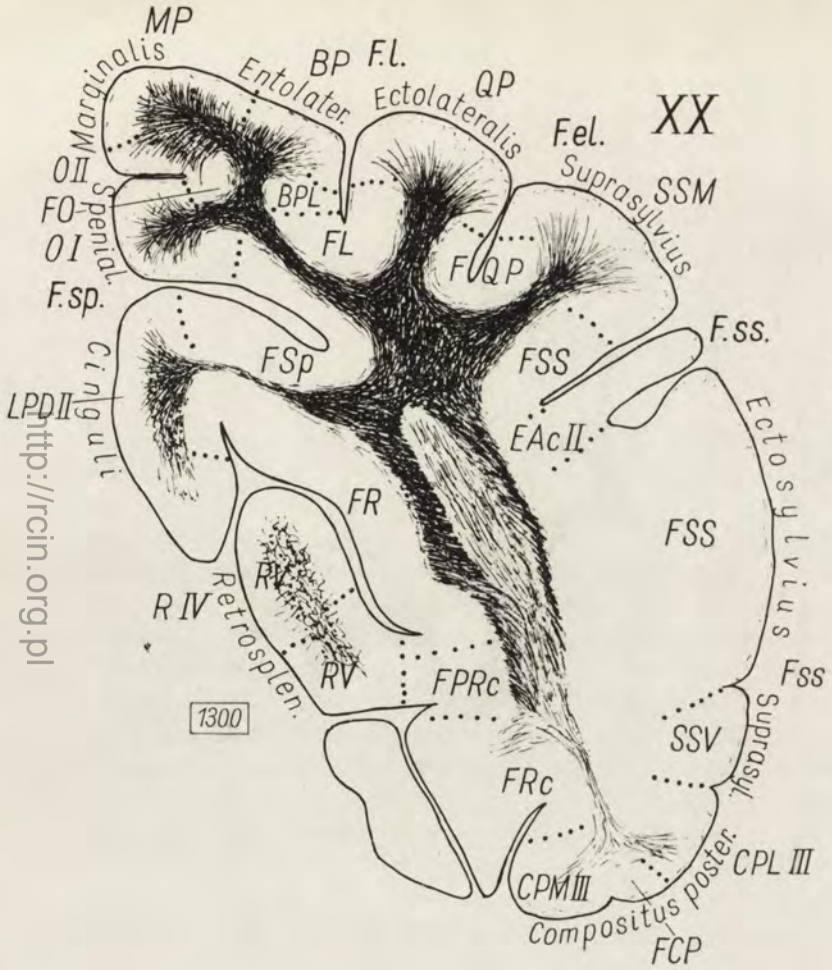


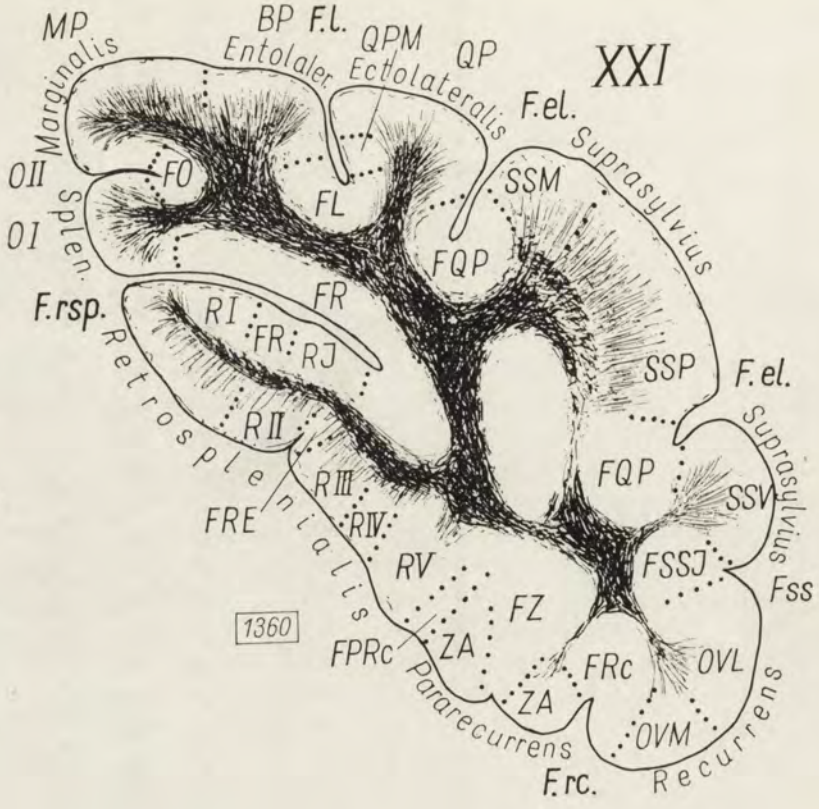


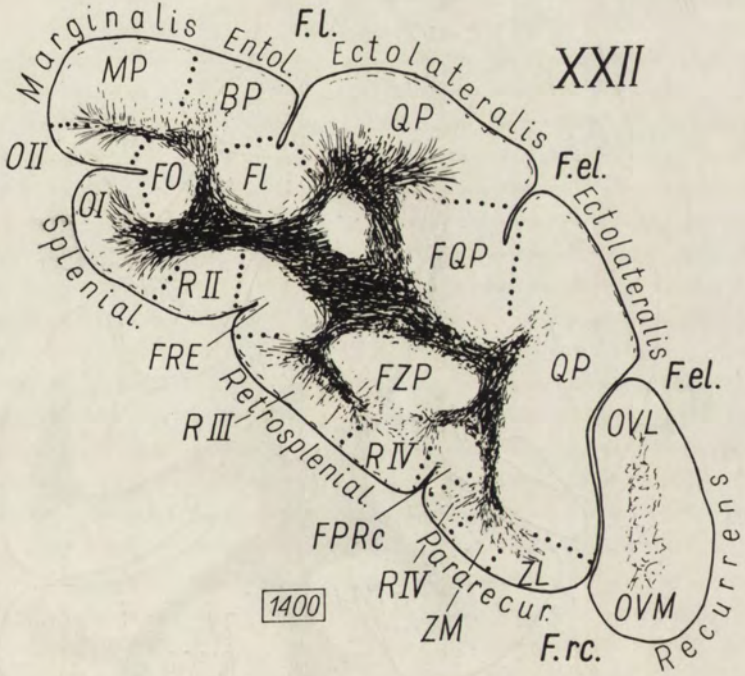


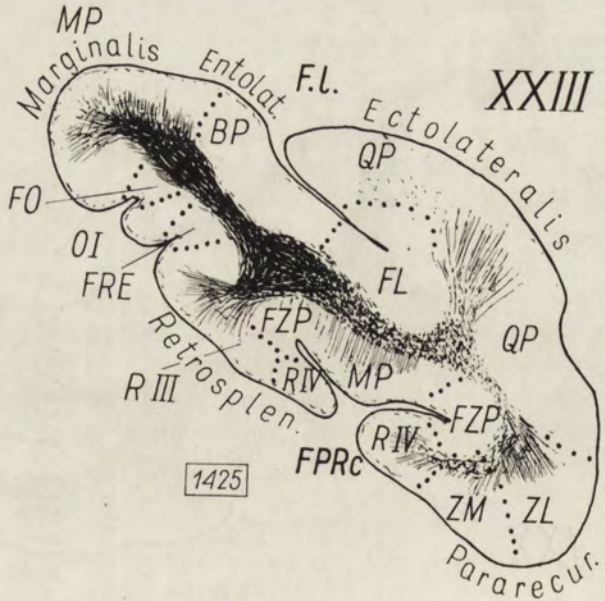


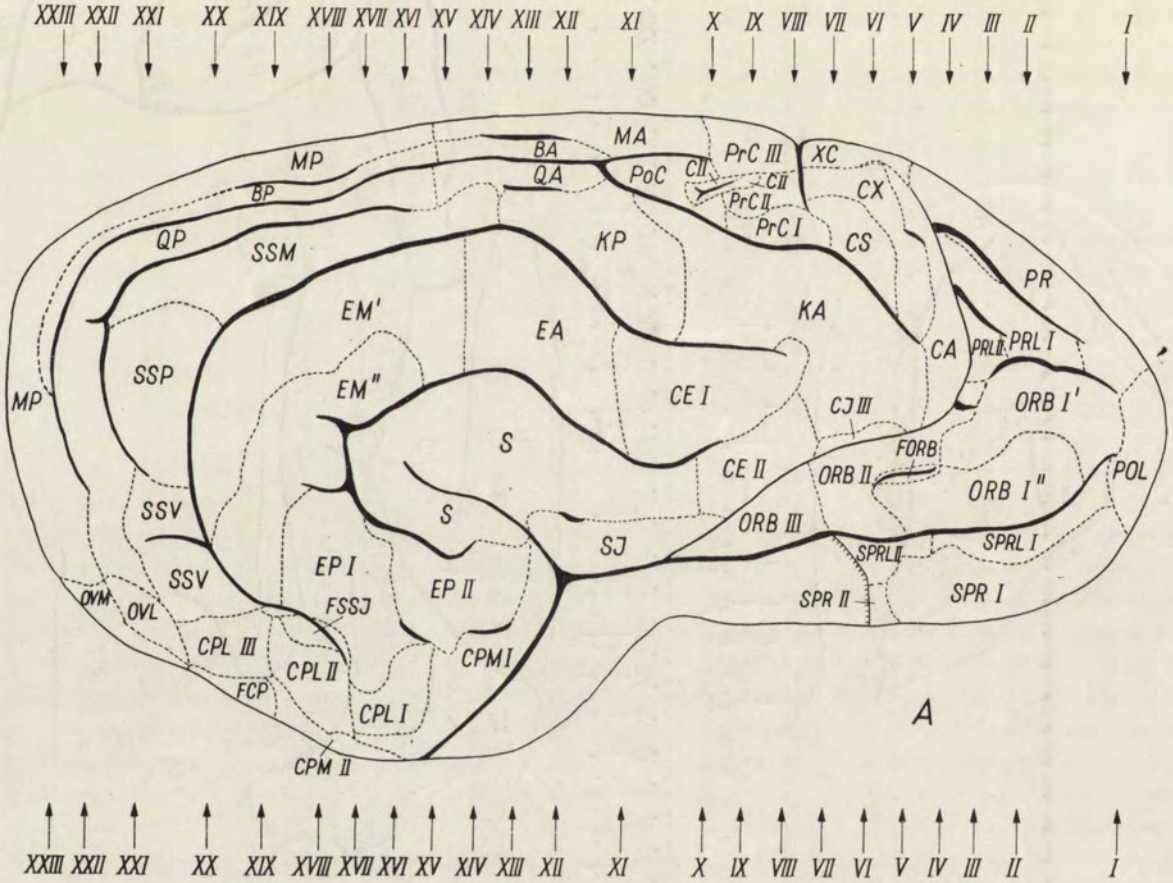


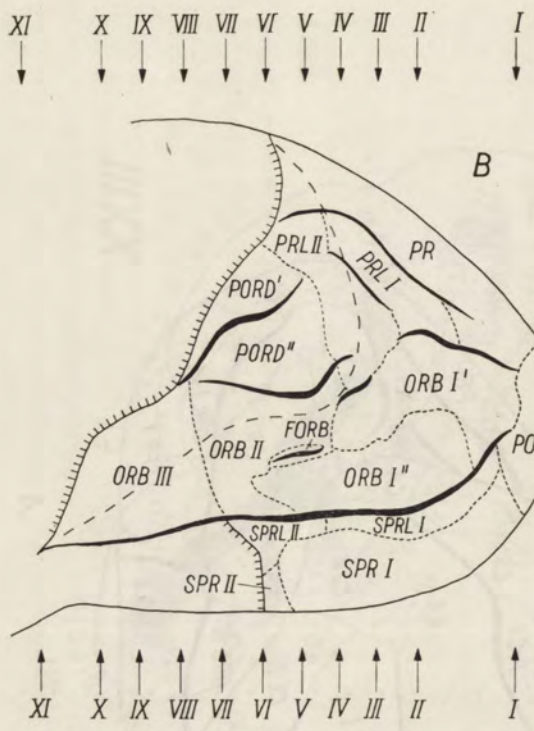


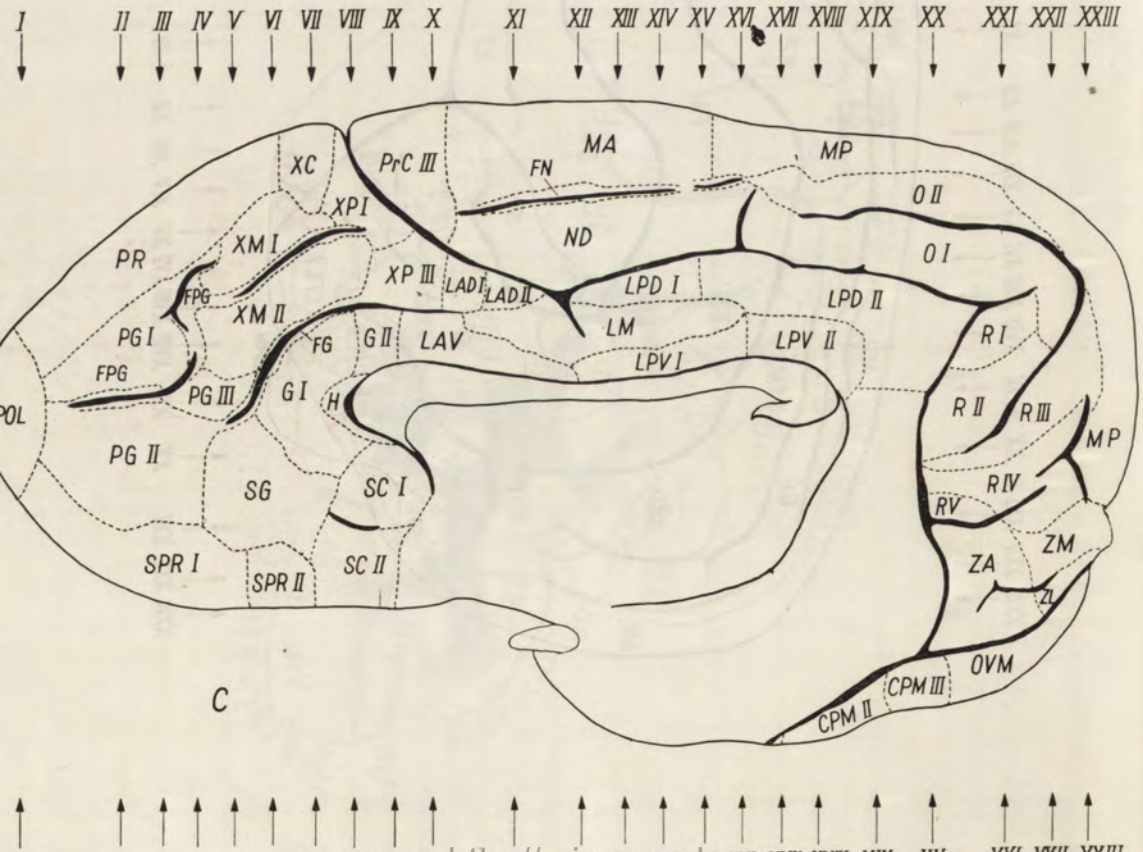


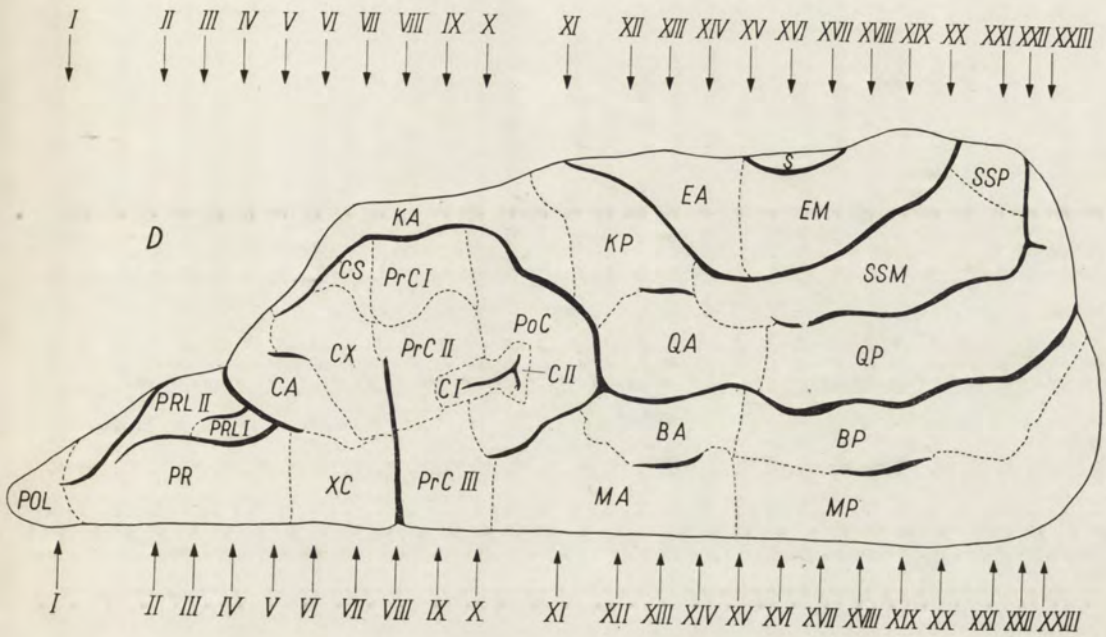


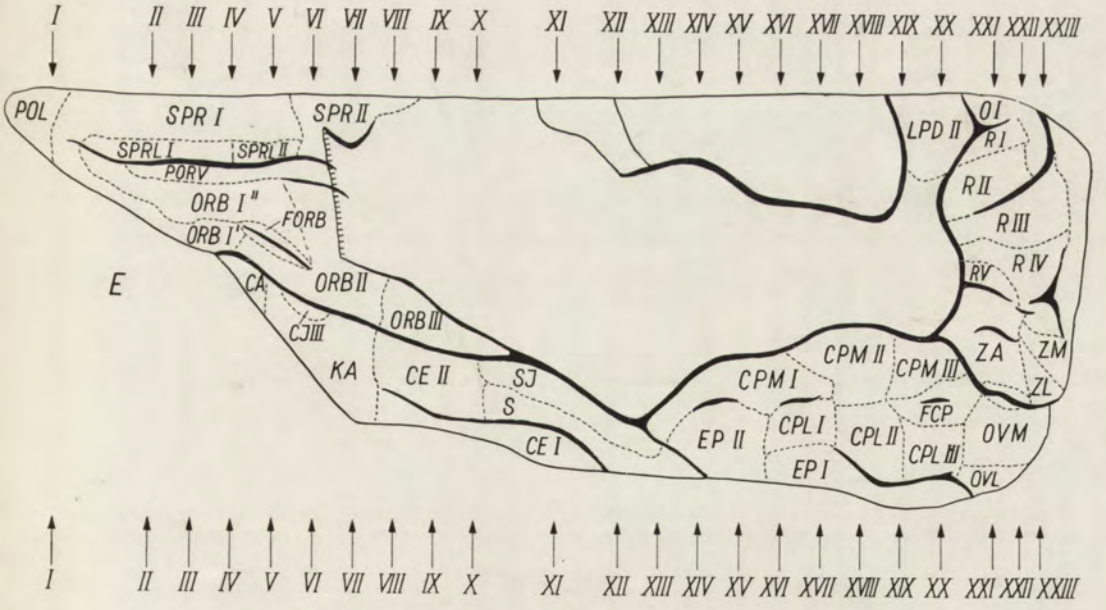


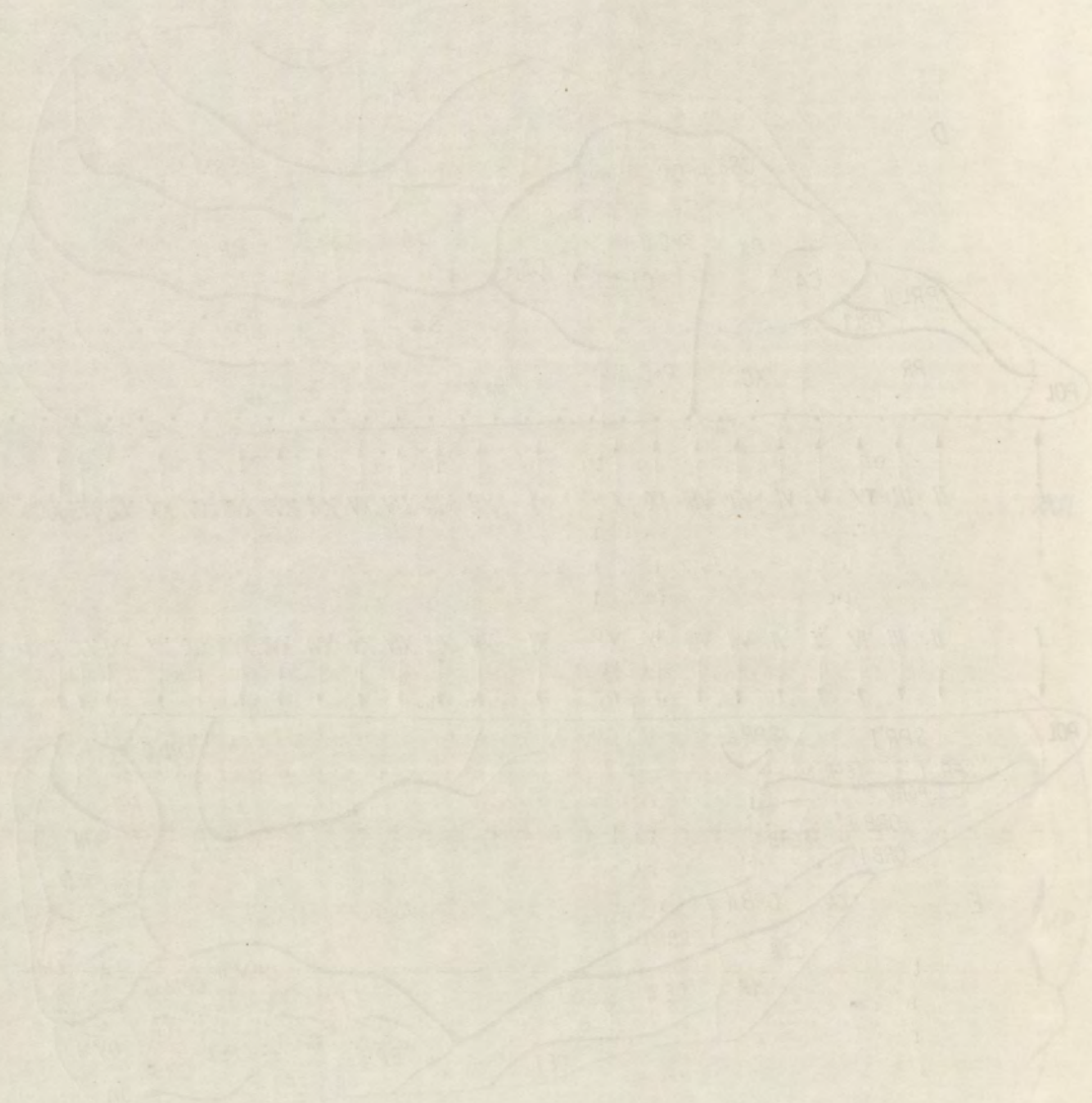












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THE EFFECT OF SATIATION ON THE VALUE OF THE CONDITIONED SALIVARY RESPONSE [1]

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(Received January 25, 1966)

In an effort to understand the role of motivation in determining behavior, various investigators have attempted to define the concept of drive in terms of the operations by which it is produced and in terms of a wide variety of response measures which are affected by the drive-producing operations. Different strengths of drive have usually been produced by varying the time of deprivation of some needed substance, although other techniques (chemical, hormonal, and surgical) of altering drive state have been employed. Among the many response measures which have been used to measure drive states are consummatory responses, resistance to extinction, response latency, number of trials or errors in reaching a learning criterion, rate of responding, and the overcoming of various kinds of obstacles to reach a goal object. These response measures, along with several others, are described by K i m b l e (1961). In general, there is very little agreement between the several response measures. For example, M i l l e r and his associates (1950, 1957) have shown that rats made hyperphagic by the bilateral destruction of the ventromedial nuclei of the hypothalamus eat significantly more food than control subjects only when the food is freely available; when the animals are required to press a lever, pull a weight down a runway, overcome electric shock or the taste of quinine in their food, the lesioned rats eat less than the normal rats. The consummatory response measure,

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amount of food eaten, does not yield the same information about the drive state of the operated animals as do the other response measures. A similar relationship using thirst motivation has been reported by Heyer (1951a, 1951b) and O'Kelly and Heyer (1951).

In conditioning situations, it is apparent that various motor responses (including consummatory behavior) do not accurately reflect changes in the drive state of the animal. A satiated dog will orient toward the food pan when the previously reinforced CS is delivered, and a puppy will continue to traverse a runway to a pan of food after it has consumed all it can retain. Since motor responses seem to be insensitive to minor changes in drive state, we have investigated the possibility of using a glandular response (salivation) for the assessment of small changes in satiation.

The effect of satiation on the value of the conditioned salivary response has been investigated in Russia, but the authors have been unable to find translation of these data (Airapetiants 1955, 1962).

MATERIAL AND METHOD

Apparatus: The experimental procedure was the same as that employed by Pavlov (1927) in salivary conditioning. The animal was placed on a table in an experimental chamber with equipment attached for recording breathing, heart rate, and the magnitude of the salivary response. The saliva was collected by a plastic cup secured over a parotid gland fistula by negative pressure. A tube running from the collecting cup to the recording equipment was filled with liquid so that as saliva was secreted, adding to the pressure in the closed system, drops of liquid were ejected on an electric recorder in an adjoining room. All records were made on an eight channel EEG machine, modified to record the response measures used.

Subjects: Three mature beagle-terrier hybrid dogs, all belonging to the well balanced type, were used in the experiments. The behavior of these animals had been consistent in the laboratory for over a period of 3 years. They have shown no tendency to extreme inhibition, and they have been used in demonstration, as well as in special studies, over this period without difficulty.

Procedure: In the experimental sessions there was a variation in the time interval between the reinforced signals (2—8 minutes), as well as the interval between the CS (tone) and UCS (food). (2—5 minutes, except for test signals of 30 seconds). This plan was followed so that the animal would not become adjusted to the time factors involved. In the usual test a 30 second signal was given in the early part of the period to determine the value of conditioned salivary response for the session. The CS was a 600 Hz pure tone well above the threshold of the animals' sensitivity. The intensity was not measured in db's. The UCS consisted of a ball of food weighing 85 grams. The balls were made by soaking commercial dry food (Jim Dandy) with an equal amount of water. It was then pressed into a rounded shape by a tube and plunger, and rolled in dry powdered food to eliminate excessive moisture. The tests were given at the regular feeding time

in the afternoon. From 12 to 15 food balls were enough to satiate the animals. In the present series instead of giving one test signal per day, three were given each session, at the beginning, the middle and the end of the series, in order to assess the change in value of the conditioned salivary response. In a few cases 4 and 5 test signals were presented during the session. The number of test signals applied to the animals were as follows: No. 282, male, 3 test signals per day for 14 sessions, and 4 tests per day for 3 sessions; No. 366, male, 3 tests per day for 17 sessions, 4 tests for 4 sessions, and 5 tests for 3 sessions; No. 348, male, 3 tests per day for 24 sessions, and 4 tests for 3 sessions. All other signals for each session were of short duration and of varied time intervals, from 2 to 5 seconds, and all were reinforced with food.

In the case of animal No. 366, 4 tests were made after injection of food directly into the stomach to determine the effect of a full stomach, in the absence of consummatory behavior, on the value of the conditioned salivary response. One test was made after the dog consumed all the food it wanted just before being placed in the laboratory for the regular daily session.

RESULTS

The gradual decrease in value of the conditioned salivary response as the session continues is indicated in Figs 1 through 5. One peculiarity in the data of No. 366 and No. 282 was a lessening of the rate of decrease toward the end of the session in the case of 4 and 5 tests per day. Table 1 indicates the average value of the first test each session, as well as the standard deviation, on each dog, to emphasize the consistency of the response of dogs of this type. Fig. 5 shows the effect of pre-feeding on the value of the dog No. 366. The highest value obtained after pre-loading, on the first test signal, was 8. This is rather low from the value of 17 drops for the first test period on the 24 tests without pre-loading. Apparently stomach loading had an immediate inhibitory effect on the conditioned salivary response. In 3 of the tests after pre-loading, the animal ate 5 balls of food (85 grams each), while on the fourth test, only 4 were consumed. In all of these tests the salivary response was extremely weak, or zero. Inhibition of the salivary response precedes inhibition of the motor response. This inhibition may be based on feedback from the stretch receptors in the stomach, as suggested by T o w b i n (1949). If the animal was given a pan of food in the kennel before the test, there was immediate inhibition of the conditioned salivary response and also of eating. This would indicate that eating plus a full stomach has a stronger inhibitory effect than a full stomach alone. Thus all the factors involved in consummatory behavior (taste, smell, chewing, swallowing, etc.) could feedback to the satiation center of the hypothalamus and add to the feedback from the stretch receptors in the stomach.

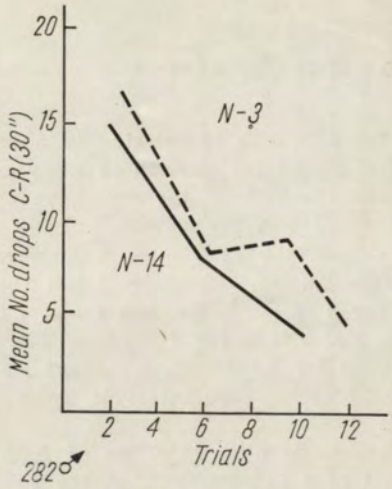

Fig. 1. Average value of the conditioned salivary response to 3 test signals per session in animal No. 282 (N-14), and for 4 tests per session (N-5) as satiation develops

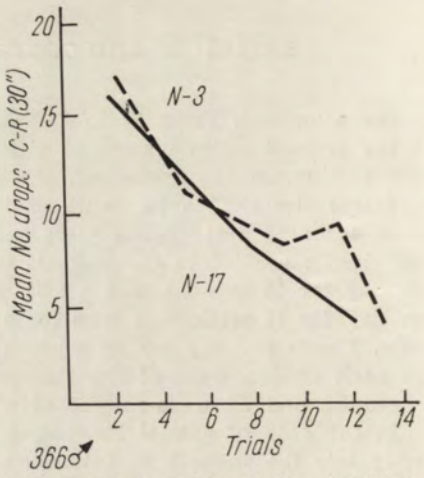

Fig. 2. Average value of the conditioned salivary response to 3 test signals per session in animal No. 366, (N-17), and 5 tests per session (N-3) as satiation develops

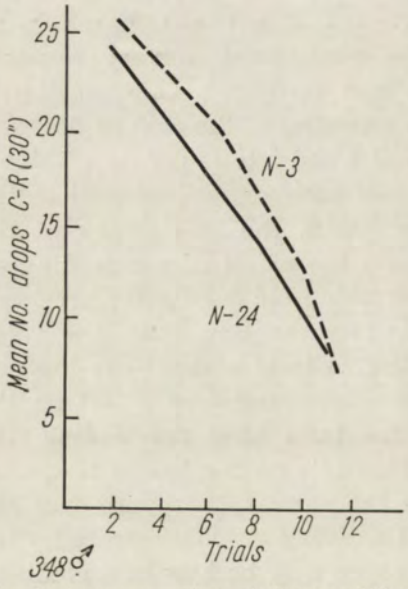

Fig. 3. Average value of the conditioned salivary response to 3 tests per session in animal No. 348, (N-24), and 4 tests per session (N-3) as satiation develops

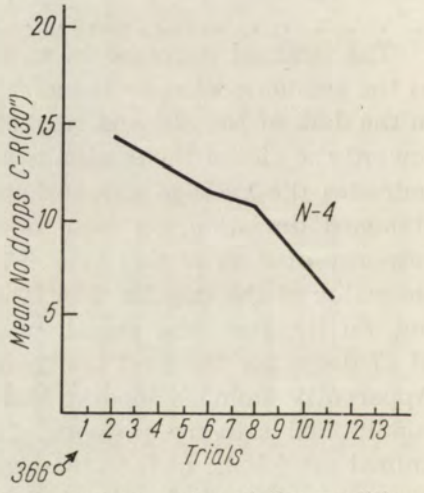

Fig. 4. Average value of the conditioned salivary response to 4 tests per session in animal No. 366, (N-4)

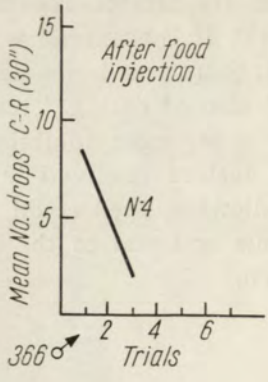

Fig. 5. Average value of the conditioned salivary response to 2 signals immediately after food injection. (N-4) animal No. 366

In an analysis of the effect of various degrees of deprivation on food intake, or learning, or the effect of a drug on food intake, it seems that a salivary response would give a finer measure than any form of motor response that may be used. As the satiation develops, the animal becomes less eager for food, but is not observed until the stomach is about full. By the use of the salivary response, however, decrease in the hun-

Table 1

The average and standard deviations of the conditioned salivary responses to the 3 test signals as satiation occurs

Dog No. 366, 17 tests			
Test	1	2	3
X	16.0	8.3	4.1
SD	2.81	2.24	3.86
The range of the value of the UCR : 48—52 drops			
Dog No. 282, 14 tests			
Test	1	2	3
X	15.5	8.2	4.1
SD	2.15	1.29	1.29
The range of value of UCR : 72—79 drops			
Dog No. 348, 24 tests			
Test	1	2	3
X	24.25	14.0	8.0
SD	2.09	1.87	1.74
The range of value of UCR : 81—90 drops			

ger drive may be indicated early in the series and long before it is indicated by the motor response. It would thus seem that in studies on the effect of various types of food, nature of intake, or physiological changes on hunger and satiation, the use of the conditioned salivary response would be practical and significant.

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PROTECTION OF THE FEAR-ELICITING CAPACITY OF A STIMULUS FROM EXTINCTION[1]

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(*Received December 1, 1965*)

Instrumental avoidance responses have often been very difficult to extinguish by the standard method of omitting the aversive UCS on all trials (Solomon, et al. 1953). Why should this be the case? Konorski (1948) and more recently Sołtysik (1960, 1963) have suggested that: (1) The feedback from the avoidance response, by virtue of being paired with the termination of an aversive stimulus, becomes a conditioned inhibitor (CI) of the classically conditioned fear reaction. (2) When a fear-eliciting stimulus is paired with such a CI (response feedback) without reinforcement, the extinction of the fear reaction is retarded, i.e. the CR is protected from extinction. Because the conditioned fear reaction provides the motivational basis for the avoidance response, extinction of the latter is also retarded.

Sołtysik and Kowalska (1960) demonstrated that a lever press avoidance response was followed by cardiodeceleration, or at least a decrease in the rate of cardioacceleration produced by the CS. If cardioacceleration is assumed to represent the occurrence of the classically conditioned fear reaction, such a result supports proposition (1) above. Furthermore, Rescorla and LoLordo (1965) have demonstrated

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[2] National Science Foundation Pre-doctoral Fellows.

that a stimulus which bears the same formal relation to shock as does feedback from an avoidance response acquires the capacity to reduce the rate of unsignalled (Sidman) avoidance behavior, presumably by becoming a conditioned inhibitor of fear.

Several experiments from the realm of appetitive behavior support a more general form of proposition (2) above, namely, that the addition of a CI to a CS will retard extinction of the conditioned reaction to that CS, relative to a control condition in which the CS is presented alone during extinction. Chorążyna (1962) established alimentary conditioned stimuli and conditioned inhibitors for both salivary and instrumental paw-placing responses in dogs. She then determined that, once the CI-CS combination had been differentiated from the CS alone, unreinforced CI-CS pairings did not contribute to the subsequent extintion of the conditioned responses to the CS alone. Sołtysik (1960), primarily interested in extinction in the avoidance training paradigm, argued that Chorążyna's experiment was not a good model of the stimulus relations in the typical avoidance training experiment, where the CI (feedback from the avoidance response) *follows* the CS. He constructed a better "alimentary model" of the avoidance reflex. After a CS and a CI had been established, a dog received 120 extinction trials on which the CS was presented for 3 sec., and was then accompanied by the CI for 10 sec. This procedure did not produce extinction of the salivary CR to the CS presented alone. Although these two experiments suggest that a properly applied CI may retard the extinction of a CR, they can provide no more than tentative support for propositions concerning the mechanisms of fear conditioning and avoidance learning.

The present experiment compares the relative efficacy of the standard extinction procedure and the "protection" procedure within the fear conditioning paradigm. The fear-eliciting capacities of two CSs are compared after one has been repeatedly presented alone in extinction, while the other has been repeatedly presented but always followed during extinction by a conditioned inhibitor of fear. Our measure of the magnitude of the fear reaction elicited by a stimulus is the increase in the rate of avoidance on an unsignalled (Sidman) avoidance schedule which the stimulus produces. Rescorla and LoLordo (1965) have shown that this measure is sensitive to differences in the level of fear.

The stimulus relations programmed in the present experiment are a model of those which occur in the trace avoidance reflex: In acquisition, a CS comes on for a brief period, after which there is a silence period of variable duration. Then either a shock occurs, or another signal (CI) occurs instead of shock. This mimics the stimulus relations in the

trace avoidance procedure in which response feedback is viewed as a CI. In such a procedure, the CS occurs and is followed either by a shock or by a response (and associated feedback). The two extinction procedures employed here correspond to (1) a standard extinction procedure in which the avoidance response is occuring consistently (CS followed by CI) and (2) an extinction procedure in which the avoidance response never occurs (CS presented alone), e.g. a response prevention procedure. Consequently this experiment enables us to ask whether the occurrence of inhibitory feedback during the extinction of an avoidance response, by preventing the extinction of conditioned fear, contributes to the great resistance to extinction typically shown by the avoidance response.

METHOD

Subjects and Apparatus: Subjects were ten mongrel dogs maintained on *ad libitum* food and water and housed individually. They were given short exercise periods before and after the daily 1-hr. sessions.

The apparatus was a two-compartment shuttlebox for dogs described in detail by Solomon and Wynne (1953). The two compartments were separated by a barrier of adjustable height and by a drop gate which, when lowered, prevented S from crossing from one compartment into the other. The floor was composed of stainless-steel grids which could be electrified through a scrambler. Speakers mounted above the hardware-cloth ceiling provided a continuous white noise background and permitted the presentation of tonal stimuli. Two fifty-watt light bulbs mounted above the hardware-cloth ceiling provided another source of stimuli. The general noise level in the box, with the white noise and ventilating fans on, was about 80 db. *re* .0002 dynes/sq. cm.; the tones added 10 db. to this level. All control and recording equipment were isolated from the animal chamber.

Procedure: The experimental procedure was similar to that described by Rescorla and LoLordo (1965). First, a baseline avoidance response was established. The dogs were trained to jump a barrier dividing the shuttlebox to avoid electric shock. An unsignalled avoidance schedule similar to that described by Sidman (1953) was used. If S did not jump the barrier, a shock was delivered to the grid every 10 sec.; each jump postponed the next shock for 30 sec. Thus the shock-shock interval was 10 sec. and the response-shock interval was 30 sec. Shock duration was 0.25 sec.; the intensity was 6 ma. on day 1, and 8 ma. thereafter. On day 1 the barrier height was 23 cm.; on all subsequent days it was 38 cm.

The S received three days of avoidance training before receiving Pavlovian conditioning. Beginning with the fourth experimental day, S was confined to one half of the shuttlebox and given five, 1-hr. Pavlovian conditioning sessions on alternate days. On the intervening days S continued to receive 1-hr. sessions of avoidance training.

During the Pavlovian conditioning sessions, S received four kinds of conditioning trials: (a) CS_1 was presented for 5 sec.; either 2, 5, or 8 sec. following the termination of CS_1, a 5-sec. 3-ma. shock was presented; (b) CS_1 was presented for 5 sec.; either 2, 5, or 8 sec. following its termination, a CI was presented for 5 sec. but no shock was presented; (c) CS_2, a tone of different frequency, replaced CS_1 (in other respects the trial was like (a) above): (d) CS_2 replaced CS_1 in a trial otherwise like (b) above. Thus the stimulus relations constitute a model of the trace avoidance reflex, in which each CS followed, after a variable interval, by either shock or a conditioned inhibitor.

On each conditioning day, nine of each of the four kinds of trial were presented in random order. The intertial intervals were 1, 1.5, and 2 min., with a mean of 1.5 min. For five Ss, CS_1 was a 400 cps tone and CS_2 a 1,200 cps tone; for the other five Ss the tones were interchanged. Turning off the lights in the shuttlebox served as the CI.

After each animal had received eight avoidance sessions and five Pavlovian conditioning sessions, extinction of the fear reactions to the two CSs was begun. Extinction sessions were the same as the Pavlovian conditioning sessions except that for one CS each of its presentations was followed after a variable interval by the CI; the other CS was followed by neither shock nor the CI. Thus, during extinction the "protected" CS was always followed by the CI; the "unprotected" CS was always presented alone, as in standard Pavlovian extinction procedures. Eighteen trials with each of the two CSs were given in each extinction session.

Two extinction sessions were given, each followed by an avoidance training day. During the avoidance session which followed the second extinction day, the two CSs were presented while the animal performed on the avoidance schedule. Each tone was presented 40 times for 5 sec. each time; the mean intertone interval was 35 sec. A second series of two extinction sessions followed by avoidance sessions (on the second of which the CSs were again presented) followed the initial test session. All sessions were 1 hr. long.

If we assume that a CS followed by a CI is protected from the effects of the extinction procedure, then there should be little extinction of its capacity to elicit fear, and this CS should produce a greater increase in the rate of avoidance than should an "unprotected" CS.

RESULTS

The baseline avoidance response was acquired rapidly and remained sufficiently stable that only a few shocks were administered during test sessions. However, two animals became ill during the experiment and had to be discarded.

Fig.1 illustrates the group mean rate of avoidance responding during the first test session as a function of successive 5-sec. intervals prior to, during, and after presentation of a CS. It is evident that the effects of the two stimuli were almost identical. An analysis of variance indicated no effect of the protection procedure ($F < 1$). Both stimuli produced an increase in rate at their onset and a further increase with their termina-

tion. Combining the effects of the two stimuli, the rate of responding was higher during the 10 sec. following CS onset than during the period prior to CS onset (Wilcoxon $T = 4$, $p < .05$).

The results of the second test session are illustrated in Fig. 2. Although the general level of responding is reduced, the results of the second test session were highly similar to those of the first session. Again the two stimuli did not differ in their effects ($F < 1$). Both stimuli produ-

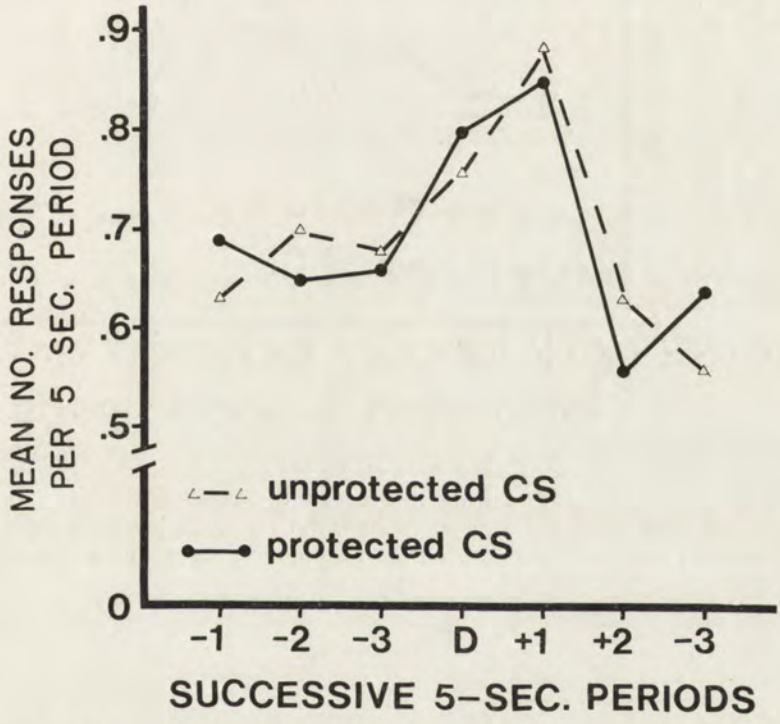

Fig. 1. Mean number of avoidance responses per 5 sec. period for successive 5 sec. periods during the first test session. Periods prior to CS onset are given a negative sign, the 5-sec. CS period is labelled "D", and periods following CS termination are given a positive sign

ced increases in the rate of avoidance, although the increases were limited to the 5-sec. period following CS termination. The response rate during the 5—10 sec. period following CS onset was reliably greater than the rate prior to CS onset (Wilcoxon $T = 1$, $p < .02$). Thus while repeated extinction trials with the stimuli weakened the effectiveness of CS onset, the effects of CS termination indicate that the stimuli retained some fearelicíting properties.

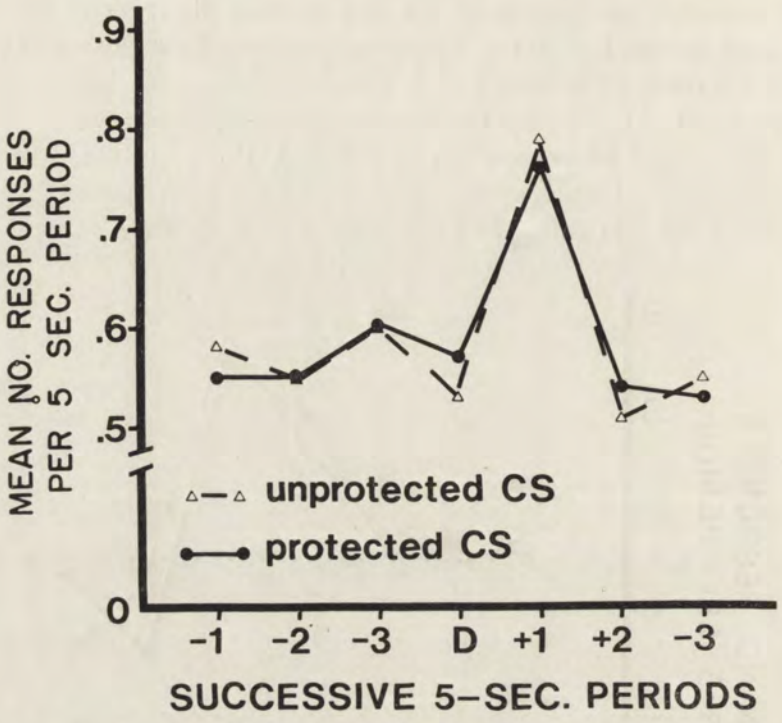

Fig. 2 Mean number of avoidance responses per 5 sec. period for successive 5 sec. periods during the second test session. Periods prior to CS onset are given a negative sign, the 5-sec. CS period is labelled "D", and periods following CS termination are given a positive sign

DISCUSSION

The protection and standard extinction procedures produced similar extinction of a conditioned fear response. In every case where the fear reaction to a previously unprotected CS had extinguished, so had the response to a previously protected CS. Our results provide no evidence that the sequence of CS and CI which is present in avoidance extinction should especially retard extinction of the fear reaction.

However, there are several reasons why the present "model" of avoidance extinction might fail to detect a protection effect which is operative in actual avoidance extinction. First, there is no direct evidence that turning off the lights in the shuttlebox actually served as a conditioned inhibitor. However, an auditory stimulus which was placed in the same temporal relation to a fear-eliciting stimulus in an earlier experiment did produce large decrements in the rate of avoidance

responding (Rescorla and LoLordo 1965). Furthermore, observation of the animals during Pavlovian conditioning indicated that they "relaxed" when the lights were turned off. Second, there may have been generalization of extinction between the two CSs, both of which were pure tones. Nevertheless, the effects of the protection procedure should not have been eliminated completely. Finally, it is possible that further extinction would have revealed differences between the effects of the two stimuli. It was not possible to test this in the present experiment since by the second test session the dogs had received so many presentations of the previously protected CS without the CI that one would not expect it to be very different from the unprotected CS.

It may be that the precise time of presentation of the CI is crucial. A CI which is presented only after the fear reaction elicited by the CS has begun may not protect that CS from extinction. In this context it should be remembered that the conditioned inhibitors used by Chorążyna and Sołtysik typically prevented the conditioned responses from occurring. On the other hand, in the present experiment, as in the avoidance learning situation, the fear reaction presumably is elicited before the conditioned inhibitor occurs, and may extinguish despite presentation of the CI.

Furthermore, in the avoidance training paradigm S has control over the occurrence of the CI, so that it may be presented just as the level of fear reaches a threshold value; the avoidance situation would then be designed to allow the minimum elicitation of fear before the CI (response feedback) prevents further fear reaction. In contrast, in the present experiment the CI was presented after the CS without regard to the level of fear which that CS elicited. It seems plausible that the degree to which fear-elicitation takes place is the important variable in determining extinction of the fear reaction to a CS. The present "protection" procedure may have been ineffective in retarding extinction because it allowed the occurrence of too much fear before CI onset, while the CI in the typical avoidance procedure would necessarily limit more severely the degree of fear aroused.

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DIFFERENTIATION LEARNING IN RABBITS WITH LESIONS OF THE FRONTAL CORTEX OR THE HYPOTHALAMUS

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This research was prompted by a series of earlier studies but, primarily, it was an attempt to extend the experiment on the fronto-hypothalamic control over food-reinforced conditioned-reflex (CR) performance and differential inhibiton in rabbits (B a l i ń s k a et al. 1966). A summary of the results of previous investigations will provide the necessary background for the current research. It has been found that both rabbits with lesions of the medial hypothalamus who are in the dynamic phase of hyperphagia and rabbits with lesions of the lateral hypothalamus who have recovered their eating behavior and conditioned-reflex performance show (a) an augmented CR performance, (b) an impairment or disinhibition of the preoperatively established differential CR, and (c) a striking food-directed activity (B a l i ń s k a et al. 1961, B a l i ń s k a 1963a, b, B a l i ń s k a et al. 1966). It has furthermore been found that these alterations in behavior patterns in rabbits correspond to those obtained after ablation of the frontal area of the medial aspect of the hemisphere in rabbits (B a l i ń s k a et a. 1966) and the upper portion of the medial prefrontal cortex in dogs (B r u t k o w s k i and D ą b r o w s k a 1963, 1966, B r u t k o w s k i 1964, 1965). A reduced reactivity followed by an increased reactivity and disinhibition of frontal operatees have been made the basis of the conclusion that the behavioral changes that occur after medial frontal lesions in subprimates reflect changes resulting from lesions of the lateral and medial hypothalamus.

In all of the experiments cited above, the retention was studied. The present research was undertaken to determine whether differentiation learning would be impaired following lesions placed within the frontal cortex or the hypothalamus. Rabbits were used for this purpose.

MATERIAL AND METHOD

Subjects. Experiments were carried out on 20 naive male and female rabbits, about 2 years old, and weighing 2.0—2.5 kg. All rabbits were offered ad-lib food, consisting of grains of oats and purée-type potatoes, in individual home cages after the time of testing session in the testing cage. Five equal groups of animals were randomly assigned: (a) a medial hypothalamic lesion (HM) group, (b) a lateral hypothalamic (HL) lesion group, (c) a medial prefrontal-premotor cortex (FM) lesion group, (d) a dorsolateral prefrontal-premotor cortex (FL) lesion group, and (e) a normal control (C) group.

Surgery and histology. Aseptic surgical technique was used. A description of the procedure for electrocoagulation of the hypothalamic regions and removal of the portions of the frontal cortex can be found in Bališka et al. (1966). The lesions were bilateral and of the type reported previously (Bališka et al. 1966).

Procedure. In groups HM, FM and FL, training was begun 11 days after surgery. In group HL, training was begun ca. 3 weeks after surgery, at which time the HL group animals accepted the food offered in their home cages. In an attempt to keep the lateral hypothalamic rabbits alive, subcutaneous injection of 100 ml. of 4 per cent glucose was administered. Glucose injection was discontinued when voluntary acceptance of food recovered. All training was conducted in a testing cage which was described elsewhere (Bališka et al. 1966). The training consisted of teaching the animals to place their right forelimb on the food box to the presentation of an acoustic conditioned stimulus (CS+), this response being reinforced with a piece of carrot, and to withhold this response to the presentation of a differential acoustic CS (CS−), which was associated with no reinforcement. Five positive (CS+) and five inhibitory (CS−) stimuli separated by 20 sec. — intervals were given daily. They occurred randomly in a successive order. The training consisted of 5 stages: (1) habituation to the experimental conditions, (2) teaching the animals to eat the reinforcement food presented in successive trials, (3) establishment of the placing response, (4) teaching the animals to perform the placing response to the presentation of CS+, and (5) proper differentiation training. The animals were trained to the criterion of 94 to 96 correct responses (failure of response) in 100 inhibitory trials (100 presentations of CS−). If this criterion was not reached within 125 days, the training was terminated. For a more detailed description of the procedure see Bališka et al. (1966).

RESULTS

Table I presents the data in terms of the number of days necessary for the individual animals to be proficient in the initial four stages of the training. Tables II and III permit comparison of error scores on po-

sitive and inhibitory trials and the number of intertrial interval responses, respectively, divided into 10-day blocks, for the individual animals.

HM group. The animals of this group were hyperphagic and ate the food voraciously. The first two stages of training took 1 day each. The placing response was acquired very promptly, and after the acquisition it was performed in a violent manner both on the positive trial and dur-

Table I

Acquisition of the CR

Rabbit No.	Stage 1	Stage 2	Stage 3	Stage 4
	Days			
	Medial hypothalamic lesions, group HM			
R— 1	1	1	5	10
R— 2	1	1	9	10
R— 3	1	1	2	5
R— 4	1	1	3	3
	Medial frontal lesions, group FM			
R— 9	4	117	119	121
R—10	29	38	41	46
R—11	27	31	41	46
R—12	31	38	45	50
	Dorsolaterel frontal lesions, group FL			
R—13	1	2	10	17
R—14	1	2	4	10
R—15	1	2	12	25
R—16	1	2	6	13
	Normal control, group C			
R—17	1	2	5	9
R—18	1	2	6	12
R—19	1	3	9	13
R—20	1	3	7	11

Stage 1, habituation; Stage 2, teaching the animals to eat the reinforcement food; Stage 3, establishment of the CR; Stage 4, teaching the animals to perform the CR to the onset of CS.

ing the intertrial interval. In contrast to the unoperated animals who learned to perform the placing response on the positive trial within 9 to 13 days, the HM animals required 3 to 10 days for learning. On the other hand, differentiation took very long in the HM animals. Moreover, it was associated with and enhanced level of emotionality, manifested by stamping feet, biting, and tearing at the food cup during the presentation of CS—. Eventually, the HM animals learned to refrain from responding on inhibitory trials.

HL group. During the first days after surgery all HL animals exhibited aphagia, a decreased motility and poor reactivity to external cues. Due to glucose injections the animals regained eating activity within 2 to 10 days postoperatively but they ate less than before. When the ability to eat returned to the preoperative level the animals became more lively and their general behavior was similar to that which had been seen prior to the ablation. Postoperative training was started 3 weeks following surgery and lasted 125 days. It was found that none of the HL animals adapted to the experimental situation. Despite the fact that they behaved normally in their home cages, they were apathetic and immobile in the testing cage throughout the entire period of the postoperative training, and were unable to learn the CR.

Table II

Errors on positive and inhibitory trials during differentiation learning

Rabbit No.	Positive trials					Inhibitory trials				
	10-day blocks									
	1	2	3	4	5	1	2	3	4	5
	Medial hypothalamic lesions, group HM									
R— 1	0	0	0	0		50	21	16	2	
R— 2	0	0	0	0		35	23	8	1	
R— 3	2	1	0	0		15	7	12		
R— 4	0	0	0			33	12	5		
	Medial frontal lesions, group FM									
R— 9	12	10	3	1	0	15	8	6	6	2
R—10	5	5	1	0	0	10	8	10	4	1
R—11	1	2	0	0		10	12	5	1	
R—12	0	0	0	0	0	25	38	30	23	5
	Dorsolateral frontal lesions, group FL									
R—13	0	0	0			23	8	0		
R—14	0	0	0	0		19	12	9	1	
R—15	4	2	0			16	8	2		
R—16	0	3	0			16	9	5		
	Normal control, group C									
R—17	0	0	0			28	12	0		
R—18	0	0	0	0		30	10	7		
R—19	0	0	0			28	10	8	1	
R—20	3	2	0			9	7	1		

FM group. Initially, these animals showed hypophagia, decreased motility, apathy and somnolence. However, they rapidly recovered their food intake and became as activite as before. Nevertheless, they dis-

played a considerable reluctance to adapt to the experimental situation for a period of 4 to 31 days and learned to associate the presentation of the reinforcement food with eating this food abnormally long. The acquisition of the positive CR did not encounted any major difficulties

Table III

Number of intertrial interval response during differentiation learning

Rabbit No.	10-day blocks				
	1	2	3	4	5
	Medial hypothalamic lesions, group HM				
R— 1	132	70	31	10	
R— 2	76	30	1	1	
R— 3	20	11	7	3	
R— 4	52	11	5		
	Medial frontal lesions, group FM				
R— 9	18	4	3	7	0
R—10	16	17	12	9	0
R—11	32	13	7	0	
R—12	54	69	29	17	2
	Dorsolateral frontal lesions, group FL				
R—13	33	0	1		
R—14	6	3	4	3	
R—15	26	15	1		
R—16	32	7	3		
	Normal control, group C				
R—17	22	7	1		
R—18	49	20	2		
R—19	34	6	0	0	
R—20	60	3	0		

but in the initial period after CR acquisition the animals occasionally failed to respond when CR+ was given. Since the HM animals did not suffer from any motor peripheral deficit, it was obvious that the occasional failure of response on positive trials was due to the incapacity to execute consistently the instrumental CR to the CS. After the recovery of food intake and the abolition of the apathetic state, the FM animals showed an increased food-directed activity. This and the high rate of responding during the intertrial interval which developed after the acquisition of the CR clearly interfered with the differentiation learning. Eventually, the animals learned to respond consistently on positive trials and to inhibit the placing response on inhibitory trials.

FL group. The animals of this group did not differ from the animals of the normal control group, except that they learned longer than normally to respond to the presentation of the CS+.

DISCUSSION

The data demonstrate the inability of rabbits with lesions of the lateral hypothalamus to acquire a response reinforced with food when these animals recovered their food intake due to subcutaneous glucose injections. One may infer that this was due to the conspicuos decrease of the drive for food, resulting from a damage to the hypothalamic "feeding center" (Anand and Brobeck 1951). Previous results obtained on rabbits, who were fed basically during training of an instrumental CR, demonstrated that lesions of the lateral hypothalamus produced a transient impairment of the preoperatively trained CR (Balińska 1963a). Balińska et al. (1966) recently showed that such lesions made in 5 rabbits disturbed the retention of the CR activity in 2 animals, permanently, and in 3 animals, temporarily; the animals, who had recovered their CR performance, later exhibited an enduring impairment of differential inhibition in association with excessive food-directed activity.

Neither lesions of the frontal cortex of the dorsolateral surface of the hemisphere nor lesions of the medial hypothalamus were found to interfere with CR learning. The demonstration that animals with lesions of the medial hypothalamus were facilitated in learning and showed a high rate of intertrial interval responding as compared with normal animals might be related to the postoperative increase in the drive for food, caused by a damage to the hypothalamic "satiety center" (Anand and Brobeck 1951). Interference with differentiation learning in animals with lesions of the medial hypothalamus might reflect the abolition of the inhibitory influence exerted on the CR performance on nonreinforced trials by the hypothalamic "satiety center" and the facilitation of the functional connections between the "feeding center" and the "CR center" which has been postulated by Wyrwicka (1952). It has previously been shown (Balińska et al. 1961, Balińska 1963a, Balińska et al. 1966) that lesions of the medial hypothalamus profoundly distrub the retention of a differentiation task.

Among rabbits with lesions of the medial frontal cortex, a marked retardation of the CR acquisition occurred. When the FM animals learned to perform the CR, they often made it during the intertrial interval and then also on some inhibitory trials, although they did not make it on some positive trials. In the later postoperative period, they appeared

to perform well on positive trials and poorly on inhibitory trials, but reached a final level of performance equal to that of the other groups. Following the initial period of apathy, the FM animals displayed an excess in food-directed activity, a symptom of direct interference with differentiation learning. As noted in previous studies (Brutkowski and Wojtczak-Jaroszowa 1963, Stępień et al. 1963, Brutkowski and Dąbrowska 1966, Balińska et al. 1966), lesions of the medial cortex in dogs and rabbits may impair temporarily the avoidance response or the instrumental response reinforced with food. When the CR is regained it occurs excessively during intertrial intervals but, at the early stages of the recovery, it often fails to occur on some positive trials. It is of interest that despite this inconsitent occurrence on positive trials the CR is performed on some inhibitory trials. In the later postoperative period, a consistent responding on positive trials returns. Moreover, the CR occurs in the majority of inhibitory trials and this impairment of differential inhibition is of long duration. Brutkowski (1965, 1966) has suggested that strong emotional activity which occurs in animals with lesions of the medial frontal cortex accounts for the elimination of the CR performance or the retardation of the CR acquisition seen in the initial postoperative period; it also accounts for the interference with differentiation learning and performance seen in the later postoperative period.

The finding that damage to the medial frontal cortex produces a retardation of CR acquisition followed by a retardation of differentiation learning suggests that behavioral changes which are associated with the medial frontal cortex ablation reflect, at first, changes arising from lesions of the lateral hypothalamus and, subsequently, changes resulting from lesions of the medial hypothalamus. This agrees with the suggestion from the earlier work concerned with the retention of a differentiation habit following lesions of the frontal cortex (Balińska et al. 1966).

SUMMARY

Rabbits with selective lesions of the frontal cortex or the hypothalamus were trained on a differentiation task. Animals with lesions of the medial hypothalamus were facilitated in CR acquisition and retarded in differentiation learning. Animals with lesions of the lateral hypothalamus were not able to learn the CR performance. Animals with lesions of the medial frontal cortex exhibited a marked retardation of CR learning. After the acquisition of CR, these animals showed a retardation

of differentiation learning as compared with normal controls. Animals with lesions of the dorsolateral frontal cortex performed essentially as normal controls. The results conformed to previous work on CR and differential inhibition retention following frontal or hypothalamic lesions.

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EFFECTS OF FRONTAL LOBE LESIONS ON URINARY BLADDER FUNCTION IN DOGS. STUDIES WITH NEW TECHNIQUES OF CYSTOMETRY

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Despite considerable research little is known on the localization and functional organization of the brain structures related to the facilitation, and particularly to the inhibition of micturation (T a n g and R u c h 1954, 1956, T a n g 1955, R u c h 1960, K u r u 1965).

A f o n a s s i e w (1869) presented evidence that a bilateral transection of the cerebral peduncle does not produce a definite impairment of vesical function. G o l t z (1892), R o t h m a n n (1923) and D u s s e r d e B a r e n n e (1937) found that dogs and cats with bilateral extensive lesions of the cortex of both hemispheres empty their bladders in typical posturing. These findings were extended by the demonstration showing that no alterations of vesical responses occur to electrical stimulation of the hypothalamus in animals with unilateral removals of the cerebral hemispheres (L i c h t e n s t e r n 1912) or bilateral removals of the frontal lobes (M a g o u n 1938). However, the majority of studies has pointed to the essential participation of the forebrain in the regulatory mechanism of micturition. B a r r i n g t o n (1914, 1921, 1925, 1928) reported that the abolition of reflex micturition occurred only after a subcollicular decerebration, that is, after a transection of the brain-stem through the posterior parts of the inferior colliculi dorsally and the middle of the pons ventrally. Since after severance of the brain-stem at the intercollicular level the reflex micturition occurred normally, the

detrusor contraction being powerful and sustained, Barrington concluded that the rostral pons contained a micturition facilitatory area (Barrington's anterior pontine detrusor nucleus) and that all reflexes essential to the performance of reflex micturition should arise caudally to the plane passing between the optic and acoustic colliculi dorsally and through the origins of the third nerves ventrally.

Facilitatory responses such as (a) a contraction of the vesical muscle with a rise of the intravesical pressure, (b) an increase in the peristaltic activity and (c) bladder emptying consisting of complete or dribbing urination, and inhibitory responses such as (a) relaxation of the detrusor, (b) a diminution of the peristaltic activity and (c) the suspension of micturition, have been elicited by the stimulation of the following brain regions: (1) the sensorimotor cortex and frontal lobe (Francois-Franck 1887, Spiegel and Hunsicker 1936), particularly (a) the dorsolateral aspect of the anterior sigmoid gyrus (Bochefontaine 1876), (b) the dorsolateral aspect of the posterior sigmoid gyrus (Frankl-Hochwart and Fröhlich 1904, Spiegel and Mac Pherson 1925), (c) the dorsolateral aspect of both the anterior and posterior sigmoid gyri (Langworthy and Kolb 1935, Gjone and Setekleiv 1963), (d) the medial aspect of both the anterior and posterior sigmoid gyri (Bechterew and Mislawsky 1888, Gjone and Setekleiv 1963), (e) the secondary sensorimotor cortex, that is, both the anterior ectosylvian and anterior sylvian gyri (Gjone and Setekleiv 1963), and (f) the motor area corresponding to the lower extremities and the trunk (Gjone and Setekleiv 1963), (2) the limbic system, particularly (a) both the anterior and posterior parts of the cingulate gyrus (Kremer 1947, Henneman 1948, Ström and Unväs 1950, Matsumoto 1957, Mukai 1959, Yokoyama et al. 1960, Ingersoll et al. 1961, Gjone and Setekleiv 1963), (b) the piriform cortex (Henneman 1948, Smith 1949, Kaada 1951, Koikegami et al. 1957), (c) the anterior olfactory lobe (Ström and Unväs 1950), (d) the hippocampus (Koikegami et al. 1957, Porter and Bors 1962), (e) the orbital cortex (Okinaka 1955, Koikegami et al. 1957, Gjone and Setekleiv 1963), and (f) the amygdaloid complex (Kaada 1951, Gastaut et al. 1951, MacLean and Delgado 1953, Kaada et al. 1954, Anand and Dua 1956a, b, Shealy and Peele 1957, Koikegami et al. 1957, Mochida 1957a, b, Ingersoll et al. 1961, Porter and Bors 1962), (3) the basal ganglia, that is, the putamen, globus pallidus and caudate nucleus (Ström and Unväs 1950, Ingersoll et al. 1961); and (4) the septal areas (Schaltenbrand and Cobb 1930, Ranson et al. 1935, Kabat et al. 1936, Hess 1936, Rioch and Brenner 1938, Hess and Brügger

1943, Gloor 1954, Grossman and Wang 1955, Nishiyama 1959, Yokoyama et al. 1960).

It has been found that during brain stimulation the facilitatory responses occur more often than the inhibitory responses. On the other hand, the inhibition of vesical function has fraquently been observed in ablation and transection experiments. Thus it has been shown that (a) the increase in the intravesical pressure to the stimulation of the hypothalamus is considerably greater when both cerebral hemispheres are removed (Lichtenstern 1912), (b) the removal of the forebrain in front of the mid-thalamic region results in a slight decrease of bladder capacity (Langworthy and Kolb 1933), (c) after transhypothalamic decerebration in a plane rostral to the superior colliculi dorsally and just rostral to the mammillary bodies ventrally the bladder empties reflexly at a markedly smaller volume than that which is required to produce micturition in normal cats (Tang and Ruch 1954, 1956, Tang 1955), (d) a transection of the brain-stem through the optic colliculi results in a marked decrease of the micturition threshold (to one half of the preoperative value), in a fall of the intravesical pressure and in diminution of rhythmic activity of the vesical muscle; the emptying contraction is strong and precipituous and not preceded by contraction waves (Langworthy and Kolb 1933), (e) a supracollicular transection of the brain-stem rostral to the optic colliculi dorsally and anterior to the pons ventrally is followed by a slight decrease of vesical reflex capacity with no signs of intravesical pressure changes (Tang and Ruch 1954, 1956, Tang 1955), (f) a decerebration at the intercollicular level dorsally and the anterior border of the pons ventrally is (Langworthy and Kolb 1933) or is not associated with hypotonicity of the detrusor (Tang and Ruch 1954, 1956, Tang 1955) and causes a more marked reduction of the micturition threshold than a supracollicular brain-stem transection; the micturition is strong and precipituous, (g) after a unilateral lesion of the motor cortex a decrease of the micturition threshold occurs; a further decrease of bladder capacity follows the lesion of the motor cortex on the other side of the brain or the transection of the brain-stem below the superior colliculi; the height of the intravesical pressure and the strength of emptying contraction remain unaltered (Langworthy and Kolb 1933), (h) after the removal of the entire frontal pole in one slice, including the proreal gyrus and the underlying olfactory cortex without opening the lateral ventricles or destroying the head of the caudate nucleus there is a definite decrease of the micturition thresshold; a further decrease follows a similar procedure on the other side; a decerebration at a later date produces no more alterations; bladder capacity reexamined

after a few months though slightly increased is still reduced to a half of the preoperative value; after a bilateral removal of the frontal lobes some micturition habits are lost (Langworthy and Kolb 1933), (i) Smith and Feldmahn (1954) established that a combined lesion of the hindleg movements area in the macaque results in a hypertonicity of the detrusor and alterations of micturition patterns, consisting of frequent urinations of small amounts of urine throughout the 24-hour period (in normal animals, one large and one or few smaller voidings occur during morning hours and then there is a 16—20-hour nonvoiding interval); lesions limited to the medial part of the cortex in the hindleg area produce no alterations of micturition patterns, (j) uni- or bilateral lesions of the pericruciate cortex or the frontal lobes in cats are not followed by any alterations of urination volume (Woolsey and Brooks 1937); variations in the volume of individual urinations are less marked than in normal animals (preoperatively, the daily urinary output is relatively constant while the volume of individual urinations varies considerably from one micturition to the other; daily micturition volume diminishes during estrus and increases significantly post partum), (k) after the removal of the entire neocortex uni- or bilaterally there is a diminution of variations in the volume of individual urinations but the average micturition volume is either unaltered for months or increased; the increase is of short duration and is followed by a longer lasting decrease; a stabilization then occurs which is however somewhat below that obtained preoperatively (Woolsey and Brooks 1937).

Thus there is to-day increasing support for the view that the cerebral cortex exerts some regulatory effect on micturition and the frontal area seems to be critical.

The present paper describes experiments undertaken to study the effects of lesions of the prefrontal and premotor areas on urinary bladder function in the dog, using the extraurethral techniques of cystometry. As has been demonstrated, parts of the medial prefrontal-premotor cortex in subprimates appear to be essential for inhibition of automatic and drive functions (cf. Brutkowski 1964, 1965).

MATERIALS AND METHODS

Six, adult mongrel male dogs, weighing from 17 to 23 kg. were used. After a period of adaptation to experimental conditions a pelvoureterocutaneostomy was carried out which consisted of removing the left kidney and implanting the left renal pelvis with the ureter into the skin. When the healing had been achieved the bladder was catheterized through the implanted ureter by means of plastic catheters (sizes 8 to 12 Charriere) and cystometric studies were performed

(transureteric cystometry, Cieśliński 1964). Since in two dogs the ureteral opening obliterated after operation, the anterior bladder wall was extraperitonized by Sauvage's (1960) technique. Postoperatively, the bladder was punctured and pressure readings were carried out (suprapubic cystometry, Sauvage 1960). During cystometry, the bladder was filled with 50 ml. increments of mild antiseptic solution, the intravesical pressures being recorded by means of a simple water manometer. All cystometric studies were carried out in unanesthetized animals. The technique of performing suprapubic cystometry was somewhat different from the original one. The bladder was filled and intravesical pressures were measured in lying position through an intramuscular needle inserted into its cavity (originally, a catheter was introduced into the bladder suprapubically through a troacar, the cystometry being done in standing position). In each dog, from 6 to 14 bladder readings were done.

After the normal bladder capacity and cystometrograms had been ascertained brain lesions were made (Figs 1 and 2) under intraperitoneal nembutal anesthesia (35 mg/kg of body weight). In dogs Nos. 1—4, the superior portions of the cerebral cortex on the medial aspect of the frontal lobe between the ramification of the cruciate fissure and the frontal pole were removed bilaterally by subpial suction. In dogs Nos. 5 and 6, both the white and gray matters of the prefrontal area in front of the presylvian sulcus laterally and the genual sulsus medially were bilaterally removed (prefrontal lobectomy). Following frontal ablations from 4 to 18 cystometrograms were obtained in each animal.

RESULTS

Cystometric studies in normal dogs. In dogs Nos. 1, 4, 5 and 6, a remarkable similarity in cystometrograms was obtained. Both the vesical capacity which varied from 500 to 600 ml. in three dogs, and was 1000 ml. in one dog, and intravesical pressures showed no greater variations (normotonicity) during successive examinations. In dogs Nos. 5 and 6, the data obtained by both the transureteric and suprapubic techniques of cystometry were wholly similar. In dogs Nos. 2 and 3, only was initially a slight hypertonicity noted; later on, the data were uniform with those obtained in other animals (normotonicity, bladder capacity 500 to 600 ml.).

Cystometric studies after frontal lesions. After the bilateral removal of the *superior parts of the medial frontal cortex* no alterations of either bladder capacity or intravesical pressures occurred in dogs Nos. 1 and 4. The decrease of the vesical capacity which was seen during the first reading on the 2nd postoperative day in dog No. 4 could be attributed to the posttraumatic shock. In dog No. 3, the bladder capacity decreased to the half of the preoperative level in the immediate postoperative period (three bladder readings on days 4, 6 and 8). Thereafter the capacity gradually increased and attained the preoperative level with the 25th day following surgery. Dog No. 2 was very poor throughout the entire postoperative period and died on the 16th day after surgery.

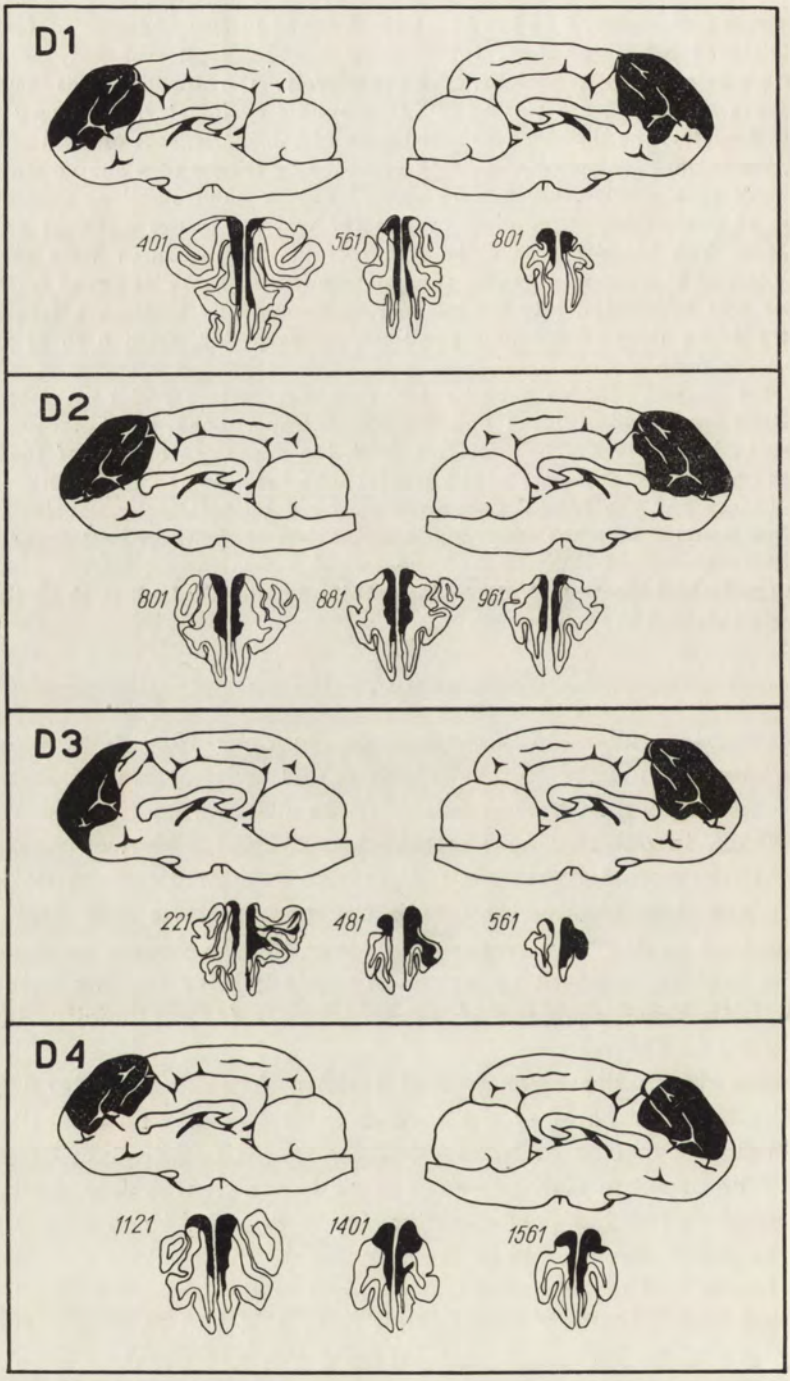

Fig. 1. Reconstructions of the lesions within the medial prefrontal-premotor region and representative cross sections of cerebral hemispheres of the dog

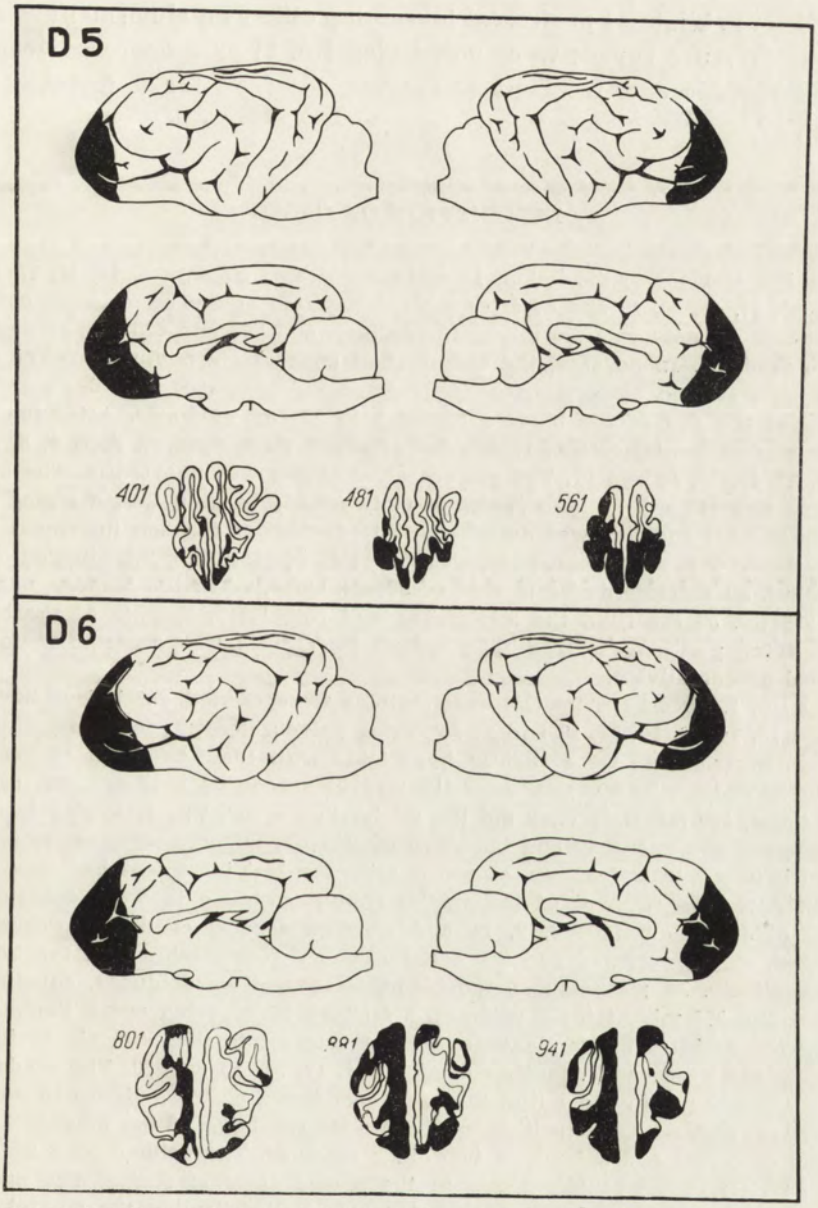

Fig. 2. Reconstructions of the total removal of the prefrontal lobe and representative cross sections of cerebral hemispheres of the dog

All bladder readings in this dog showed a markedly reduced volume at which micturition was seen to occur.

After the bilateral *prefrontal lobectomy* either no abnormalities of the volume-pressure curves were noted (dog No. 5) or a decreased capacity of the bladder and frequent urinations occurred for a fortnight (dog No. 6).

DESCRIPTION OF CASES

Dog No. 1. Prior to the lesion, 14 cystometrograms on days 7, 10, 14, 17, 21, 24, 28, 31, 35, 37, 41, 49, 60 and 67 after the implantation of the ureter were obtained. The vesical capacity was 600 ml. in 12 readings, and 700 and 625 ml., respectively, in two readings. In no case, the examination ended by a micturition. The cystometrograms showed normotonicity. The intravesical pressures ascended slowly, attaining at the end of the bladder filling 7 to 25 cm. of water. After the lesion of the medial frontal cortex, 11 bladder readings were done on days 4, 8, 11, 14, 17, 23, 27, 34, 39, 45 and 51. The postoperative course was uneventful. The bladder capacity was 600 ml. in nine readings. Only in one reading was a strong desire to void present and this was during the introduction of the last increment of the fluid. Once, the vesical capacity was 625 ml. (during the introduction of the last 25 ml. an extreme desire to void occurred). Once, when the bladder was filled with 650 ml. of the fluid the micturition and complete emptying of the bladder in the lying position occurred. The cystometrograms were very similar to those obtained preoperatively.

Dog No. 2. Seven cystometric examinations were carried out before the lesion on days 10, 14, 16, 20, 24, 28 and 34 following pelvoureterocutaneostomy. The 5 initial cystometrograms were slightly hypertonic with the final limb of the curve steeply ascending. The vesical capacity was in successive readings 400, 600 and, three times, 500 ml. Only once did the micturition occur. The following two readings showed normotonicity and bladder capacity 600 ml. (the intravesical pressure ranged between 18 and 22 cm. of water after the last increment had been introduced). After the removal of the medial frontal cortex only were 4 cystometric curves obtained, on days 3, 9, 11 and 13. The general condition progressively worsened, stupor occurred and the dog succombed from cachexy on the 16th day postoperatively. In all readings, the bladder capacity was reduced, equaling 100, 350, 200 and 375 ml. At these volumes, a micturition occurred every time.

Dog No. 3. After the implantation of the ureter into the skin, six cystometric examinations were carried out on days 10, 14, 17, 24, 28 and 30. The bladder capacity was 500 ml. in the initial four readings (once, a strong desire to void was manifested, and, once, micturition with complete emptying of the bladder in lying position occurred) and 600 ml. in two other readings. The volume-pressure curves were flat, sometimes rapidly rising in the second stage of filling. The pressures did not exceed 30 cm. of water. The dog was well following the frontal lesion. Apathy and sleepiness were transient. Eighteen bladder readings were performed postoperatively, on days 4, 5, 6, 8, 11, 14, 18, 22, 25, 29, 32, 38, 42, 49, 54, 60, 66 and 156. Initially, the vesical capacity was markedly reduced to 200, 250 and 350 ml. Later on, between the 11th and 22nd postoperative days, it increased slightly, equaling 3 times 400 ml., and, once, 450 ml. In all examinations, except

that which was done on the 11th day, a micturition occurred. Examinations on days 4, 6, 8, 11 and 14 showed hypertonic curves with intravesical pressures surpassing 40 cm. of water. Cystometry on days 18 and 22 showed a normotonic curve though the capacity was still decreased to 400 ml. Commencing with the 25th postoperative day, the cystometrograms became stabilized at the preoperative level. The bladder capacity was 500 ml. 3 times, on days 42, 49 and 66; 600 ml. 6 times, and, once, 550 ml. In six examinations, however, in spite of the normotonic appearance of the volume-pressure curve the micturition occurred after introducing of the last increment of the fluid.

Dog No. 4. Transureteric cystometry was done 10 times prior to the frontal lesion, on days 3, 7, 10, 14, 17, 21, 23, 27, 38 and 45 after the pelvoureterocutaneostomy. The vesical capacity was remarkably large and constant, equaling 1000 ml. (only in the second reading it was 800 ml., the bladder filling being probably discontinued to early). All the volume-pressure curves were normotonic. Signs of the desire to void were noted only 3 times after the bladder had been filled with 800 ml. of the fluid. Following the frontal lesion eleven bladder readings were obtained on days 2, 5, 8, 11, 17, 21, 28, 33, 39, 45 and 48. During the first postoperative week, the dog was sleepy, apathetic (almost without contact) and lost his appetite. Subsequently, the dog's condition progressively improved so that, in the third week, he was well in every way and behaved normally. Only did the first postoperative cystometric examination on the second day after surgery show a markedly reduced vesical capacity. A strong desire to void was manifested at the volume of 200 ml. At 300 ml. a micturition and complete evacuation of the bladder took place in the lying position. The structure and appearance of other 9 cystometrograms did not differ from those obtained preoperatively. On the 48th postoperative day, the status epilepticus had occurred, and the animal was sacrificed 3 days later. The last reading on the 48th day done in a lucid interval showed a decrease of the bladder capacity; the micturition occurred at 500 ml. of the volume.

Dog No. 5. Prior to the brain operation 4 transureteric and 3 suprapubic cystometric studies were carried out. The findings obtained with both techniques were remarkably similar; they showed normotonicity and bladder capacity equaling 600 ml. in all the readings. A desire to void was present only just before the limits of the bladder capacity had been reached. After the prefrontal lobectomy, 9 readings by a suprapubic route were obtained on days 3, 5, 8, 18, 24, 28, 32, 36 and 128. The initial 3 cystometrograms showed a marked decrease of the bladder capacity (the volume at which the micturition occorred was 200, 250 and 400 ml.) and hypertonicity. The postoperative course was uneventful: the animal recovered promptly, displaying no signs of a neurological deficit; urinations, however, were small and frequent. In the 4th reading, there was slight hypertonicity and the capacity was 500 ml. Further cystometric studies showed that both the bladder capacity and the intravesical pressures returned to the preoperative level; the pollakisuria subsided.

Dog No. 6. Four transureteric and four suprapubic cystometrograms were obtained. Seven readings showed normotonicity and bladder capacity = 600 ml. Only in one reading (done on the second day after the implantation of the ureter into the skin) was the bladder capacity reduced (400 ml.) and the intravesical pressures elevated. A desire to void was manifested at 500 ml. of the volume. After the prefrontal lobectomy, the recovery was rapid and uneventful, and no alterations relative to those obtained preoperatively were noted. Four cystometric studies were performed postoperatively, on days 2, 7, 9 and 12. The bladder capacity was

600 ml. and only once did, at this volume, the micturition occur. At a successive attempt to perform a suprapubic cystometry, the fluid was introduced outside the bladder. The dog died on the 20th postoperative day.

DISCUSSION

The transureteric cystometry has proved a satisfactory technique for investigating the effect of the experimental chronic intracranial hypertension on the function of the urinary bladder (Cieśliński 1965). As seen from the present paper, it is also suitable in experimental studies on the cerebral control of micturition. While applying this technique one (a) avoids both anesthesia and urethral catheterization, (b) observes both facilitatory and inhibitory segments of the volume-pressure curves, (c) estimates the bladder sensation and micturition as a whole, (d) may perform several accurate pressure readings at any desired time before and after the operation on the brain, and, finally (e), obtains several times in succession normal cystometrograms comparable in each animal and from animal to animal.

The suprapubic cystometry proves somewhat inferior to the transureteric cystometry. The data obtained with both techniques are similar but when the suprapubic cystometry is used the danger of complications such as extravasation or introduction of the fluid outside the bladder exists.

A number of experiments has been reported which has been concerned with assessing the bladder tonus defined as a contraction or relaxation of the vesical muscle during the stimulation of the brain. Much of the research has been done with bladder manometry, recording bladder contractions (under conditions of a wide laparotomy) in response to the stimulation of the brain (Lichtenstern 1912, Spiegel and MacPherson 1925, Kaada 1951), or observing the waking animal's behavior during micturition elicited from the stimulation of the hypothalamus (Hess and Brügger 1943). Manometric studies have been performed with a catheter introduced into the bladder connected by means of a T-tube with a water, mercury or electromanometer and a syringe which allows to add or to withdraw the fluid to rise or to diminish the intravesical pressure during the examination. The catheter or a cannula have been introduced (a) per urethram from the external urethral orifice (Langworthy and Kolb 1935, Kremer 1947, Ström and Uvnäs 1950, Gellhorn 1959, Ingersoll et al. 1961, (b) per urethram, after suprapubic incision of the urethral wall at its junction with the bladder (Lichtenstern 1912, Barrington 1925, Monnier 1939, Wang and Harrison 1939,

Koikegami et al. 1957, Kuru and Kamikawa 1960, Kuru et al. 1961), and (c) through a cystotomy opening with a vesical neck ligation to avoid leakage of urine (Bechterew and Mislawsky 1888) or without it (Frankl-Hochwart and Fröhlich 1904). In order to record alterations of rhythmic activity or intravesical pressures the bladder was filled with varying amounts of fluid. Kuru et al. (1961) held the pressure at low and high levels to record excitatory and inhibitory responses, respectively. Langworthy and Kolb (1933), and Kremer (1947) observed the most striking poststimulatory bladder contractions when the bladder was filled to the limits of the capacity. Ingersoll et al. (1961) showed that the height of the intravesical pressure plays no major role in the type of response obtained.

Koikegami et al. (1957) used a pneumatic transmission by balooning the bladder with a condom instead of an aqueous transmission system.

Micturition reflex has been studied rarely in ablation and transection experiments, using cystometry. A transurethral route and anesthesia have been used despite the fact that anesthesia profoundly influences the bladder tonus, rhythmicity of the detrusor and the micturition reflex. Tokunaga and Kuru (1959) have found that the effect of anesthesia on bladder responses elicited from the stimulation of the medulla oblongata is negligible. On the other hand, Kabat et al. (1936), Koyama et al. (1962) and Gjone and Setekleiv (1963) have presented evidence, indicating that bladder capacity, micturition threshold, intravesical pressures and vesical effects of brain stimulation are greatly influenced by the depth and the nature of anesthesia. Barrington (1925), Langworthy and Kolb (1933) and Hesser et al. (1941) found nembutal to inhibit bladder function and increase bladder capacity, dial to decrease micturition threshold, and ether to rise intravesical pressure and inhibit a complete emptying of the bladder. Kabat et al. (1936), Magoun (1938), and Wang and Harrison (1939) have established that, under barbiturate anesthesia, points in the brain whose stimulation results in bladder relaxation (inhibitory points) are difficult to find, and have concluded that when anesthesia is used the observations are not reliable. Hess and Brügger (1943) have found that bladder responses on the stimulation of the hypothalamus can be more quickly and easily elicited under anesthesia than in a waking state due to the loss of cortical inhibition. Kremer (1947) could not obtain bladder responses on cingulate gyrus stimulation when anesthesia was deep. Koyama et al. (1962) classified pressure recordings of the resting bladder in 3 types: (1) high pressure recording with

irregular undulations (when the anesthesia was light), (2) rhythmically undulating recording, and (3) low pressure recording (when the anesthesia was deep).

In discussing the results of Langworthy and Kolb (1933), Hesser et al. (1941) have stated that due to the retardation of the micturition reflex with nembutal the cystometric studies cannot demonstrate well the effects of the removal of the motor cortex on urinary bladder activity. Our own experience shows that the vesical capacity varies with the depth of the anesthesia and is inferior to that in the waking state. A comparison of cystometric findings under and after anesthesia is virtually impossible. To obtain reliable data the depth of the anesthesia is to be held constant.

Tang and Ruch (1954, 1956) and Tang (1955) were the first to carry out cystometric studies in unanesthetized cats. Bladder readings were performed with the animals immobilized in woolen sacks or in a plaster of the Paris cast. Light ether anesthesia was used to insert the urethral catheter into the bladder. After the animals had recovered from anesthesia it was practically impossible to obtain a normal cystometrogram since bladder capacity vàried in wide ranges with the emotional excitation of the animals. The examination due to the presence of a catheter in the urethra led to a conspicuous irritation and was associated with annoying movements which resulted in artefacts in the cystometrograms. Therefore, the cystometrograms obtained before and after the brain lesion could hardly be compared. Comparisons were only possible after decrebrations at different levels.

Langworthy and Kolb (1933) have indicated that the evaluation of changes of the bladder volume, initiating reflex micturition, is difficult since the bladder capacity of the cat is too small. Most convincing results can be obtained in animals in which the preoperative volume at which the bladder empties is more than 100 ml.

Cystometry under anesthesia shows only the reflex bladder capacity. The inhibitory segment of the volume-pressure curve cannot be evaluated. Anesthesia limits also time and the number of bladder readings. Thus, it is clear that, under chronic conditions, the alterations of the bladder function arising from brain lesions may be convincingly assessed only when animals with a large bladder capacity are used and neither anesthesia nor urethral catheterization are employed.

The present data showed that, after a bilateral removal of all the cortex of the superior portion of the frontal lobe extending from the cruciate fissure to the frontal pole medially in three dogs (dog No. 2, who was in a very poor health and died on day 16 postoperatively, is

not considered here), the cystometric curves were unaltered in two animals, and in the third animal, the bladder capacity was reduced for a period of about 2 to 3 weeks with a tendency to a slight hypertonicity toward the end of the bladder filling. The data further showed that, after a bilateral prefrontal lobectomy, the cystometrograms remained in unaltered in one dog, and in the second dog, a temporary hypocapacity and frequent urinations occurred.

Bladder capacity of the normal, healthy dog is large and constant, the intravesical pressures during bladder filling being low. Alterations of cystometrograms (the decrease of the capacity and hypertonicity) due to such factors as pain, excitation, estrus, or infection occur infrequently. Therefore, the postoperative changes of the cystometrograms which were found in several successive readings of the present work are not likely to be a chance occurrence. They may be attributed to the surgical procedure rather than to the frontal lesion itself. Since in 3 out of 5 dogs with frontal lesions the bladder function was found to be unaltered, and in two others, it was found to be affected slightly and transiently, it may be concluded that a lesion of the medial frontal cortex or a prefrontal lobectomy produce little or no impairment of the bladder function. This is in agreement with the common observation, indicating that the effects of lesions confined to the prefrontal and premotor areas upon autonomic functions are temporary or escape experimental analysis (cf. B r u t k o w s k i 1964, 1965).

SUMMARY

The present study was undertaken to ascertain what further knowledge could be obtained about the function of the urinary bladder after bilateral removals of the superior parts of the medial cortex of the frontal lobes or prefrontal areas, by applying new techniques of bladder readings in unanesthetized dogs. A search of the literature showed no reference to such investigations. Previous experiments have primarily been concerned with the study of bladder tonus or micturition reflex.

It was demonstrated that a lesion of the medial frontal cortex located between the cruciate fissure and the frontal pole, or a prefrontal lobectomy in the dog produced little or no impairment of the bladder function.

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IS THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS A "SATIATION CENTER"?

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The application of the term "satiation center" for the ventromedial nucleus of the hypothalamus (NHvm) has been based on opposite effects, obtained by electrolytic lesions and by electrical stimulation of the ventromedial region of the hypothalamus. Numerous findings have confirmed the results of Anand and Brobeck (1951) who showed that hyperphagia and, consequently, obesity were produced by the lesion of NHvm. Experiments in which, following the electrical stimulation of NHvm (Anand and Dua 1955, Wyrwicka and Dobrzecka 1960, Morgane 1961a, Smith 1961, Hoebel and Teitelbaum 1962), the food intake was reduced or inhibited, have also confirmed the hypothesis on the inhibitory role of NHvm in relation to the food intake. On the other hand, it is well-known that the effects obtained by the method of electrical stimulation of NHvm are not confined to the feeding behavior only. Wheatley (1944) found that NHvm is implicated in the defensive behavior. Stimulating NHvm and adjacent regions, many authors observed the symptoms of aggression and flight, rage and fear. The localization of these reactions in the medial region of the hypothalamus, which also includes NHvm, in cats and rabbits, has recently been shown by Romaniuk (1962, 1963, 1965). In the medial part of the hypothalamus in goats, Wyrwicka and Dobrzecka (1960) have also found — in addition to "inhibitory" points — a number of points which, on stimulation, yielded defensive reactions, fear and restlessness. The predominance of defensive reactions, occurring when NHvm was stimulated in cats. has been showed by Robinson (1964).

It has been found by Karcz et al. (1957), as well as by Traczyk (1962) that, in rabbits, the act of eating is differently influenced by the stimulation of the medial areas of the hypothalamus. The orienting reflex, gnawing, sniffing, eating and flight were evoked. In addition, stamping feet which, in rabbits, is a symptom of a strong emotional excitation, has been observed by Lewińska (unpublished). These reactions contradict the state of satiation and, moreover, as pointed out by Robinson (1964), an area, yielding so different reactions, is contradictory to the concept of a "center" which implies a full uniformity and "purity" of the response. In view of these discrepant reports, one might presume that overlapping systems with different functions exist in the NHvm and the localization of the "purely" feeding inhibitory points should be found. For this reason, an attempt was undertaken at the electrical stimulation of various points of NHvm during a CR performance which allows one to observe the effects of such stimulation on both drive and motivation for the food.

MATERIAL AND METHOD

Experiments were performed on 20 adult cats. All of them were trained in an instrumental (Type II) conditioned reflex (CR), manifested by placing the right foreleg on the foodtray. This movement, performed in response to the conditioned stimulus (CS) was reinforced by food. The CS was a sound of a bell. Small bits of meat were used as reinforcement. The experimental compartment was a wooden box, 50 × 50 × 40 cm. with a low (5 cm.) foodtray bearing several bowls. An automatic device enabled the presentation of one bowl at a time. Every experimental session consisted of 15 trials with 1 to 3 min. intervals. When the CR was firmly established, i.e. when the animals responded with the trained movement to every CS on 10 succesive experimental days, the chronic electrodes were implanted in the NHvm.

The operation was made in a Horsley-Clarke stereotactic apparatus, under a narcosis, produced by Nembutal, injected intraperitoneously (40 mg/kg.) and under semiaseptic conditions. The skin was cut along the midline and holes were drilled in the skull through which, according to the Delgado technique (1955), unipolar electrodes were introduced to the NHvm. The cordinates of the NHvm were taken from Jasper and Ajmone-Marsan's atlas (1954). Each cat was implanted with one electrode in each hemisphere. The electrodes were made of steel wire 150μ in diameter and Teflon coated over their entire length, except for a 0.5 mm tip. Introduced to the brain, the electrodes were fixed to the skull surface with self-polymerizing resin (Duracryl). The extracranial ends of the electrodes were soldered to miniature sockets which in turn were fastened, also with Duracryl, to the skull surface. The indifferent silver electrode was attached with one end to the crista of the occipital bone and, with another, to the socket. Then, the skin was sutured in such a manner as to leave the socket slightly protruding over the skin surface.

One week after the surgical implantation of the electrodes the testing experiments were started in which the effect of the NHvm stimulation on the perfor-

mance of CR, and on food intake was studied. Each experimental session consisted of 15 trials of which there were 10 CR trials and 5 trials with NHvm stimulation. In some cases, the electrical stimulation of the NHvm started 1 sec. before the CS, in others, when the cat was putting its paw on the foodtray and, in still others, when the cat was eating. One or two NHvm were stimulated. There were the following parameters for brain stimulation: 50 cy/sec. rectangular impulses, 1 and 10 msec. impulse widths, and 0.1 to 2.0 mA amplitude during 5 to 10 sec. Impulsing was monitored on the screen of the cathode oscillograph. Several testing experiments were made on each animal at intervals of a few days.

Since the aim of the first stimulation experimental session was to settle the threshold and optimum parameters of stimulation for each point and to observe changes in animals' general behavior, it had a checking character, while, in subsequent experimental sessions, the stimulation of particular points was occasionally used during the routine CR trials, made to investigate the effect of stimulation on the conditioned and unconditioned feeding behavior.

After termination of the experiments, the points explored were coagulated for 15 sec. with a 3 mA D. C. After 20 days, the animals were killed and their brains perfused with a 10 per cent formalin. Paraffin sections 20μ thick were made in frontal plane. Every tenth of them was stained by Nissl method.

RESULTS

As a result of the experiment, involving the electrical stimulation of different points within the range of NHvm and those situated in its vicinity, four groups may be individualized which are different in the localization and in the responses evoked. The histological verification of the localization of particular electrodes, correlated with the reactions obtained is presented in Fig. 1.

G r o u p I contained 6 points, situated in the dorsal part of the NHvm.

Checking stimulation: The first perceptible symptoms, evoked by stimulation, were manifested by the reactions of attention, motionlessness, sometimes looking around the cage and dilation of pupils. Restless locomotor movements and rambling about the cage were observed after a prolonged stimulation. A more intensive stimulation resulted in the reactions of flight, jumps and attempts to get out of the cage. Furthermore, after a few seconds of a latent period, mewing was evoked by the stimulation of these points and it was particularly intensive when point 36-B was stimulated.

Stimulation during the CR experiment: During a slight stimulation that did not exceed 0.5 mA, the cats displayed both the CR and UR feeding behavior irrespective of the stimulation being switched on prior to the CS, during its action or when the animal was eating, as well as regardless of the symptoms of fear. Placing the foreleg was often performed in a crouching position, with a bent down back and somewhat stealthily. The gnawing movements were mostly hasty and very inten-

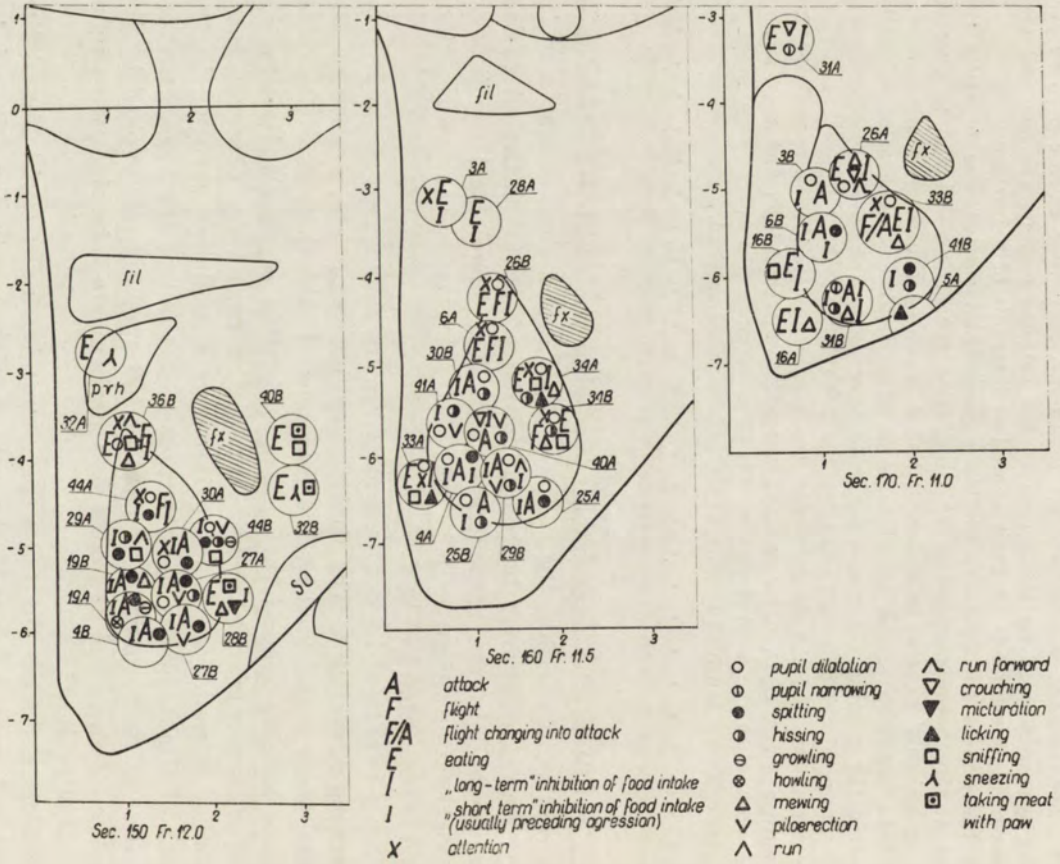

Fig. 1. Localization of stimulated points in the ventromedial nucleus of the hypothalamus (NHvm). Large circles show the stimulated areas. Numbers with letters A and B indicate numbers of cats

sive. Sometimes, when gnawing movements were particularly fast, the bites of meat were dropped from the animal's mouth. Mostly, however, the morsels were rapidly swallowed. Nosing at the bowl and smelling the meat was additionally obtained by stimulating point 36-B. When the stimulation was prolonged, this reaction changed into the flight which took place very quickly if an intensive stimulation was applied at once. In this case, neither CR nor UR came about. In the majority of cases, even after a relatively slight stimulation during which the cats ate quickly and voraciously, an afterinhibition occurred and exerted its influence on subsequent trials, that is, the animals responded neither to CSi nor USi. During the afterinhibition, the looks and behavior of the cats were changed. Usually, they lay low, mopish and drowsy, in the furthermost part of the experimental chamber. Depending on the intensity of stimulation, the afterinhibition lasted for from a few seconds to several minutes and, sometimes, even a few hours. A very interesting case was presented by points 6-A. When a 0.5 mA stimulation was applied a while after the performance of a CR, a pupil dilation occurred in the cat which stopped eating, then hastily continued and, after finishing, became motionless, displaying a complete lack of any responses to two successive trials. The CR reappeared in three further trials but the cat refused to eat. In a successive trial, the correct CR and UR were observed in turn and, hereafter, until the end of the experimental session, there were no responses to any stimuli. It shoud be emphasized that also on the following day this cat refused to respond in the experimental chamber, both to the CS and to US despite the fact that it ate normally in its home-cage. The aggression behavior was evoked by stimulating point 3-B, situated at the apex of the nucleus but in the caudal plane (Fr. 11.0), that is, lower than in the remaining planes and fairly medially.

G r o u p II contained 11 points, situated in the ventral part of the NHvm.

Checking stimulation: The immobility, piloerection, back arching, pupil dilation (in the case of point 31-B, narrowing) and, with a slightly intensified stimulation, hissing or spitting and showing teeth, frequently combined with o forward jump, were the most frequent reactions, produced by the threshold stimulation. Sometimes, these reactions were accompanied by growling and howling (point 19-A), mewing (points 19-B and 31-B) and licking (point 19-A).

Stimulation during the CR experiment: The feeding CR and UR reaction was as a rule inhibited by the threshold stimulation. If the stimulating current was switched on after the CR performance, the cat

sometimes snaped the food and either kept it in its mouth, or dropped it. The hasty eating and gnawing movement did not occur at all. The aggressive reaction appeared with a longer or a somewhat more intensive stimulation. The attack was usually aimless, although the animal mostly jumped at a glass wall of the chamber behind which the experimenter was visible. In the majority of cases, directly after the stimulation was stopped, the cats pitched into the food and rapidly ate it. The attack did not exert any effect on subsequent trials, the animals normally responded to successive CS′ and ate the food offered.

Group III contained 10 points, situated in the central part of the NHvm.

Checking stimulation: Mixed reactions, containing the elements of both flight and active defense, were evoked by the stimulation of these points. In general, cats withdrew to the furthermost parts of the chamber. Sometimes, contralateral turns (point 29-A) were observed or a flight, accompanied by hissing (points 34-B, 40-A and 41-A), hissing and spitting (points 29-A and 44-B), as well as only spitting (point 30-A). In some cats, the flight did not come about and the reaction was limited only to a backward jump, followed by hissing and arching the back (point 41-A) or spitting and growling (sometimes, hissing), combined with piloerection (point 44-B). Points 6-B, 30-B and 40-A which are mostly situated in a more medial part, yielded an aggressive behavior marked by forward jumps. As compared with the aggressive reaction, obtained from ventral points, this behavior occurred after a slightly longer latency period, reaching 8 sec. and that from point 40-A was preceded by a crouch. The reaction of hissing also followed a latency period a few seconds long, its threshold being also higher than that of the growling reaction.

Stimulation during the CR experiment: When slightly stimulated, points 33-B, 34-B and 41-A yielded the hasty feeding behavior. Particularly noteworthy and interesting was point 34-B which pronouncedly intensified the feeding reaction. To stop eating, a 1.5—2.0 mA current intensity was required but, on its application, the animal started to hiss. When a stronger stimulation (~1.0 mA) was applied to the remainig points, the reaction of anxiety was developed such as, for instance, rapid looking to the right and to the left or withdrawing which, sometimes, passed into the flight. In several instances micturition was also observed and, occassionally the reaction of running and searching occurred. The flight was often accompanied by hissing, less frequently, by baring teeth and spitting. Spitting, combined with aggression was evoked in point 33-B by a more intensive stimulation. The afterinhibition was, in

general, longer, particularly so from points, yielding the flight (0.5—3.0 minutes). Upon the stimulation of the points of aggression, the behavior of the cat was similar to that, described in the previous group.

Group IV contained 11 points, situated outside the NHvm area, of which 3 points were ventromedially located in the frontal planes of the brain, Fr. 11.0 and 11.5 (16-A, B and 33-A), 4 points ventromedially above the NHvm in the Fr. 11.0, 11,5 and 12.0 planes (3-A, 31-A, 28-A and 32-A), 3 points laterally to the NHvm in the Fr. 12.0 plane (28-B, 32-B and 40-B) and one point — ventrolaterally below the NHvm in the Fr. 11.0 plane (5-A).

Checking stimulation: No defensive reaction was evoked by the stimulation of all the 11 points. The points, located medially sometimes yielded the reaction of looking about, mewing (16-A), sniffing (16-A, B and 33-A), locomotor behavior and pricking ears (33-A), sneezing (32-A) and turns (31-A). The stimulation of lateral points resulted in gnawing movements (28-B), quivering of the mouth (32-B, 40-B), CR feeding behavior (28-B) and licking (5-A).

Stimulation during the CR experiments: Neither CR nor UR were, in general, interrupted by the stimulation with a slight (threshold) current of the points, situated ventromedially (Fr. 11.0 and 11.5). An increased rate of eating, as well as sniffing, licking, crouching, pricking the ears and dilation of pupils occurred sometimes. The inhibition of the feeding behavior occurred with the application of a stronger current (1.0—2.0 mA). In such cases, the cats stopped eating but did not display any accompanying reactions. The afterinhibition was, in general, brief, although a longer one (1 and 3 min.) was yielded by point 33-A. Similar effects of inhibiting the feeding behavior were obtained when the points, situated medially above the NHvm, were stimulated.

A very lively feeding behavior was yielded by the points, situated laterally to the NHvm. The cats mostly reached the food with their paws which they subsequently used to place it into their mouths. This reaction was sometimes accompanied by sniffing. Such motor reactions as quivering of the mouth which disturbed the function of eating were observed when the stimulation was intensified. In all cases, however, when the stimulation was stopped, the cats continued eating normally. Sometimes, micturition and mewing were evoked by the stimulation of point 28-B which is situated on the boundary of the NHvm. On a more intensive stimulation, this cat jumped backwards from the foodtray, performed gnawing movements which caused dropping meat from the mouth and, afterwards, threw with its paw the bits of meat out of the bowl.

DISCUSSION

It has been shown by the present results that several somatic and autonomic symptoms are evoked by the electrical stimulation of the NHvm during CR performance. The majority of these symptoms are accompanied by defensive reactions. Moreover, the stimulation of dorsal points intensified the act of eating, sniffing and searching. With a more intensive stimulation, defensive reactions were obtained from all points. It should be emphasized that always, when the intensity of stimulation of the NHvm was increased, the inhibition of the food CR performance and stopping the act of eating came about sooner or later but this effect appeared against the background of defensive reactions. There were no neutral points or those in which the effect of stimulation would be confined only to the inhibition of the conditioned or unconditioned feeding behavior patterns.

The reaction of flight was evoked by the stimulation of dorsal points of the NHvm, situated in the Fr. 12.0 and 11.5 frontal planes and the aggression was yielded by the stimulation of ventral parts of these areas. The aggressive behavior also was observed when the upper part of the nucleus in the Fr. 11.0 plane was stimulated. In the middle part of the NHvm some points produced the aggressive reaction and, some others, the flight which was usually combined with hissing. Mewing was often evoked when the dorsal parts were stimulated, while spitting, growling and, sometimes, howling were the effects of the stimulation of the ventral parts of the NHvm. These reactions were mostly accompanied by the pupil dilation, pilo-erection and baring of the teeth. The micturition was also observed in a few cases. No "pure" ejection reaction was ever obtained and vomiting, a reaction so typical of the amygdaloid nucleus stimulation was lacking completely. On the other hand, "pure" inhibition of the feeding behavior which was not accompanied by any other perceptible symptoms was evoked by the stimulation of the ventromedial areas, closely adjoining the NHvm, as well as of the remote points, located above the NHvm and close to ventricle III. The points, situated laterally to the NHvm produced on stimulation positive feeding reactions and strongly interfered with the CR instrumental reaction. The stimulation of these lateral points resulted in drawing out the food from the bowl and placing it in the mouth by means of the paws. Such a behavior in cats is, under normal conditions, very rare and, if it occurs at all, it is caused by the inaccessibility of the place in which the food is offered and by the difficulties in reaching it. Perhaps it should be assigned, therefore, to the symptoms of the intensified motivation. This seems to be justified by the fact that the medial forebrain bundle

to which the capability of the activation of different motor complexes related with securing food (Morgane 1961b) is ascribed, runs nearby this area.

In the light of the results we obtained, the role of the NHvm as a "satiation center", that is, a center which inhibits the feeding reaction, is unacceptable because the "inhibition" of the CRs und URs which we observed when different points of this nucleus were stimulated either occurred as a consequence of the flight reaction, or it formed an introduction to the aggressive reaction. The afterinhibition, occurring after the flight reaction, continued to bear the traces of the fear experienced, that is, the cats crouched down in the corner of the chamber and, even provoked, did not approach the foodtray. They responded neither to the CS, nor to the food. Sometimes, such a state was prolonged over the next few days. In the case of a slight stimulation of ventromedial points, the inhibition of the feeding behavior, although evoked, did not ever last longer than the action of the stimulus. Even this reaction cannot be, however, considered an inhibition caused by the state of satiation which is always marked by calming down and drowsiness. It was, as a rule, accompanied by the pupil dilation and the intensified muscle tension which made up an introduction to the aggression, actually obtained from these points by the increase in the intensity of stimulation. After the aggressive reaction a brief afteraction was observed. Almost immediately after the stimulation was stopped, the animals rushed to the food and ate more voraciously than before stimulation. Certain papers in which the use of the electrocoagulation method is described, may also arouse reservations as to the localization of the "satiation center" within the range of the NHvm. It has been shown by Poirier and ass. (1962) in monkeys and by Romaniuk (1962) and Lewińska (1964) in rabbits that hyperphagia is obtained not only by lesions of the NHvm, but also of other areas of the medial hypothalamus.

On the basis of our present investigations, we conclude that either the localization of the points, inhibiting the feeding behavior in the NHvm is inaccurate, or the applied method of the electrical stimulation does not allow for their individualization. It seems that, in the case of nervous elements of different systems, interlacing each other within the region of the NHvm, the separation of the feeding and defensive reactions by the method of the electrical stimulation, now in use, is probably imposible. May be that the application of a more concentrated stimulation, for instance, by the use of micro-electrodes, would allow one to select these points since, according to Krasne

(1962), the expected results cannot be obtained by a slight but long-lasting stimulation. It turned out that, for every electrode, the cessation-of-eating threshold was at least as high as the escape threshold. Krasne (1962) believes that the ventromedial stimulation prevents from feeding and drinking behavior by upsetting or distracting animals.

The character of responses obtained from the hypothalamus was also determined in the experiments in which the self-stimulation method was applied to show if a reinforcement of the type of reward or punishment is supplied by a given area. It was shown that, in the hypothalamus, there are extreme areas where the escape without the approach or the approach without the escape or both these effects may be provoked. This third ambivalent area may represent a system in which a mild or brief stimulation rewards, while a strong or long one punishes (Olds 1960). With regard to the medial area of the hypothalamus, the views of different authors are not fully unanimous. Hoebel and Teitelbaum (1962), as well as Wilkinson and Peele (1962) believe that it is only a negative reward that is supplied by this area. Grastyan et al. (1965) also obtained only crouching-withdrawing movements from these areas. Reynolds (1958) maintains that the medial hypothalamus is provided with such points in which rewarding effects decline as the intensity of the current increases, and Olds (1960) claims that this ambivalent area is situated mesially and the points, yielding only the escape, are situated medially and they occupy the ventromedial nucleus region.

The reactions to the food, which we observed when the NHvm was stimulated, cannot be, however, put in the same class. The responses from the dorsomedial parts represented a sort of a negative of those, evoked from the ventromedial ones. Three stages each might be differentiated in the development of these reactions. At first, a hastened eating was evoked by the slight stimulation of the dorsomedial parts. In some cases, this reaction gave the impression of a positive feeding behavior. In the second stage — during the escape — and in the third — after the stimulation was stopped — the response to the food was negative. The stimulation of the ventromedial parts yielded different and opposite effects. Both in the first stage which was an introduction to the attack and in the second, in which the attack was developing, the reaction to the food was negative, while in the third — after the stimulation ceased — it was positive. This "off effect" which was observed by Wyrwicka and Dobrzecka (1960) only from the "inhibitory points" and which was considered by them to be a sort of a rebound phenomenon was — in our experiments — evoked by the stimulation of both the ventromedial points of the NHvm, yielding the aggressive beha-

vior and the ventromedial points, situated outside the NHvm and inhibiting the feeding behavior.

In comparing our results, obtained by the electrical stimulation with those, obtained by the use of chemical stimulation applied to different areas of the hypothalamus, a certain similarity occurs between the reactions, coming from upper areas of the nucleus and those, yielded by the adrenergic stimulation, as well as between the reactions observed when the ventromedial areas were stimulated and the symptoms of the cholinergic stimulation. Grossman (1964a) showed that adrenalin, applied in moderate doses, evokes the food intake response. However, the excess of such a dose over an optimum magnitude evokes hypoactivity, drowsiness and a general apathy. And contrariwise, small doses of cholinergic agents inhibit the feeding behavior, while supramaximal doses are the cause of a considerable hyperactivity, growling, sniffing and hyperexcitation. On the other hand, according to Myers (1964) who investigated the emotional and autonomic responses, the cholinergic effects are allegedly manifested by fear, rage and sympathetic symptoms (pupil dilation, tachycardia), while the adrenergic effects lead to the drowsiness and have a character of parasympathetic reactions. Likewise, Grossman (1964a, b) considers that the cholinergic drugs interfere with the avoidance reaction but exert no effects on the escape which would support the hypothesis of the decrease in fear, while the capability of an active defense is retained.

This problem, concerning the NHvm area, would require further studies. Moreover, some interest may be aroused by the areas adjacent to the ventromedial part of the NHvm itself, from which we obtained only the feeding inhibition without any other, externally perceptible reaction.

SUMMARY

During the food CR experiments, 38 points were occasionally stimulated within the cats' NHvm and adjacent regions. During 5 to 10 sec. periods, the electrical current was used with a 0.1 to 2.0 mA intensity, 1 to 10 msec. pulse duration and 50 cy/sec. frequency. The effect of stimulation on the food CRs and URs depended on the place of the electrode implantation, duration and intensity of stimulation. The threshold stimulation of the dorsolateral part of the nucleus inhibited neither the CR nor the UR feeding behavior and mostly evoked a hastened eating. A more intensive stimulation resulted in the reaction of fear and flight followed by the inhibition of the feeding reaction which lasted a few to several minutes. The threshold stimulation of the ventromedial part of the nucleus mostly caused a brief inhibition of both the food CRs

and URs and was accompanied by a tense posture, dilation of pupils and immobility. A more intensive stimulation led to the aggressive reaction which, in most animals, was followed by the instant response to both the CSi and USi.

The complex character of the reactions, evoked by the stimulation of the NHvm among which the defensive and feeding ones predominate, while the feeding inhibition seems to be an aftereffect cused by the negative influence of fear is discussed.

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THE EFFECTS OF OVERTRAINING AND SEPTAL LESIONS ON THE ABILITY TO SWITCH ATTENTION BETWEEN CUES

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Sutherland 1964a, b) and Mackintosh (1965) have presented much evidence that discrimination learning is mediated by at least two separate mechanisms: one which determines the cues or dimensions that are attended to (which analyzers are switched in, using Sutherland's terminology) and another which determines the response, approach or avoidance, that is attached to each cue. According to the theory, overtraining a visual discrimination in the rat primarily has the effect of increasing the strength with which the relevant analyzers (those which mediate the discrimination are switched in. Thus overlearning should make simple reversal learning easier since the same cues must be attended to and only responses must be changed. On the other hand, overtraining should make transfer to a discrimination which requires attention to a different dimension more difficult since previously irrelevant analyzers must be switched in. A number of experiments seem to support these conclusions (Mackintosh, 1962, 1963, 1964).

The present experiment provides a further test of this second point. All animals were trained to discriminate between a white vertical (positive) and a white horizontal (negative) form. Thus the form cues were relevant and brightness cues irrelevant. They were then transfered to a brightness task which required the discrimination of one of the previous stimuli, either positive or negative, and another stimulus of the

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same shape but black instead of white. The positive or negative value of the form that was the same in both tasks was also left unchanged. According to Sutherland's theory (1964b) overtraining should hinder the transfer from the first to the second to task since, although one of the cues is identical and of identical value (positive or negative) in both tasks, correct performance in the second problem requires the switching in of a different analyzer or dimension (brightness) from that which was previously dominant (form). The theory predicts that overtraining should hinder the transfer both when the positive and when the negative stimulus is unchanged from the training to the transfer test.

Previous experiments have demonstrated that rats with septal lesions are very poor in reversing active and passive avoidance responses but perform as well as normals when required to reverse a simple simultaneous or successive brightness discrimination (Liss, unpubl. PhD thesis, 1964). One may attempt to explain these results by assuming that septal lesions impair the ability to switch attention between cues and yet have no effect on the ability to switch responses to the same cue. It is possible that the normal animal solves the active-passive reversal problem by switching its attention between different cues, in which case septal animals would be poor. On the other hand, the only way to solve the brightness-reversal problem is to switch responses to the same cues, and thus (according to the above hypothesis) septal lesions should produce no deficit.

If it is true that septal lesions produce a general deficit in the ability to switch attention from one cue to another, then, like normal overtrained rats, they should perform poorly when required to transfer from a form to a brigthness discrimination. Again it should make no difference whether the positive or negative stimulus is unchanged from the training to the transfer test.

This experiment, then, is designed to test the hypothesis that both overtraining and septal lesions decrease the ability of rats to switch attention from a dominant cue to a previously irrelevant one and thus impair the transfer from a form to brightness discrimination. This deficit should exist whether the positive or negative stimulus is the same in both the training and the transfer test.

MATERIAL AND EXPERIMENTAL PROCEDURE

Experiments were performed on 24 naive male albino rats, weighing from 210 to 330 grams at the start of testing. Septal lesions were made in 8 rats and the others were not operated on. The lesions were made stereotaxically with the animal under nembutal narcosis. Bilateral electrocoagulation of the septum was performed by passing a direct current of 2.0 mA for 15 seconds through a stainless-steel wire

7 mm in diameter insulated except for 0.5 mm at the tip. Training began 2 months postoperatively after the gross hyperreactivity characteristic of the "septal syndrome" disappeared. After the testing had been completed, the animals were sacrificed and their brains removed. The loci of lesions were verified on sections cut at 20 mi in the area of coagulation and stained with the Klüver-Barrera technique.

The apparatus was a modified version of one first described by Thompson and Bryant (1955). It consists of a V-shaped choice chamber with a grid floor, and a goal chamber with a wood floor. Two windows, each measuring 14 cm×14 cm, and separated by a partition which protruded 10 cm out into the choice chamber, allowed entrance from the choice to the goal chamber. The grid in the choice area and the separately wired grids under each window delivered 4.0 mA of shock. The 16 cm × 16 cm stimulus cards were painted grey and black or white horizontal or vertical bars were pasted onto them. These forms measured 16 cm × 3.5 cm.

On the first day of training, each animal was taught a simple active avoidance response. S was placed in the choice chamber with his back to the goal chamber. If the rat did not enter the goal chamber through either of the open windows within 5 seconds he was shocked until he did enter. He was allowed 3 minutes in the goal chamber and then was placed in a waiting stand for 15 seconds before the next trial began. Training continued until three avoidances occurred successively.

On the second day, each S was taught to go through the stimulus cards. Cards with identical white vertical forms were at first placed a few centimeters behind each window. Thus on the first trial, the animal could enter the goal box without having to push through the cards. After each trial the cards were gradually brought closer to the windows until each window was completely covered by a stimulus card. Therefore the animal had to push away one of the stimulus cards in order to enter the goal box. Shock was administered if no avoidance was made within 5 seconds and training continued until three successive avoidances occurred with the cards in their final position. No attempt was made to break position preferences. On this and the preceding day any trial in which shock was administered was counted as an error.

On the third day and all following days discrimination training was given. The incorrect (negative) card was always locked in place from behind and the grid under it was continuously charged. An error now consisted of an approach to the incorrect window which resulted in shock to the front paws. As before, only one error per trial was counted. Each trial terminated with the correct response and immediately upon entering the goal chamber the animal was placed in the waiting stand while other animals were tested. Twelve animals were tested at a time with the resulting intertial interval being about 4 minutes. Ten trials a day were given with the correct side (left or right) randomized. The training criterion for each task was either 18 errorless trials in two days or a maximum 100 errors.

All animals were first trained to discriminate a white vertical (positive) from a white horizontal (negative) form. After reaching criterion one normal group of 8 animals was given an extra 10 days (100 trials) of overtraining on the form discrimination before being transferred to the brightness task. The other normal group of 8 animals and the septal group began the brightness task the day following criterion performance on the form discrimination.

Each main group (normal criterion, normal overtrained, and septal criterion) was divided into two subgroups "Positive" and "Negative" (of four animals each). The "Positive" subgroups were given the brightness task in which the positive stimulus was the same as in the previous form task (white vertical-positive vs. black vertical-negative); the "Negative" subgroups were given the brightness

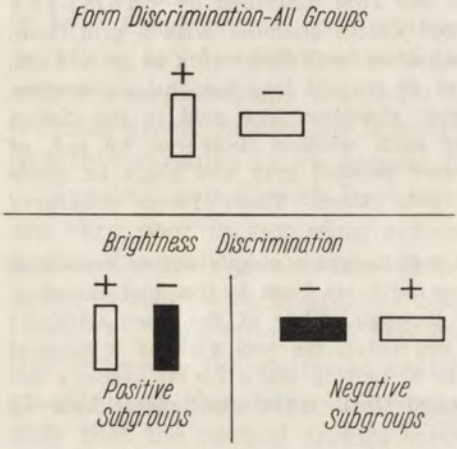

Fig. 1. Stimuli used in the training of the form and brightness discriminations

task in which the negative stimulus was the same as in the previous form task (white horizontal-negative vs. black horizontal-positive). Fig. 1 diagrams the stimuli that were used.

All statistical analysis, except where noted, was by the Mann-Whitney U test, 2-tailed.

RESULTS

Table 1 shows that the rats with septal lesions were slower than the normal group in learning the original avoidance response ($p < .02$) and in learning to go through the stimulus cards ($p < .02$). Four of the septal rats performed poorly on both tasks while the other four lesioned animals performed within normal range on both problems. The typical behavior before making correct active avoidance responses of the im-

Table I

Mean and range (in brackets) of errors in original avoidance learning and learning to go through the stimulus cards

	N	Avoidance	Go-through cards
Normal	16	1.6 (1—3)	5.9 (2—11)
Septal	8	4.1 (2—10)	17.9 (5—46)

paired rats was to the open window and stay there until the shock forced them to enter the goal chamber. This behavior was rarely seen in normal rats. While learning to go through the cards, the septal rats would run to the window till their whiskers touched the card. This produced a strong "arrest reaction" and the rat might often run back into the choice chamber rather than push away the card. A similar tendency to stop at the card was observed in normal rats but it was overcome much more quickly.

As Table 2 shows, the rats with septal lesions made significantly fewer errors than the normal rats in the original learning of the form discrimination ($p < .002$). The eight septal animals made from 12—31 errors (median 20.5) while the sixteen normal rats made from 23—72 errors (median 42). The median and range of trials to criterion for the septal and normal groups were 75 (50—130) and 125 (70—170) respectively.

Table II

Median and range (in brackets) of errors to criterion in the form and brightness discriminations (4 animals per subgroup)

	Normal Criterion		Normal Overtrained		Septal Criterion	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Form	43.0 (25—58)	45.4 (29—72)	41.5 (41—67)	38.5 (23—66)	22.0 (18—31)	20.5 (12—30)
Brightness	29.0 (18—76)	29.0 (25—45)	79.5 (35—100)	26.0 (5—34)	51.0 (27—89)	9.0 (4—13)

The raw data from the brightness task were subjected to a square-root transformation ((Meredith and Wong 1961) and an analysis of variance was performed. Table 3 shows that the effect of tasks (Positive vs. Negative) was significant while the effect of groups (normal criterion vs. normal overtrained vs. septal) was not. Furthermore, the interact-

Table III

Summary of analysis of variance of errors made in the brightness discrimination

Source of Variance	df	MS	F	P
Tasks	1	196.83	18.14	.001
Groups	2	20.57	1.89	not. sign.
Tasks x Groups	2	39.19	3.61	.05
Residual	18	10.85		

ion between tasks and groups was significant. Inspection of Table 2 shows that this interaction is due to the fact that the normal criterion group performed as well on the positive as on the negative tasks whereas for the normal overtrained and septal groups the negative task was much easier than the positive task ($p < .05$ in both cases). In fact the septal group trained on the negative task performed significantly better than the corresponding normal criterion group ($p < .05$), a finding in direct contradiction to the original hypothesis.

Histological examination showed that the lesions consistently destroyed almost all of the lateral septum and most of the medial septum. Typically the lesion extended from the anterior portion of the hippocampal commissure to the genu of the corpus callossum. The medial aspects of the caudate, especially in the anterior part, were usually slightly damaged. No damage to the corpus callossum or anterior commissure was ever observed. In six animals the post-commissural fornix

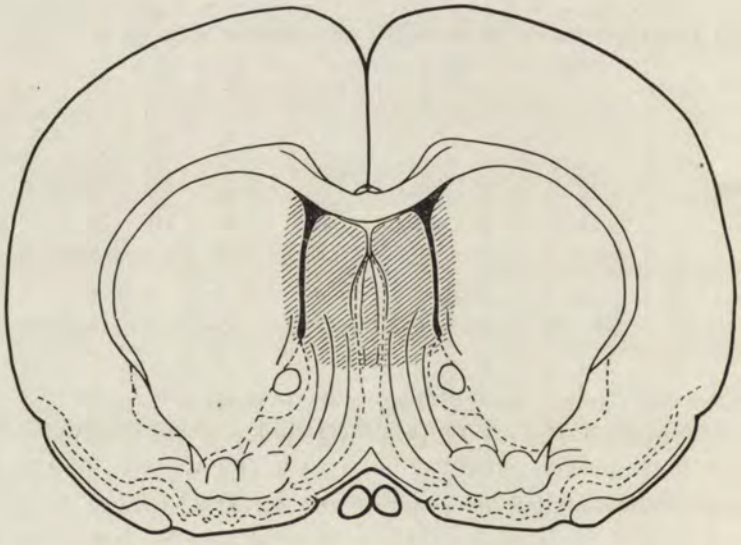

Fig. 2. Maximal extent of a typical lesion of the septum. (From: J. F. R. König and R. A. Klippel, The rat brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem, The Williams and Wilkins Co., Baltimore, 1963, Fig. 166)

was cut, in two it was spared. The lateral ventricles were usually somewhat enlarged from hydrocephalus. No behavioral differences within the lesions group was found to correlate with either the size or location of the lesion. The maximal extent of a typical lesion is shown in Figure 2.

DISCUSSION

Before discussing the results of the transfer from the form to the brightness discrimination, it is necessary to point out that the rats with septal lesions made considerably more errors than the normal group in the original learning of the active avoidance response and in learning to go through the stimulus cards. On the other hand, the septal rats made considerably fewer errors in learning the original form discrimination than did the normal animals.

The poor active avoidance learning of septal rats in a simple one-way situation confirms the results of Kenyon (unpubl. PhD thesis, 1962), Vanderwolf (1964) and Liss (unpubl. PhD thesis, 1964). The generally superior performance of both the normal and septal groups in this experiment as compared to the previous ones is most likely due to the use of a 3-minute intertrial interval in the goal box rather than the more conventional 30-second or 1-minute interval. Apparently, it takes 2 or 3 minutes before a rat relaxes in the goal box after escaping from shock. If given this opportunity to relax, the normal rat learns an active avoidance response after making only one or two escapes (cf. Denny and Weismann 1964).

Both the relatively poor active avoidance learning and the deficit in learning to go through the stimulus cards demonstrate that, at least in some conditions, rats with septal lesions may show a stronger freezing or arrest reaction than normal animals. These results should be contrasted with the decrease in freezing behavior shown by septal rats when tested in shuttle-box and passive avoidance tasks (Kaada et al. 1962, King 1958, Krieckhaus et al. 1964).

As for the transfer test, neither Sutherland's prediction that overtraining, nor our prediction that septal lesions, consistently decreases the rat's ability to transfer from a form to a brightness discrimination was confirmed. Overtraining did seem to hinder performance in the transfer test in which the positive stimulus was the same. However, when the negative stimulus was the same, the overtrained animals performed at least as well as the criterion group. Similarly the rats with septal lesions performed poorly when the positive stimulus was the same but made significantly fewer errors than the normal group when the negative stimulus was the same. Whether similar results would be found if the discrimination were based on appetitive (food) rather than aversive (shock) motivation is not known. In general, then, it is still not clear whether there really is an "ability to switch attention between cues" which may be modified by specific training or ablation procedures.

There are at least two possible explanations why the overtrained and septal rats performed better in the brightness task when the negative stimulus of the previous form discrimination was the same than when the positive stimulus was the same. One hypothesis is that these animals learned more about the negative than the positive stimulus. This may be due either to an increased restriction of attention to the negative cue (perhaps most applicable in the case of overtraining) or to an increased fear of the negative stimulus (perhaps most apllicable in the case of septal lesions).

The second hypothesis is that these animals were attracted to the new stimulus with the unfamiliar or novel brightness. They would then prefer the wrong new stimulus in the "positive" case and the correct new stimulus in the "negative" case. This hypothesis seems especially promising when applied to the septal group since it has already been demonstrated that frontal lesions in monkeys produce an analogous effect (P r i b r a m et al. 1964). However, one may still wonder why a presumed increased reactivity to novel stimuli should here lead to an approach response and other times to an arrest or withdrawal reaction as appeared to be the case when the septal rats had to learn the original active avoidance or to simply go through the stimulus card.

SUMMARY

Normal rats and rats with septal lesions were trained to discriminate between two forms using the motivation of fear of shock. One normal group was overtrained while the other normal group and the septal group were just trained to criterion. Then all animals had to learn a brightness discrimination in which either the positive or negative stimulus of the previous form discrimination remained the same ("positive" and "negative" tasks). The main results were:

1. Rats with septal lesions were worse than normal animals in the original active avoidance learning and in learning to go through the stimulus cards but better than normal animals in learning the form discrimination.

2. The normal criterion group found the "positive" brightness task as easy as the "negative" task while both the normal overtrained and the septal groups found the "positive" task more difficult than the "negative" task.

3. The original hypothesis that both overtraining and septal lesions decrease the ability of the rat to switch attention between cues was

not confirmed. Other possible explanations of some of the present results were suggested.

The authors would like to thank Dr. K. Zieliński for suggesting and performing the analysis of variance.

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DELAYED RESPONSE DEFICIT IN DOGS AFTER SELECTIVE ABLATION OF THE PROREAL GYRUS[1]

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Delayed-response impairment after bilateral prefrontal lobectomy, a phenomenon first discovered in the monkey (Jacobsen 1936), has recently been demonstrated also in cats and in dogs (Ławicka and Konorski 1959, 1963). In the monkey, however, it is now known that this behavioral effect can be produced by a cortical removal far more selective than prefrontal lobectomy. Thus, severe deficits have been found after lesions limited to the lateral as opposed to the ventromedial surface of the prefrontal area (Pribram et al. 1952) and even more specifically, after lesions confined to the midlateral as opposed to the dorsolateral or ventrolateral regions (Blum 1952, Mishkin 1957). The conclusion that the monkey's midlateral frontal granular cortex, i.e., the cortex in and around the sulcus principalis, constitutes a focal area for delayed response functions has recently been confirmed both by stimulation (Weiskrantz, Mihailovic and Gross 1962) and ablation techniques (Gross and Weiskrantz 1962, Gross 1963).

The present study was undertaken to determine whether or not a focus for delayed response functions could also be demonstrated within the prefrontal cortex of the dog. Positive results would prove helpful in suggesting the location of homologous prefrontal areas in the two species.

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MATERIAL AND METHODS

Animals and experimental procedure: Eighteen naive male mongrel dogs were used. The animals were trained in a rectangular room, 8×4 m., with three widely separated food dispensers arranged on the floor as shown in Fig. 1. Each food dispenser consisted of a box which enclosed ten food cups mounted on a rotating disc. A small opening in the top of each box provided access to whichever food cup was in position directly below it. The cup that was accessible at the beginning of the experiment was empty; the others were baited with food and could be brought into position, one at a time, by a partial rotation of the disc. Rotation of the disc was activated remotely by the experimenter, who was seated at a table placed against one wall of the room (see Fig. 1). The experimenter could also activate remotely an electric buzzer mounted on the top of each food box.

On the first day of preliminary training, the animal was allowed to explore the testing room. Whenever it drew near one of the food boxes, the experimenter delivered a reward at that position. Each delivery produced a clearly audible click, which the animal soon learned to associate with food presentation. At the beginning of the next day's session, the animal was induced to approach and stand at the starting place which was located next to the experimenter. A buzzer on one of the food boxes was then sounded for 3 sec., and this was followed immediately by delivery of the food reward at that dispenser. After approaching the box and eating, the animal was permitted to explore the room for a short time, and was then induced to return to the starting place again. After a few such trials, the animal learned to approach the correct box at the sound of the buzzer alone, i.e., prior to food delivery. In the rare case of an incorrect choice the food reward was nevertheless presented at the box signalled by the buzzer. Training was continued in this manner until the animal made 15 correct choices in succession.

On the following day, training with 0-sec.-delay trials was begun. This stage involved leashing the animal at the starting position near the end of each intertrial interval. Only after the buzzer had sounded for the full 3-sec. period was the animal unleashed. A single 15-trial session was usually sufficient to assure fully correct responding at 0-sec. delay. The entire sequence of preliminary training, including the time for adapting the animal to the experimental situation, took approximately 8 days.

For 14 animals, the subsequent training involved a series of three problems, each presented to a criterion of 90 percent correct choices in 60 consecutive trials. Problem I, consisting of trials at 15-sec. delay, was followed by problem II, with trials at 60-sec. delay. In problem III, testing was continued at 60-sec. delay but an intradelay distraction was added, which consisted of offering the animal a fixed ration of food at the starting place. The food was presented 15 seconds after the start of the delay period, and ingestion of the food took approximately another 15 seconds.

The four remaining animals were tested by a different method. Following preliminary training, these dogs received daily sessions consisting of trials with 5 delay intervals (30, 60, 90, 120, and 180 sec.) presented in random order. One animal received all of its training without intradelay feeding, whereas the three others were trained both without and with this distraction. Since length of delay did not prove to be a critical variable, either before or after operation, only the

data gathered on 60-sec. delays will be presented, and for convenience, the problems given these animals will be referred to as problems II and III.

Throughout training for all animals, the buzzer was presented for 3 sec. on each trial and the animal was unleashed only after the specified delay. Fifteen trials separated by 2-min. intertrial intervals were given daily, and the three food boxes were signalled for 5 trials each, in random order. Correction technique was used; i.e., after an error, the animal received additional buzzer presentations from the same food box with 0-sec. delay. Self-correction within a single run was not rewarded.

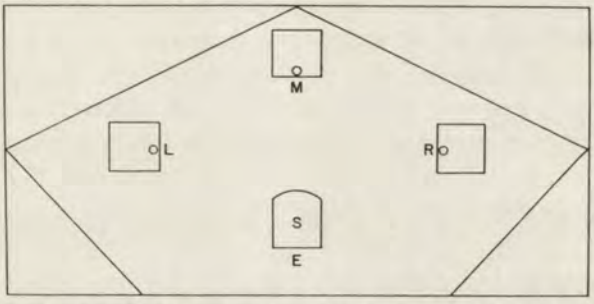

Fig. 1. Schematic diagram of the experimental room

After achieving criterion on the final problem, the animals were given a rest interval of one week and were then retested on problems II and III, for one day on each.

At this point the animals were operated, and after a recovery period of one week all animals were retested on the delayed response problems under the same conditions as before; i.e., whichever problems the animals received before operation were presented once again for a block of 60 trials on each. Generally, if an animal failed to reattain criterion immediately on either of the problems with 60-sec. delay, it was given additional training on problems II and III, presented in alternating 60-trial blocks.

Surgery: Following preoperative retention testing, each animal was subjected to one of three different single-stage bilateral lesions of the prefrontal cortex. In 9 dogs (proreal group), the lesions included the cortex of the proreal gyrus, both laterally and medially. In 7 dogs (orbital group), the lesions involved the cortex of the orbital gyrus. Finally, in 2 dogs (presylvian group) an attempt was made to remove the cortex situated in the anterior bank and depth of the presylvian sulcus. In contrast to the lesions in the proreal group, those in the orbital and presylvian groups were confined to the lateral aspect of the hemisphere.

The general surgical procedure was the same for all groups. The animals were anesthetized with Nembutal (35-38 mg/kg) and aseptic technique was used. The scalp was incised and retracted, the frontal sinuses were entered, and the conchae nasales were tightly packed with wax. A large bilateral opening was then made in the bone overlying the prefrontal region, with the posterior boundary of the opening located approximately 3 mm. caudal to the presylvian sulcus. The dura was opened, the appropriate cortical region was removed by suction, and the wound was sutured in anatomical layers.

Histology: Following postoperative testing, the animals were anesthetized and perfused, and their brains were removed and placed in formalin. The brains were

then embedded in paraffin and cut into sections 20 microns thick. Every 20th section was stained alternately according to the Nissl, Kluver-Barrera and Woelke techniques.

RESULTS

Anatomical Findings

Sections through each animal's lesion are illustrated in Fig. 2. Reconstructions of the lesions were prepared from the serial sections, and were then transferred to standard diagrams of Kreiner's (1961) myeloarchitectonic map of the prefrontal cortex. A representative reconstruction of each of the three types of lesion-proreal, orbital and presylvian — is shown in Fig. 3. To ilustrate the communality and variation of the lesions within a given operated group, all the lesions of that group were superimposed, yielding the composite diagrams shown in Fig. 4.

For purposes of individual analysis, the myeloarchitectonic areas invaded in each animal are given in Table 1 (see Fig. 4 for location of the designated areas).

Proreal group: The lesions in this group of dogs (Pr 1—9) involved area prorea (PR), although they also invariably invaded immediately surrounding areas. The adjacent subdivisions most commonly damaged were area polaris (POL) and area pregenualis (PG I), but still others were occasionally encroached upon. In addition to the instances listed in the table, area precruciata centralis (XC) was partially damaged in dogs Pr 5 and Pr 8, area precruciata medialis (XM I) in dog Pr 6, and the ventral segment of area pregenualis (PG II) in dog Pr 7.

Orbital group: The animals in this group (Or 1—7) had lesions of area orbitalis (ORB I′ and ORB I″), although, as in the preceding group, the damage frequently extended outside the intended locus. The additional areas invaded were orbitalis posterior (ORB II, located in the anterior bank of the presylvian sulcus immediately posterior to ORB I″), and that portion of area prorea (PR) situated on the lateral surface of the hemisphere immediately dorsal to area orbitalis (ORB I′). In dog Or 3, the lateral part of area prorea was entirely removed. In addition to these instances, which are listed in the table, area subprorea lateralis (SPRL I) and area subprorea (SPR I) were partially damaged in dogs Or 2 and Or 5.

Presylvian group: The two dogs in this group (Ps 1—2) had lesions of the anterior bank and depths of the presylvian sulcus (PRL I, PRL II and PORD). There was little or no extension of this lesion into other myeloarchitectonic areas.

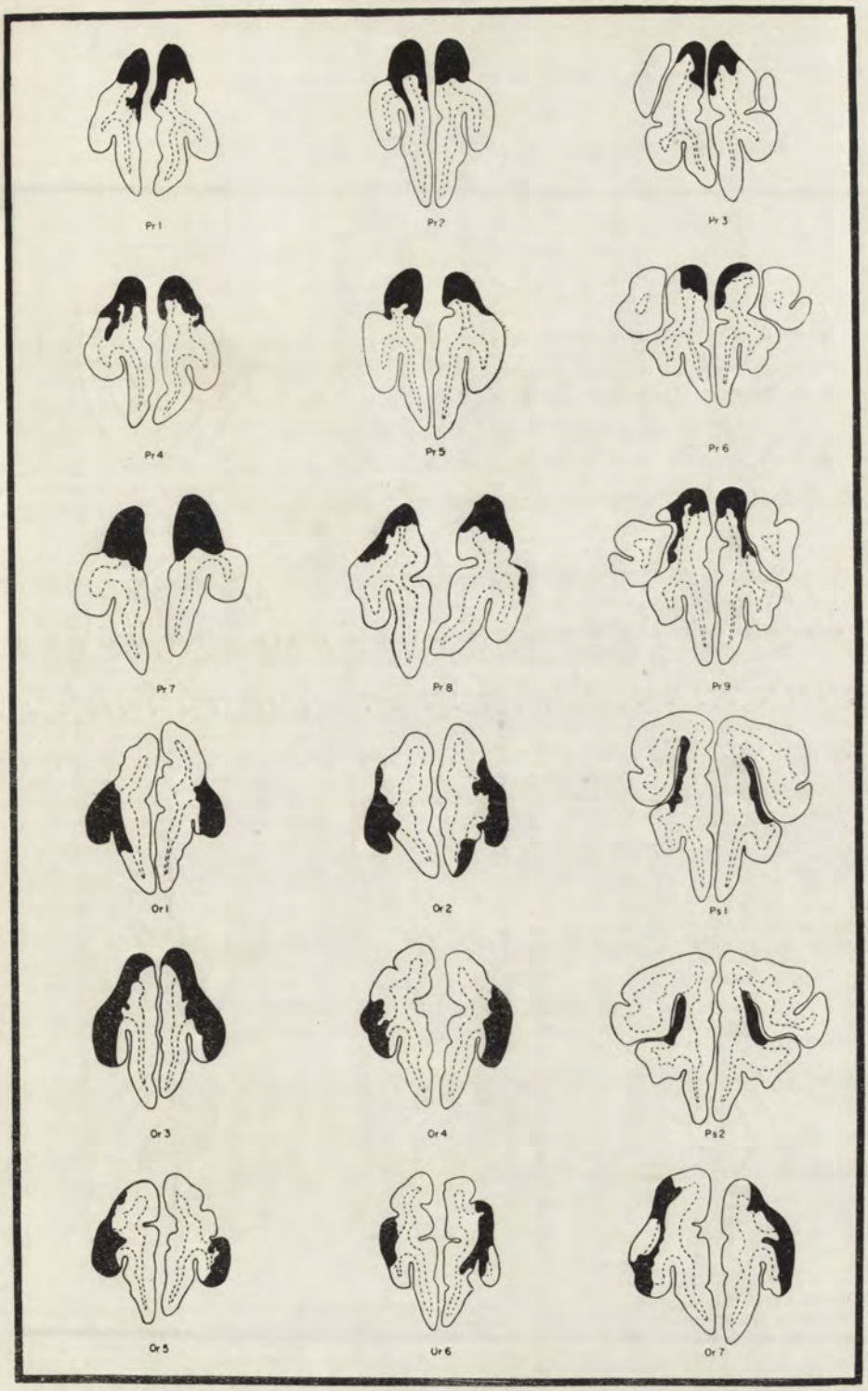

Fig. 2. Cross sections through each animal's lesion, indicated in black. To facilitate comparison among animals and groups, rostrally located sections have been placed to the left, caudally located sections to the right. Pr=proreal; Or=orbital; Ps=presylvian

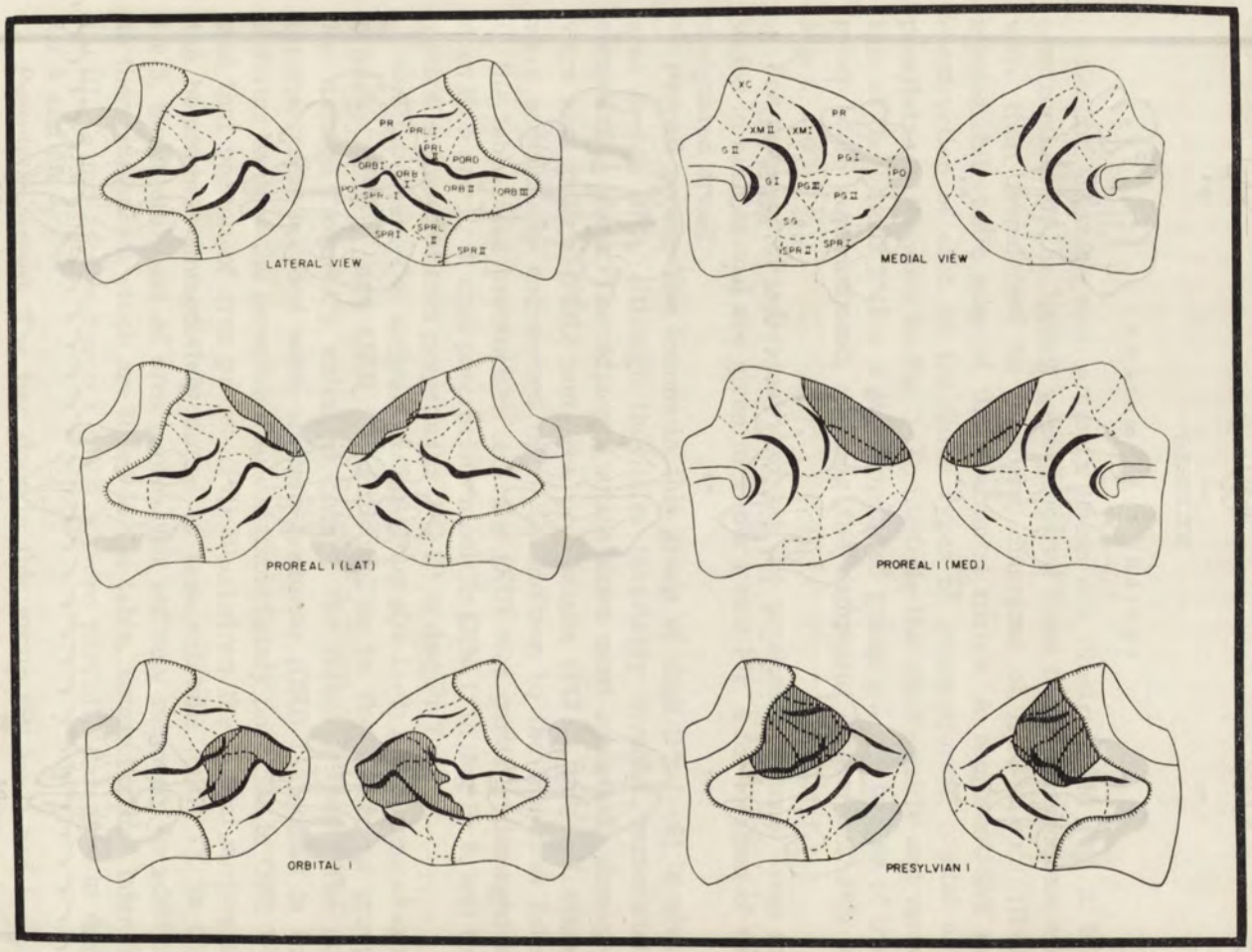

Fig. 3. Representative reconstructions of each type of lesion (dogs Pr1, Or1 and Ps1), transferred to standard diagrams of Kreiner's myeloarchitectonic map of the prefrontal region. Lesions indicated by shading. In the lateral views, the posterior

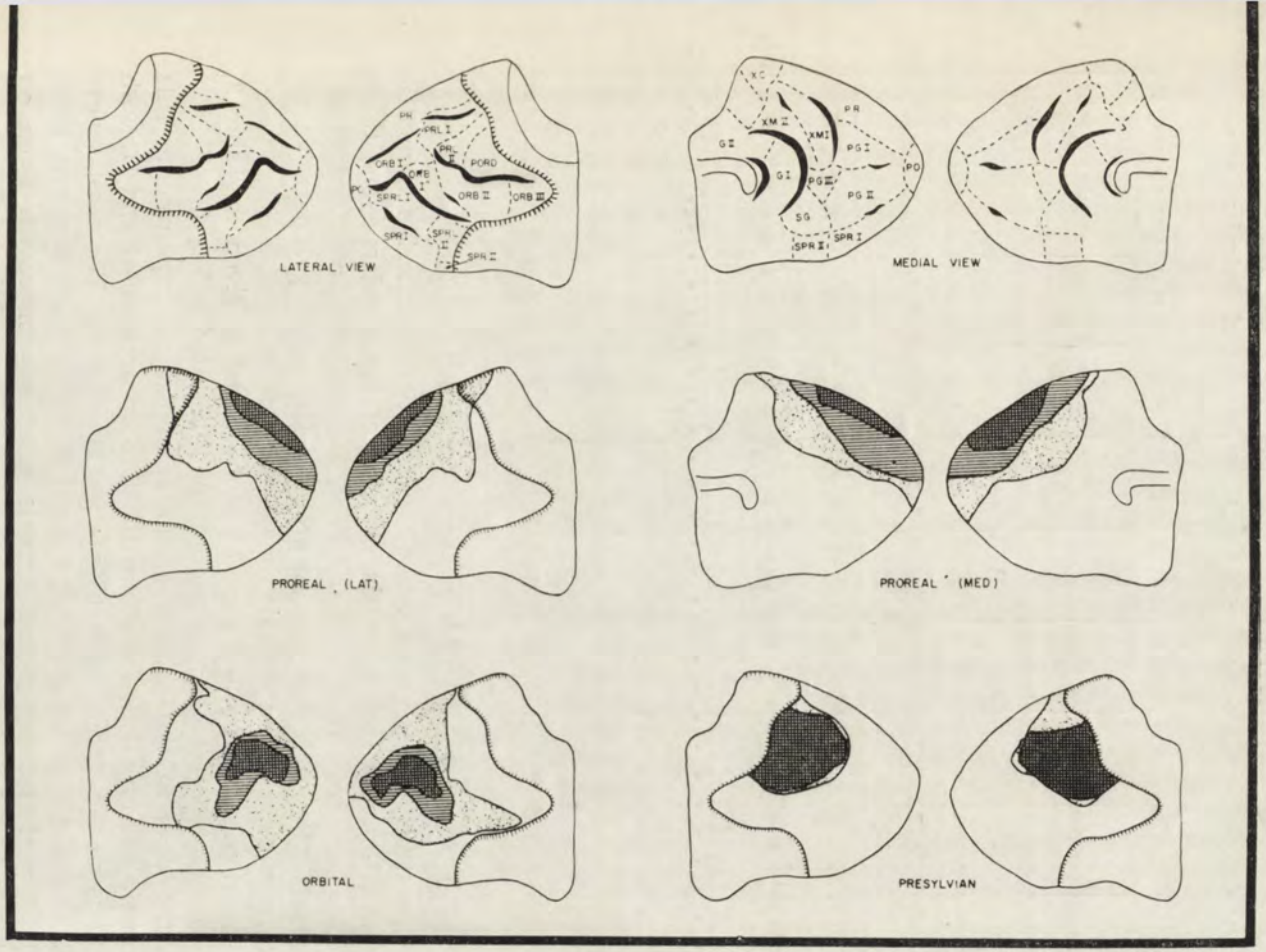

Fig. 4 Composite diagrams of the lesions in each group. Outlines of the lesion were overlapped to yield the following: (a) the inner perimeter of the overlapped lesion, which encloses the damage common to all dogs, shown by crosshatching; (b) the median perimeter of the overlapped lesions, which encloses the area damaged in a fictitious "median" dog, shown by shading (this particular composite is absent from the diagrams for the presylvian lesion, since there were only two dogs in this group); (c) the outer perimeter of the overlapped lesions, which marks the maximal extent of all lesions combined, shown by stippling

Table I

The myeloarchitectonic areas damaged in each dog. Pr = proreal; Or = orbital; Ps = presylvian. X indicates bilaterally complete, or nearly complete, damage and P denotes partial damage. See Fig. 4 for location of designated areas

	PR	POL	PG I	PRL I	PRL II	PORD	ORB II	ORB I''	ORB I'
Pr 1	X		P						
Pr 2	X	P	P						P
Pr 3	X	X	P						
Pr 4	X	X	P						P
Pr 5	X			P					
Pr 6	X		P						
Pr 7	X	X	X						X
Pr 8	X	P	P						X
Fr 9	X	P		X	P	P			
Or 1								X	X
Or 2	P						X	X	X
Or 3	P								X
								X	X
Or 4									
Or 5	P							X	X
Or 6							P	X	X
Or 7	P			P				X	X
Ps 1				X	X	X			
Ps 2				X	X	X			

Behavioral Findings

Preoperatively, all animals except three attained criterion immediately, i.e., within the first 60 trials, on each of the problems. The three exceptions were dogs Pr 5, Pr 6 and Pr 9, each of whom required 30 additional trials to reach criterion on problem III. In the preoperative retention tests, none of the animals showed any losses.

The postoperative performance of each animal is given in Table 2, and the average performance of the groups illustrated in Fig. 5.

All the dogs in the proreal group exhibited an impairment. This impairment was greater on problem III than on problems I and II, with the deficits on the latter two not differing appreciably. The most severe disturbance was seen in the first 60-trial block on each problem, after which the performance of the animals gradually improved. Even on the third block of testing, however, some of the animals in this group still failed to reattain their preoperative level of performance. It may be noted that the dogs who required additional training on problem

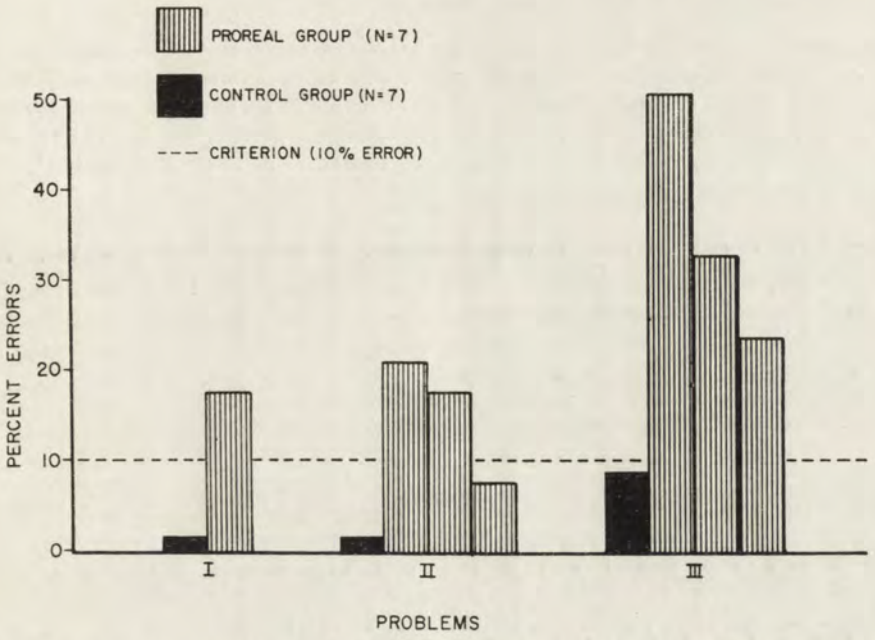

Fig. 5. Average performance of the proreal group and of the combined orbital and presylvian group for blocks of 60 trials on th three delayed response problems, Each group consists of 7 animals that received training on all three problems I = 15-sec. delay; II = 60-sec. delay; III = 60-sec. delay with intradelay feeding

III prior to operation were not consistently different from the others on any of the postoperative problems. The differences in the extent of lesions within the proreal group, noted in the preceding section, did not appear to be correlated with differences in the severity of impairment.

Of the 7 animals in the orbital group, 5 were unimpaired postoperatively. Of the two remaining animals, one (dog Or 7) showed a deficit in performance at 60-sec. delay with distraction, whereas the other (dog Or 2) was deficient even at 60-sec. delay without distraction. Evaluation of the impairment in dog Or 2 is rendered difficult by his deviant training history; in the case of dog Or 7, on the other hand, where a direct comparison with the performance of other dogs is possible, it would appear that the impairment was less severe than in any of the dogs with proreal lesions. It should be noted that both of these animals had lesions which extended into the lateral portion of area prorea; however, this area was also invaded in 2 other animals of the orbital group who showed no impairment.

Table II

Performance on three delayed response problems after selective lesions of prefrontal cortex. Scores are errors in each block of 60 trials, a score of 6 or less indicating criterion. Successive blocks on Problems II and III were presented in alternating order Pr = proreal; Or = orbital; Ps = presylvian. I = 15-sec. delay; II = 60-sec. delay with interadelay feeding. — denotes "not tested"

	Problems						
	I	II			III		
Pr 1	9	18	11	1	40	11	0
Pr 2	—	25	—	—	37	—	—
Pr 3	3	11	15	4	24	24	15
Pr 4	7	6	0	1	29	29	19
Pr 5	19	16	13	9	23	12	12
Pr 6	1	4	2	2	32	14	9
Pr 7	—	2	—	—	29	—	—
Pr 8	30	17	26	14	39	36	30
Pr 9	9	5	6	0	24	10	14
Or 1	0	0			3		
Or 2	—	33	25	11	—		
Or 3	1	2			4		
Or 4	2	2			3		
Or 5	1	1			4		
Or 6	—	1			2		
Or 7	1	0	0	0	19	17	9
Ps 1	0	0			1		
Ps 2	1	0			2		

Neither animal with removals in the presylvian area showed any postoperative disturbance.

The general behavior of the animals did not appear to be affected by the operation. Hyperactivity and accentuation of food-directed activity, observed previously in dogs with extensive lesions of the medial prefrontal area, were not observed in the animals of the present study.

DISCUSSION

Comparison of the effects of the three types of prefrontal lesions on delayed response indicates that the lesions of the proreal group were clearly the most effective in producing an impairment. Although two animals in the orbital group were also impaired, it seems reasonable to conclude that their performance was aberrant and not representative

of the effects of orbital lesions; five other animals with similar lesions were completely unimpaired, as were the two animals with presylvian lesions.

The negative findings following presylvian lesions are of interest since the results of a comparative study of autonomic functions had demonstrated that the effects of stimulating the presylvian area in the dog were similar in certain respects to those followig stimulation of the principalis area in the monkey (Delgado and Livingston 1948). The findings of the present study indicate that this similarity does not extend to the behavioral effects produced by damage to these areas. The absence of impairment following lesions of the presylvian region is compatible rather with a suggestion based on anatomical considerations that the presylvian region in the dog may be comparable to the simian arcuate region (Kappers et al. 1960) damage to which also is relatively ineffective in producing impairment on delayed response (Pribram 1955, Gross 1963, Gross and Weiskrantz 1962).

In contrast to the results obtained following orbital and presylvian lesions, all animals with proreal lesions were markedly impaired. Although there appeared to be no correlation between severity of deficit and variations of the lesions within the proreal group, the question may still be raised as to whether or not all parts of gyrus proreus are equally important for performance on delayed response. Damage extending into the lateral portion of area prorea occurred in four dogs of the orbital group, but only two of these animals exhibited an impairment. Furthermore, the animal that sustained the most damage to this area (dog Or3) was completely unimpaired. It would appear from this that a lesion of the lateral portion of proreal cortex, without involvement of the medial portion, is relatively ineffective in producing an impairment. Whether the entire gyrus must be removed, however, or whether removal of the medial portion is sufficient, cannot be determined from the data of the present study.

The second of these alternatives, namely, that the medial proreal area is particularly important for delayed-response performance, gains some indirect support from some recent work of Stępień and Stępień (unpublished experiments). In that study, severe deficits in delayed response were produced by damage to the medial segment of the precruciate region (Area XM), but not by damage to the lateral segment. Since the medial proreal area is adjacent to the medial precruciate area, it may be that the former, also, is intimately concerned in delayed-response performance, and that these two areas together comprise the

focal area for the function measured by the test. As indicated earlier, there is evidence which suggests that the focal area for delayed-response functions in the monkey is located in and around the sulcus principalis. One possible implication of the present results, then, is that the dorsomedial prefrontal area of the dog is homologous to the midlateral prefrontal area of the monkey.

It should be pointed out that the suggestion advanced here concerning the location of functionally aquivalent prefrontal areas in the dog and monkey can only be a tentative one. First, all portions of the prefrontal area in the dog have not yet been investigated. Thus, as opposed to the evidence in monkeys, which is based on a study of the effects of selective lesions throughout the prefrontal cortex, no evidence is available in dogs on the effects of selective lesions within the ventral and medial regions (i.e., the subproreal and pregenual areas). At present, the only areas which can be considered to lie outside the focal region are those which were removed in the animals of the orbital and presylvian groups.

In addition to the need for data on the unexplored regions, further behavioral information must be obtained. For example, both in dogs and in monkeys extensive prefrontal lesions lead not only to delayed response deficits but also to deficits on differentiation (Brutkowski et al. 1956, Ławicka 1957, Brutkowski 1959, 1964, 1965, 1966, Battig et al. 1962) and other go-no go discrimination tasks (Weiskrantz and Mishkin 1958, Gross 1963). If the lesion in the monkey is confined to the principalis area, however, performance on these various go-no go tasks is either unaffected (Brutkowski et al. 1963, Ławicka, Mishkin and Rosvold (1966) or affected comparatively less than it is by other partial prefrontal removals (Gross and Weiskrantz 1962, Gross 1963). The possibility that proreal lesions in the dog lead similarly to a selective delayed response impairment receives support from studies by Szwejkowska et al. (1963), Brutkowski (1964), and Brutkowski and Dąbrowska (1966) who found that lesions confined to the proreal area tended to produce little or no impairment in differentiation. While these data are suggestive, any conclusion concerning the functional equivalence of the proreal and principalis areas must await the outcome of further behavioral analyses of this sort.

Finally, the question of homologies cannot be settled without comparative neuroanatomical evidence. A start on this problem has already been made by Akert and his colleagues (Warren et al. 1962, Akert 1964), but comparative data are not yet available for the selective areas which appear to be critical for delayed response.

SUMMARY

Eighteen dogs were trained pre- and post-operatively on a three-choice delayed response task. The task was presented with short (15″) and long (60″) delays and with and without intradelay distraction. Half of the dogs received bilateral lesions of the proreal gyrus, while the others received bilateral lesions of either the orbital gyrus or the anterior bank and depths of the presylvian sulcus. All 9 dogs with proreal lesions exhibited an impairment, which was particularly severe on the trials with intradelay distraction; length of delay, on the other hand, did not appear to be a critical variable. Of the remaining 9 dogs, only two (both with orbital lesions) showed an impairment. The relationship found in this study between delayed-response deficit and proreal damage is discussed in terms of the possibility that the proreal gyrus in the dog is functionally equivalent to the monkey's principalis area, i.e., the area which is considered to be the focus for delayed-response functions in this species.

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DISTURBANCES OF MOTOR CONDITIONED BEHAVIOUR FOLLOWING BILATERAL ABLATIONS OF THE PRECRUCIATE AREA IN DOGS AND CATS

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In the last few years we focused our attention on the disturbances of motor CRs following bilateral ablations of the cortical areas lying just in front the so-called motor cortex in dogs.

It has been shown that ablation of this area produces severe disturbances in both manipulatory and locomotor CRs, as well as evident changes in the general behaviour of the animals (S t ę p i e ń et al. 1960, 1963, 1965). In all operated dogs two very characteristic disturbances were observed, i.e. conditioned motor dysreflexia or areflexia and strong food CS magneto-reaction The former symptom consisted in the disruption of the link between the alimentary CS and the instrumental response trained to it. The latter symptom was manifested by the increased tendency to run to the source of the positive (but not to the inhibitory) CS. On the basis of these results we concluded that the premotor dysreflexia was due to the interference between the magneto-reaction and the preoperatively trained motor reaction.

As for the general localisation of lesions producing this syndrome it has been shown that the crucial area lies on the medial aspect of the hemisphere just in front of the cruciate sulcus. The lesion situated in the dorso-lateral part of the precruciate area did not contribute essentially to the syndrome described (S t ę p i e ń and S t ę p i e ń 1965).

It was felt that the problem of the premotor dysreflexia and magneto-reaction requires further analysis. First, it was interesting to examine whether indeed these two symptoms are causally interrelated. Then, the

problem arose as to precise localization of lesions producing these symptoms. Lastly, it was worthwile to repeat analogous experiments in cats in order to see whether in these animals the same disorders result from similar lesions.

MATERIAL AND METHOD

The experiments were carried cut in 22 mongrel adult dogs and 3 cats in a large experimental room in which the animals could move quite freely. The experimental chamber (Fig. 1) contained the starting platform and 2 food trays situated on the left and right sides at a distance of 2 meters from the platform. The sources of acoustic stimuli (metronomes, buzzers) were placed about two feet from both food trays. The experimenter was sitting just near the platform and was

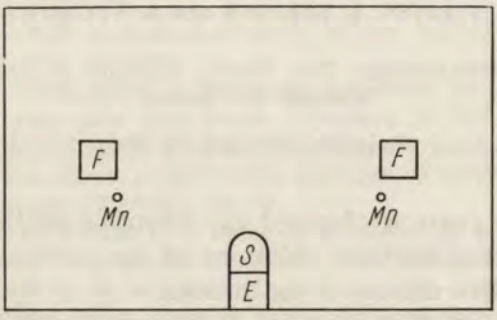

Fig. 1. Experimental setting. S, Starting platform; E, experimenter; F, food tray; M, metronomes

visible to the animal during the testing sessions. The animals were brought from the animal house into the experimental room six times a week.

During the first several days, the subjects were adapted to the experimental chamber, were given experience in eating the food, and were trained to associate the food presentation with the operation of the food trays. During these sessions the animals learnt to run from the plaform to the food tray when the food was given, this running being the response to the sound of moving bowl with the food. After this reaction was well established, i.e. the animal ran to the correct food tray without any error in 60 consecutive trials, the conditioning training proper began.

The task required the animal to run to the right food tray when the metronome from the left was given and vice-versa, to run to the left food tray when the right stimulus was in action. In the preliminary experiments, the sound of metronome was given together with presentation of food (bread and meat) in the appropriate food tray. In some trials, the stimulus was presented alone. After some days of such experimentation the animals learnt to run from the platform to one of the food trays when the CS was given. The noncorrection method was used, i.e. the food was offered only when the animal ran to the proper food tray. The food was not presented when the subject ran first to the wrong and only then to the correct food tray.

The daily experimental sessions consisted of 12 to 20 trials, the duration of the CS was 5 seconds, and the intertrial intervals lasted 60 seconds. Food was offered an equal number of times in each food tray during each session. The training was carried out until the animals attained the criterion level of at least 95 correct reactions in 100 consecutive presentations of CS and overtraining was continued for another 100 or more trials. The intertrial responses occurring in the first sessions disappeared in the course of further training. In the last period of preoperative training, a one week interval in experimentation was given and afterwards some more sessions were added.

When the training was over, all the animals were given one-stage bilateral surgical operation under general Nembutal anaesthesia (35 mg per kg of weight) and received cortical lesions done by subpial aspiration in aseptic conditions. One week was allowed for recovery from operation and then the experiments were resumed.

In the postoperative period, the experiments were performed six times a week and the CSi were presented the same number of times as before surgery in the following animals: D-3, D-4, D-7 and D-11 to D-22. The testing sessions were performed only once a week and consisted only of few trials in order to avoid retraining in the following animals: D-1, D-2, D-5, D-6, D-8, D-9, D-10, C-1, C-2 and C-3. However, when the conditioned reactions in these dogs were spontaneously restored, or when the retraining began, experiments were performed every day and CSi were presented in the same number as before surgery.

In 5 dogs and one cat (D-1 to D-5 and C-1) which were not able to solve the task during several weeks (4—6) after surgery, a special retraining was applied analogous to that used in the preoperative conditioning. In 2 dogs (D-4 and D-7) who manifested an exceedingly strong magneto-reaction this reaction had to be extinguished by a special experimental procedure. Since in the early postoperative period most animals failed to approach the food tray in response to the CSi, food was offered "gratis" several times in the intertrial interval, and only then the CS was repeated. This procedure helped the animals to perform conditioned reaction.

Extent of lesions. For the purpose of surgery all the animals were divided into two groups: In the first group including 16 dogs and 3 cats the precruciate area in the medial aspect of hemispheres was ablated (the precruciate group). The extent of lesions was limited by: s. cruciatus, s. cinguli and s. subproreus (Fig. 2). In 2 dogs (D-3 and D-10) the damage included also the dorsal part of the precruciate area. In the second group including 6 dogs different parts of the frontal lobe were removed: g. proreus in 2 animals (D-17 and D-18); g. orbitalis in one (D-19); motor area in one (D-20) and anterior part of cingulum in 2 (D-21 and D-22) — the control group. The extent of lesions in the control group is presented in Fig. 3.

RESULTS

Preoperative training showed that the task was not difficult both for dogs and cats (S t ę p i e ń, in preparation). The animals usually attained the criterion level in 20 to 25 experimental sessions. After the task was mastered, all the animals behaved in roughly the same way: during the intertrial intervals they usually waited on the starting platform and only occasionally they walked around the room sniffing objects.

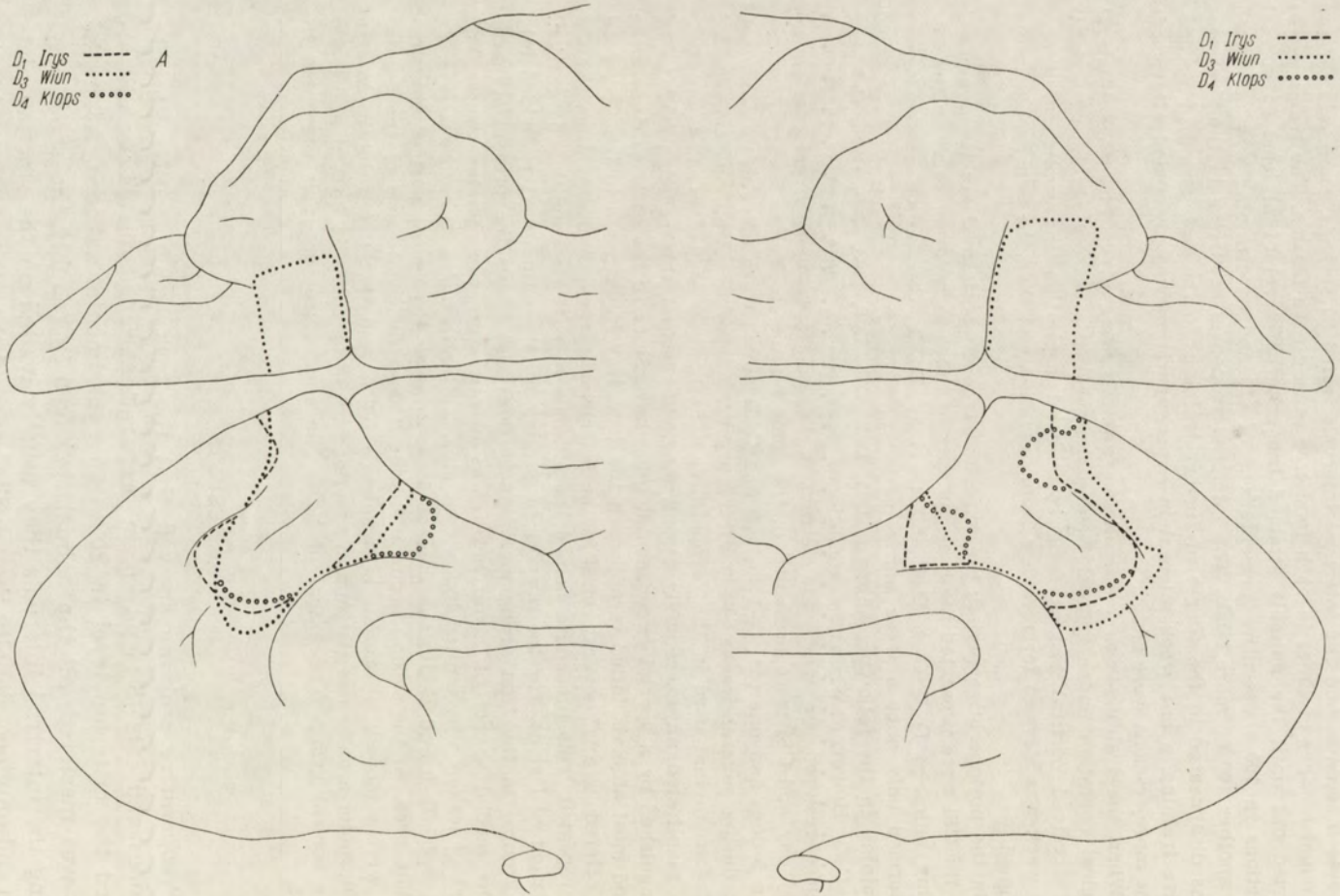

Fig. 2A. Extent of brain lesions

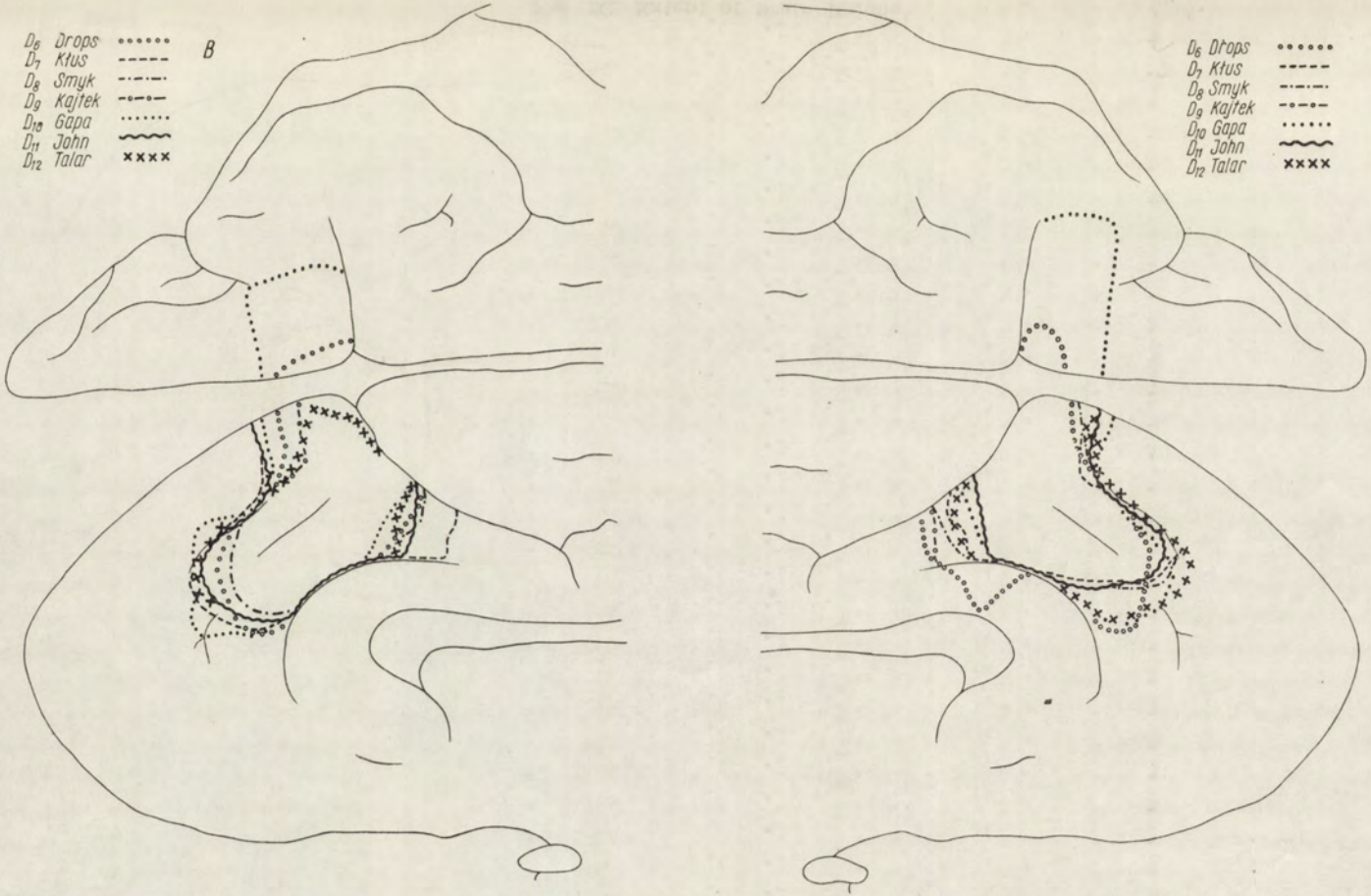

Fig. 2B. Extent of brain lesions

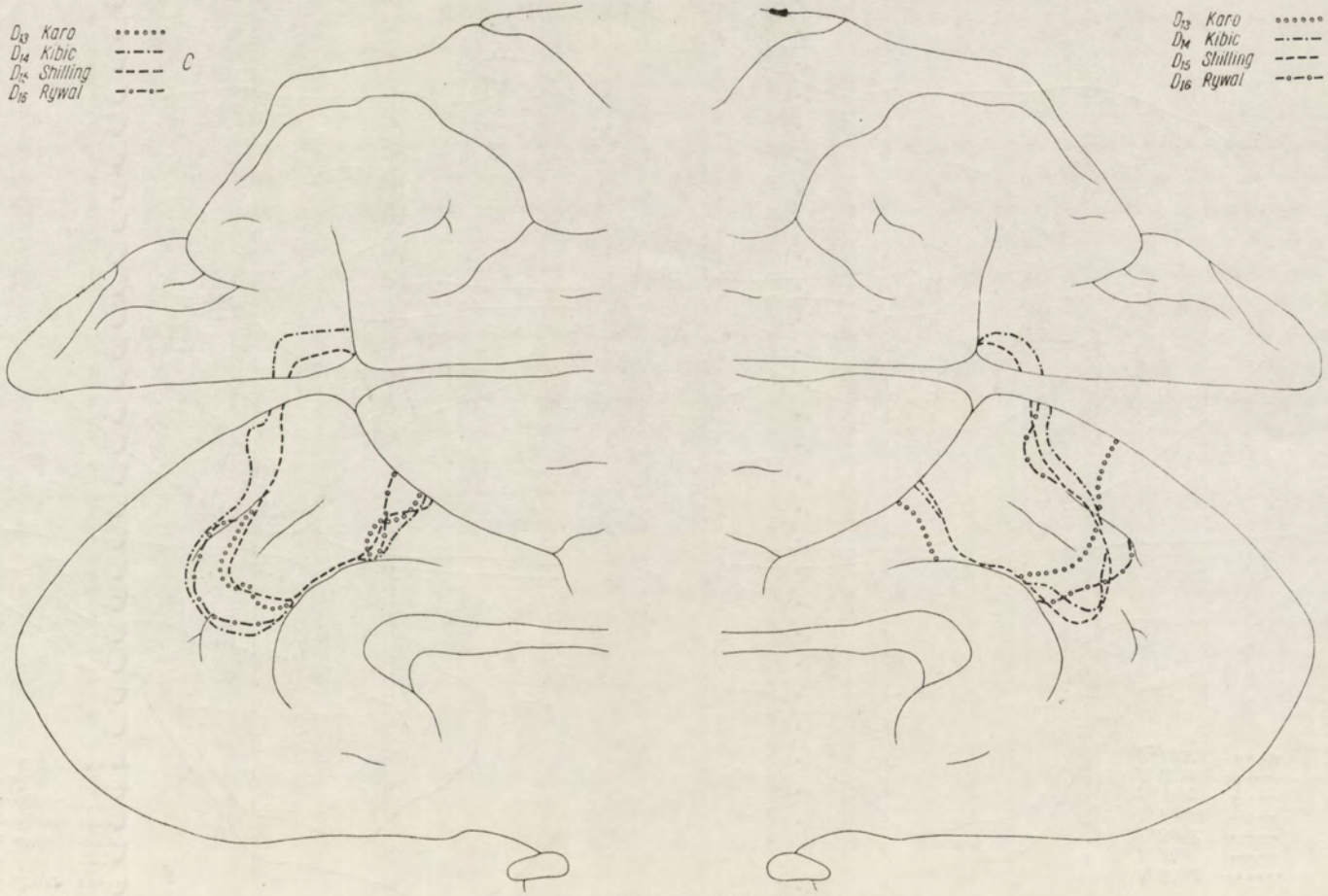

Fig. 2C. Extent of brain lesions

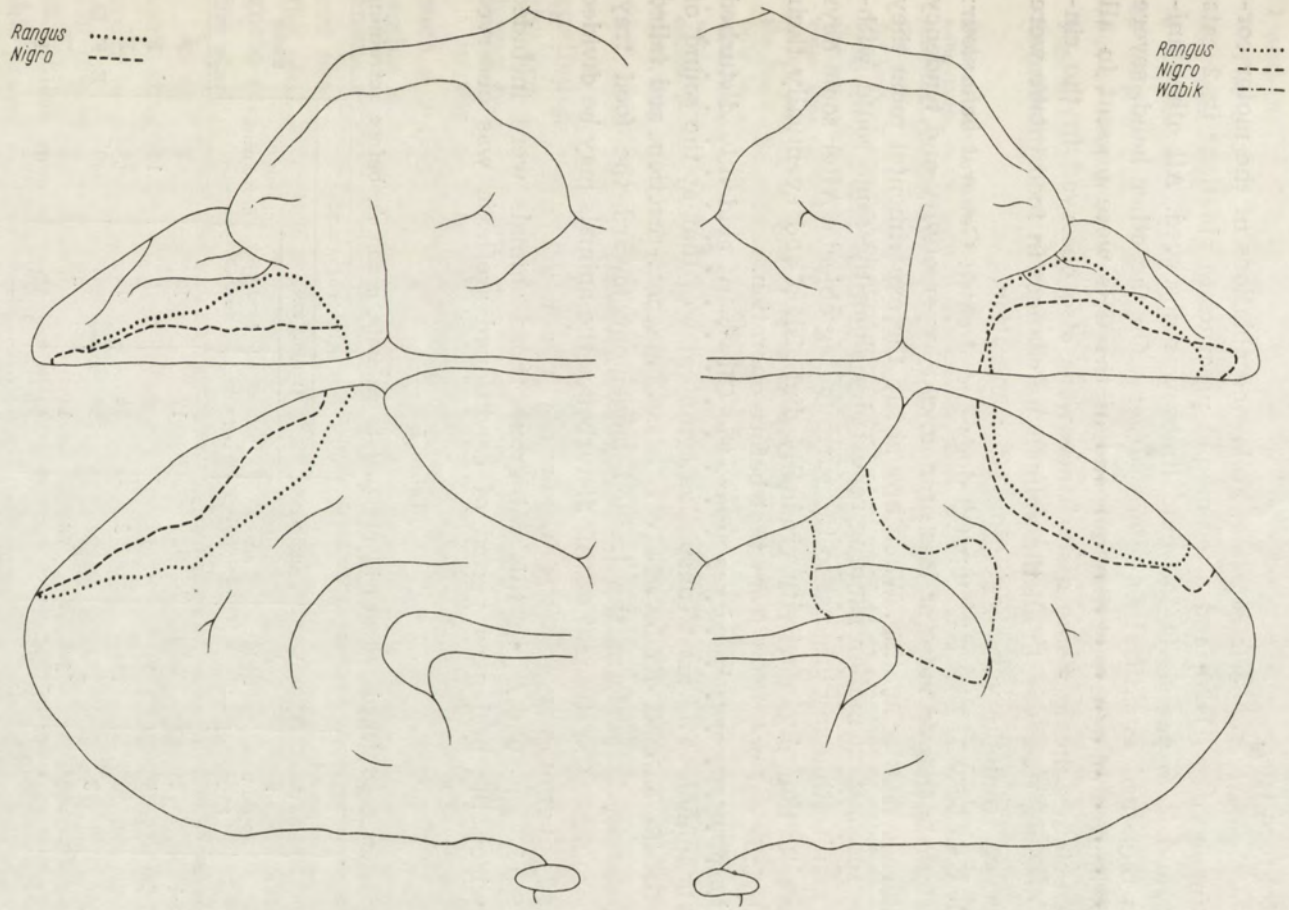

Fig. 3. Extent of brain lesions

When the CS was given the animal turned his head or only his eyes toward the source of the stimulus and ran immediately to the proper (opposite) food tray.

Postoperative experimentation. Following ablations of the motor cortex in one dog (D-20) and after medial precruciate ablations in 2 cats (C-1 and C-3) paresis in the hind limbs was observed. All other animals remained as skillful as before surgery. On the other hand, severe disturbances of general behaviour and of the CRs were present in all precruciate animals. Some disturbance were also observed in the cingulate dogs. However, in all the animals no changes in food intake were observed from the very beginning after operation.

I. Precruciate group (16 dogs and 3 cats): *General behaviour*: all animals showed more or less pronounced hyperactivity and tendency to stereotyped locomotion. When brought to the experimental room they were in constant motion running repeatedly along the same route, without manifesting any interest to the surrounding objects. After some days or weeks their hyperactivity gradually diminished and eventually their behaviour did not differ from that before operation.

Locomotor conditioned reactions: the CRs were evidently disturbed in all animals. The most conspicous symptom was that at the sound of the CS the animals displayed a very strong magneto-reaction, and failed completely to perform the trained movement toward the food tray. According to the strenght of this disturbance the animals may be divided into 3 subgroups.

Sub-group A. In this sub-group those animals were included in which the prolonged period of conditioned areflexia was observed

Table I

Locomotor conditioned reflexes in precruciate animals, group A, before retraining

Animal	period of time after operation (days)	number of trials	number of errors	analysis of errors:			tricks
				running to:		lack of movement	
				source of CS	food tray near CS		
D-1	42[a]	30	29	12	10	7	F
D-2	35[a]	14	14	4	0	10	F
D-3	30[b]	111	98	11	13	74	F
D-4	28[b]	280	271	136	85	40[c]	E, F, D
D-5	28[a]	34	28	14	7	7	—
C-1	49[a]	80	69	34	35	0	F

a, experiments were performed once a week; b, experiments were performed every day; c, lack of movement following extinction; F, food was presented several times in both food trays; E, extinction of magneto-reaction; D, the animal on leash during the CS.

and the locomotor CRs appeared only following a postoperative retraining (5 dogs: D-1 to D-5 and one cat C-1). The results are presented in Tabl. 1 and in Fig. 4.

The conditioned behaviour of 3 dogs (D-1, D-4, D-5) and the cat was very similar. When the CS was presented the animals ran immediately to the source of the stimulus and remained there sniffing the apparatus as long as the CS was in action or even longer. Very often the subject

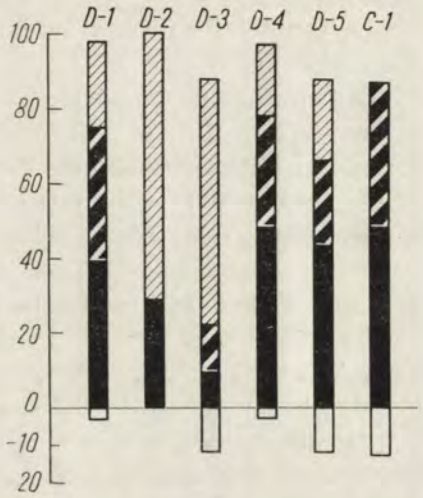

Fig. 4. Locomotor conditioned reflexes in precruciate animals, group A, before retraining

Each column represents the conditioned behaviour of one animal. Above the zero-line the erroneous reactions and below it the correct responses to CSi are indicated (in percentage). The white area in the column shows that the animal ran directly to the correct food tray; the dotted area indicates that the dog ran first to the source of CS and then to the proper food tray; the black area indicates that the animal ran only to the source of CS; the black area with white strips shows that the subjects ran to the food tray situated nearby the source of CS; the striped area shows that there was no movement and the animal remained on the plaform

continued to sniff the apparatus even while being struck in the nose by the arm of the metronome. When the CS was discontinued the animals began to walk round the room and showed no interest in the food trays.

In the course of experimentation the animals behaviour changed in such a way that in some trials they ran first to the source of the stimulus nad then to the food tray situated nearby it. In other trials the animals ran immediately to the food tray, but only to that wich was placed near the metronome. Thus, all motor reactions were directed toward the source of the CS. In the later period the animals inhibited the magneto-reaction and in some trials they did not run at all, remaining on the starting platform.

In one dog (D-4) in which the magneto-reaction after operation was very strong special training was applied from the very beginning in order to extinguish this reaction. The task appeared to be difficult and 230 presentations of CS without reinforcement by food were necessary to inhibit this reaction in 10 consecutive trials. During the course of this procedure, first to be inhibited was the movement toward the metronome, then the running to the nearby food tray, and at the last the animal remained on the platform when the CS was acting. Now, when the

magneto-reaction had been extinguished, the food was offered to the animal in both food trays several times, and then CSi were given again. It appeared that the animal began to run again to the food tray placed near the acting CS.

Then, another series of experiments was performed with the same dog (D-4). The animal was attached on the leash on the platform before the CS was presented and was released only when the CS was discontinued. This procedure helped the animal to solve the task and from then on he ran to the proper food tray even when he was not attached to the platform.

In two dogs (D-2 and D-3) the magneto-reaction to the CS was rather weak from the very beginning after operation, but nevertheless the locomotor CRs were absent. The dog D-2 sometimes ran to the source of the CS but more often remained on the starting platform or continued walking round the room as he used to do during the intertrial intervals. The second animal (D-3) when brought to the experimental chamber ran continuously round the room and didn't pay any attention to the acting CSi. However, when in the course of retraining the food was presented several times, a very strong CS magneto-reaction appeared and the dog began to run to the source of CS during it's action.

Retraining: When the period of areflexia was prolonged and there were no sings of spontaneous restoration of the CRs, the special retraining in all animals was applied: the CSi were accompanied by presentation of food in the proper food tray, and in some trials the CS was applied without food reinforcement.

The number of trials in which the CS was combined with food plus the number of errors performed by the animals before they attained the criterion level was more or less the same before and after surgery in animals D-2, D-3 and D-4, and it was smaller in postoperative retraining in D-1, D-5 and C-1 (Table II).

Table II

Postoperative retraining in precruciate animals, group A

Animal	Period of areflexia before retraining (days)	Number of trials in which CS was combined with food until the criterion	
		before operation	after operation
D-1	42	82	45
D-2	35	163	267
D-3	30	81	88
D-4	28	122	128
D-5	28	178	92
C-1	28	282	112

Thus, although the task appeared rather difficult for precruciate animals al subjects attained the criterion level.

S u b-g r o u p B. To this sub-group 7 dogs (D-6 to D-12) and cats (C-2, C-3) were included. In these animals the conditioned areflexia changed into dysreflexia without postoperative retraining. However, in all animals but one (D-8) the CRs appeared only when special procedures were applied. The results obtained in this group of subjects are presented in Tabl. III and Fig. 5.

During the period of areflexia the animal's reactions to the CSi were much the same as in subjects of group A. When the CS was given the animals usually ran to its source or to the food tray situated near it. Very often they sniffed the apparatus even when the arm of metronome struck them in the nose. In the later period after surgery the subjects

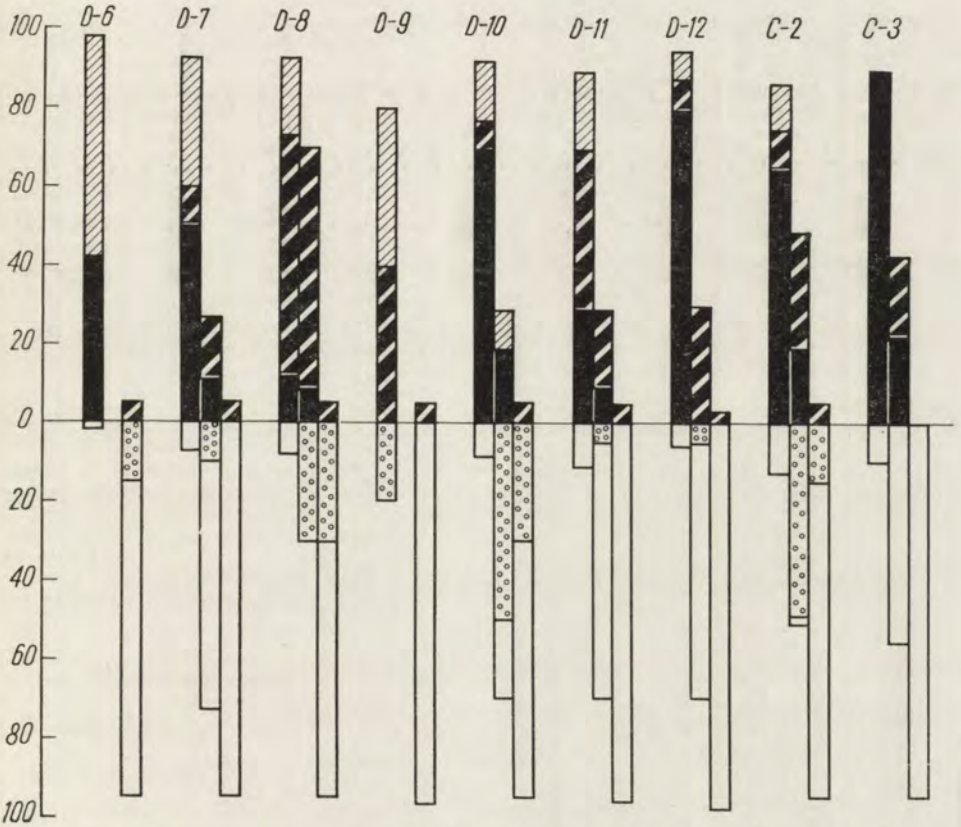

Fig. 5. Locomotor conditioned reflexes in precruciate animals, group B

Each block represents the conditioned behaviour of one animal in the period of areflexia (first column), dysreflexia (second column) and criterion level (third column). Note that there was no period of dysreflexia in dogs D-6 and D-9. Other connotions as in Fig. 4.

more-or-less-often inhibited the running toward the source of the CS and remained on the platform. Sometimes the animals ran to the correct food tray but this happened very rarely (once in many trials). Although these correct responses were reinforced by food it did not help them to react correctly in the next trials.

In all animals but one (D-8) the procerdure of offering the food in both food trays during the intertrial intervals was applied. To the sound of the moving bowl the subjects always responded with running immediately to the proper place. Following several presentations of food the areflexia changed into dysreflexia, i.e. when the CS was given the animal ran to

Table III

Locomotor conditioned reflexes in

Animal	Areflexia						
	period of time after operation (days)	number of trials	number of errors	analysis of errors: running to: source of CS	analysis of errors: running to: food tray near CS	analysis of errors: lack of movement	tricks
D- 6	33[a]	46	45	20	0	25	F
D- 7	29[b]	298	280	153	33	94	E, F, D
D- 8	28[a]	62	57	8	37	12	—
D- 9	26[a]	51	40	2	18	20	F
D-10	20[a]	43	39	30	3	6	F
D-11	15[b]	145	130	47	56	27	F
D-12	14[a]	40	38	32	3	3	F
C- 2	28[b]	30	26	20	3	3	F
C- 3	14[b]	20	18	18	0	0	F

a, experiments were performed once a week during the period of areflexia; b, experi- during last experimental session of this period; E, extinction of magneto-reaction; D, the

Table IV

Locomotor conditioned reflexes in

Animal	Areflexia					
	period of time after operation (days)	number of trials	number of errors	analysis of errors running to: source of CS	analysis of errors running to: food tray near CS	analysis of errors lack of movement
D-13	7	10	10	10	0	0
D-14	7	10	10	3	2	5
D-15	-------	-------	-------	-------	-------	-------
D-16	-------	-------	-------	-------	-------	-------

the food tray, but very often he approached that situated near the source of CS. The number of errors was very high: 27 to 30 per cent in dogs D-7, D-10, D-11, D-12, near 50 per cent in cats C-2 and C-3, and about 70 per cent in dog D-8 in which the trick with food was not applied.

The correct responses performed by animals in this period differed very much from those in normal animals. In some trials the subjects ran first to the source of the CS or looked for several seconds toward the CS and only then they turned to the correct food tray.

After a shorter or longer period of dysreflexia the conditioned responses of animals gradually improved and the subjects attained the crite-

precruciate animals, group B

Dysreflexia						To criterion level	
period of time after	number of trials	number of errors	analysis of errors			period of time after operation (days)	number of errors in 100 consecutive trials
			running to:		lack of movement		
			source of CS	food tray near CS			
— — —	— — —	— — —	— — —	— — —	— — —	34—45	5
30—35	52	14	6	8	0	36—45	5
29—31	35	25	3	22	0	32—40	5
— — —	— — —	— — —	— — —	— — —	— — —	27—37	5
21—42	170	49	32	17	0	44—55	2
16—17	40	12	1	11	0	19—28	5
15—21	80	24	0	24	0	22—30	2
30—40	70	34	13	20	1	40—50	5
15—20	77	33	18	15	0	22—30	5

ments were performed every day; F, food was presented several times in both food trays animal on leash during the CS.

precruciate animals, group C

Dysreflexia						To criterion level	
period of time after	number of trials	number of errors	analysis of errors			period of time after operation (days)	number of errors in 100 consecutive trials
			running to:		lack of movement		
			source of CS	food tray near CS			
8—10	40	11	11	0	0	12—17	0
8	20	3	1	2	0	10—16	0
7—10	60	30	6	22	2	11—17	5
7—9	56	26	5	15	6	10—17	0

rion level. However, some of them still ran first to the source of the CS and then to the food tray (D-8, D-10, D-11, C-2).

Sub-group C. — This sub-group included the animals in which the periods of areflexia and dysreflexia were very short (D-13, D-14) and those in which areflexia was not observed at all and dysreflexia was short (D-15, D-16). The behaviour of these animals was similar to those belonging to group B (Tabl. IV and Fig. 6). All the dogs displayed a more or less pronounced magneto-reaction consisting in running only to the source of the CS or to the nearby food tray. When improving they began to run towards the opposite food tray and thereafter they turned to the proper one. About 2 weeks after operation the criterion level was reached by all animals.

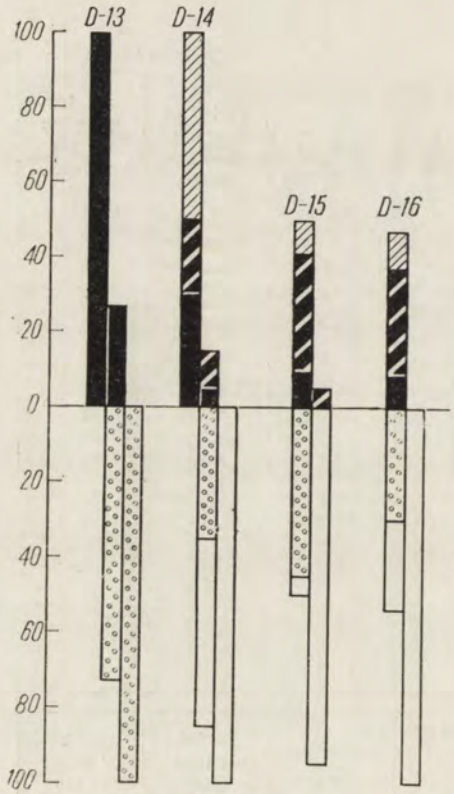

Fig. 6. Locomotor conditioned reflexes in precruciate animals, group C

All connotations as in Fig. 5. Note that there was no period of areflexia in dogs D-15 ond D-16.

II. Control group — the effects of other lesions. In 6 dogs different parts of the frontal lobe were bilaterally removed in one-stage (Fig. 3). No disturbances were observed following such ablations as removal of g. proreus (D-17, D-18) g. orbitalis (D-19) or motor area (D-20). All these dogs responded to the CSi in the same way as before surgery.

Slight impairment of conditioned reactions was observed in 2 dogs (D-21, D-22) in which the anterior part of the cingular region was damaged. In the two first postoperative sessions the animals in some trials ran to the wrong food tray, or remained on the platform. On the third day all their conditioned responses were correct.

DISCUSSION

In our previous studies on the effects of precruciate ablations on the alimentary motor conditioned behaviour in dogs the preoperative training was much simpler and easier than that which was used in the present investigation. In the first experiments (Stępień et al. 1960, 1963) the subjects were trained to put the right foreleg on the food tray in response to the CSi acting in front of the animal (instrumental CRs). In a later series of experiments simple locomotor CRs were established to acoustic stimuli in a special experimental room (Stępień and Stępień 1965). The animals were trained to run from the starting platform to the food tray situated in front of it; the sources of the CSi were placed on the left and right side at a distance of 2 meters from the platform. In the latter experimental conditions the disturbance of motor conditioned behaviour in form of CS magneto-reaction was clearly demonstrated in precruciate animals. However, as the locomotor CR was very simple it returned spontaneously after a short period of postoperative experimentation. In order to make the precruciate syndrome even more demonstrable and longlasting the preoperative conditioning in the present experiments was more difficult and the locomotor CR was more complicated: the animals were trained to run to the right food tray in response to the CS acting from the left side and to run to the left when the stimulus to the right was in action. Indeed, in such experimental conditions the precruciate syndrome appeared more clearly distinctly.

In almost all animals the magneto-reaction, consisting in running toward the source of the CS or to the food tray nearby, was very pronounced and longlasting. In some subjects this reaction was so strong that it kept the animal sniffing the apparatus for a long period of time. This was most clearly seen in trials in which the CS was kept operating as long as the animal was sniffing its source (30—50 seconds). The results of the present study support our previous opinion that magneto-reaction is strongly connected with the positive alimentary CSi. In fact, in dogs D-2 and D-5, the magneto-reaction was not seen during the period of areflexia; however, when retraining was applied and the CSi

were reinforced by food several times, a very strong and prolonged magneto-reaction appeared.

The other precruciate symptom — a disapearance of well established locomotor CRs — was also clearly manifested. In 6 animals the CRs didn't return spontaneously even after a long period of time and their retraining lasted almost as long as the preoperativ conditioning. In 13 subjects the CRs returned after a shorter or longer period of time but often the animals had to be prompted by occasional presentations of food or by being released from the platform only after the CS was discontinued.

It is worthwhile to mention that althgough the paralelism between magneto-reaction and conditioned areflexia was noticed in many precruciate animals, nevertheless, in some subjects it was not observed. For example, dog D-2 showed a very week magneto-reaction and a longlasting areflexia. On the contrary, in dog D-13 the period of areflexia was very short, while the reaction toward the CS was strong and longlasting. Thus, these two symptoms seem to be separate though closely interrelated.

The data presented in this paper seem to show that the magneto-reaction and conditioned areflexia are specific phenomena connected with removal of the medial precruciate region. Ablations of other areas, lying in the vicinity of precruciate cortex (prefrontal or motor), did not provoke such disturbances. Thus, bilateral removal of the dorsomedial part, of the prefrontal area which produces severe impairment of delayed responses (Ławicka and Konorski 1959) and pronounced disinhibition of inhibitory CRs (Brutkowski et al. 1956) does not produce either magneto-reaction or dysreflexia. Only lesions in the anterior part of the cingulate area resulted in a very slight impairment of CRs which disappeared after a very short period after operation. Those disturbances may be explained by the fact that the lesions included the cortical areas lying in the immediate vicinity of the medial precruciate region.

It seems that there exists a correlation between the extent of a lesion in the medial precruciate area and the strength of the provoked syndrome. For example, in dog D-2, in which the lesion was deliberately more deeply performed, both symptoms, i.e. magneto-reaction and areflexia were more clearly manifested and lasted for a longer period of time than in other animals.

The data obtained in the present study do not confirm our previous assumption that the symptom of areflexia may be explained by the interference of two antagonistic reactions, i.e. magneto-reaction and con-

ditioned reaction. Indeed, in these experiments it was found that the locomotor CR may be performed in spite of the existence of a strong magneto-reaction. Three dogs (D-2, D-10 and D-13) and all three cats (C-1, to C-3) solved the task in such a way that they ran first to the source of CS and only then to the proper food tray. Moreover, in two dogs (D-3 and D-7), in spite of the extinction of the magneto-reaction, the CRs didn't appear.

Thus, the question arises again as to the mechanism of conditioned areflexia. The possibility that the animals are not able to perform the CR because of their motor incapacity or some changes in drive for food may be ruled out. In fact, no motor impairments as well as no changes in food intake were observed following the precruciate lesions. It seems, therefore, that the most reasonable explanation of conditioned areflexia is a damage of connections between brain representations of the CS and motor CR. Different degree of impairment of CRs may be explained by the more or less severe damage to these connections within the precruciate area. This area seems to play the same functional role in motor alimentary conditioned behaviour in cats as in dogs because quite similar disturbances were observed in both species of animals after precruciate lesions.

Although the precruciate area is very important for motor alimentary CRs its function is not indispensable for their performance since the CRs improved in all precruciate subjects either spontaneously or after a special retraining. The cortical or subcortical brain structures which mediate this compensation is not known.

SUMMARY

The locomotor conditioned reflexes were established in 22 dogs and 3 cats. The experiments were performed in a large room with two food trays. The animals were trained to run to the right food tray when the metronome from the left side was given and to run to the left when the right stimulus was in action.

In 16 dogs and 3 cats the medial precruciate area was removed bilaterally. Following this lesion the CRs were more or less disturbed in all animals. To the CSi the subjects displayed a strong magneto-reaction (running toward the source of the CS or to the food tray nearby) and failed to perform the trained movement toward the food tray (areflexia or dysreflexia). In 6 animals with longlasting areflexia a special retraining had to be applied in order to restore the CRs (sub-group A). In 13 subjects the CRs returned spontaneously after a shorter or longer

period of time but often special procedure were applied which helped the animals to performe the conditioned reaction (sub-group B and C).

Bilateral ablations of g. proreus (2 dogs), g. orbitalis (one dog) or motor area (one dog) did not disturbe the conditioned behaviour of animals. Bilateral lesion in the anterior part of the cingulate area produced a very slight disturbances of CRs which disappeared after 2 or 3 experimental sessions.

It is concluded that the magneto-reaction and conditioned areflexia or dysreflexia are specific phenomena connected with removal of the medial precruciate area. The possible mechanisms of these disturbances are discussed.

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STEREOTAXIC METHODS IN HUMAN BRAIN SURGERY

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Since the separation of the neurosurgery as an independent medical discipline attempts have been made to employ operative procedures in the diseases of the nervous system in which the prophylactic treatment was insufficient or failed at all. This has concerned the diseases with the following symptoms: (1) disturbance in volitional movements and occurrence of involuntary movements instead, (2) tremor of rest, considerably intensified with volitional movements, (3) intensional tremor, often intensified with a volitional movement to such an extent that the performance of this movement is impossible at all, and (4) abnormal tension in different groups of muscles, causing the loss of the movement smoothness, the occurrence of movements performed in a stepwise manner and, in extreme cases, resulting in excessive muscular tensions and, consequently, in a considerable pain, accompanying the attempts to overcome these tensions; this eventually leads to a complete immobility or to distorted positions of the head, extremities and the entire body.

Since the etiology and pathogenesis of many diseases of this type have been unknown and most of them have been associated with similar symptoms, they could hardly be classified. However, with the advance of the clinical and pathomorphological knowledge, the causes of these diseases have been related with some abnormalities within the central nervous system. Nevertheless, the anatomopathological changes, found in the brain, have often been so diversiform that they could hardly be regarded as a real cause of a given disease. On the other hand, in some entirely defined nervous diseases, no macro- or microscopic changes have been found in the brain. These ambiguities delayed the introduction of neurosurgical treatment, although in many injury or tumor cases, in

the encephalitic and post-encephalitic states and, finally, when measures had to be taken for the relief of intractable pain, the operative approach has occurred to be appropriate. A search after surgical methods which could bring relief to the patients who were ready to some sacrifices to get rid of an incessant tremor or excessive hyperkinesias has been continued. In 1937, K l e m m e, and later, B u c y (1943) removed the premotor and motor areas with complete relief of the involuntary movements. However, some side effects also followed this operation. These were a paresis and a considerable spasticity of extremities. The unsatisfactory results of cortical operations induced a series of surgical treatments at the spinal cord level (C-2 pyramidotomy). Unfortunately, this has also turned out unsatisfactory. Hereafter, operative procedures have been applied to the cerebellum, peripheral nerves and sympathetic nervous system but they did not exert a beneficial effect on the intensified tension and involuntary tremor either. To sum up, all efforts with the use of the "classical" neurosurgery, proved to be unsatisfactory in treating the diseases of the so-called extrapyramidal system.

In recent years, the increasing interest in neurophysiology has allowed one to consider the possibility to apply — for the neurosurgical purposes — the stereotaxic method which has been successfully used in animals. The problem was to reach a "point" within the brain so as to be able to intervene in any subcortical structure without the necessity of a classical craniotomy. Thus, the idea has been developed of applying the stereotaxis to man. It turned out, however, that the neurophysiological experience arising from the experimentation on animals could not be entirely utilized in the human clinic.

For a successful use of the stereotaxy the following three factors have had to be considered: (1) neuroanatomy, (2) instruments, and (3) neurophysiology. Firstly, it was necessary to have a system of "marking" brain structures, making up the target of treatment. The difficulty has arisen with the localization of intracerebral structures which varies individually to a considerable extent. Secondly, proper instruments were required to reach intracerebral structures. Finally, neurophysiology has been a factor of control. The stimulation and electrophysiological recording methods were aimed at showing whether or not the electrode or the probe, introduced to the brain, was implanted in an appropriate place. If not, its position was to be changed and only then, the lesion, planned in advance, might be carried out.

The first stereotaxic treatment was applied to man by Spiegel et al. in 1947. It aroused a great interest in all neurosurgical centers all over the world. Many ideas have been introduced of constructing ever new types of stereotaxic instruments and stereotaxic atlases for man have been published. Currently, the stereotaxic therapy has been introduced to many neurosurgical clinics. In this country, we have also started to apply this type of treatment. The stereotaxy which has been used in Poland is based on methods which underwent and stood the test of time, their results being favorable. The values, brought in by this new surgical trend, together with different methods of applying it, deserve much interest and, therefore, the introduction of the readers to these problems and, in particular, to the most recent achievements in the field of the stereotaxy, appears to be advisable.

Anatomical marking of intracerebral structures

The anatomical marking of intracerebral structures is, for a neurosurgeon, of a fundamental importance. To accurately locate any point in the brain, a system of references is to be used. The X-rays control the accuracy. There are different types of marking, as: (1) a system of marking on the bone of the skull, the "bony" system; (2) a system of marking, based on cerebral structures (Talairach et al. 1954 and most authors who apply them nowadays); (3) a system of combined marking (Spiegel et al. 1951, Spiegel and Wycis 1952, Cooper 1961, 1962). A system of combined marking may concern a single structure or a small group of structures.

The system of "bony" marking refers to only one intracerebral structure. Anatomical variability of the brain vs. the skull restricts an accurate marking. Considering the morphological differentiation of the skull and, consequently, the deficiency in the bony system of marking, Delmas and Pertuiset (1952, 1959) have published an atlas of the craniocerebral topometry and worked out a combined (osteocerebral) method of marking, destined for some brain structures.

The cerebral marking has been based on anatomical points, situated close to the deep cerebral structures looked for. The calcified pineal gland and the anterior commissure were the first points of reference on the basis of which the first atlas of the stereotaxic anatomy was prepared by Spiegel and Wycis (1952). Talairach's (Talairach et al. 1957) system of marking has been presented in a very clear manner in his atlas of the stereotaxic anatomy. It is based on the system of the basal line and auxiliary lines between the anterior and posterior com-

missure (Fig. 1): (1) The basal, bicommissural line CA-CP separates the thalamic from the hypothalamic region. (2) The top line, perpendicular to the CA-CP line, passing through the anterior commissure. (3) The top line, passing through the posterior commissure. This system of marking allows one for a general orientation as to the situation of the basal

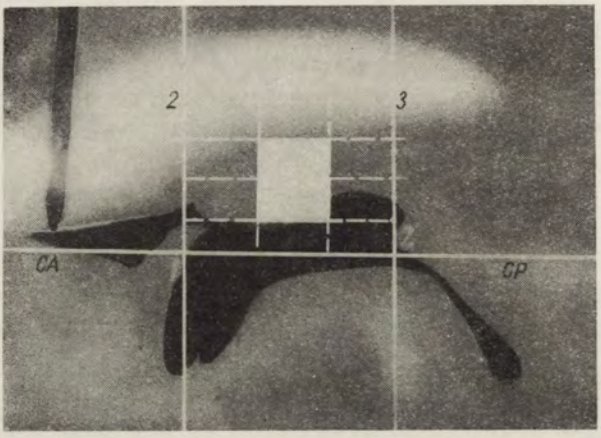

Fig. 1. Marking system of basal ganglia according to Talairach

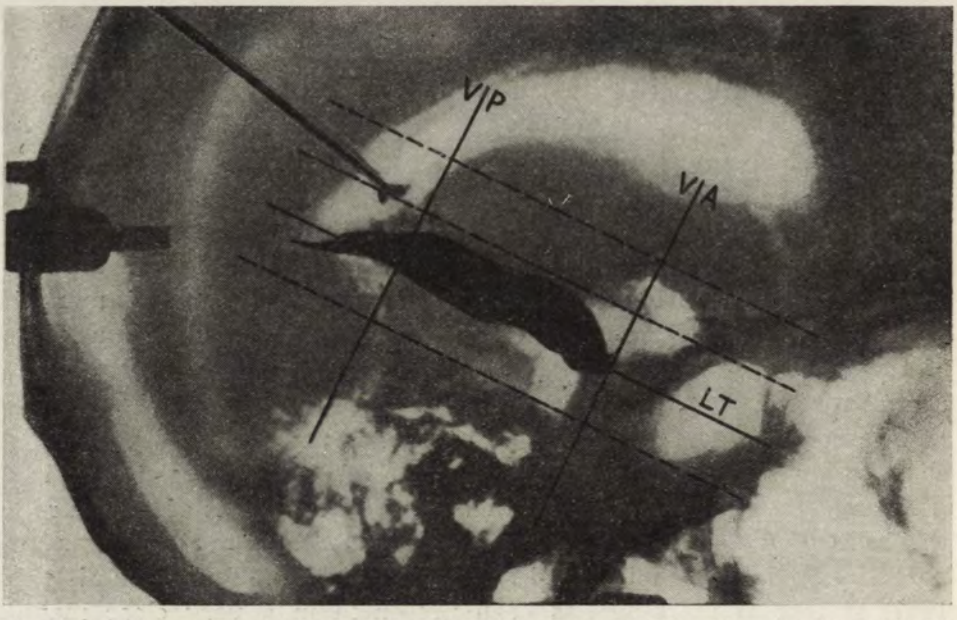

Fig. 2. Marking system of the structures of the temporal lobe according to Talairach
LT, basal line and auxiliary line, based on the lipiodoloventriculography of the temporal horn

ganglia. It also allows one for a proportional localization of the thalamic nuclei in the case of an increase or decrease in the mass of these nuclei. This system of proportional marking may also comprise deep brain structures, located outside the thalamus, as well as other areas of the brain hemispheres. However, for the structures within the temporal (Fig. 2) and parietal lobes, separate systems of marking have been prepared by T a l a i r a c h et al. (1958). Although, with regard to the localization of the electrode in a point in the brain, the anatomical marking does not yield an absolute accuracy but the performance of an operation is possible, under these conditions. The accuracy is increased by a control in the form of the electrophysiological recording or electrical stimulation of intracerebral structures to evoke motor or sensory effects. Unfortunately, the present state of knowledge of the neurophysiology of man does not allow one for obtaining adequate responses from many deep structures of the brain. In view of this fact, the localization of the position of the electrode is, for the most part, based on the system of anatomical marking. The physiological tests make up an auxiliary checking factor.

Stereotaxic methods and instruments

The Guiot method (G u i o t and B r i o n 1953) and apparatus are adapted only to the treatment of diskinesias. The instrument (Fig. 3) consists of a holder and a mobile part. The bone fixer has three points of attachment to the skull in the medial line which, considering the pneumocephalographic picture, makes up a medial plane of the brain. The mobile part has two extensible slideways which revolve on a cylindrical tube, fastened perpendicularly to the holder. Both

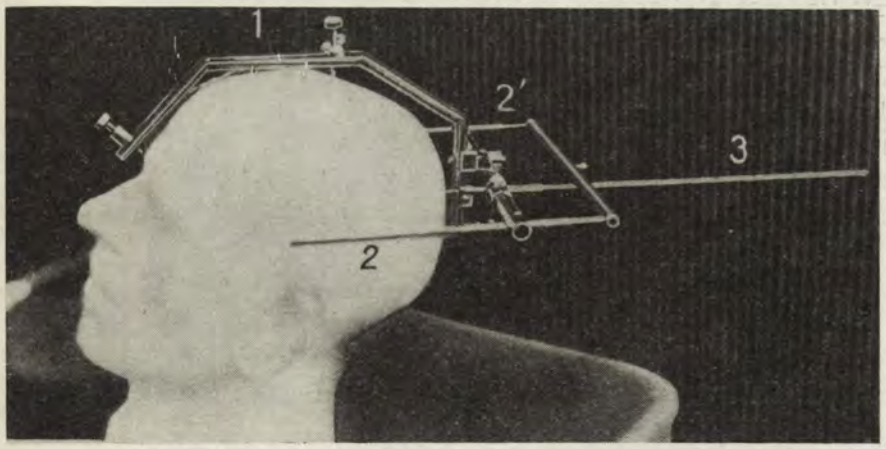

Fig. 3. The Guiot instrument for the treatment of dyskinesias
1, holder; 2, mobile part; 3, coagulating probe

slideways always shift simultaneously in the horizontal plane and draw, also during their revolutions, two planes parallel to the medial plane of the brain. The anteroposterior and angular (upwards and downwards) shifting of the slideways allow one to find the position of their end pieces in the X-ray picture at any point of the skull. The position of the coagulating probe is determined by a final disposition of the slideways and their superposition is verified by a picture, viewed in a shining amplifier and on an X-ray plate. The treatment is usually carried out in two stages. The electrophysiological part of the treatment has recently been extended by G u i o t and his associates (1962). The experience, acquired by them, allows one for an accurate estimate in which nucleus of the thalamus the probe tip is placed, this being accurately recorded by the instrument and, likewise, for an accurate determination of the range of lesion, made in the VL nucleus.

The S p i e g e l and W y c i s (1962) method and instrument (Fig. 4). The base of the apparatus facilitates the gradation of the electrode position in point 0 which is either perpendicular to the horizontal plane of the instrument or is determined by the intersection of the biauricular and sagittal lines. The focus-film distance amounts to 115 cm. The rays are concentrated in the sagittal or biauricular plane. The ratio of real dimensions, read from the instrument scale to the dimensions,

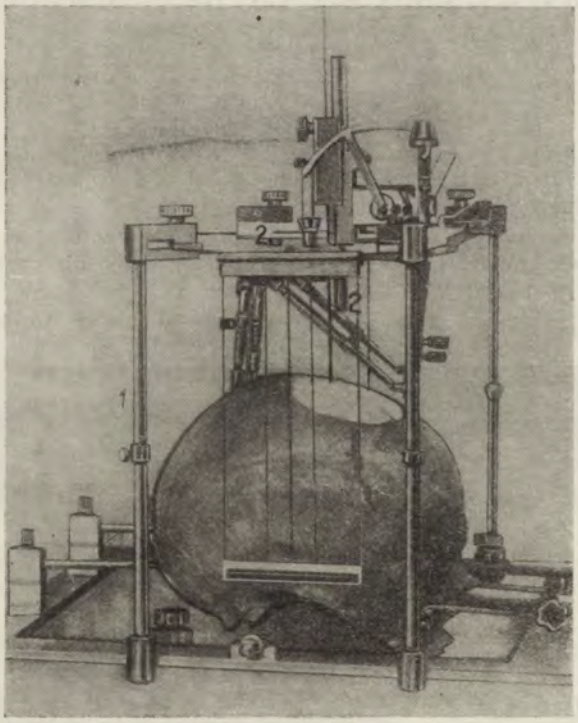

Fig. 4. The Spiegel and Wycis instrument (recent model)

1, frame for putting on the instrument; 2, an instrument with electrode which after fastening to the bone, allows one to remove part 1

obtained on the X-ray plate makes up a coefficient of magnification for all structures in the sagittal plane. The marking of structures is carried out on the basis of a pneumoencephalographic picture of different parts of the ventricular system and of the calcified pineal gland, most frequently, however, in the posterior commissure — Monro's foramen relation. The calculations are either relatively simple, or complex, depending on the apical or oblique approach way. More complex calculations are, in particular, required by the latter. An atlas, containing the cross sections through the brain, adapted to the needs of this method, serves as an aid.

The Leksell (1957) method and instrument (Fig. 5). This instrument consists of a calibrated arch whose ends are connected with a metal frame, fastened to the skull. A sliding device which moves along the axis of the arch facilitates concentrating the probe or rays in the medial point of the arch. In addition, the arch may revolve forward and backward without changing the convergence with the point aimed at in the brain. The X-rays, fall perpendicularly on the backslide from a distance of 80 cm. In this case, the coefficient of magnification depends on the distance between the plane, taken into account and the plane of the plate. The marking of structures is carried out on the basis of an encephalographic picture with consideration of different orientation points of the ventricular system, in particular, how-

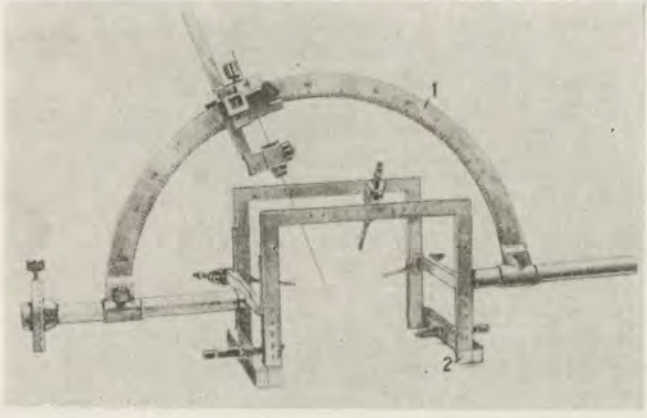

Fig. 5. The Leksell instrument (recent model)
1, calibrated arch with electrode; 2, instruments frame

ever, of the anterior and posterior commissure of the brain. A scale, mounted on the edge of the instrument frame facilitates a direct calculation of coördinates. Both the anteroposterior and vertical coordinates are settled on photographs. Identical operations but carried out by means of frontal photographs and frontal sections of the brain (from the atlas) allow one to settle the transverse coördinate.

The Riechert (1961, 1962) method and instrument (Fig. 6). In this method the coördinates for a point within the brain in a three-dimensional system are determined by: (a) the plane of a ring which makes up a base of the instrument, (b) the biauricular plane, (c) the sagittal plane. In preparing the radiograms, the central ray should always be placed at the level of the ring and, simultaneously, oriented in the diagonal anteroposterior and biauricular axis. Centering the bundle of rays is helped by optical viewfinders on both sides of the ring and on the tubing of the X-ray tube. The distance of the X-ray unit from the focal point amounts to 3 m.

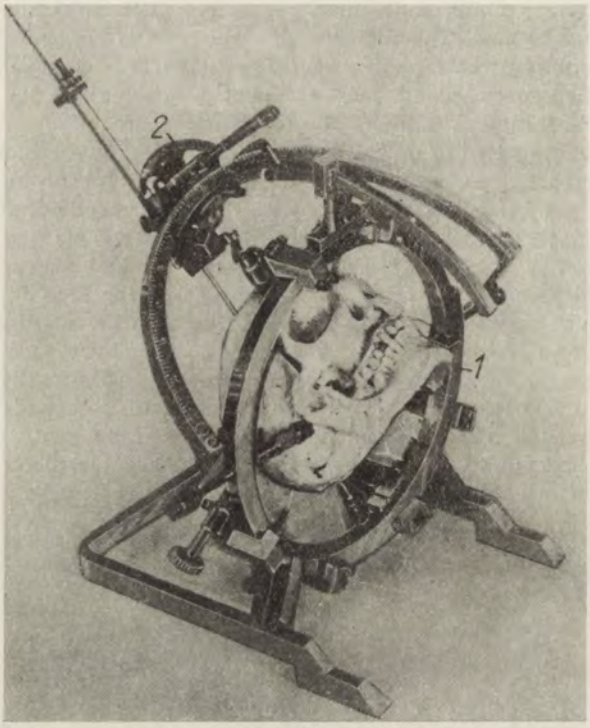

Fig. 6. The Riechert instrument (new model)
1, basal ring; 2, stereometer with electrode

The comparative analysis of the magnitudes obtained and real ones which are determined in relation to the correct scale on the instrument may be made on the encephalographic picture of the skull. The magnitude coefficient obtained amounts to $Mc = \frac{10\ cm}{11\ cm} = 0.91$. Now, by means of this coefficient it is enough to settle all lengths, measured on the film in the frontal and lateral projection, to obtain the real dimensions of a given brain.

The Remond et al. method (1958) and instrument (Fig. 7). The instrument is furnished with a special optical device and a stereotaxic frame, corresponding to the operational frame. The phantom treatment is carried out by means of the optical device. The scattering of light beams and the focal point — film holder distance provide conditions similar to those under which the X-rays are used. The marking of structures and location of the electrode takes place on the phantom photographs. Here, it should be born in mind that the shadow of the electrode, projected on the film through the light beams, should be superposed upon the radiological marking. Thus the correction of the scattered X-rays takes place automatically with the correction of the scattered light beams.

The Cooper method (1961, 1962). At present, this method is used in Poland with the stereotaxic instrument somewhat modified. At first, there were three points of attachment to the skull in the instrument. Since this did not assure a sufficient stability, following other authors' example, an instrument with a base having four

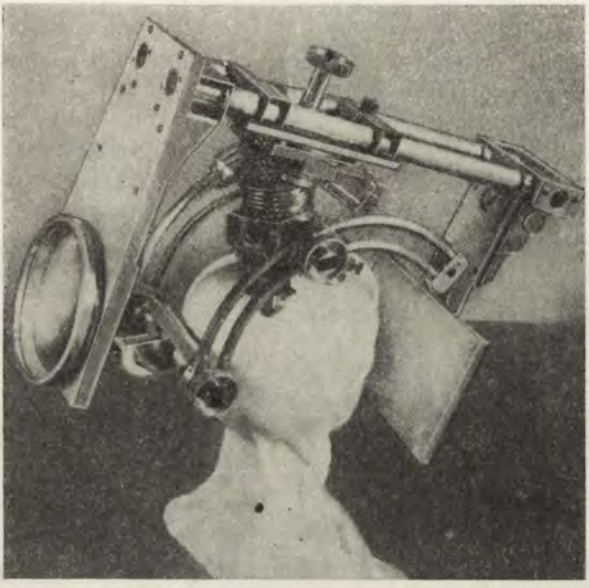

Fig. 7. The Remond and Houdart stereotaxic instrument (recent model)

points with which it was attached to the skull was applied. The film holders with X-ray plates are placed under the head and at the side of the instrument base. After making sure that, in the X-ray picture, the head is placed in a symmetrical position, the holder, also having four propping points, is put on the skull. This device is similar in working to the stereometer. The anteroposterior and profile pictures are taken by the X-ray unit from a distance lesser than 1 m. The position of the freezing probe is found radiologically on the basis of the encephalographic picture and of the systems of references of the ventricular (Monro's foramen, posterior commissure, pineal gland) and osseous (for instance, the ridge of the sella turcica) elements.

The T a l a i r a c h et al. method (1955, 1958) and instrument (Fig. 8). The instrument, now in use for stereotaxic treatments on hemispheres and basal ganglia is the most recent version of the 1948 original. It consists of a quadrangular stereotaxic, duralumin frame, of a view finding system for the teleradiography and of a set of auxiliary instruments, adapted to various treatments. The basal frame allows one to apply a certain number of different instruments for radiological marking or for both the uni- and bilateral treatment of different regions of the brain. The instrument is also provided with devices which facilitate its repeated use, always under identical conditions in relation to previous applications. The latter is particularly important in the cases of many-stage or bilateral operations. The head is attached to the instrument in four points, the fixers being deeply embedded in the skull bone and reaching always the same depth. On the side of the quadrangular frame and on its posterior edge, there are screws which keep the X-ray film holders in position. The optical view finders serve for finding the optimum position of the X-ray unit in relation to the patient's head. Marking structures in the contrast picture of the brain is facilitated by double metal networks which, in different

positions, are applied to the instrument frame. An accurate superposition of the meshes of these double networks assures a parallel trace of the X-rays with a distance between the plate and the X-ray tube amounting to 450 cm. The X-ray tube, taking the anteroposterior pictures of the skull always from an identical distance, moves along a metal arch. Its optimum adjustment is controlled in the picture of a TV monitor. The application of two X-ray tubes which facilitates frontal and lateral view pictures of the skull being taken almost simultaneously, considerably cuts down

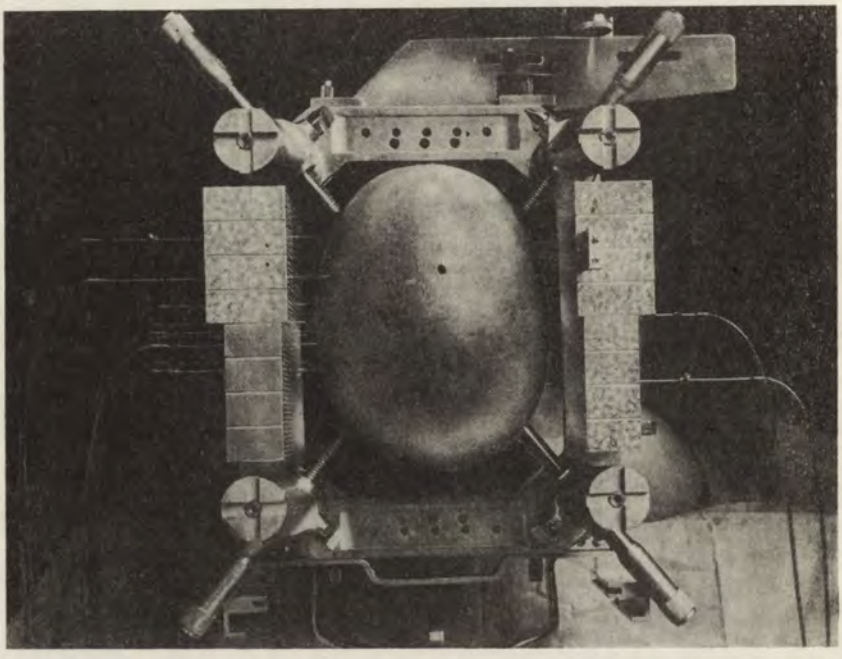

Fig. 8. The Talairach instrument for treatments of brain hemispheres

the duration of the treatment. A single X-ray tube may also be used. In such a case, however, it is necessary to change the patient's position together with the entire operating table in relation to the X-ray tube. Marking deep brain structures is carried out by means of a light (fractional encephalography) or heavy contrast (lipiodol). The lipiodoloventriculography produces a very distinct picture of the ventricular system of the brain. It helps avoiding doubts and difficulties met with by some authors who base their work on marking cerebral structures in the pneumographic picture. In some types of treatment, the necessity occurs of yet more complex contrast examinations. They ae carried out in the following oder, the arteriography, the fractional encephalography, the lipiodoloventriculography. The superposition of the X-ray plates in these contrast examinations supplies us with many data, indispensable for a deep exploration of the brain.

The Talairach preoperative and operative techniques affords a possibility of applying also preliminary calculations, made on the phantom table, as well as allows one to use the stereometer and to treat simultaneously different regions of both brain hemispheres.

This brief review of the most frequently used modern stereotaxic methods allows one to evaluate different manners in which they have been solved by particular authors.

The relatively simplest solution of the stereotaxic method has been found by Guiot. Its assumptions, however, as well as the possibilities, related with the instrument, introduced by this solution, predispose the Guiot method to the treatment, restricted only to the thalamus and the globus pallidus. A relatively wider range of operations is offered by the Riechert, Leksell, Spiegel and Wycis methods which are based on the use of the stereometer. However, they require fairly laborious angular calculations, necessary to place the electrode in a point, aimed at, with consideration of the three-dimensional spatial system of the brain. In addition, the existence of the magnification coefficient in the radiograms of the skull should be taken into account which also requires the conversion of all magnitudes of the structures, we are interested in, on these pictures.

The Talairach method deserves particular attention. Owing to the application of the teleradiography, that is, a technique in which the bundle of X-rays runs parallel when reaching the X-ray plate, the radiological pictures obtained correspond to real dimensions of the skull and brain. This considerably facilitates marking structures on contrast pictures of the brain in a direct image and thus, decreases the possibility of committing errors in accurate localization of even the smallest intracerebral areas. Furthermore, the Talairach instrument, like none other, richly furnished with different accessories and auxiliary instruments, provides possibilities of a most versatile performance of a variety of stereotaxic operations.

Means and methods of stereotaxic lesions

It is extremely difficult to carry out a perfectly demarcated lesion in a non-uniform structure which occupies small space and is irregular in shape.

Mechanical lesion. Moniz (1936) was the first to apply the leucotome to lesions of this type. Obrador and Driessen (1958) have made mechanical lesions by means of a metal loop, revolving in a cannula. The lesions, thus obtained, are 1 cm. in diameter. The possibility of a hemorrhage from the blood vessels, mechanically injured, and, consequently, different types of side-effects, make up the drawback of this method.

Chemical lesions. The anesthetics of the novocain group or the alcohol are the most often used means of lesion which are, however, irre-

gular in shape. On account of non-uniform diffusion of these substances, the dimensions of lesions cannot be envisaged. Moreover, the damaging agent frequently happens to return through the cannula track. With these disadvantages in mind, a wax-oil suspension with an admixture of novocain has been applied by Narabayashi (1956). At a temperature of 45°C, this mixture is liquid and may easily be injected, while at the human body temperature, it acquires a pasty consistency. A lesion 1 cm. in size is produced by a single injection. A mixture of alcohol with ethyl-cellulose, the latter making difficult the diffusion of the former, has been used by Cooper (1960). In addition, Cooper's cannula has been provided with two tubes and, at its end, a small balloon, filled by the injection of a contrast agent. The balloon has played a triple role. Filled with a contrast medium, it has allowed one to check the correctness of the place in which the lesion was intended. The swelling of the balloon has yielded a temporary physiological lesion which might serve as a test and, moreover, it has supplied the space for the injected medium, performing the chemical lesion. For more than four years now, a system of freezing the brain tissue has been used by Cooper (1962). We shall discuss it below.

Lesions by physical agents. The stereotaxic lesions of the brain are carried out by most neurosurgeons by means of the electric current, the D.C. and the high frequency current being the most often used. The dimensions of lesions of this type are difficult to envisage with a full accuracy. This has been shown by experiments which yielded contradictory results. The propagation of the current is different and depends on a medium, for instance, the propagation in the white substance differs from that in the grey matter and it takes place in contact with blood vessels in a different manner than in spaces, filled with a liquid. It also depends on the type of electrodes and of the current supplying device. Spiegel and Wycis (1962) prefer electrolytic lesions by means of the D.C. with the use of a unipolar electrode. However, the high frequency current is applied by most neurosurgeons who, in the majority of cases, use an instrument of the surgical electrocoagulation with an amount of the current consumed for lesions empirically tested on the basis of the coagulation capability peculiar to a given instrument. The dosage of current from these sources is not, therefore, exactly the most accurate. This was the reason why a control by means of the thermoelectric element has been introduced by Leksell (1957). It indicates the temperature between two electrodes with the application of a high frequency current. This temperature fluctuates between 55 and 60°C. The dimensions of the lesion depend on the electrode implantation direction and on the length of its uncoated tip. Wyss (1960) makes use of

a coagulator which displays an amount of the energy emitted, depending on the resistance of a medium in which the coagulation takes place. Despite this apparently satisfactory device, the shapes of lesions, even with the application of bipolar electrodes, not always coincide with each other under experimental conditions. At present, Wyss' coagulator assures, however, the most stable results of lesions, performed by means of the electric current.

The ultrasonic lesions. As shown by experiments, made by Fry et al. (1954), a lesion of any size may be performed in a definite region of the brain by means of a concentrated bundle of ultrasounds. These experiments have been made use of by Meyers and associates (1959) who performed similar lesions in man, suffering from parkinsonism and diskinesias, and obtained satisfactory results. However, the skull bones and even the dura matter, make up an obstacle in the penetration of ultrasounds into the brain and, therefore, the classical craniotomy and even opening of the dura are necessary. Although Leksell (1957) has tried to avoid craniotomy, applying ultrasounds on the side of the temporal bone, where the bone layer is very thin, the dosage of ultrasounds has not been accurate under such conditions. In the light of these considerations, this method requires continued experiments.

Lesions by ionizing radiation. There are two methods of applying this radiation to obtain lesions within the brain tissue. One of them consists in the action in situ in the form of the radioactive isotopes and another — in the form of radiation sources placed outside the skull. In the latter method, it is important to determine the depth of the radiation action. The accelerators of alpha particles, protons and deuterons (synchrocyclotrons) serve as radiation sources. The bundles of radiation may be 1 to 2 mm in width, may converge on a given structure and have several input directions. The lesions of this type have been performed by Leksell (1961) (thalamotomy) and Tobias (1961). By means of a stereotaxic instrument and a crossed bundle of protons, a lesion 3×7 mm in size and with 20,000 rads in the point aimed at has been performed by Leksell (1961). The lack of any physiological control in this method and the impossibility to display the place of lesion in the X-ray picture have been pointed out by the critics of lesions of this type. The method of external irradiation requires a very expensive equipment and is still in the experimental stage.

The application of *radioactive isotopes* is of great importance to stereotaxic lesions. Two types of isotopes are used, one, with a pure beta radiation and another, with a mixed beta-gamma radiation. The isotopes with the beta radiation have a short but definite range of action and,

therefore, they are used in the functional stereotaxic neurosurgery which requires definite sizes and shapes of lesions, planned in advance. The functional neurosurgery comprises the treatment of parkinsonism, diskinesias of different types, intractable pain, psychosurgery and surgery of the epilepsy, in particular of the temporal epilepsy. The element Yttrium (Y^{90}) is a representative of the group of isotopes which emit beta rays. The energy of the beta particles corresponds to 2.4 MeV and, therefore, it is advantageous as a rapid radiotherapy. With a 1 mC grain a necrotic dose, equalling approximately 100,000 rads, reaches a distance of 3.5 mm. One mm deeper, the nervous tissue remains unchanged at all. A period of a partial disintegration of yttrium amounts to 64 hrs and the lesion of the tissue takes place, in practice, during this period. This type of slowly performed lesions is especially important when elderly people are operated. The Y^{90} isotope is used in the form of cylindrical grains 1.13 mm in diameter. These cylinders are 3 to 5 mm long and have a porous surface which allow them for a close adherence to the tissue, and, at the same time, prevents them from changing position. The fact that they are well-contrasting on the X-ray plates, which facilitates the control of the grain position, also makes up one of their advantages. A space, destroyed by a grain of Y^{90} 5 mm long and 1.13 mm in diameter, is strictly determined and amounts to 0.8 cu cm. This considered, one may easily calculate a wider range of lesion, combining the action of a few isotope grains.

The radioactive isotopes, emitting mixed beta and gamma rays, present identical advantages, as well as identical dangers with those, corresponding to the X-rays. The point is here that late, secondary changes occur at a certain distance from the implanted isotope grain. The isotopes with a mixed emission are used for destroying cerebral tumors.

The interstitial Curie-therapy has been developed as a treatment method, supplementary or, in some cases, completely replacing the classical neurosurgery in oncology. The cerebral tumors, developing in such areas, as the motor region, the vicinities of the Sylvic fissure, or the parieto-temporal region of the dominant hemisphere, cannot, in practice, be removed surgically. There are also such tumors which develop towards the basal ganglia and which are only partially accessible to the surgical treatment. In these cases, no satisfactory results may be obtained by the external irradiation with the X-rays since the greatest doses, applied at present to the cerebral tumors, fluctuate between 6,000 and 10,000 rads which is insufficient for a full destruction of the neoplasm. The application of a large necrotic dose in situ which completely destroys the tumor is therefore, a more suitable and effective method. This

may be obtained owing to radioactive isotopes with a mixed radiation, directly introduced to the tumor (the interstitial Curie-therapy) by the stereotaxic method. The isotope of the radioactive gold, Au^{198}, is a representative of this type of therapy. The period of its partial disintegration amounts to 2.7 days. It emits beta rays with an energy, equalling 0.96 MeV and gamma rays with 0.41 MeV. The doses of a single treatment reach millicuries. This isotope, with a high degre of activity, is used in the form of threads and is rather inexpensive. A short period of its radiation allows one to leave it over permanently in the tumor tissue where it represents no danger to the patient. The element iridium, Ir^{192}, is also a well-tried isotope, suitable for the purposes of the interstitial Curie-therapy. It is used in the form of wires 5 to 15 mm long and 0.5 mm in diameter. Its single dose should not exceed 50 to 70 mC. Iridium emits an energy, equalling 0.66 MeV and its spectrum of gamma rays exceeds the action of the radioactive gold. The application of the radioactive iridium is, according to Reichert, tolerated by the body system of both elderly people and small children. The optimum action of this isotope is recorded after 4 to 5 weeks.

Other radioactive isotopes such as, those of phosphorus, tantalum, strontium, palladium and cobalt have also been applied to the interstitial Curie-therapy. However, different inconveniencies, related with the use of these isotopes, have caused them to be gradually discarded by most authors.

It is worth mentioning that most neurosurgeons use the radioactive isotopes only for the treatment of the brain and hypophysis tumors, while the electrocoagulation is most often applied to the stereotaxic lesions. It is of interest, however, that Talairach et al. (1961) have based their stereotaxic method entirely on the application of the radioactive isotopes, assuming that, for the purposes of functional neurosurgery, Y^{90}, producing strictly limited lesions which may easily be controlled radiologically, is the best damaging medium, while all indispensable requirements of the interstitial Curie-therapy are successfully met by the isotope of radioactive gold, frequently, in combination with the isotope of iridium.

Cryonecroses are lesions, caused by freezing of the brain tissue. The first experimental cooling of the brain has been performed, as early as in 1950, by Baumgartner and Balthasar (Balthasar 1957). However, it was only in 1961, that this technique acquired a greater importance in practice when applied by Cooper to his stereotaxic method. The cryosurgical lesions, performed within the brain, have definite dimensions, resulting from two functions, a degree of a lowered temperature at the tip of the cooling probe and a duration of a period during which

the tissue is frozen. The cryosurgical lesions are advantageous since they do not cause cerebral hemorrhages. Moreover, in the initial stage of the tissue cooling to a certain limit, they also produce a reversible cutoff of the brain functions. This is a sort of physiological checking test. In the case of an unsatisfactory result of such a test, cooling may be interrupted without any neurological disturbances and, after changing the position of the cooling probe, cooling of an appropriate brain structures may be resumed once more. The lesions of this type are gaining an ever increasing number of followers in the U.S.A., Italy and France. In the last 3 year period the cryosurgical lesions by the Cooper stereotaxic method have also been performed at the Department of Neurosurgery of the Polish Academy of Sciences in Warsaw.

Indications of the stereotaxic treatment of the brain and hypophysis

The indications of the stereotaxic treatment have become ever more extensive. The first treatments have been performed in patients who suffered from Parkinson's disease. The range of these interventions has gradually been extended to other diseases: (1) Within the range of functional neurosurgery, these treatments comprise: (a) Parkinson's disease; (b) dyskinesias as, athetosis, ballismus intentional tremor (congenital cerebellar tremor, tremor of the medial line, writers' cramp) and myoclonias; (c) dystonias as, torsion spasm, torticollis, dystonia musculorum progressiv deformans and idiopathic distortion of the spinal column; (d) intractable pain (of the central origin); (e) temporal epilepsy; (f) psychosurgery.

Within the range of Parkinson's disease, the best prospects are presented by the postencephalitic or post-traumatic cases, particularly those with the rigidity of the abduction type and the periodical tremor, with the contralateral associated movements retained and without the psychical disturbances and disturbances in the body equilibrium. These treatments are best born by middle-aged patients or, at any rate, those who are below 65, although the possibility cannot be excluded of applying this treatment even to persons over 70.

Stereotaxic surgery for pain. From the standpoint of both the theoretical considerations and curing practice, stereotaxic surgery for pain is an open question. All kinds of classical neurosurgical treatment, performed below the thalamic level, fail to bring any relief to the patients. It was but natural that quite justified hopes have been aroused of applying the stereotaxic methods to relieve pain.

There are two types of stereotaxic treatment for the relief of pain: (1) the interventions on the thalamic nuclei, performed in many stereotaxic neurosurgery centers; (2) the interventions on the corticothalamic fibers, within the internal capsule at the level of the Sylvic fissure. These types of treatment have been initiated by T a l a i r a c h (1959).

Stereotaxy in temporal epilepsy. In contradistinction to typical cases of the temporal epilepsy, displaying focal EEG changes in temporal regions, there are other cases in which the clinical symptomatology is also temporal but the epileptogenic foci may be located outside this region. In such cases patients are not relieved of epileptic fits after surgical interventions in temporal regions. Considering the difficulties in the localization of these foci, as well as revising a former approach to the Jacksonian epilepsy, T a l a i r a c h and his associates (1958) elaborated a stereotaxic method of examining the activity of the brain which makes up a neurophysiological approach to the surgical treatment of epilepsy. According to the assumptions of T a l a i r a c h and his group, each form of this disease constitutes a focal epilepsy. The aim of this method is to discover the epileptogenic foci, existing in different regions of the brain which, so far, have been undetectable. This system of examination consists in recording, by means of depth electrodes, the action potentials. These examinations are carried out after an accurate anatomical marking of a patient's brain by means of contrast methods. The data, obtained from this stereoelectroencephalographic exploration (SEEG) are considered from the standpoint of the possibilities of surgical treatment.

Stereotaxic psychosurgery helps to perform lesions of the frontothalamic pathways. The range of these lesions depends on the disease and aim of the treatment. In psychalgia, persistent pains with a distinctly psychical component, as well as pains resistant to all other manners of treatment, anterior cingulectomy (Fig. 9) is performed. In psychotic patients with attacks of rage and aggression, cingulate leucotomy is performed with the section of the frontothalamic fibers. In contradistinction to the frontal leucotomy, generally applied, this type of treatment does not evoke clear-cut changes in the patient's personality. Lesions are made uni- or bilaterally.

In *neurosurgery of the hypophysis*, the indications concern:

(a) endocrine syndroms, produced by chromophobe adenomas, acidophylic adenomas, craniopharyngiomas and those associated with Cushing's disease;

(b) hypophysectomy in the cases of the cancer of the breast, in selected cases of the cancer of prostate and thyroid, producing secondary me-

tastases on bones, in the cases of malignant exophthalmus, in hypophysic thyroidic disorders, as well as in the cases of a grave and drug-resistant diabetes.

The classical neurosurgical operations of hypophysis have been made to remove tumors associated with vision or endocrine disorders. More recently, hypophysectomy has become a treatment for the cancer of the breast, forming secondary metastases on bones. These operations have yielded a fairly high mortality rate. In addition the postoperative course of the disease has often been very trying for the patients due to the trauma and edema of the frontal lobe, as well as due to hypothalamic disorders. Therefore, a surgical approach has been worked out to the hy-

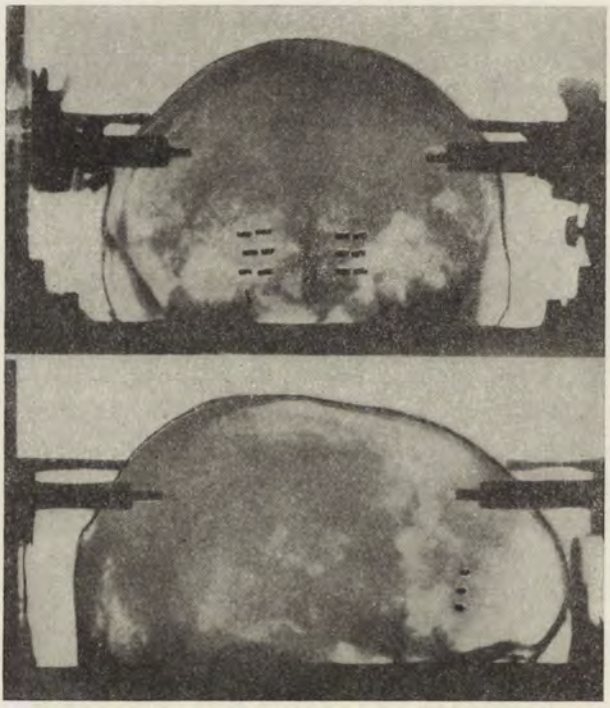

Fig. 9. Anterior cingulectomy; distribution of isotopic grains in the frontal lobe

pophysis through the nose and the bottom of the sella turcica. With the application of the stereotaxic apparatus, this way of approach is incomparably advantageous for the patient. In most cases of the tumors of hypophysis, even those inaccessible to the classical neurosurgery through its backward expansion from the cella turcica, the performance of a stereotaxic treatment does not afford any considerable difficulties. The stereo-

taxic hypophysectomy by means of the radioactive isotopes of Y^{90} is more radical than that, performed by any other method.

The instrumental problem in the stereotaxic surgery of the hypophysis has been solved in different manners. Usual stereotaxic instruments, based on stereometric principles have been used by some authors (Riechert and Mundinger (1961), Spiegel and Wycis (1962) and others). Others as, Talairach et al. (1962a, b) or Rand et al. (1963) have used for this purpose specially constructed stereotaxic instruments (Fig. 10). The stereotaxic interventions in the hypophysis are born by the patients very well. They are bloodless, their postoperative course is undisturbed and the duration of the treatment itself is considerably shortened.

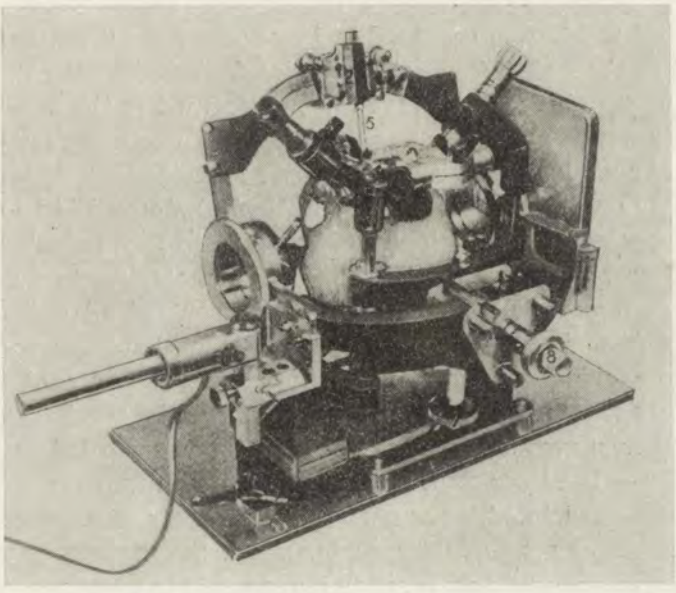

Fig. 10. The Talairach stereotaxic instrument for treatments of hypophysis

The stereotaxic treatment is primarily applied to the slowgrowing *tumors* in the medial region of the brain, to the tumors which infiltrate various areas of the dominant hemisphere and to those whose course is marked by scanty symptoms but their removal is frequently followed by serious disorders. This treatment is also applied to the tumors, developing on the brain base, inaccessible to classical surgery. In such cases, by the stereotaxic method, radioactive isotopes may also be introduced to the brain to destroy the tumor.

DISCUSSION ON THE STEREOTAXIC SURGERY

On the basis of Cooper's material which, to 1960, comprised 1,000 cases, the mortality rate amounted to 2.4 per cent (and, after introducing cryosurgical lesions, it dropped to 0.8 per cent). A favorable effect of treatment on the muscular rigidity in cases of parkinsonism was recorded in 80 per cent of the total number, the involuntary tremor retreated in 60 per cent of all cases and the improvement in the capability of performing alternate movements reached 70 per cent of all patients operated. In cases in which the symptoms were noticed only on one side of the body, the satisfactory results were still more numerous, reaching — in the range of the muscular rigidity and involuntary tremor — 90 per cent of all cases. Of the postoperative complications, hemiparesis amounted to 3 per cent, disorders in sensory functions — to 6 per cent and disorders in speech, after bilateral treatment, occurred in 10 per cent of all patients and were short-lived in character. Cooper's statistics deals only with the treatment of the globus pallidus and the thalamus, performed in the cases of parkinsonism, dyskinesias and muscular dystonias. In their January, (1963a, b) statistics, Mundinger and Riechert state that a total number of stereotaxic operations, performed by them, amounted to 1,891. Of 477 operations, performed on patients suffering from Parkinson's disease, the mortality rate amounted, in their operative material, to 2.69 per cent. The permanent paresis remained in 0.8 per cent of all cases, while the secondary effects in the form of pneumonia or vascular blocks, which attacked only elderly persons, were found in 2.9 per cent of patients. The improvement in the cases of muscular rigidity reached 88 per cent, of this number, a very evident improvement was recorded in 49 per cent and a distinct — in 31 per cent. In the cases of involuntary tremor improvement was observed in 91.5 per cent of all patients and, in 42.4 per cent of them, the tremor completely disappeared. Such state caused at once a general improvement in performing movements in 59 per cent of patients and a distinct improvement in speech in 55 per cent of all cases of parkinsonism subjected to neurosurgery. After operative treatment, temporary disorders of speech were observed in the form of dysarthria or aphasia in 3 cases but, after 6 weeks, these disorders disappeared. Of 76 patients on whom 110 treatments were performed to remove hyperkinesias and dystonias, very good results were obtained in 23 cases of involuntary movements, in 3 cases of hemiballismus in 11 cases of athetosis and in one case of myoclonia.

During stereotaxic operations of the tumors of brain and hypophysis, 196 patients were implanted by Riechert and Mundinger (1961), Riechert (1962) and Mundinger and Riechert (1963a, b) with

radioactive isotopes up to 1962. In this number, there were 131 cases of tumors in the brain hemispheres, 108 cases of adenomas of hypophysis and 22 other cases. Comparing the numbers of patients who previously were operated in a classical manner and, hereafter, irradiated with X-rays or only conventionally treated with patients who — between 1951 and 1959 — were stereotaxically implanted with radioactive isotopes, Mundinger and Riechert (1963a, b) conclude that the latter method extends the lifetime of 20 per cent of patients suffering from malignant gliomata and of 30 per cent of those, having oligodendroglioma. Continuing the comparison of the results obtained in the surgery of hypophysis in 87 patients, operated on stereotaxically and in 74 operated on in a conventional manner, these authors believe that more satisfactory results are yielded by the stereotaxic operations. The latter, performed for the first time, yielded no mortality at all, while the mortality rate after conventional treatments came to 19 per cent.

In their 1962 statistics, concerning 71 patients suffering from parkinsonism, Spiegel and Wycis present the mortality figures, amounting to 2 per cent, while the temporary pareses reached 5.6 per cent and monoplegia — 3 per cent.

In the material of Talairach and his associates (1962a, b) concerning 145 cases of hypophysectomy in patients of 25 to 73 years of age, satisfactory results of abolishing pains in persons who suffered from the breast cancer metastasis on bones, were obtained in 80 per cent of these cases. In the assemblage of objective symptoms, the improvement concerned, in 33 cases, the calcification of metastasas on bones, in 3 per cent, the disappearance of pulmonary metastasis and, in 2 per cent, the cicatrization of local relapses.

With regard to epilepsy, the Talairach's material (Talairach et al. 1958) comprises the SEEG examinations of 42 patients in whom, in 37 cases, surgical interventions were undertaken. A complete or partial decline of epileptic seizures was attained in 79 per cent of all cases and a decrease of mental disorders — in 60 per cent.

An improvement, obtained in the behavior of epileptic and retarded children displaying hyperactivity, at the same time, was described by Narabayashi (1961). These children, of 5 to 14 years of age, on the one hand, suffered from frequent epileptic seizures and, on the other, were troublesome and their behavior was unbearable to other people. In 21 cases, the abolition of violent behavior followed a unilateral amygdalotomy which allowed these children to adapt themselves to the society. A bilateral amygdalotomy, performed in 7 cases did not, according to Narabayashi, produce any undesirable results such as, for instance, the Klüver-Bucy symptom.

With regard to the stereotaxy for pain, Riechert (1961) described an immediate improvement in 80 per cent of his patients in whom the thalomeotomy had been carried out. Of this number, however, a half of all cases displayed a relapse of pains which occurred a few months after the treatment. Similar observations have been made by Spiegel and Wycis (1962) who, on the basis of his material, consisting of 54 cases, stated that the relapses of pains of the central origin may occur even 4 years after the operation. The cases in which no relapses are observed after a few years have passed may be considered permanently cured.

Many instances may be cited of satisfactory treatment results, obtained owing to stereotaxic operations in different disease cases. According to other authors, the results they attained are more or less similar. Particular attention should, however, be paid to positive results following such treatment in patients, suffering from Parkinson's disease, dyskinesias and dystonias, as well as those, obtained in the surgery of hypophysis.

Nowadays, the stereotaxic neurosurgery reaches a period of its full development. Completely new curing prospects have been opened by this operative method with regard to many diseases which, so far, failed to respond to all former treatment methods. The indications of the stereotaxic treatment are formulated ever more boldly and become ever more extensive. The elaboration of new and still better forms of treatment, as well as yet more accurate formulation of operative indications exert a distinctly positive influence on the decrease in the number of complications and side-effects, as well as on the improvement of operative results.

The future development of the stereotaxic neurosurgery depends on the advance in neurophysiological investigations. Data in this field are constantly supplied by the experimental neurophysiology, as well as by the results of the stereotaxic operations, performed in man. It seems that, owing to a new methodology, introduced by Talairach to the studies on epilepsy, the diagnosis and surgical treatment of epilepsy will be considerably advanced and developed in future. We may also expect a progress in the surgery for pain. The forthcoming period will, however, show which trends in the neurophysiological investigations will develop most rapidly and — in their wake — new types of stereotaxic treatment will be invented.

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Book Reviews

Conference on Learning, Remembering, and Forgetting. Vol. I: *The Anatomy of Memory.* Ed. Daniel P. KIMBLE. Palo Alto: Science and Behavior Books, Inc., 1965, 451 pages. Price: $ 4.35.

The first of a series of five annual interdisciplinary conferences on Learning, Remembering, and Forgetting was held in Princeton during the fall of 1963. This soft-covered book contains the record of the conference plus some "afterthoughts" provided by some of the participants. The main contributors were Eccles, Kruger, Hydén, McGaugh, Uttley, and von Foerster, while Pribram who was chairman and Bureš, Chorover, Feigenbaum, John, Krech, McConnell, Miller, Roberts, Sperry, Teuber, and Weiskrantz, among others, participated in the discussion. This review will indicate some of the more interesting points made during this high-powered attempt to localize the engram.

First Eccles made clear his conviction that permanent memory must be mediated by changes in the efficacy of synaptic functioning. He reviewed the rather confusing results he and his collaborators obtained when they tried to demonstrate what they considered a spinal analogue to learning (increased efficacy of spinal reflexes with use and decreased efficacy with discuse) and concluded that memory must be related to aspects of synaptic functioning not found in the cord but only or primarily in higher levels. He emphasized that higher-level neurons are different from spinal neurons in showing greater frequency potentiation, stronger and more lasting inhibition, and special synapses on dendritic spines, and speculated that these properties may somehow be related to memory changes. The discussion concentrated on the problem of which neural system would be optimal in finding anatomical changes correlated with learning, with Teuber emphasizing the advantages of studying bilaterally symmetrical systems which are modified on only one side.

Kruger then described his experiments which demonstrate that fibers of cortical neurons clearly grow into a lesioned area as long as glial scars do not form. He argued that subtle growth and degeneration of fibers occur all the time and may be the basis of learning and forgetting. In the discussion, Krech reviewed his studies which demonstrate an increase in the size and weight of the rat's visual cortex following experience in an "enriched" environment. This increase appears to be completely extra-neural.

In reaction to the strong connectionist bias of the first two presentations, Sperry pointed out in the first summation the fact that there are poorly understood "intrinsic" properties of cells which greatly influence their pattern

of firing, and he emphasized the possibility that memory changes may be due to modifications of such intrinsic properties rather than changes at the synapse. There followed a general discussion of the idea and evidence for and against reverberatory activity as the mechanism of short-term memory. Teuber pointed out that present evidence of long-lasting central activity makes the notion of neural reverberation more of historical than logical interest. Sperry then argued that the search for a structural engram may be an impossible task as long as the complex spatial-temporal neural patterns which correspond to any simple psychological event is completely unknown. Finally he repeated his old point that learning should be thought of as changes in "contextual set" rather than as the formation of associations between elements.

Hydén next gave a detailed description of his experiments which demonstrate that RNA base rations of neurons and glia from many parts of the brain are generally very stable despite considerable changes in the quantity of RNA produced. Only in a rope-climbing learning task were changes in the RNA base ratios of vestibular neurons an glia observed. These changes appear to be due to changes in chromosomal RNA. The effect was clearly observed one hour after the performance of a well-learned task but was not apparent 24 hours later. The significance of these results was hotly disputed with most of the criticism being directed to the lack of adequate controls. Hyden's general theory of the mechanism of learning is based on the assumption that neurons become sensitized to particular frequency ptterns of input rather than to particular neurons.

McGaugh followed with a summary of quite a few studies of the effect of various drugs and ECS on the consolidation process. Despite considerable variations due to task, species, and even strain differences, the experiments do seem to demonstrate that "excitatory" drugs such as strychnine may facilitate while "depressant" drugs such as pentobarbital may depress the consolidation process. Attempts to estimate the time of consolidation using the ECS technique gave results varying from a few seconds to a few hours.

After a summation and "afterthought" by Roberts in which he elaborated a greatly detailed synaptic theory of memory which attempts to integrate data and ideas from the behavioral to the molecular level, Uttley described some interesting experiments which he performed with Burns. Uttley's argument was that if the synapse transmits "Shannon" information, then not only should the synaptic efficiency between X and Y be increased if an impulse in X is followed by an impulse in Y but it also should be decreased if X fires without Y following or Y fires without X preceding. A preliminary attempt to discover whether cortical synapses actually work according to these principles involved the use of single-cell recording and stimulation near the cell. Although, as Eccles repeatedly emphasized, the results were difficult to interpret, many interesting relations were demonstrated. One incidental result worth mentioning was that less than a microamp of DC polarization apparently produces a permanent shift in the rate of firing of a cell if the polarization is applied for 10 minutes but not if applied for only 4 minutes. This effect could be canceled by applying the opposite polarization for a similar length of time. The similarity and relevance of this finding to Albert's recent demonstration of the role of DC polarization in the consolidation process (Neuropsychologia, 1966) is obvious. The excellent discussion following Uttley's presentation included a description of some stimulation and recording experiments by Bureš and John on the role of the reticular formation in conditioning. Feigenbaum then described

his computer model of human serial learning and demonstrated how new learning could produce "forgetting" without disrupting or weakening any previously-established connections.

In the final presentation, von Foerster gave a mathematically sophisticated description of hypothetical complex neural network which may be generated by a simple genetic command and which categorizes the input. He argued that memory does not consists in the simple recording of past events but rather produces a structure or schema which can predict the future and thus reduce uncertainty.

The considerable conflicts and disagreements apparent in this record of the conference of the Anatomy of Memory demonstrates once again that the engram will be hard to find. However, recent advances seem to promise a real increase in our understanding of how we remember.

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The control of chromatophores. International Series of Monographs on Pure and Applied Biology. By M. FINGERMAN. Division: Zoology. General editor: G. KERKUT. Oxford: Pergamon Press, 1963, pp. 184.

This book is a most valuable approach to the recent developments in studies on color changes in different groups of animals. It surveys in comprehensive form the entire field of present knowledge of chromatophores as revealed by morphological and physiological studies.

In the first chapter the author gives a broad introduction to the subject with much emphasis devoted to background information concerning the different types of chromatophores, changes of the chromatophore response, chemistry of chromatophoral pigments and biological significance of color changes in animals. In the five further chapters a detailed review of chromatophores in crustaceans, insects, cephalopods, echinoderms and vertabrates is given. Throughout the whole book the role of evolution of nervous and endocrine control mechanism is stressed.

As a well written account of recent advances in studies on structure and function of chromatophores, this book should prove extremely useful to comparative physiologists and to specialists interested in the morphological and physiological aspects of color changes in animals. It can be also recommended as esential reading for all those engaged in other fields of experimental zoology.

The book is well illustrated and printed. The references and indexes are comprehensive.

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Structure and Function of Connective and Skeletal Tissue. Proceedings of an Advanced Study Institute organized under Auspices of N.A.T.O., St. Andrews, Scotland, 15—25 June 1964. Edited by S. FITTON JACKSON, R. D. HARKNESS, S. M. PARTRIDGE and G. R. TRISTRAM. Published by Butterworths Co. Publishers, London, 1965, pp. 537+XXII.

The book comprises a series of lectures presented on "Structure and Function of Connective and Skeletal Tissue" at the Advanced Study Institute. The lectures are preceded by a list of participants of the Study Institute. The lectures, though

dealing with some more detailed problems, cover together a very wide scope of investigations on connective tissue physiology and metabolism. The structure, chemistry and interaction between intercellular macromolecules as well as experimental, comparative, morphogenetic and some other aspects are discussed. The material presented is conveniently gathered and arranged in 12 chapters.

In chapter 1 on Strucure and Chemistry of Collagen, R. E. Burge discusses the basic structure of the collagen triple-strand coiled-coil and K. A. Piez, P. Bornstein, M. S. Lewis and G. R. Martin — the lateral association of chains within the coiled-coil. K. Hanning and A. Nordwig and S. Seifter, C. Franzblau, E. Harper and P. L. Gallop present amino acid sequence studies of collagen which suggested, that in the primary structure of collagen particle, segments can be distinguished, which are composed mainly of neutral amino acids, e.g. glycine, proline and hydroxyproline, and others with a large number of polar amino acids. This probably explains the cross-striation of collagen fibrils observed in the electron microscope. A. J. Hodge, J. A. Petruska and J. A. Bailey discuss the probable arrangement of tropocollagen macromolecules in the native-type fibril and the consequences of the hypothesis of subunit structure of the tropocollagen macromolecule. A. Connel and G. C. Wood describe the modification of collagen structure by molluscan enzymes and P. W. Heaps, R. Reed and G. Stainsby as well as J. Manahan and I. Mandl the effect of trypsin on collagens. Trypsin action does not change the gross structure of collagen fibres in the electron microscope, but the number of bonds susceptible to the action of other enzymes is increased.

Chapter 2 is devoted to the Structure and Metabolism of Elastin and Resilin. S. M. Partridge, J. Thomas and D. F. Elsden present results of experiments on isolation of new amino acids from elastin hydrolysates. The amino acids are called desmosine and isodesmosine. The authors suggest, that they are synthesized from lysine and might be involved in the cross-links of the elastin molecules. L. Gotte, V. Meneghelli, and A. Castellani report, that elastin from the normal human aorta is composed of a fibrous component resolvable in the electron microscope into filaments of about 30 Å diameter and second protein which can be easily removed by hot alkali. The papers of T. Weis-Fogh and S. O. Andersen are concerned with resilin, a rubber-like protein found in Arthropoda. Two unusual amino acids were noticed in acid hydrolysates of resilin. These are synthesized from C^{14}-labelled tyrosine, and it is suggested that they are dityrosine and trityrosine. Attempts of purification of elastoproteinase and various elastomucases on DEAE Sephadex columns are presented by W. A. Loeven. It is suggested by D. A. Hall that the stabilization of the elastin molecule is at least in part dependent on intermolecular cross-links which consists of EDTA-resistant but elastase-sensitive coordinately bound calcium atoms.

Chapter 3 is entitled Structure and Chemistry of Ground Substance. The first paper by M. Schubert is devoted to entanglement and excluded volume of protein polysaccharides. Albumin is excluded from the solution of protein polysaccharide in the presence of salts of 15 M or more. At lower salt concentration the excluded volume effect is completely abolished. Some similarity is suggested between water-insoluble Sephadex which can exclude large molecules from its domain and protein polysaccharides solutions. However, in the case of Sephadex, the excluded volumes are additive because the domains are rigid and not interpenetrable, but in the case of hyaluronate or protein polysaccharide the domains are compressible or interpenetrable so that the excluded volume increases slower

than the concentration. A. A. Castellani found that aspartic acid is present in high concentration in keratosulphate-peptide from beef cornea but it is not known how carbohydrate is linked to peptide in this complex. H. Muir isolated heparan from normal human aortas and gives its biochemical analysis. L. Roden discusses the structure of glycopeptides liberated from chondroitin 4-sulphate. Galactose, xylose, xylosyl serine are found as well as the terminal trisaccharide unit glucuronic acid-galactosamine-glucuronic acid. Heparin also occurs in native state covalently bound to protein. It is suggested, that the carbohydrate-protein linkage region in the polysaccharides may have identical or closely similar structure. M. J. Barnes describes the isolation and characterisation of muco-protein from thoracic aorta and suggests, that it is closely associated with the elastic membranes. C. Cessi and G. Bernardi describe results of experiments on kinetics of enzymatic degradation of the protein polysaccharide complex supporting its comb-like structural model with a central core of protein. S. Fitton Jackson describes electron microscop studies on the protein polysaccharide complex. S. Partridge, A. H. Whiting and H. F. Davis fractionated under mild conditions and in the absence of proteolytic enzymes the low-molecular-weight protein polysaccharide fraction (PP-L) of cartilage. The authors report that by this procedure the protein content of the complex can be reduced to 7.5 per cent, significantly lower than it was obtainable up till now. The suggestion is advanced, that the protein polysaccharide fractions of cartilage are aggregates f- d
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Jerzy Kawiak, Warsaw, Poland

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domain and protein polysaccharides solutions. However, ...
the excluded volumes are additive because the domains are rigid and not interpenetrable, but in the case of hyaluronate or protein polysaccharide the domains are compressible or interpenetrable so that the excluded volume increases slower

than the concentration. A. A. Castellani found that aspartic acid is present in high concentration in keratosulphate-peptide from beef cornea but it is not known how carbohydrate is linked to peptide in this complex. H. Muir isolated heparan from normal human aortas and gives its biochemical analysis. L. Roden discusses the structure of glycopeptides liberated from chondroitin 4-sulphate. Galactose, xylose, xylosyl serine are found as well as the terminal trisaccharide unit glucuronic acid-galactosamine-glucuronic acid. Heparin also occurs in native state covalently bound to protein. It is suggested, that the carbohydrate-protein linkage region in the polysaccharides may have identical or closely similar structure. M. J. Barnes describes the isolation and characterisation of muco-protein from thoracic aorta and suggests, that it is closely associated with the elastic membranes. C. Cessi and G. Bernardi describe results of experiments on kinetics of enzymatic degradation of the protein polysaccharide complex supporting its comb-like structural model with a central core of protein. S. Fitton Jackson describes electron microscop studies on the protein polysaccharide complex. S. Partridge, A. H. Whiting and H. F. Davis fractionated under mild conditions and in the absence of proteolytic enzymes the low-molecular-weight protein polysaccharide fraction (PP-L) of cartilage. The authors report that by this procedure the protein content of the complex can be reduced to 7.5 per cent, significantly lower than it was obtainable up till now. The suggestion is advanced, that the protein polysaccharide fractions of cartilage are aggregates formed by the physical complexing of a protein with a molecule containing polysaccharide chains linked covalently by peptide chains. K. Meyer, B. Anderson, N. Seno and P. Hoffman present evidence, that in a variety of chondroitin 4-or 6-sulphate-peptides, carbohydrate is only linked via O-glycosidic bonds to serine. Keratosulphate-peptide of bovine cornea is mainly resistant to alkali and its carbohydrate moiety most likely is bound by N-glycosidic bonds with the amide group of asparagine. Keratosulphate of human costal cartilage and bovine nasal septum showed mostly bonds through serine and threonine. P. W. Kent and F. K. Stevenson describe two fractions, which they found in the chondroitin sulphate-peptide complex coupled with 1-fluoro-2, 4-dinitrobenzene. One fraction was a chondroitin sulphate free from peptide and the second contained all the DNP residues as well as glucosamine and galactose. A. Dorfman in concluding remarks proposes that in the Hurler syndrome, the linkage region between polysaccharide and protein may be defective.

Further chapter are devoted to comparative aspects of supporting tissues, experimental techniques, interaction between intercellular macromolecules, biosynthesis and morphogenesis, mineralization, mechanical properties of connective tissue, immunochemistry of connective and skeletal tissues, tissue maintenance and breakdown and medical aspects of connective tissue physiology and wound healing. About 100 different lectures are included in the book and it is beyond the scope of this review to deal with all of them.

The reader will find the book a good record of recent trends in studies on structure, chemistry and metabolism of connective tissue components as well as of their interactions.

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