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KAZIMIERZ BIAŁASZEWICZ

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PAŃSTWOWE WYDAWNICTWO NAUKOWE
WARSZAWA 1961

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POISON SUSCEPTIBILITY OF CILIATES (*PARAMECIUM CAUDATUM* EHRBG., *STYLONYCHIA MYTILUS* EHRBG.), ADAPTED TO THE HYPERTONIC MEDIUM

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(Received 30 April 1960)

Many investigators have attended to the problem of animals adaptation to the medium of a different osmotic pressure. Protozoa have very often been the subject of the investigations (Massart 1889, Hafkine 1890, Davenport and Neal 1896, Daniel 1909, Jollos 1921, Crane 1921, Jahn 1934, and others). There exist, however, some divergences between the results of their works. Finley (1930), for instance, states that *Paramecium caudatum* keeps very well the addition of 10—20% of sea water into the culture, and, when gradually got accustomed, it even may remain in 100% sea water corresponding to about 3% salt solution. Yocom (1934) confirms, that *Paramecium caudatum* undergoes no changes in 10% sea water, but in 20% sea water it already perishes within 5 min. At the gradual addition of sea water into the culture paramecia perish when the concentration is near to 60% of sea water. The results obtained by Frisch (1939) are similar. According to him, paramecia perish in 33—52% sea water solution.

This work is the consequence of my earlier experiments concerning adaptation of paramecia to the ferrous chloride, potassium bichromate, lithium chloride and sodium chloride (Rostkowska unpubl.). In those experiments protozoa have been placed in a solution of a chemotactic positive concentration. The changes of the chemotactic positive concentration under the influence of adaptation have been observed, in a more concentrated solution. The most distinct adaptation have been obtained to the 0.1—0.5% sodium chloride solutions.

METHODS

In this work the modified method of adaptation has been applied: protozoa have been transferred every 48 hours, within 25 days, from the lower to the higher concentrations increasing in a geometrical progress, the initial being 0.125‰ and the final 1.457‰ sodium chloride solution. The experiments have been conducted with *Paramecium caudatum* Ehrbg. and *Stylonychia mytilus* Ehrbg. Protozoa have been bred in an 1 liter jar, filled with non distilled, clean water (left quiet for several hours before use). Every 3—4 days, when the culture became clear, they were fed on the yolk medium. The cultures remained at a room temperature 20—22°.

Paramecia from the tigmotropic ring, appearing in the upper part of the vessel, and *Stylonychia* from the homogeneous culture have been used for the experiments. Protozoa have been centrifuged in a hand-centrifuge (18—20 rpm) and washed twice with tap water. The volume of the experimental culture, placed in a 50 ml. beaker, was 50 ml. Condensation: 2000 individuals per 1 milliliter. Four cultures of protozoa have been led in the same time — two controls and two of protozoa adapted to the sodium chloride. Experimental protozoa have been fed every 48 hours after having been transferred into the medium of a higher concentration. Sodium chloride solutions, Ringer's fluid and toxins solutions have been prepared on distilled water. The counting of protozoa in the experimental cultures have been realised as follows: 1 ml. of protozoa culture fluid has been taken by means of a pipette, divided dropwise into ten drops on a slide, killed by the action of 0.001‰ HgCl_2 solution and counted. During the observation of the contractile vacuoles, paramecia have been placed between a slide and a cover-glass and fixed by means of the cotton wool fibres. The amount of contractions at three paramecia has been counted within 5 min. The sensitiveness of protozoa on toxins was determined as follows: 0.1 ml. of the solution has been placed on a slide by means of a calibrated pipette. Then, 0.02 ml. of the centrifuged paramecia have been introduced into this solution, and the slide has been placed in moist chamber. The behaviour of infusoria has been observed after 10 and 100 min. The number of plasmolized individuals has been estimated on a chequered slide. The experiment having been finished, protozoa have been killed with 0.001‰ mercuric chloride solution and all individuals counted. The statistical significance of the difference have been calculated using Fisher's test of significance.

RESULTS

The carried out experiments showed that low concentrations of the sodium chloride solutions, concluding from the number of populations, accelerate the fission-rates, whereas higher ones — inhibit this process. According to Herfs, the sodium chloride concentrations of 0.25‰ to 0.5‰ accelerate the fission rate likely to the addition of sea water in the amount of 6‰ (Frisch), or 10—13‰ (Finley). The quoted data found no confirmation in the work described in this paper. It was stated in this work, that the fission-rates are already inhibited by the sodium chloride concentration of 0.477‰. In higher concentrations the culture condensation decreased below the start point. The results presented in Table I are average from two determinations.

Table I

Number of control and adapted paramecia in the culture

NaCl concentration in the culture in ‰	Adaptation time hrs.	Average number of paramecia adapted in 1 ml.	Average number of control paramecia in 1 ml.
0.125	48	2 040	2 000
0.305	264	4 627	2 860
0.477	360	4 487	3 923
0.745	446	3 038	7 016
0.932	504	1 793	9 009
1.457	600	887	9 787

It is well known, that the dimensions of the contractile and food vacuoles are smaller in cultures of higher salt-concentrations and their contractions are less frequent. Likely, the gradual disappearance of contractions with the increase of sodium chloride concentration in the culture was stated in this work (Table II). Kamada's observations (1935), that the frequency of contractions is inhibited in paramecia transferred into the sodium chloride solution, and after two hours it becomes normal, are not confirmed by these experiments.

Some experiments have been made, too, aiming at the adaptation of paramecia to the Ringer's fluid of the following composition: 8.5 g. of NaCl, 0.25 g. of KCl, 0.3 g. of CaCl₂, 100 ml. of H₂O. The method applied in this case was the same as that for the adaptation of protozoa to the sodium chloride. Paramecia have been adapted to the Ringer's fluid, beginning with the ninefold and finishing with 68-fold dilution

in relation to the initial solution, taking the Ringer's mother fluid for 100%. The particular concentrations of Ringer's fluid have corresponded to the osmotic pressure of 0.125—0.932% sodium chloride solutions. Salt concentrations in cultures numbered from I to V corresponded to the osmotic pressures of the following sodium chloride solutions: I — 0.125% NaCl, II — 0.305% NaCl, III — 0.477% NaCl, IV — 0.745% NaCl, V — 0.932% NaCl.

Table II

The influence of sodium chloride concentration on the contraction frequency of the contractile vacuole

NaCl concentration in %	Temperature	Time of formation of contractile vacuole in sec.	
		average	range
0.0	20—22	8.60	8.3 — 8.9
0.125	20—22	11.65	11.3 — 12.0
0.305	20—22	12.95	12.0 — 13.9
0.477	20—22	17.70	16.7 — 18.7
0.745	20—22	28.00	26.0 — 30.0
0.932	20—22	57.50	55.0 — 60.0
1.457	20—22	250.00	200.0 — 300.0

The influence of the Ringer's fluid upon the behaviour of the contractile vacuoles was much more distinct than that of the sodium chloride solution (Table III). The number of contractions of the contractile vacuoles decreased more rapidly than in the case of paramecia adapted to the sodium chloride.

Table III

The influence of the diluted Ringer's fluid upon the contraction frequency of the contractile vacuole

Cultures of paramecia adapted to the diluted Ringer's fluid.	Temperature	Time of formation of contractile vacuole in sec.	
		average	range
Controls	20—22	8.78	8.57—9.0
I	20—22	12.00	12.00—12.00
II	20—22	13.85	14.70—15.00
III	20—22	19.75	19.50—20.00
IV	20—22	57.50	55.00—60.00
V	20—22	110.00	100.00—120.00

Table IV

Reaction on poison of paramecia adapted to the sodium chloride and of control paramecia

NaCl concentration in the culture in %	Number of plasmolysed individuals after 10 min.																								
	Atebrin 0,001 %					Methylene blue 0.01%					Chinosol 0.02%					Mercuric chloride 0.0002%					Phenol 0.2%				
	Adapted protozoa		Control protozoa		The statistical significance of the difference	Adapted protozoa		Control protozoa		The statistical significance of the difference	Adapted protozoa		Control protozoa		The statistical significance of the difference	Adapted protozoa		Control protozoa		The statistical significance of the difference	Adapted protozoa		Control protozoa		The statistical significance of the difference
	Abso-lute number	%	Abso-lute number	%		Abso-lute number	%	Abso-lute number	%		Abso-lute number	%	Abso-lute number	%		Abso-lute number	%	Abso-lute number	%		Abso-lute number	%	Abso-lute number	%	
0.125	13	6.8	108	79.46	p > 99%	199	43.8	183	47.32	p < 80%	54	2.7	62	46.63	p > 99%	43	8.9	129	59.5	p > 99%	33	12.49	195	42.27	p > 99%
0.305	3	1.3	373	96.9	p > 99%	50	42.67	186	42.78	no difference	5	3.5	39	18.1	p > 99%	12	6.5	16	8.3	p < 80%	167	46.4	128	45.6	no difference
0.745	0	0	203	81.48	essential	43	31.6	186	71.23	p > 99%	3	1.66	66	36.5	p > 99%	16	9.7	81	23.44	p > 99%	143	44.46	130	64.83	p > 99%
0.932	0	0	184	83.83	essential	30	17.34	171	75.89	p > 99%	0	0	124	53.81	essential	7	4.11	90	43.72	p > 99%	40	24.96	129	60.59	p > 99%

REACTION OF PROTOZOA ON POISONS

In the attainable literature, I have found no mentions about the susceptibility of protozoa, remaining in the hypertonic medium, on poison. The sensitiveness of protozoa has been examined on paramecia and stylonychia adapted to the sodium chloride solutions (concentrations 0.125—1.457‰) and Ringer's fluid in the corresponding dilutions, and on the control protozoa (Table IV).

The results of the experiments, presented in Table IV, show augmented resistance of paramecia adapted to the hypertonic medium against poisons, in comparison with the control paramecia. The lower percentage of the adapted paramecia undergoing plasmolysis may be the proof of it. The protective influence of the hypertonic medium appeared in paramecia adapted to the sodium chloride, for, with increasing salt concentration in the medium, paramecia showed higher resistance against poison. The resistance of paramecia against the action of atebtrin, methylene blue, chinisol and sublimate has been stated in this work. This fact seems to be a rather interesting one, especially in the case of protozoa living in stinks and of parasitic protozoa, since both kinds are living in the hypertonic medium. In order to explain whether it is due to the sodium chloride, or perhaps to the influence of the osmotic pressure of the salt solutions, the sensitiveness of paramecia adapted to the Ringer's fluid has been examined (Table V).

The Ringer's fluid proved to be more efficacious than the sodium chloride solution. Paramecia adapted to the Ringer's fluid, demonstrated increased resistance against poison already in the lower concentrations, whereas in the higher concentrations of Ringer's fluid in the culture, they did not react at all on the poison concentrations, mortal for the control paramecia. In order to examine the behaviour of other protozoa, some experiments have been conducted with *Stylonychia mytilus* species, adapted to the sodium chloride of the same concentrations as *Paramecium* (Table VI).

Stylonychia have been adapted to the sodium chloride of the concentrations of 0.125—0.745‰. The lower sodium chloride concentrations in the culture prove to be suitable, since they are favourising better growth and make stylonychia resistant against the action of toxins. The increase of the population may be the proof of it. The sodium chloride concentrations higher than 0.477‰ become injurious. *Stylonychia* fell on the bottom of the beaker, move very slowly and gradually perish. *Stylonychia* living in the hypertonic medium of 0.305‰ NaCl show higher resistance against atebtrin, chinisol, sublimate and methylene blue in comparison with controls. After 10 min., plasmolysis does not

Sensitiveness against poison of paramécia adapted to the Ringer's fluid and of control paramécia

Number of plasmolysed individuals after 10 min.																
Kind of substance concentration in %	Paramecia adapted to the diluted Ringer's fluid Culture II		Control protozoa		The statistical significance of the difference	Paramecia adapted to the diluted Ringer's fluid Culture III		Control protozoa		The statistical significance of the difference	Paramecia adapted to the diluted Ringer's fluid Culture IV		Control protozoa		The statistical significance of the difference	
	Abso-lute num-ber	%	Abso-lute num-ber	%		Abso-lute num-ber	%	Abso-lute num-ber	%		Abso-lute num-ber	%	Abso-lute num-ber	%		
Alebrin 0.001	12	9.75	75	64.1	p > 99%	0	0	119	83.4	essential	0	0	83	72.79	essential	
Methylene blue 0.01	42	32.84	74	54.26	p > 99%	13	8.67	79	57.65	p > 99%	0	0	73	56.6	essential	
Chinosol 0.02	2	1.47	83	48.25	p > 99%	0	0	41	45	essential	0	0	57	50.81	essential	
Phenol 0.2	37	27.67	73	48.34	p > 99%	7	0	98	71	p > 99%	0	0	64	52.32	essential	

appear at all, and after 100 min. the percentage of plasmolysed individuals is rather low. Phenol proved to be more toxic for *Stylonychia*, than for *Paramecium* (Table IV and VI), since, after 10 min, ca 50% off the adapted individuals perish, and after 100 min. practically all are dead.

Table VI

The reaction of *Stylonychia mytilus* adapted to the sodium chloride (0.305% of NaCl) and of control *Stylonychia* of poison

Kind of substance concentration in %	Number of plasmolysed protozoa after 10 min.					Number of plasmolysed protozoa after 100 min.				
	Adapted protozoa		Control protozoa		The statistical significance of the difference after 10 min.	Adapted protozoa		Control protozoa		The statistical significance of the difference after 100 min.
	Absolute number	%	Absolute number	%		Absolute number	%	Absolute number	%	
Atebrin 0.001	0	0	0	0	no difference	12	6.59	78	53.79	p > 99%
Methylene blue 0.01	8	7.01	21	13.18	p = 95%	69	42.70	84	65.9	p > 99%
Chinosol 0.02	0	0	0	0	no difference	20	11.1	165	100	p > 99%
Phenol 0.2	104	58	53	34	p > 99%	191	100	158	100	no difference
Mercuric chloride 0.0002	0	0	0	0	no difference	49	36.84	161	100	p > 99%

I have found no data in the literature, concerning stability of the acquired resistance to the sodium chloride solutions. There is no evidence, whether within the course of adaptation of individuals the lasting, adaptative modifications appear and whether the stated deviations are permanent and hereditary. The opinions of many investigators on this subject are different. According to some of them, the resistance is preserved under normal conditions even within a long period of time. The others' opinion is, that it declines when the conjugation appears, or, immediately after transferring into the normal medium (Jollos 1913, 1934, Górski 1938, Neuschlosz 1919, 1920 and others).

It has been examined, in the course of these experiments, for how long a time the acquired resistance to the sodium chloride is preserved in *Paramecium caudatum* after transferring of protozoa into the medium with no increased salt concentration, using the same toxins in the same concentrations. The behaviour of infusoria has been examined after 24.48 and 72 hours (Table VII).

Table VII

The reaction of paramecia adapted to 1.165% sodium chloride, placed in water for 24, 48 and 72 hrs.

Kind of substance concentration in %	Number of plasmolysed individuals after 10 min.											
	Reaction of paramecia after 24 hrs.				Reaction of paramecia after 48 hrs.				Reaction of paramecia after 72 hrs.			
	Adapted protozoa		Control protozoa		Adapted protozoa		Control protozoa		Adapted protozoa		Control protozoa	
	Abso-lute num-ber	%	Abso-lute num-ber	%	Abso-lute num-ber	%	Abso-lute num-ber	%	Abso-lute num-ber	%	Abso-lute num-ber	%
Atebrin 0.001	39	23.46	87	65.25	97	61.1	89	62.66	101	62.34	89	62.67
Methylene blue 0.01	68	42.76	79	55.24	63	46.13	79	52.98	69	53.48	78	53.42
Chinosol 0.02	36	23.68	92	72.45	95	52.2	72	57	101	55.49	104	55.91
Phenol 0.2	72	46.85	96	64	99	52.38	92	58.22	103	59.88	89	59.13

Paramecia adapted to the sodium chloride, when transferred into water, lose gradually their acquired resistance. After 24 hours the number of plasmolysed control individuals is greater. After 48 hours the difference decreases, and after 72 hours there is no difference between the control paramecia and paramecia deriving from the hypertonic medium. It may therefore be assumed, that the acquired resistance against the sodium chloride solutions is not related with the lasting modifications in the organism of Paramecium, and that it is independent from the selective elimination of individuals, more sensitive to the changes of the medium osmotic pressure.

CONCLUSIONS

1. The number of paramecia in the culture decreases with the raise of sodium chloride concentration. The contractile vacuoles gradually disappear, especially in Ringer's fluid (Table II, III).

2. Increased resistance against the following poisons atebirin, chinolol, methylene blue, mercuric chloride, phenol, is observed in paramecia living in the sodium chloride medium (concentration: 0.125—1.457% of NaCl), or in the Ringer's fluid (Table IV, V).

3. Lower sodium chloride concentrations in the culture (up to 0.477%) make resistant *Stylonychia mytilus* against atebirin, chinolol, methylene blue, mercuric chloride (Table VI). Higher concentrations prove to be noxious.

4. Paramecia adapted to the sodium chloride solution (1.165%) lose their acquired resistance against poisons within 72 hours, after having been transferred into water (Table VII).

I am greatly indebted to Prof. R. Kadłubowski for help and kind suggestions of the theme of this work.

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RECEPTION AREAS AND POLARIZATION OF CILIARY MOVEMENT IN CILIATE DILEPTUS

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(Received 12 May 1960)

1. INTRODUCTION

According to Alverdes (1922) stimuli are received by cilia located in the anterior region of the ciliate cell, thus causing the motor reaction of the whole ciliary apparatus. He assumed that in the anterior part of the Paramecium cell there existed some specific receptors which received chemical and thermic stimuli. This assumption gave rise to the well known polemics between Alverdes and Koehler (1934, 1939) who ascertained, that the whole surface of Paramecium was of equal value as far as the ability to receive stimuli was concerned. From that time many authors have expressed their opinions on this subject, attributing manifold receptive functions to various structures of the ciliate; however due to lack of new conclusive experimental material a deadlock was reached and the problem stopped drawing the attention of the protozoologists. Nevertheless the general importance of this problem is unquestioned and it may be worth while to restart the researches. Recently Parducz (1956) in a series of detailed experiments examines the ciliary movement of Paramecium and brings new facts concerning reactivity of this ciliate. The author of the present paper does not discuss the existence of receptors as elements of the reflex arch in protozoa, neither does he attempt to extend the exact neuro-physiological terminology to this group of animals. We merely try to supply an experimental base for further works on the subject, using provisional terminology and simple interpretations.

It seems, that one of the most important factors in the study of the problem of receptors in ciliates is the choice of suitable material.

Paramecium is not very suitable as a subject for study because of its comparatively small size and the relative uniformity of the surface of its cell. Besides, the fragments of individual Paramecium are easily destroyed and have only a slight ability to regenerate. It is necessary therefore to find a larger ciliate with a non-uniform cell surface and great ability to regenerate. It is also necessary to choose a reaction which is comparatively simple and easy to detect which can reveal the receptors and also a stimulus whose action can be localized.

2. EXPERIMENTAL MATERIAL

All the above mentioned conditions are fulfilled in the ciliate *Dileptus*. It is of large dimensions, sometimes exceeding 1 mm and its morphology is non-uniform. On the anterior part of the cell there is a long sprout, called proboscis and just behind the base of the proboscis is the round opening of the cytostome. The posterior part of the ciliate is pointed. The whole surface of the cell is evenly covered with cilia and on the proboscis are also rows of trichocyst bodies. Behind the cytostome of the *Dileptus* there is a certain organ stained with iron-haematoxyline, described by Vissher (1927) as the neuro-motoric apparatus. Two small fibres connect this with the tip of the proboscis; other fibres surround the gullet and several delicate fibres run towards the surface of the cell; their connection, however, with the ciliae was not ascertained. On the proboscis of the *Dileptus* Gellert (1934) discovered the existence of a certain number of forms, which he assumed had the function of osmo-receptors. The silverline system of the *Dileptus* has according to Klein (1942) a character of a fine grating. The cytological structure, the cycle of development, the role of the trichocysts and the cultivation of this ciliate were described by Vissher (1924) and Jones (1951).

The described ciliate leads a carnivorous way of life. *Dileptus* kills smaller ciliates with his trichocysts and swallows them. The movement of his cilia has a metachronic character, but the ciliary waves are more difficult to observe than in *Paramecium*. During the forward movement the individual ciliates turn round their longer axis, making circles with the proboscis, which is the first to make contact with objects lying in the way of the ciliate.

The easiest type of reaction to stimuli to be observed is the sudden reversion of the ciliary movement, that is a change in the direction of their beating of about 180° and the withdrawal of the ciliate. It withdraws a distance of from a half to some three times its body length and then starts forwards again, often at a different angle.

The appearance of this reaction was accepted by the author as the criterion of reception of the stimulus.

A natural breeding ground of *Dileptus anser* (Müller) was found near Warsaw in a roadside, where at certain times they appear in great quantities in water with decomposing alder leaves. The author together with K. Golińska elaborated a culture method based on feeding the *Dileptus* with *Colpidium*, which in turn were bred on grains of rice.

3. REACTIONS OF A NORMAL CILIATE

A. Testing of reaction to mechanical stimulation

For mechanical stimulation of the ciliate a pneumatic micromanipulator of de Fonbrune's construction was used. In each series of experiments a dozen or so individual ciliates from the culture were placed under a slide in drops of water. The whole was closed in an oil chamber as described by de Fonbrune. The size of the drops of water was so regulated as to give the ciliate freedom of movement in the horizontal plane, the diameter of the drops being about twice its length (Fig. 1). For stimulation glass needle made in a micro-forge were used.

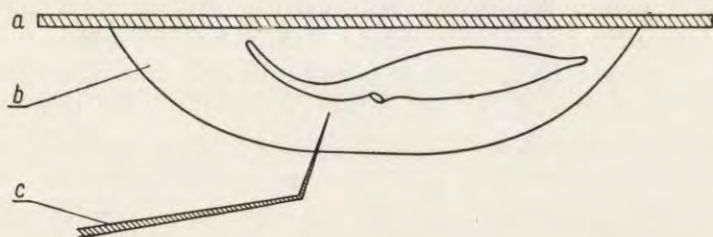


Fig. 1. Stimulation of *Dileptus* using micromanipulator

a — slide; b — drop of water containing ciliate; c — micro-needle.

When examining the reaction to touching of a ciliate in motion a needle was moved along the cilia and along the body of the ciliate. When the area near the proboscis was stimulated, the reverse reaction occurred and the ciliate retreated for a distance of up to twice its body length. It should however be mentioned that this reaction did not occur in all the examined individuals. On the other hand in all cases where during the control experiments, other parts of the ciliate's body were

stimulated in a similar way, the reaction did not occur. If the ciliates were moving in a backward direction, the application of the needle to the posterior end of the body caused them to change the direction of their movement to a forward one. Similar experiments were made also without the micromanipulator, using needles made of glass or steel minute needle. In this case the ciliate had a complete freedom of movement. The results obtained were similar to those from experiments with stimulation applied by means of a micromanipulator.

Use was also made of the fact that in the experimental situation with a large drop the ciliate has its own normal speed. In the next series of experiments a needle was used as a stationary obstacle and placed in the ciliate's way (Fig. 2). The moment the proboscis of the ciliate

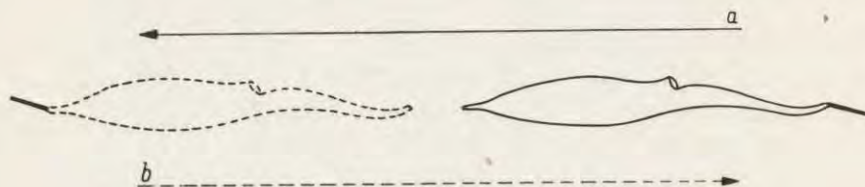


Fig. 2. Stimulation of *Dileptus* without the micromanipulator

a — Direction of movement after stimulating the proboscis b — Direction of movement after stimulating the posterior end of the body.

touched the needle, the ciliate immediately retreated. If during this backward movement another obstacle was placed in front of its posterior end, the ciliate reacted by retreating again, that is by re-starting the forward movement. This could be repeated several times, so causing successive changes of direction of about 180°.

For control the stimulating of the proboscis during the backward movement and the stimulating of the posterior part of the ciliate during forward movement was applied by both methods that is by using the micromanipulator and by free hand. No withdrawing reaction or acceleration of forward movement was observed. During all the described experiments the ciliate was in constant motion, that is no stimulation was applied to a ciliate with immobile cilia, because it was not observed that the *Dileptus* was ever completely immobile.

In order to interpret more clearly the described experiments, the reaction to traumatic effect and water sheak was examined. The mechanical injury of the proboscis by pricking during the forward movement caused in every case a reversal of movement and the retreat of the ciliate. The injury to the posterior end of the ciliate's body during

backward movement caused a movement forward. These were however certain differences in the reaction to the tactile stimuli. Namely, the reversion of the ciliary movement after pricking of the proboscis appeared in all examined cases and lasted longer (the ciliate retreated about twelve to fifteen times its own length). Besides, in a few cases, the pricking of other parts of the body could evoke reaction.

It was ascertained that the *Dileptus* can react by reversion of the ciliary movement to water sheak caused by shaking of the scale with the culture or by striking the water with the needle in the vicinity of the ciliate. It was however discovered that slower movements of the needle made during tactile stimulation did not give rise to reaction if the ciliae or the surface of the ciliate's body was not directly touched.

B. Examining the reaction to ultra-violet radiation*

In order to expose the ciliate to ultra-violet rays an apparatus was used working on the wave-length 2790—2805 Å which enabled obtaining of a radiation beam of less than 50 microns in diameter.

The examined ciliates were placed on a quartz plate mounted in the slide with a round incision. Before every experiment the ciliates were introduced into the drop of water and covered with a slide. The layer of water was so regulated as to give the ciliate the possible free movement needed for the experiment. For observation top and side lighting was used. The ultra-violet beam passing from the magnesia-arch through the optical system of the apparatus and through the special condenser of the microscope could be observed indirectly thanks to the fluorescence of the layer of fat on the glass. The micro-beam was regulated by moving of the bottom slide together with the ciliate.

As compared with the experiments with mechanical stimuli this method had the advantage of facilitating the handling of the ciliate in motion and gave no difficulty in separating the effect of direct touching from the accompanying water sheak which was the cause of inaccuracies of the previous experiments. However the main drawback of using the micro-beam of ultra-violet rays is the artificiality of this agent. This will be discussed later.

In the first series of experiments the beam was placed in front of the moving ciliate just as the mechanical obstacle was before (Fig. 3).

* The author expresses his best thanks to prof. Z. Kraczkiewicz and dr. I. Duszyńska for having allowed him to make use of the apparatus build in the Department of Cytology of the Warsaw University for his experiments.

In all the cases reversion of the ciliary movement occurred and the individual ciliates withdrew. This reaction did not however occur immediately that is just at the moment when the anterior tip of the proboscis met the micro-beam but a fraction of a second later, when about $\frac{1}{4}$ of

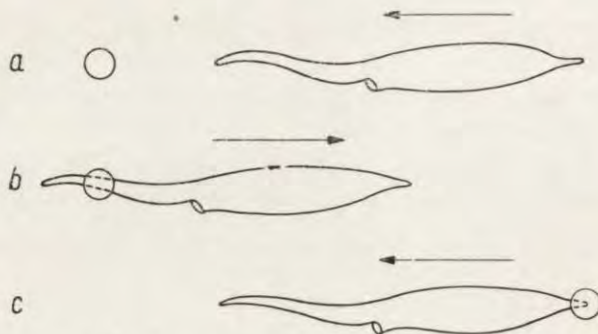


Fig. 3. Stimulation the Dileptus with ultra-violet radiation by placing the beam in the ciliates way.

a — the ciliate before stimulation; b — the ciliate after stimulation of the proboscis; c — the ciliate after stimulation of the posterior and of the body.

the proboscis had already passed the micro-beam. This reaction occurred regularly in all the examined cases.

During the backward movement of the ciliate the ultra-violet beam was placed before its posterior end. When this pointed posterior end had passed under micro-beam, the Dileptus changed to the forward movement. Just as in the experiments on mechanical stimulation this action could be repeated many times, thus keeping the ciliate in alternating movement.

In the next experiments other parts of the Dileptus were exposed to ultra-violet rays, both during the forward and backward movement (Fig. 4). It was ascertained that only exposure of the proboscis during forward movement or of the posterior end of the body during backward movement caused a reversion of the movement and no reaction occurred when other parts of the ciliate's body were exposed to radiation. However a certain difference in the length of the reaction could be observed if either the proboscis or the posterior end of the ciliate were stimulated. In the first instance the ciliate retreats more than twice its body length, in the second — less than one. Unlike the reaction of proboscis to exposure, the reaction of the posterior end of the body is not reliable and may not occur if the backward movement of the ciliate is very quick.

In order to establish the location of the responsive part more accu-

rately a slightly different technique was applied (Fig. 5). During the forward movement exposure was begun from the posterior end of the body and the micro-beam was gradually shifted forwards towards the anterior end of the body. Reaction occurred after the beam passed over

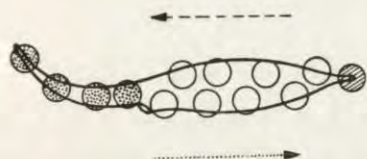


Fig. 4. Stimulation of the Dileptus by directing the beam of in various parts of the cell. The dotted positions of the beam are those which, if the ciliate is moving forward, cause the reaction of withdrawal; the dashed positions of the beam are those which, if the ciliate is moving backwards, cause the reaction of moving forward.

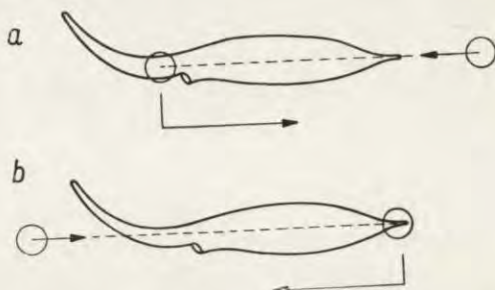


Fig. 5. Stimulation of the Dileptus by moving the beam along the cell

a — Moving the beam from the posterior end of the body towards the front during the forward movement of the ciliate. The head of the arrow shows the position of the beam when the reaction occurs; b — Moving the beam from the anterior to the posterior end of the body during the backward movement of the ciliate.

cytostom and the posterior end of the proboscis was exposed. The limit of the part of proboscis responsive to stimuli was about 40 microns from the anterior edge of the cytostom. Similar exposure of the ciliate was carried out during the backward movement, beginning from the tip of the proboscis, moving the beam towards the posterior end of the ciliate. The reaction occurred at the moment when the beam touched the base of the pointed sprout at the end of the Dileptus body. However some error in the exact localization of the responsive part could occur because of the necessity of leaving the ciliate a certain possibility of movement.

In the described experiments the exposure of any part of the ciliate's body to the micro-beam lasted a short time, a matter of a fraction of a second. In order to enable a proper interpretation of these experiments, and in particular to examine the traumatic effect of the ultra-violet radiation, further experiments were carried out with longer exposure. The ultra-violet beam was stopped on some definite spot of the proboscis. After a time of over a hundred twenty seconds the proboscis would break off at the exposed spot and at both sides of the cut small effusions of cytoplasm could appear; the edges of the pellicula

on both sides folded over. If during the exposure the ciliate had freedom of movement, it continued the backward movement all the time. If the middle parts of the cell were thus exposed, the whole body after several seconds began to twist to the sides and quiver in all directions until the outflows of cytoplasm occurred.

4. POLARIZATION OF CILIARY MOVEMENT IN FRAGMENTS OF CILIATE

A. Behaviour of fragments after operation

Before considering the reactions of fragments of *Dileptus* it was necessary to examine certain aspects of behaviour of these fragments. An operational method was sought which would enable to make in the quickest way possible a cross section of the ciliate while it was moving freely, without causing any additional injuries to the cell. It was also essential to perform operations on ciliates placed in widely spread drops, so that they could move without obstacles for as long a distance as possible. It was found that the manual cut was the most suitable for this purpose. Steel dental needles or silver minute needles were used, shaped appropriately and sharpened on unglazed porcelain until their ends were scalpel-like. The needle was put into the water and when the ciliate was right underneath it a quick cut was made.

In the first series of experiments the ciliate was cut across approximately in middle of its body, not counting the proboscis. In every case posterior part of the ciliate immediately started moving backwards, away from the anterior fragment. The back fragment moved in a straight line, turning at the same time round its longer axis. After reaching the edge of the drop of water the fragment started to circle round it with the posterior end leading, sometimes it stopped or slightly withdrew, but generally the backward movement was maintained.

The anterior fragment of the ciliate maintained the forward movement, but immediately after the cut it circled several times, often in the backward direction. After a few seconds the forward movement returned and the fragment re-started moving like a normal ciliate. If any backward reactions occurred then they took their normal course and the forward movement returned again.

Experiments were also carried out performing sections at other levels of the ciliate, as shown on Fig. 6. The results obtained were similar.

Sectioning was also performed during the backward movement following after the cutting off of the proboscis. In these cases the

morphologically posterior fragment continued to move backward and the anterior fragment started to move forward, that is with the anterior part leading-like a normal ciliate.

Further operations consisted in consecutively cutting off the opposite ends of the ciliate. Consecutive changes in the direction of movement

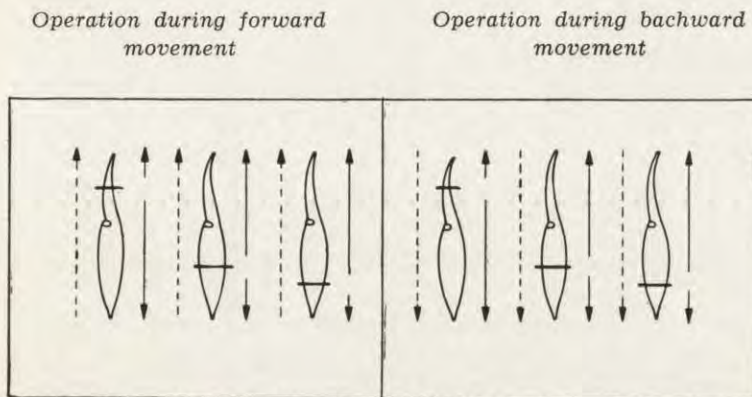


Fig. 6. Diagrams of separation of the fragments of *Dileptus* after operation. The arrows drawn with continuous line show the main direction of movement of the fragments after operation.

of the middle fragment were obtained and the two fragments which resulted after sectioning moved in opposite directions. Up to five consecutive operations were performed in this way on one individual ciliate.

No observations were made on the behaviour of the small fragments of the proboscis, because with continuous changes in the directions of movement, proper morphological orientation of these fragments could not be easily detected. Also no examinations were done as to the small middle fragments which folded into a ball or took on the shape of an ellipse. Also the very end section of the ciliate's body was not examined.

If an operation was performed too slow, sometimes a definite dislocation of the fragments occurred as a result of the action of the needle; then the fragments could separate at a different direction. The same could happen if as the result of a badly performed cut outflows of cytoplasm occurred, retaining temporarily the fragment on the spot. In such cases the posterior fragment started to move in the forward direction.

Summarising the obtained results it is possible to state that the fragments of the ciliate after sectioning start moving in the opposite directions, the posterior fragment moving in the direction as shown by the morphological posterior end of the body.

B. Further behaviour of the fragments and their regeneration

As was said before, if reversion occurs when the posterior fragments are moving backwards, it is only of short duration and the backward movement soon returns.

Then any fragment stops it re-starts moving in the direction of the morphological anterior end.

After about five minutes it was observed that all fragments moved towards their morphological anterior end irrespective of their previous direction. If reversions occur at this stage, the main direction of movement i.e. the forward direction always returns. The return to this direction after a previous backward movement mostly occurred after the ciliate had stopped at the edge of the water drop while restarting the move.

The fragments which contained proboscis and cytostom began already to kill and normally swallow *Colpidium* after 10 minutes, the posterior part beginning to slowly regrow. Also on posterior fragments regeneration follows comparatively quick. On ciliates sectioned behind the cytostom the killing of *Colpidium* by the trichocysts of the newly regenerated proboscis could be observed at the earliest after 40 minutes, and normal feeding with the regenerated cytostom could be observed after 61 min. Regeneration in *Dileptus anser* was described by Sokołow, (1924).

5. REACTIONS OF FRAGMENTS OF CILIATE

A. Reactions to mechanical stimulations

Fragments stimulation was done in a similar way as stimulating of the whole ciliate. When working with a micromanipulator, sections were done with a glass needle; when manual stimulation was applied, the ciliates were sectioned in the way described in the previous part of this paper. The surrounding areas of both poles of the ciliate were stimulated by touching with a needle — their side surfaces being stimulated during the control experiments.

Three kinds of experiments will be described: firstly with stimulation of the posterior fragment, then of the anterior one, and lastly with the fragment deprived of both the anterior and the posterior part (Fig. 7).

In the first case the individual ciliate was sectioned in the middle of its length, not counting the proboscis, that is in the middle of the

distance between the base of the proboscis and the posterior tip of the body. During the backward movement of the posterior fragment various parts of it were successively stimulated. When the areas in the immediate vicinity of the morphologically posterior pole of the fragment were stimulated, the individual as a rule receded, that is moved in the

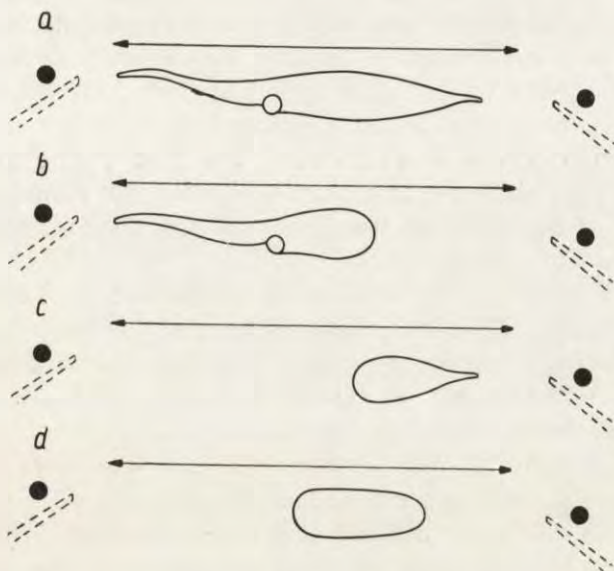


Fig. 7. Diagram of stimulation of the fragments of *Dileptus* and their reaction. The arrows show the direction of movement before and after stimulations, the black spots show the positions of the ultra-violet micro-beams, and the lines — the positions of the needle used for stimulating

a — the ciliate before stimulation; b — the ciliate after stimulation of the proboscis; (the movement was difficult to evoke); c — the posterior fragment; d — the middle fragment.

direction as pointed by the morphological anterior pole. During the forward movement of the fragment, stimulation was applied in a similar way. If the vicinity of the anterior pole was stimulated (that is the area adjoining the cut), the reaction also occurred. Stimulating of the fragment's other areas did not produce reaction.

Just as in the experiments on a whole ciliate, alternative stimulation of the poles was applied, thus keeping the fragment in constant forward and backward motion. In the case of the described posterior fragment no essential differences in the reactivity of the two polar areas were detected. Thus it was ascertained that a responsive area was formed also on the morphologically middle part of the ciliate, on surfaces

formed by the pellicle from the sides of the ciliate, which immediately closed over the cut. It was not found that this sensibility lessened with time after operation.

Next the response of the anterior fragment which resulted from the cut described above was examined. Stimulating was applied in a similar way and the results obtained were also similar, i.a. stimulation of its anterior part during the forward movement caused withdrawals and the stimulation of its posterior part during the backward movement caused the reversal into a forward movement. However a difference in the effects of stimulation was, that in this case the whole area of the proboscis was responsive just as in a whole ciliate, which means that it covered a comparatively much greater area than in the previous case. Unlike with the posterior fragment, response to stimulation of the posterior end of the anterior fragment did not always occur, that is it was not evoked by every touch.

Further, series of experiments were performed on *Dileptus* devoid both of the anterior and of the posterior part of the body. Cross sectioning was performed by the described method. The anterior cut was made immediately beneath the cytostom, the posterior cut — in the place where the body begins to narrow.

The same procedural method as in the previous cases was applied, namely stimulating at the poles and of the side areas of the fragment. Touching of the side areas did not evoke any response. When the areas of the poles were stimulated reversions occurred if the pole towards which the movement was directed was stimulated, notwithstanding the morphological orientation of the fragment. The areas of the poles were repeatedly stimulated and the fragment was kept in alternating motion, backwards and forwards. Reactivity was seen only in the immediate vicinity of the poles.

B. Examining the reaction to stimulation by ultra-violet radiation

Stimulation of the ciliate by means of a micro-beam of ultra-violet rays was performed using the described apparatus and similar methods. The advantage of these experiments over mechanical stimulation is that the radiation does not produce water sheak which can play a certain role in mechanical stimulation; besides this method assures more stable experimental conditions. In the first experiments the fragments were sectioned off by long exposure to radiation, using the method described by Tchakhotin (1937). However this caused outflows of the cytoplasm and the cuts were not as precise as in mechanical operations.

Before every experiment some 10 to 15 individual ciliates were sectioned with the forged steel needle at pre-determined levels, leaving some individuals untouched for the purpose of control. The material so prepared was covered with the slide and the depth of the water was so regulated as to leave the ciliates the necessary freedom of movement; then the proper experiment began. In every experiment the ultra-violet micro-beam was directed to the wanted spot on the fragment of the ciliate, or it was placed directly in front of it, so that the ciliate had to meet it on its way. The sections were made at the same levels as in previous experiments on mechanical stimulation, described above.

In the posterior fragment reversion occurred if the pole exposed to radiation were anterior in regard to the direction of the movement. Exposure of the side areas did not produce any reaction. Repeated stimulation was also applied and the results obtained were the same as for mechanical stimulation. Certain differences in the results occurred when the anterior fragment was exposed while moving backwards. Evoking reversion was more difficult than with the mechanical stimulation; it occurred often but after several trials. For these reasons repeated stimulations of the anterior fragment were not performed although its reactions during the forward movement were quite regular and appeared just as in a normal ciliate.

Exposure of the ciliate fragment with both ends eliminated produced very regular reversion reactions, although of short duration, if the area adjoining the pole (at this particular moment — the front one) was stimulated. Alternative stimulation was applied, producing normal reactions. Exposure of other parts of the fragment in this same way did not produce reaction. In this series of experiments middle fragments smaller than those described above were also examined and stable reactions were found even in fragment already taking the elliptical form.

In each of the above mentioned series of experiments some 100 to 500 ciliates or their fragments were examined.

6. DISCUSSION

Let us now try to present the results of the described experiments in a methodical way, not taking into consideration for the time being certain details which will be dealt with later in this discussion.

Stimulation of the anterior polar area of the ciliate during its forward movement and of the posterior pole during the backward movement evokes reversion of the ciliary movement. Let us assume that we always stimulate the anterior pole i.e. the anterior pole with regard to the

actual direction of the ciliate's movement and not necessarily the morphologically anterior pole. Let us call this pole the „anterior motor pole” and determine it by a „+” sign. Let us then call the other pole the „posterior motor pole”, determined by a „—” sign. (Fig. 8). Stimulation of the area of the front motor pole causes a change of 180° in the direction of the movement i.e. a change of localization of the motor

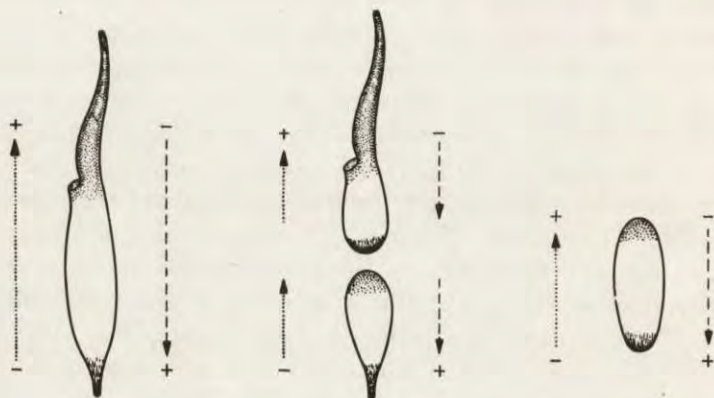


Fig. 8. Drawing of the location of the reception areas in *Dileptus*. The dotted areas denote the reactive part during forward movement; the dashed areas denote the reactive parts during the backward movement.

poles. Now the other morphological pole will become sensitive to stimulations that is the new anterior motor pole. Let us now call the area which, if stimulated at a given moment, produces a motoric reaction of the ciliae of the whole body (i.e. the present responsive area of the ciliate) the reception area. This reception area is always formed round the anterior motor pole. The introduced definitions concern only the functional aspect of the phenomena; they do not take into consideration at all the permanent structures of the ciliate. We will try now to verify if stating the problem in such a way holds also with regard to the results of the experiments on fragments of the ciliates. When sectioning the ciliate, the posterior fragment moves in the opposite direction to the anterior fragment. We can say that when this occurs we are faced with the formation of two motor poles with the same sign at the site of the cut. The inversion of the motor poles in the posterior fragments in relation to the morphological polarization has a more permanent character than was the case with tactile stimulation. Now by stimulating the anterior motor poles of the fragments we evoke successive reversions of the ciliary movement i.e. the inversion of the

poles and the change in position of the receptive areas. In this case it becomes specially clear that the areas of reception are not necessarily connected with a particular place in the ciliate cell, since they can appear in many places where it was sectioned.

This proves that the problem as set out at the beginning of these deliberations was correct. Of course the cell of the ciliate is not symmetrical at both ends as regards the polarization of the ciliary movement. The role of the morphological polarization of the cell is here seen clearly from the very beginning. As a rule the movement of the ciliate is directed towards the anterior morphological pole; the reversed polarization of the ciliary movement is not stable and there is always a tendency to conform the location of the anterior motor pole with the morphological pole. When the ciliate is sectioned, certain of its fragments may attain a state of unstable motor polarization contrary to the morphological polarization. Of course after a certain time all the bigger fragments of the ciliate move forward, which means that the morphological polarization always dominates in the long run.

If the matter is put like that it would follow that the actual direction of the ciliate movement and the position of its reception areas depend on the tug of forces between the morphological polarization and the factors which temporarily disturb it. Conformity of the motor and morphological poles would constitute a state of stable balance and should this state be disturbed, some factors would appear trying to restore it.

Let us now look a little closer at the localization of the reception areas. In a normal ciliate the sensitive part comprises the proboscis and the vicinity of the cytostom, although there is a certain difference of the extent of the area of responsivity to ultra-violet radiation and to mechanic stimulation. This difference is difficult to be explained; may be it lies in the difference in the location of some specific reactivity, appearing however only in this case. But it can equally be maintained that we may have to deal here with certain changes in localization of reactivity with regard to stimuli of different strength.

The extent of the reception area round the posterior morphological pole is smaller and comprises only the posterior sprout and its immediate vicinity. In the case of the ciliate being sectioned, the reception areas could be formed at the site of many of the cuts, but as was already mentioned, not all the possible combinations were examined. The weaker responsivity of the posterior end of the anterior fragment, which appears very plainly with exposure to ultra-violet rays, does not however appear on the fragment devoid of both the anterior and the posterior ends. In this case no difference is detected; reactions appear every time

when either of the poles is stimulated. The formation of the reception area at the site of the cut could perhaps be explained by the greater sensibility of the injured spot, if it was not for the fact that the reactivity increases together with the process of regeneration. Although it was possible to obtain reaction to the stimulation of every anterior motor pole, nevertheless we lack sufficient exact information to be able to state that this reactivity is of equal value irrespective of the site of the cut.

The side areas of the ciliate and their fragments, apart from the vicinities of the poles are not sensitive to touching or to short exposure to ultra-violet rays, but they can react by reversion to the obvious traumatic injury. As has been said, the reaction to traumatic injury is more lasting than the reaction to touching. When sectioning the ciliate we are dealing with the reversing of the ciliary movement in one of the fragments. It is possible that apart from the traumatic shock a certain role is being played by the separation of the fragment from the entity of the ciliate and thus becoming free of the direction dominating at this particular moment. The posterior morphological pole can temporarily take on the functions of the anterior one.

Following this conception, the hypothesis could be put forward that the effect of traumatic stimulation is in principle a strong direct disturbance of the state of polarization; this could explain the localization the reactivity to this stimulus over the whole of the ciliate's cell.

The experimental results described above do not say yet anything about the role of the neuromotoric apparatus, about the fibres which issue from it and about other similar structures for the reception of stimuli, transmitting and coordination. It should however be pointed out that the experiments dealt only with the effects of certain stimuli and one of the possible reactions, namely the reversion of the ciliary movement. It is possible that the role of the neuromotor apparatus would appear in some other phenomena, it is however a fact that the existence of the neuromotor apparatus is not the essential condition of the appearance of the reversion reaction, because these fragments which did not possess this apparatus also reacted. It would follow that the elements which are present in every examined fragment, such as the cilia and the ectoplasm must be responsible for the described phenomena. On the other hand it is known that the nucleus and the endoplasm do not play a direct role in the described phenomena.

The results of the experiments described in this paper do not point towards the necessity to attribute a different background to the ability to receive and transmit stimuli. The author shares the view of Par-
duc z as to the dominant role of polarization of the ectoplasm in the

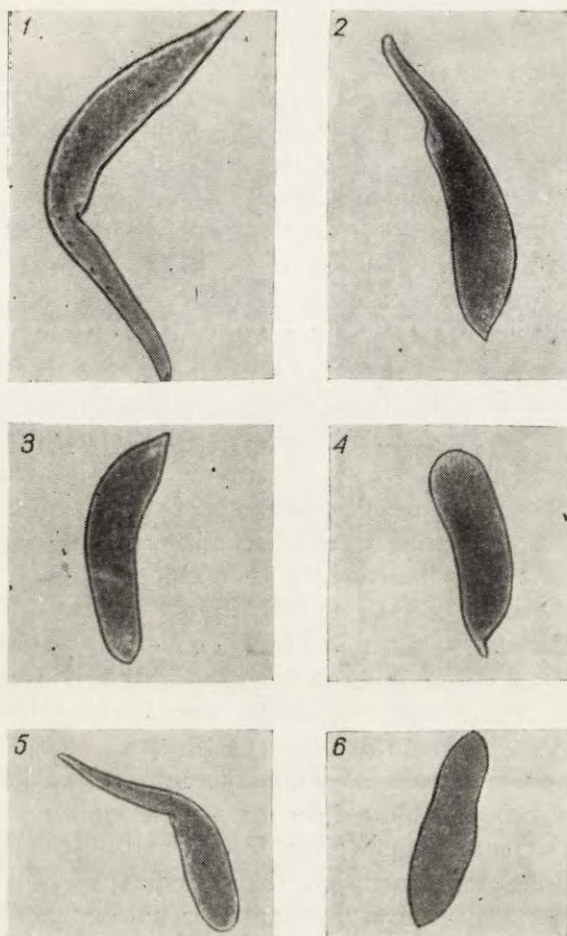


PLATE I

Photomicrographs taken of living specimens. Enlarged around 100 times

1. The normal individual of *Dileptus* used for experiments. 2. The individual with the anterior part of proboscis cut off. 3. The posterior fragment of *Dileptus* cut immediately after the cytostome. 4. The posterior fragment of *Dileptus* cut in the middle of the body. 5. The anterior fragment of *Dileptus* cut in the middle of the body. 6. The middle fragment of *Dileptus*, the anterior and posterior parts cut off.

coordination of the ciliary movement. Perhaps in future the application of stimuli of an exactly defined strength and the precise registration of the changes in the ciliary movement will bring some new developments in this field. The demonstrated important role of polarization of the cytoplasm would speak in favour of the fact that electrical phenomena play an important role in reception and transmission of stimuli in a ciliate. It should also be stressed that the reception areas form in ciliates in places which normally do not perform these functions. Compensation of the receptive functions appear before the regeneration of the structures normally responsible for them. It is not impossible that in this case the function preceeds here the formation of its base.

Let us now discuss briefly the problem of the quality of the applied stimuli and the specialized function of the receptive areas. We deal here with two groups of stimuli: mechanical stimuli, such as tactile and traumatic and the ultra-violet radiation, harmless when applied in small doses, but in big doses causing injury. The mechanical stimuli are without doubt natural stimuli. The role of the tactile stimulus is in natural conditions undoubtful, but we also have reasons to maintain that the traumatic stimulus constitutes in ciliate another factor of great importance. The great sensitivity of the ciliate to injury and their considerable regenerating abilities give us reason to assume that as these injuries often happen in normal conditions, these animals possess in respect to them certain adapting mechanism. Perhaps the results of the above described experiments on the reactivity of ciliate fragments and their reactions after sectioning should be interpreted in this way that ciliate has apart from the ability to regenerate another mechanism enabling them to adapt themselves to traumatic stimuli — a mechanism of compensation of reactivity. The biological role of the localization of the reception area round the anterior motor pole seems understandable because this pole is the first to contact the factors of the environment during the movement of the ciliate. It is also not impossible that the phenomenon of moving apart of the fragments after sectioning has a certain importance as an endeavour to withdraw from the noxious stimulus (the place in which the injury occurred). The water shock could be also considered as a natural factor. Contrary to these factors it can not be ascertained that ultra-violet radiation, at least of the intensity as used in our experiments is a factor acting on the ciliate in natural conditions. Such statement might be refuted if only on the ground of the weak conductivity of water to ways of this type. It is possible that the comparatively close conformity in the localization of the reception areas sensible to ultra-violet rays and of the areas sensible to tactile stimulation is the result of the sensibility of these areas to non-typi-

cal stimulation. In any case it would be too early to talk about any specialisation of the reception areas in *Dileptus* before examining of the effects of other stimuli. In his previous paper yet unpublished the author described the reactions of the fragments of *Dileptus* to chemical factors, finding the reverse reaction in all the examined fragments, irrespective of the place of the sectioning.

It should be mentioned that the *Dileptus* is a ciliate seldom examined up to now because of the difficulty in breeding and that so far no experimental examinations have been carried out on its ability to react to stimuli and transmit impulses. The author of this paper did not carry out similar experiments on other ciliates, so that the conclusions of this paper concern for the time being only the *Dileptus*. In the general discussion between Alverdes and Koehler the author would like to take a view different from both these authors. The receptive ability in ciliates is located in determined areas of the cell, but this localization depends from the actual state of polarization of the ciliary movement, and on the fragments of the ciliate a substitute location may appear.

SUMMARY AND CONCLUSIONS

1. In a normal *Dileptus* the reaction of reversion of the ciliary movement to touching and ultra-violet radiation appears if the stimulus is acting on the area round the pole which is at that particular moment the anterior one with regard to the direction of movement. The author calls this pole the „anterior motor-pole”.

2. In fragments of *Dileptus* the receptive functions are taken over by the anterior motor poles of the fragment.

3. The location of the receptive area depends on the actual state of polarization of ciliary movement; this area is situated round the anterior motor pole.

4. A state of equilibrium is established when the location of the motor pole and the morphological pole conform.

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EXPERIMENTAL STUDIES ON THE SELECTION AND ADAPTABILITY IN *PARAMECIUM CAUDATUM*

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In the cultures of *Paramecium caudatum* different types of self-defence against the noxious changes in the chemical properties of medium are observed. The self-defence of the culture by the secondary action of Protozoa on the chemical character of medium has been studied before (Grębecki and Kuźnicki 1956). The present study concerns the adaptation of *Paramecium* to the medium and the concomitant selection. The simultaneous study of adaptation and selection may be considered as an attempt to follow the conception initiated in protozoology by Gause and his school. The result of their research was summarized by Gause (1934, 1942) in his theoretical works.

The self-defence of Protozoa by action on the medium chemical character is influenced by the overdensity of population i.e. by the protective effect of accumulation of individuals (Grębecki and Kuźnicki 1956). This may be of no less importance for the self-defence arising as effect of the adaptation of Protozoa, since this process is involved by a continuous selection and overdensity is commonly considered as a selection factor. In view of the above theory, the present research was initiated by an analysis of cultures of various density degree, then of the selection process itself and finally the adaptation problem was studied.

OVERDENSITY AND UNDERDENSITY OF CULTURES

In biology two concepts concerning the effect of overdensity of the same species individuals are competing. According to the first — the overdensity action is inhibiting, to the second — it stimulates the deve-

lopment. The inhibiting effect is proved by the bent of the growth curve after a certain population number has been reached (Maupas 1866 and 1888, Coster 1936, Hall 1941). Nevertheless another data suggest a mutual stimulating action of protozoa individuals (Robertson 1908, 1921, 1922, 1923, 1924 a, b, c). These contradictory results require an experimental revision.

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Experiments analogous to Robertson's fundamental experiments (1921) were carried out. Paramecia were placed in 0.1 ml. of medium deriving from a low density culture ($\text{pH} = 6.8$, $t = 25^{\circ}\text{C}$) in 3 series, each consisting of 50 samples. In each sample of the series I a single specimen was placed, in the series II — 2 specimens, in the series III — 2 sister specimens deriving from 1 mother paramecium kept before division in the same drop of medium. For 72 hrs the number of paramecia was recorded at intervals of 12 hrs. The mean results are visualized in the Table I.

Table I

Division rate of paramecia kept single and by
2 in isolated drops of culture liquid

Time in hrs.	Number of paramecia deriving from		
	single	two alien	two sister
—	—	—	1.0
0	1.0	2.0	2.0
12	1.3	3.2	3.3
24	1.9	5.0	5.2
36	2.6	7.9	8.2
48	3.7	11.2	12.0
60	5.5	16.3	17.6
72	8.1	22.9	24.3

Single paramecia divide at a slower rate indeed. Although a fourfold numerical dominance over the single specimen progeny as found by Robertson (1921) in *Enchelys* and *Colpidium* was not observed, yet the dominance was threefold which corresponds to the acceleration of division rate by 50%. The results support the general conclusion of Robertson (1921) that the rate of multiplication increases in presence of other individuals of the same species.

Mass cultures were also used for experiments. The effect of accumulation of specimens upon the intensity of life processes (rate of food consumption) and on growth of the culture (changes in the number of individuals per unity of volume) were studied.

5 series of cultures were prepared:

- I — 1000 ml. culture of the density 100/ml.
- II — 200 ml. culture of the density 500/ml.
- III — 100 ml. culture of the density 1000/ml.
- IV — 50 ml. culture of the density 2000/ml.
- V — 20 ml. culture of the density 5000/ml.

Each of the 5 cultures contained initially 100.000 individuals (total number). 2.5 g. of powdered milk in 100 ml. of H_2O was added to each culture. Finally the cultures differed by their volume, density of protozoa accumulation and by the concentration of food supply. The absolute number of protozoa and the food supply ratio for each individual were the same in all the cultures. For 7 days, nephelometric measurements of the opacity of the centrifuged medium were executed every day. The relative opacity was defined, from which the food supply was calculated in % of the initial value. The results are visualized in the Table II.

Table II

Fall of milk nutrient concentration in cultures of various density in percentage of the initial concentration

Time in days	Density of culture — ind/ml.				
	100	500	1000	2000	5000
0	100	100	100	100	100
1	43	62	68	71	72
2	11	36	39	43	43
3	—	18	24	30	32
4	—	7	9	12	13
5	—	—	3	6	7
6	—	—	—	2	4
7	—	—	—	—	—

The rate of food consumption and, as consequence, the general rate of the vital processes of paramecium, falls gradually with the rise of the density of the culture. This conclusion is not fully comparable with the data known presently (especially Chejfec 1929), concerning either individual cultures with a food supply calculated for 1 indi-

vidual, or mass cultures — per unity of volume. In the present experiments mass cultures with food supply calculated per 1 individual were used.

A rise of population number in the culture was also observed in the 5 series of cultures. After 7 days, paramecia in 1 ml. of liquid were counted and percentage, related to the initial number was calculated. Results approximate to 100/ml. are represented in the Table III.

Table III

Changes in the density of cultures depending from the initial density (measured by individuals number/ml)

After 7 days,	Initial density of culture				
	100	500	1000	2000	5000
Absolute density	1300	2000	1600	1300	1200
% of initial density	1300	400	160	65	24

The rise of the culture density does not enhance divisions. This conclusion is not compatible with the results of Robertson's (1921) experiments which were repeated in the course of the present study with a positive effect (Table I). Presumably the discrepancy is involved by a different character of the culture which in the former experiments was individual and in the latter — a mass culture. To prove this supposition the method of Dimitrowa (1932) was applied, based on using the medium without the protozoa. For 5 days the division rate of single protozoa was observed: 1) in tap water, 2) in the medium of a culture 500 individ./ml., 3) in the medium deriving from a culture 5000 individ./ml. ($t. = 20^{\circ}\text{C}$). The medium of a moderate density culture gives the best rate of division. Probably there exists an optimal density of culture. Both overdensity and underdensity may be unfavorable, which was already pointed out by Allee (1931, 1934 and 1941).

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Factors inducing the inhibitory effect of overdensity are discussed by authors who study the so called „ageing of cultures”, and make the autointoxication responsible for the inhibition of growth (for detailed references see Hall 1941). As to the underdensity of cultures, the postulation of Robertson (1923) is widely accepted that the negative effect of the culture underdensity is the result of the deficiency of

autocatalytic or allelocatalytic substances in the medium. Consequently the factors of the culture density are looked upon as phenomena involved by the metabolism of protozoa or by secretion of some unknown specific substances.

The most part of Robertson's followers could not find a full confirmation of his results. Some authors tried to interpret them without accepting the theory of special substances enhancing the increase of population number of the culture, suggesting the change of pH (Darby 1930), rH (Jahn 1934) and of food supply (Chejfec 1929) as factors responsible for the density of culture. This interpretation seems to be the most adequate. If the so called phenomena of autocatalysis really depend on the conditions of medium — the possibility of replacing the action of partners living in the same drop of medium by the optimal and stabile conditions of medium — should be expected.

Cultures of isolated paramecia were kept for 3 days in 0.1 ml. of liquid. Differentiation of experiments concerned the temperature, ratio of nutrition added, its kind and method of preparation, and the value of pH. The medium was buffered. When one of the milk nutrients was applied (pH = 6.8, $t = 25^{\circ}\text{C}$) results were positive. In this series amounting 100 isolated infusoria, after 3 days, instead of 800 individuals as should be expected, 1927 were present. The acceleration of divisions was similar to that obtained in Robertson's experiments (1921) although it was evoked with exclusion of the eventual allelocatalysis. In the case of optimal and stabile medium conditions, the increased initial number of protozoa exerts no further stimulating action.

Thus the unfavorable action of the overdensity of the culture is not involved by the deficiency of some specific substances, but the factors of underdensity are the conditions of medium. A suggestion arises that they are also the decisive agent of the noxious influence of the culture underdensity. This supposition may be tested by mass cultures with an artificial regulation of physico-chemical properties of the medium, and of food reserves. If it was possible to rule in this way the process of the so called „ageing of culture” — the experiment would give the affirmation of the theory.

In a usual container the food reserves, but not the physico-chemical properties of the liquid, may be regulated. A special glass container — author's own device — described in another paper (Grębecki 1961a) as the in- and outflow vessel was used.

The advantage of the in-outflow vessel is the possibility of a uninterrupted renewal of the medium. Stability or any changes in the medium of the culture are available by means of a suitable choice of pro-

perties of the introduced liquid. The changes occur continuously. The action of protozoa may be evaluated (consumption of food, influence on the chemical composition of the medium) by confronting the liquid flowing in and out of the container.

For the analysis of the overdensity factors, both usual glass vessels and the in-outflow container were used. Experiments were run in 4 se-

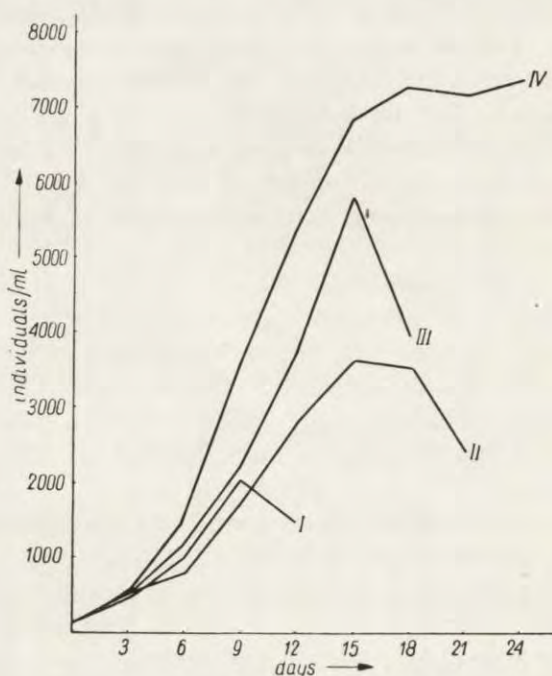


Fig. 1. Growth curve of the culture: inhibited by physico-chemical factors and by food deficiency (I), inhibited by physicochemical factor only (II), inhibited by food deficiency only (III), non-inhibited culture (IV).

ries. In cultures (volume = 50 ml., initial density = 100/ml.) the density was measured every 3 days. The medium was not buffered, the pH fluctuated about the value of 7.

The cultures of the I series in the ordinary vessels were fed only in the beginning of the experiment with 50 mg. of powdered milk. The maximal density up to 2000/ml. was reached on the 9th day of experiment, then the rate of development fell.

In the II series of cultures food was supplemented after it had been consumed. In the series I the development was exposed to inhibition by food deficiency as well as by the physico-chemical changes of medium, whereas in the II series only the second possibility of inhibition

could occur. A period of a relative equilibrium is followed by the bent of the development rate only after 15 to 18 days. Then the density exceeds 3500/ml.

The cultures of the III series were grown in in-outflow vessels. 500 ml. of nutrient passed every 24 hrs. through each culture, which corresponds to 12.5 mg. of powdered milk.

In this way the inhibition by physico-chemical changes of medium was excluded but food reserve was limited since its 24 hrs. dose was stabile and corresponded to the requirement of a 5000/ml. culture. Cultures of this series reached their peak density also about the 15th day but it rose to a much higher value amounting about 6000/ml.

The IV series cultures differed from the III in that the daily food dose was supplemented in the case when the former dose became insufficient. In this way both inhibiting factors were eliminated. On the 18th day or so of the experiment the culture density was 7300/ml. and fluctuated about this level till the 24th day. Then the experiment was stopped because of some technical difficulties.

Fig. 1 shows the growth curves of all the culture types. The curves support the hypothesis of two fundamental factors of the overdensity of culture. The role of the feeding factor requires no discussion. The role of physico-chemical changes in the medium should be correlated with the changes in the liquid of culture evoked by the passive influence (Grębecki and Kuźnicki 1956). The changes run in one respect and concern mainly the pH and rH shifting. For that reason in the scarce initial cultures they lead to the optimal medium conditions. Their influence is in this case stimulating and the underdensity — interfering with it — proves to be unfavorable. The changes continue to occur, exceed the optimum and gradually approach to the pessimal conditions. Their action becoming inhibitory is strengthened by the overdensity. This gives evidence that the overdensity is unfavorable.

OVERDENSITY AS A SELECTION FACTOR

In protozoology of the 30th a research trend of interspecies competition was developed (Gause 1934 a, b, c, d, e and 1935). The intra-species competition and the problem of „overpopulation” were not studied.

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To analyse the role of overdensity in selection it is necessary to study the reaction of cultures of different density after two competing groups had been differentiated in them and after the competition factors

had been stabilized i.e. when the changes in the medium characters which are to evoke the selection, occurred. In all the experiments, cultures of the over all density 200, 1000, 5000/ml. were used. Marking the competing groups was unnecessary in the case of interspecies competition. When intraspecies competition was concerned, the protozoa of one group were placed for 2 h. in the solution of neutral red (1:200 000). Protozoa treated by the stain neither decolorize nor exhibit any pathological symptoms (Grębecki and Kuźnicki 1956). To exclude the eventual influence of the stain, both competing groups were marked in turn in the successive experiments. The study was started by the competition in the cultures of *Paramecium caudatum*. As the competition factor the toxic substances (NaCl or quinine) were applied. The first group was previously made resistant against the action of those compounds, the second was treated with them first time. The resistant paramecia were able to tolerate the concentration of 100 mM NaCl, or 1:50 000 quinine. For normal paramecia the toxic value is 50 mM NaCl and 1:120 000 quinine. Normal paramecia were mixed with the resistant at a ratio 1:1, so that the total density of culture was 400, 2000, 10 000/ml. NaCl (90 mM) solution or quinine (1:70 000) was added to each culture in volume ratio 1:1. In conclusion, each culture contained 200, 1000 or 5000 resistant and normal individuals in 1 ml. of 45 mM NaCl solution or of 1:140 000 concentration of quinine solution. The percentage density of both groups was recorded every day till the state of a relative equilibrium was established. Mean values are given in the Table IV.

The difference of the selection process in both solutions concerns the fact that in the case of quinine the elimination is more complete, so that after a week the unresistant infusoria disappear almost completely, whereas in the case of NaCl on the fourth or fifth day, about 10% of their number are present, and this state tends to persist. Presumably the adaptation process to NaCl depends rather upon the selection of protozoa, and to quinine — on the adaptation in the proper meaning. In the case of quinine the competition between the adapted and unadapted groups is observed, and after the NaCl treatment — between the previously selected and unselected.

The expected acceleration of selection evoked by the overdensity of culture is not observed in any of the two solutions. Consequently it should be assumed that overdensity cannot be in this case the factor of selection.

The interspecies competition of *P. caudatum* and *P. aurelia* was examined. The competition factor was NaCl in the medium. According to Bullington (1930) *P. aurelia* is more resistant to the action of sea

Table IV

Elimination of normal individuals of *Paramecium caudatum* by the resistant in NaCl and quinine solutions (% of normal infusoria)

Days of experiment	Solution of NaCl			Solution of guinine		
	initial density			initial density		
	200	1000	5000	200	1000	5000
0	50	50	50	50	50	50
1	42	42	43	48	47	48
2	30	31	29	42	40	40
3	18	17	16	30	31	31
4	12	10	11	15	17	16
5	10	9	12	4	6	6
6	11	9	10	2	1	3
7	9	10	8	1	1	2
8	8	9	9	1	1	1
9	9	9	10	1	1	1
10	10	8	10	1	1	1

water than *P. caudatum*. It was also stated that in the NaCl solution *P. caudatum* was eliminated by *P. aurelia*. The former experiment was repeated to prove whether this kind of selection depends on the density of culture. The medium was continually renovated. In all the cultures the elimination of *P. caudatum* by *P. aurelia* proceeded at an approximately even rate; the difference in the initial density caused no deviations. Also in this case overdensity proved not to be the factor of selection.

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In the subsequent experiments, the mixed cultures of *P. caudatum* and *P. aurelia* were used and the competition factors were altered. As the first factor, physico-chemical changes of medium in a not renovated culture were applied, as the second — the food deficiency. Gause, Alpatov and Nastukova (1934) applying the medium of *P. aurelia* with no infusoria to the *P. caudatum* culture and vice versa, stated that *P. aurelia* is much more resistant to physico-chemical changes of culture liquid bound to the metabolism of protozoa. Yet *P. caudatum* utilized sooner and more completely the food reserves of the cul-

ture. Although in the normal mixed culture *P. aurelia* dominates in the competition, but in a continually underfed culture the domination of *P. caudatum* may be involved.

Experiments with the mixed cultures of both species were repeated applying the procedure of the culture which was discussed in the former chapter, as inhibiting of development by natural physico-chemical changes in the medium. All the 3 series of cultures (200, 1000, 5000/ml.) were kept in a never renovated medium but were well fed. This time the difference in the initial density involved the differentiation of the selection rate, which is visualized by the curve of diagram 2 (the full line). The percentage share of *P. caudatum* in the culture falls the quicker, the higher was the initial density. In average it falls after 21 days from 50% to about 10% in a culture 200/ml., after 18 days in 1000/ml. and already after 10—12 days in a 5000/ml. culture.

In the next series of experiments, cultures were placed in the in-

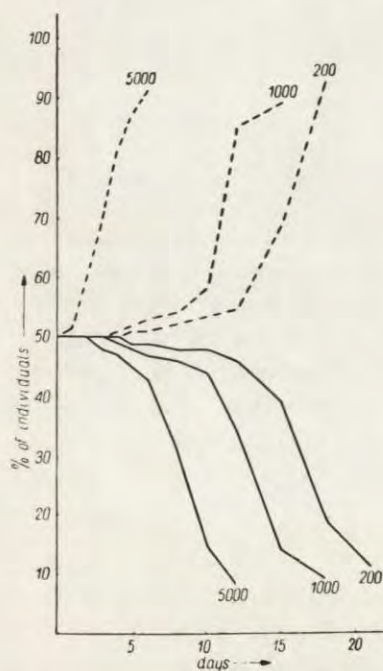


Fig. 2. The course of competition between *P. caudatum* and *P. aurelia* in the culture: inhibited by physico-chemical factors (full line), — by food deficiency (interrupted line) and of various initial density (5000, 1000 and 200 ind/ml.).

outflow vessels in which the medium was changed 10 times in 24 hrs., but the food ratio was supplied in a constant amount = 12.5 mg. of powdered milk per 24 hrs. This time the competition factor was the inhibition by food deficiency instead of the physico-chemical agents. As follows from the curve (Fig. 2 — interrupted line) the intensity of

selection depends also in this case upon the degree of initial density, although the result of competition is opposite to the previous. The percentage share of *P. caudatum* in the mixed culture rises the quicker the higher was the initial density. In average it reaches 90% from the initial 50% after 18 days in the culture of 200/ml., after 15 days in 1,000/ml. and after 6 days in 5,000/ml. culture. The detailed study of quantitative data are discussed elsewhere (Grębecki 1961b).

Overdensity may be or may not be the factor of selection. It may accelerate it or may not influence its course. The action of overdensity does not depend on intraspecies or interspecies character of competition since in the experiments with NaCl as the competition factor, the influence of overdensity on the rate of selection was not observed. Neither was it detected in the case of competition between the resistant and non-resistant individuals of *P. caudatum* nor between *P. caudatum* and *P. aurelia*. Difference in the action of overdensity becomes on the contrary evident when experiments in which the factor of competition is NaCl or quinine in the medium are confronted with those in which food deficiency or physico-chemical changes in the medium caused by the metabolism of protozoa, involve the competition. It should be stressed that in both cases the competition factors were of a different nature. NaCl and quinine are in the medium artificial and alien factors, not connected with the overdensity of the culture. Food deficiency and accumulation of the products of metabolism in the medium are decisive factors of the development in the normal culture, pointed out in the preceding chapter as factors of overdensity. Thus in the process of selection in the culture of protozoa, the overdensity seems to promote selection if the competition factor results from the overdensity of the culture itself, but it does not influence selection when the factor of competition is not connected with the degree of overdensity.

SELECTION AND ADAPTATION IN THE CULTURES OF *PARAMECIUM CAUDATUM*

The selection of individuals with different isolation capacities may occur after a noxious chemical factor had been introduced to the culture of *Paramecium caudatum*. Consequently selection seems to be the factor interfering with adaptation. This fact is manifest also in the specificity of the adaptation process and in its either continuous or abrupt character. For the study of the specificity of adaptation it was necessary to adapt a culture to the presence of a substance introduced into the medium. The adaptation to NaCl of a 100/ml. culture was executed.

In the recent literature no attempt of adaptation of protozoa to NaCl have been reported, but experiments concerning the adaptation of protozoa to sea water or to the Osterhout medium are known. Finley (1930) obtained the adaptation to 100‰ oceanic salinity, Yocom (1934) — to 20‰, and Frisch (1939) — to 40‰ which corresponds to 35‰, 7‰ and 14‰ respectively. The results of Gause (1939) show difference in various kind of clons, but correspond approximately to that of Yocom (1934). All the authors mentioned applied the passaging method, except Frisch who supplemented the medium with a solution of double concentration when 50‰ of the liquid was evaporated.

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In the present study protozoa were first adapted by the passaging method. Cultures were placed in the 25 mM solution of NaCl which corresponds to 0.5 of the toxic value. The cultures of infusoria were centrifuged at intervals of 2 days and placed in a fresh medium. The concentration of NaCl was raised by 10‰ of the initial toxic value. Passaging was repeated until the density of culture started falling below the initial value. In this way the procedure of adaptation could be continued for a month reaching the medium concentration of NaCl in the liquid = 100 mM. This result indicates the rise of resistability by 200‰ of its initial value and lies in the limits of Yocom (1934) and Gause (1939) findings.

For proving the specificity of the adaptation process which occurred in the culture, resistability of its infusoria against other substances was tested. Metals chlorides were used: LiCl, MgCl₂, KCl, CaCl₂, MnCl₂, CoCl₂, NiCl₂, CuCl₂, ZnCl₂, SrCl₂, CdCl₂, BaCl₂, HgCl₂, PbCl₂, as well as sodium salts: NaF, NaBr, NaJ, Na₂S₂O₃, Na₂SO₃, Na₂SO₄, Na₂CrO₄, Na₂WO₄, NaNO₂, NaNO₃, NaAsO₂, Na₃AsO₄, Na₂CO₃, NaBO₂, NaCN, NaCNS, Na₂Fe(CN)₅NO. In a watch glass, 1 ml. of liquid containing 500 specimens of rinsed protozoa was added to 1 ml. of the tested substance in the concentration double to the desired. As the toxic value — like in the former research (Grębecki and Kuźnicki 1956) — the concentration killing half of the culture individuals after 24 hrs. was considered.

The adaptation process proved to be not fully specific, as the required resistability against NaCl involved also the resistability against some other salts. The highest rise of resistability (over 150‰ of the initial) was found for: KCl and NaBr, NaJ, Na₂SO₄, NaNO₂, NaNO₃. Somewhat lower (120—150‰ of the initial) was the rise of resistability against CaCl₂ and Na₂CrO₄, Na₂WO₄, Na₂Fe(CN)₅NO. A low resistability

was stated for LiCl and SrCl_2 as well as for NaF and $\text{Na}_2\text{S}_2\text{O}_3$. Toxicity values of all the other salts remained without detectable alteration.

As follows from the above observations, the rise of resistability occurs more frequently and is stronger against the sodium salts than in the case of chlorides. This fact allows to assume that ion regularities stated for the phenomena of toxicity (Grębecki and Kuźnicki 1956) and chemotropism (Dryl 1952) hold to some degree true also for the adaptation. The action of cations is more effective for the adaptation of a culture than that of anions.

On the other hand, the toxicity of the Na salts for the infusoria adapted to NaCl was not diminished in the case of all the basic salts raising pH of the medium. The majority of chlorides keeping their primary toxicity, contain metals of a high atom weight. It seems therefore that the adaptation of a culture to a compound exerting osmotic action (NaCl) is non-specific to another substance osmotically active. Yet it is specific if another substance is concerned, the action of which is of a different physico-chemical nature.

In another series of experiments, adaptation to quinine was tried by placing the culture in a 1 : 240 000 (in weight ratio) — 50% of the toxic value. After 19 passages infusoria remained alive in a concentration 1 : 50 000 i.e. the resistability = 240% of the initial was obtained. The procedure described above was applied for testing the resistability of infusoria (adapted to quinine) against osmotically active substance (NaCl and KCl), acting on the pH of the medium (Na_2CO_3 and NaCNS), containing an ion of heavy metal (HgCl_2 and CuCl_2) or other plasmatic drugs (strychnine, Nile blue). The experiments proved that the resistability against quinine fails to involve the resistability against the other substances used in the experiments. The insignificant resistability against strychnine lies within the limits of error.

*

All the attempts of adaptation of protozoa to chemical factors reported in the literature were executed by introducing sudden changes in the concentration of the substance added to the medium since the passing procedure was applied and every passage is a sudden transfer of the culture into a new medium.

Experiments with NaCl and quinine carried out with the aid of this method have been described in the previous chapter. A problem is to be solved whether the sudden alterations of an external factor are a favorable or inhibiting agent for the adaptation of the culture. In the subsequent experiments continuous changes of concentration were

applied. All the other factors remained unaltered. 100/ml. cultures were placed in a liquid with 0.5 toxic value concentration, the daily rise of concentration was 5% of the toxic value. These conditions were procured by using the in-outflow vessel mentioned in the preceding chapter. Every day at a fixed hour dropping of NaCl or quinine was started in a solution exceeding that of the last day by 5% of the toxic value. In this way the old medium was not replaced by the new one, but penetrated by drops to the culture, diffused in it and gradually eliminated the old liquid. The change of medium was complete when 200 ml. of liquid has passed through the vessel. Within 24 h. 500 ml. of liquid were passing. After the exchange of the medium liquid, a rather long period of stability of concentration was established. Next day the exchange of medium liquid was repeated. The daily dose of food dropped in together with the experimented substance was adjusted to the dose applied in the method of ordinary passage. At some intervals 1 ml. of culture was taken for the count of individuals. Initially the number of specimens/ml. rises, then a period of fluctuation follows and at last an abrupt fall is observed. The day when the number/ml. of the culture fell below the initial value of density, was considered as the limit of adaptation. In the case of NaCl the continuity or abruptness of changes of the external factor acting on infusoria is not essential. By means of both methods, adaptation of cultures to the concentration of 100 mM was reached. In the experiments with quinine, if the concentration increases continuously, the infusoria remain viable in the concentration corresponding to 295% of the initial toxic value, i.e. containing nearly 1 : 40 000 quinine in weight ratio, whereas the parallel data of experiments in which the abrupt rise of concentration was applied, amounted only 240% i. e. 1 : 50 000 of quinine content.

The difference in the adaptation of culture to NaCl and to quinine consists not only in this fact that the adaptation to quinine seems to be fully specific and to NaCl relatively non-specific. Another difference consists in the fact that the continuity of concentration changes of quinine is favorable, and in the case of NaCl the adaptation process is not influenced by the continuous or abrupt character of changes in the medium. The comparison of effects after the abrupt and continuous passaging of the culture is not solving the problem, because the course of adaptation itself may prove to be continuous or abrupt independently from the character of changes in the acting external factor. The data established till now indicate that the adaptation process is abrupt (Chęjfec 1939). It is proved by the existence of critical periods in its course characterized by inhibition of quantitative growth and even by a periodical fall of density.

A problem arises as to the essential character of processes developed in the critical periods. It may be assumed that some fundamental physiological changes occur, involved by the developing adaptation, which enable the infusoria to tolerate a higher concentration of the toxic substance. Another possibility is that the intensification of the toxic factor rises to such a high level which cannot be tolerated by a considerable number of individuals; then a selective mortality begins and only the surviving infusoria are subjected to the adaptation.

Chejfec (1939) after applying in his experiments CaCl_2 noticed that the initial concentration of the compound in the medium — relatively low or high — is of a minor importance, if it is lower than the toxic value. The results of Chejfec speak in favour of a rather selective than adaptative character of the critical periods. This problem deserves an experimental revision.

The adaptation treatment by continuous passaging was repeated. At 2 days intervals the protozoa number/ml. of the culture was counted; the culture of the same population density and the same concentration of the substance was prepared and the simultaneous treatment of adaptation was started. The factors of experiment were the same, the infusoria differed only by their past treatment till the initial moment of experiment. Differences in the adaptation process to NaCl and to quinine became evident again. As for NaCl the observation of Chejfec (1939) were confirmed. Below the toxic value the level of initial concentration has no effect on the course of the adaptation process. The experiments with quinine are more successful when adaptation begins with a possibly low initial concentration.

These results indicate that the process of adaptation to quinine seems to be the adaptation *sensu stricto* and adaptation to NaCl occurs with the cooperation of selection processes. Indeed, the treatment like the continuous passaging, begun with a very low concentration, may enhance the resistability of infusoria but cannot influence the survival of those which were more resistant than the others since the beginning of the experiment.

SUMMARY

Overdensity as well as underdensity may be noxious for the cultures of *Paramecium caudatum*. The stimulating or inhibitory influence of individuals upon one another is not effected by autocatalysis or allelocatalysis but follows from the influence of each individual upon the common medium. Infusoria shift the pH and rH of the culture liquid (Grębecki and Kuźnicki 1956) creating more favorable condi-

tions for the development of culture (the stimulating influence — underdensity is noxious). The subsequent changes in the culture medium exceed the optimal value (the influence becomes inhibiting — overdensity is noxious). The essential role is also played by the food conditions which are another fundamental factor of overdensity.

The concept of two fundamental factors of overdensity may be proved experimentally. The alternate elimination of the density factor increases the maximal possible density of the culture.

The subsequent series of experiments were an attempt to prove whether overdensity may be a selection factor. Selection was evoked by involving the competition between the individuals with the previously acquired resistance against NaCl or quinine and without it. Non-resistant individuals were eliminated but the intensity of competition effect proved to be independent from the initial density. In this case overdensity is not the factor of selection.

In the competition between *Paramecium caudatum* and *P. aurelia* in a mixed culture in NaCl solution, the density of culture has no influence upon the selection rate. It should be concluded that the interspecies or intraspecies character of the competition is not decisive.

Overdensity enhances the selection when the competition of *Paramecium caudatum* and *P. aurelia* is involved by a factor of another nature. In a culture subject to food deficiency *P. caudatum* eliminates *P. aurelia*; there is an opposite effect when the culture development is inhibited by physico-chemical factors. This time the selection proceeds the quicker the higher is the density of the culture. The difference depends on the character of the competition factor.

The competition factor may be extrinsic, not bound to the degree of density (f. ex. addition of NaCl or quinine). In this case overdensity does not enhance the selection.

The competition factor may be involved by the overdensity itself (food deficiency or action of the metabolism products upon the medium). Then overdensity perceptibly accelerates the rate of selection.

Adaptation phenomena were studied as connected with selection. The process of adaptation to an osmotically active substance (NaCl) proved to be non-specific, but it was found fully specific when a plasmatic drug (quinine) was applied.

The adaptation of infusoria was evoked by using the vessel with a continuous flow of liquid through the culture which established a continuous change in the concentration of the passaged liquid.

In the case of NaCl, the continuity or abruptness of passing are of no importance; for adaptation to quinine the continuity of the concentration rise is more effective. The same results were observed when

infusoria were quickly placed in the sublethal limit concentration of NaCl. This result was not found after the application of quinine.

Presumably the plasmatic drugs acting upon the cell body may evoke the real adaptation, whereas the osmotic drugs, inflicting before all the pellicula, involve rather the selection process in the culture.

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SUR L'ÉTHOLOGIE GUERRIÈRE DE *FORMICA SANGUINEA* LATR.
(HYMENOPTÈRE, FORMICIDAE)

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INTRODUCTION

Les descriptions de la stratégie de la *F. sanguinea* que l'on trouve chez Huber (1810), Forel (1874), Escherich (1917) et d'autres auteurs donnent l'impression que la stratégie de cette fourmi est carrément géniale. En tant qu'exemple, voici la description bien caractéristique de Forel: « Le 14 juillet 1901 [...] je vois un petit peloton de *F. sanguinea* sortir de son nid situé dans un pré et se diriger dans l'herbe vers un arbre. Arrivé à deux mètres du nid ce peloton hésite, s'éparpille un peu et envoie quelques émissaires au nid pour appeler d'autres ouvrières. Puis les sanguinea avancent de nouveau peu à peu jusqu'à environ cinq mètres de chez elles, suivies d'un second peloton analogue. Entre les deux pelotons, les fourmis vont et viennent, se frappant vivement les antennes les unes les autres, et ainsi de suite. Un troisième peloton suit le second, puis un quatrième; tous envoient des émissaires en arrière. La marche est tortueuse, toujours hésitante; les fourmis paraissent chercher et n'avancent que fort lentement. A dix heures elles sont à peine à vingt mètres de leur nid et les premiers pelotons deviennent indistincts; mais les ouvrières continuent toujours à sortir du nid dans la même direction en formant de nouveaux pelotons. Arrivée vers l'arbre à 30 mètres environ du nid vers 10³/₄ heures, cette armée éparpillée, marchant irrégulièrement par soubresauts, fait un léger angle et accélère un peu sa marche; elle doit avoir quelque chose en vue. Je cherche et je découvre un nid de *S. glebaria* situé à près de 40 mètres de celui des *F. sanguinea*. Vers 11¹/₂ heures les premières ouvrières des voleuses s'en approchent ce qui alarme les *glebaria*; celles-ci font même une prisonnière qui s'était trop avancée. Mais

aussitôt que les sanguinea ont reconnu le nid, elles se retirent et courent en hâte alarmer leurs compagnes en les frappant vivement; pas accéléré en avant, marche! »

Presque tous les auteurs reviennent surtout d'une façon opiniâtre à la question des éclaireurs et des émissaires (d'après Forel). Forel écrit que quand des *F. sanguinea* découvrent un nid de *F. fusca*, elles communiquent ce fait à leur armée en frappant le front de leurs compagnes. Ghighi (1951) affirme que *F. sanguinea* envoie des avant-gardes peu nombreuses qui cherchent les endroits faibles du front de l'ennemi.

Personne n'a d'ailleurs prouvé d'une façon expérimentale l'existence des « éclaireurs » des « émissaires » ou de « l'avant-garde ». Ces notions ont été adoptées après Wheeler (1910) et Escherich (1917) qui affirment que l'on ne peut pas expliquer autrement le fait que *Formica sanguinea* tout comme *Polyergus rufescens*, va directement vers le but.

Les descriptions de Brun (1924) donnent une impression quelque peu différente: „An einem schwülen Julimorgen herrschte auf der grossen Durchgangsstrecke, welche meine Sanguinea bis zu ihrem etwa 8 m vom Nest entfernten Lieblingsblattlausstrauch zu benutzen pflegten, eine ungewöhnliche Bewegung. In kurzen Zwischenpausen verliessen zahlreiche einzelne Ameisen, dann wieder ganze kleine Trupps von 10 bis 20 Individuen das Nest, um schrittweise in der genannten Richtung vorzurücken. Bald war es offenkundig, dass sie diesmal in ganz anderes Ziel vor sich hatten, nämlich eben jenes Rufa-Nest, das von ihrer eigenen Siedelung mehr als 22 m entfernt. Schon gegen 10 Uhr war das erste Peloton der Räuber vor dem Nest der Rufa angekommen und mit deren Vorposten aneinander geraten. Ohne erst Verstärkungen abzuwarten, griffen die Sanguinea mit dem gewohnten Ungestüm ihrer Rasse an, sahen sich aber bald ihrerseits in die Verteidigung gedrängt, nachdem es den Rufa anfänglich dank ihrer Übermacht gelungen war, einige Sanguinea zu umzingeln und gefangen zu nehmen. Nun begann ein äusserst interessanter Kampf, der etwa 2½ Stunden währte, und in dessen Verlauf die Rufa Vorposten durch die in Haufen heranrückenden Sanguinea allmählich gegen das Nest zurückgedrängt wurden”.

D'après une opinion qui est aussi généralement admise (Forel 1874, Escherich 1917 et d'autres auteurs) les attaques ont lieu très rarement, 2 ou 3 fois par an, ce qui explique (Forel) l'insuffisance de nos connaissances à ce sujet. Raignier estime que la rapidité de l'armée est égale à 1 m/min. (ce qui est en désaccord avec la description de Forel et d'autres auteurs) et que la distance qu'elle peut atteindre est de l'ordre de 80 m.

Dans la lutte *F. sanguinea* essaie d'intimider son adversaire et de

provoquer sa fuite (Lubbock 1884), elle n'attaque les individus qu'en cas de défense de ces derniers. D'après Huber, *F. sanguinea* arrache les nymphes aux fourmis en fuite mais elle ne les attaque pas si ces dernières se laissent prendre les nymphes sans combat.

Comme il en résulte des descriptions qui viennent d'être citées, l'interprétation du comportement des fourmis pendant les expéditions guerrières de *Formica sanguinea* n'est pas satisfaisante. Le postulat concernant l'existence des éclaireurs ne possède pas de preuves convaincantes.

Le but de mon travail était de vérifier si vraiment les expéditions de *F. sanguinea* se basent sur des mécanismes aussi compliqués. Des observations préliminaires m'ont démontré que cette marche directe vers le but dont Escherich et Wheeler faisaient mention n'existe pas. Cette conclusion résulte d'ailleurs déjà d'une analyse attentive des descriptions de Forel et de Brun.

La myrmécologie admet certaines expressions comme par exemple « l'armée » les « esclaves » et autres, qui pourraient susciter certaines réserves. Ne pouvant m'occuper dans ce travail de formation d'une nouvelle terminologie, j'utilise donc ce genre d'expressions en faisant toute réserve contre leur compréhension trop littérale.

PARTIE EXPÉRIMENTALE

Expériences ayant pour but de trouver les ouvrières *F. sanguinea* assumant les fonctions d'éclaireurs

Expérience du type 1.

Pendant l'attaque naturelle du nid de *Formica fusca* je marquais la plus grande quantité possible de *F. sanguinea* qui y prenaient part. Un jour ou deux après la fin de l'expédition je ramassais les ouvrières marquées et je les transportais sur des nids de *F. fusca* assez proches, mais pas encore pénétrés (un de ces nids se trouvait à une distance de 9 m du nid de *F. sanguinea*, l'autre à une distance de 12 m). Ces nids étaient assez proches pour que les individus transportés puissent retrouver le chemin de leur nid natal. La majorité a d'ailleurs vraiment retrouvé son chemin et tout au moins la moitié a retrouvé son nid sans grandes hésitations. Pendant les quelques jours suivants les nids ont été constamment observés. Si l'attaque avait eu lieu cela évidemment n'aurait pas encore été une preuve suffisante en faveur de l'existence des éclaireurs. Mais les nids ne furent pas attaqués jusqu'à la fin

de la saison, c'est à dire pendant un mois pour l'un des nids, et pendant deux pour l'autre. En somme 170 individus qui prenaient sans aucun doute part dans des attaques, ont été mis en contact avec des nids de *F. fusca* sans susciter aucune attaque. Il est évidemment bien possible que le transport des fourmis constitue pour elles un puissant stimulus négatif, qui les empêche de retourner vers les nids sur lesquels ils furent transportés. Il faut signaler que contre cette possibilité pourrait témoigner le fait que trois des individus déplacés ont été plus d'une fois remarqués dans les environs de ces nids de *F. fusca*.

Le fait que pendant toute une saison un nid d'une espèce esclave se trouvant très près d'un nid de *F. sanguinea*, qui entreprenait en même temps des expéditions à une centaine de mètres de distance, n'a pas été une seule fois attaqué, est assez remarquable en soi-même.

Expérience du type 2.

Pour éviter ce genre d'inconvénient j'ai commencé à marquer les sanguinea qui se trouvaient d'eux-mêmes dans les environs des nids de *F. fusca* en question et à les transporter sur le nid même. Dans les environs des deux nids pendant cinq semaines je n'ai trouvé que 23 individus. Pendant le transport j'essayais de les inquiéter le moins possible en les transportant avec la feuille ou le bâton sur lesquels elles furent trouvées; dans ce cas les fourmis ne trahissaient aucun trouble, le terrain ne leur étant pas étranger, et elles continuaient à circuler aux alentours du nid de *F. fusca*, sans démontrer de tendance à prendre fuite. Ce genre d'expériences n'a également pas donné le résultat attendu sous forme d'attaque du nid de *F. fusca*. Ces deux genres d'expériences n'ont pas donné de réponse à la question posée.

Dans le cas du premier type, les individus transportés prenaient part dans des expéditions ayant pour but le ravissement des nymphes, mais ils n'étaient pas forcément des éclaireurs, étant donné, que cette fonction peut être spéciale et que pas tout membre de l'expédition peut être capable de l'assumer.

Dans le deuxième type d'expériences il est évident que les fourmis en pâturage dans un terrain donné peuvent être privées d'instinct de pillage des nids étrangers.

Ces expériences ont prouvé l'insuffisance de cette méthode: il faut rejeter les tentatives directes de prouver l'existence ou l'absence des éclaireurs. Il faut employer une autre méthode requérant bien plus de temps: l'observation des attaques naturelles ou artificielles des nids étrangers par *F. sanguinea* et le marquage individuel des membres de ces expéditions.

Observations et expériences ayant pour but d'étudier d'une façon exacte, le cours des attaques

Pour investiguer tout le cours d'une expédition à partir du premier moment, j'observais un nid de *F. sanguinea* sans interruption pendant des semaines entières. C'était absolument nécessaire car les attaques sont rares quoique bien plus fréquentes que l'estimait Forel. Aucun moyen plus économique n'était possible: des observations moins systématiques pourraient laisser échapper certains des phénomènes qui précèdent l'attaque; d'autre part, avant d'entreprendre des essais ayant pour but d'obtenir des attaques artificielles, il fallait évidemment connaître auparavant le cour typique de l'attaque naturelle pour avoir une possibilité de comparaison.

Pendant toutes ces observations il était nécessaire de noter constamment le comportement des fourmis car on ne pouvait jamais prévoir le moment de l'attaque.

Je pense que ces difficultés sont la cause du manque d'une description complète et précise d'une attaque de *F. sanguinea* quoique un grand nombre d'auteurs éminents ait investigué cette espèce. C'est aussi pourquoi toutes les descriptions des attaques connues commencent à une étape déjà assez avancée. C'est justement grâce à cette partie la moins expérimentale de ce travail que j'ai pu obtenir l'image de la situation typique précédent l'attaque et de l'attaque même.

Ce résultat se base sur l'observation de quelques dizaines d'expéditions.

Résultat typique de l'observation.

Le nid en question est de dimensions moyennes, les esclaves sont de l'espèce *F. fusca*. Ce nid possède une route permanente menant à des pucerons, situés sur un arbre éloigné du nid d'une dizaine de mètres. Comme les esclaves ne s'occupaient pas des pucerons cette route était pratiquement fréquentée uniquement par *F. sanguinea*. L'affluence sur cette route augmentait de jour en jour, la route elle-même s'allongeait et s'élargissait dépassant largement l'arbre portant les pucerons. Les individus qui dépassaient l'arbre ne s'occupaient pas des pucerons. D'autre part les individus qui s'occupaient des pucerons venaient vers eux sans hésitations et n'ont jamais été aperçus en dehors de l'arbre ou du chemin reliant directement ce dernier avec le nid.

Le chemin de *F. sanguinea* diffère entièrement des chemins de *F. rufa* ou de *F. pratensis*, ayant 3 ou 4 m de largeur.

12 jours après le début des observations le chemin atteignit un nid de *F. fusca*. Une partie des fourmis qui pâturaient dans les environs pénétra alors à l'intérieur du nid et se mit à enlever les nymphes. Au bout d'un certain temps la quantité des ravisseuses augmenta aux dépens des individus pâturent dans le voisinage. Il me semble que c'est justement ce fait qui a donné l'impression d'opérations stratégiques, d'arrivée de renforts, etc. En plus il est possible que les nombreuses fourmis circulant sur le même chemin et ne prenant pas part au pillage ont été prises pour des unités exécutant des missions spéciales, pour des agents de liaison, des émissaires etc.

L'attaque a une autre allure dans le cas d'une rencontre d'un nid de *F. rufibarbis*. Je ne présente que les traits caractéristiques.

Tout d'abord les premières *F. sanguinea* qui découvrent le nid de *F. rufibarbis* ne peuvent pas pénétrer immédiatement dedans en raison de la résistance présentée par le nid attaqué. Elles commencent donc une lutte, dont la violence croît progressivement car, comme dans le cas précédent, les *F. sanguinea* se concentrant aux environs du nid deviennent de plus en plus nombreuses. Dès la rupture de la résistance, l'ensemble de l'attaque ne diffère du cas précédent que par le fait qu'une partie des agresseurs est obligée de se défendre contre les *rufibarbis* qui défendent encore leur nid.

Expérience du type 3.

Avant d'entreprendre les expériences avec les nids artificiels il a fallu déterminer le comportement de *F. sanguinea* à leur égard car dans nos travaux antérieurs — (Dobrzańska, Dobrzański 1960) — nous avons remarqué que la réaction de *Polyergus rufescens* envers les nids artificiels des espèces esclaves diffère entièrement de leur comportement envers les nids naturels des mêmes espèces.

Dans le cas présent, j'ai établi que les nids artificiels des espèces esclaves suscitent chez *F. sanguinea* la même réaction que les nids naturels. Il était donc possible d'utiliser ces nids artificiels dans des nouvelles épreuves.

J'ai accompli 32 expériences de ce genre. Le nid artificiel de *F. fusca* était préparé tout au moins un jour d'avance pour permettre aux fourmis de s'y habituer et de reconstruire les corridors. Ce genre de nid était alors enterré avec son cristalliseur dans la direction du développement du chemin des *F. sanguinea*. Quelques heures plus tard, les

premières ouvrières *F. sanguinea* découvrent le nid artificiel. A partir de ce moment jusqu'au commencement de l'attaque générale chaque ouvrière obtenait un numéro d'ordre correspondant à l'ordre de son arrivée. Pour éviter tout effarouchement les fourmis étaient marquées après avoir quitté le nid artificiel à l'aide d'un brin d'herbe sans être enlevées de leur chemin. Voilà une description typique d'une de ces expériences: Les numéros 1 et 2 sont arrivés simultanément à une distance de 2 cm du nid et ont rapidement pris fuite. Le N° 3 vint un peu plus tard et s'enfuit également. Le N° 4 tâta un certain temps le bords du cristalliseur et s'en alla tranquillement. Quelques minutes plus tard le N° 3 revint, circula un moment autour du nid, pénétra ensuite à l'intérieur et en sortit aussitôt après en emportant une nymphe. Le N° 5 s'approcha du nid et s'en alla sans démontrer aucune réaction caractéristique. Le N° 6 pénétra aussitôt à l'intérieur du nid, en sortit un peu plus tard, y entra de nouveau et en sortit en tenant une nymphe. Les N° 7 et 8 entrèrent dans le nid et le quittèrent détenant des nymphes. Le N° 9 arriva au nid et le dépassa sans y prêter aucune attention. Le N° 10 circula un certain temps autour du nid et finit par y pénétrer. Le nombre des ouvrières prenant part au pillage augmente rapidement et avant que les N° 3 et 6 reviennent après avoir déposé les nymphes dans leur nid natal, 18 ouvrières prirent déjà part au pillage du nid. Au bout d'une demi-heure l'attaque devient générale.

Expérience du type 4 (10 expériences).

Un nid artificiel de *F. fusca* est enterré dans un endroit assez éloigné du chemin de *F. sanguinea* où l'on ne voit que des individus isolés. En général les fourmis s'arrêtent près du nid. Une certaine partie dépasse le nid sans démontrer aucun intérêt, quelques unes le tâtent. La majorité prend la fuite. Il n'y eut jamais d'attaques.

Expérience du genre 5.

L'absence d'attaque dans les expériences du type 4 pourrait être expliquée par le fait que, en accord avec l'opinion généralement admise, les attaques des *F. sanguinea* sont très rares. Pour vérifier cette supposition, j'ai répété une partie des expériences du type 3 jour par jour, ou bien même 2 fois par jour: chaque fois j'ai pu provoquer une attaque normale du nid artificiel situé sur le chemin de *F. sanguinea*.

Expérience du type 6 (5 expériences).

On aurait pu également supposer que la composition des individus rencontrés dans les expériences du genre 4 n'est pas la même que dans le genre 3, que les premières ne prennent pas part dans les expéditions guerrières. C'est pourquoi je me suis mis à ramasser les individus marqués, qui les jours précédents, avaient pris part aux pillages et je les ai transportés individuellement dans les alentours d'un nid artificiel de *F. fusca* situé comme dans le cas des expériences du type N° 4. Le transport était effectué sur une feuille ou un brin d'herbe de façon à éviter tout effarouchement. Je n'ai remarqué aucun indice de trouble chez les individus transportés. Dans une expérience typique, 3 des 17 fourmis transportées ont pénétré dans le nid et ont enlevé des nymphes. Ce fait n'a suscité aucune excitation chez les fourmis se trouvant sur le chemin et n'a pas provoqué d'attaque.

Expérience du type 7 (2 expériences).

Dans ce cas le nid artificiel ne se trouvait pas à l'extrémité du chemin de *F. sanguinea*, mais entre la fourmilière et l'arbre sur lequel se trouvaient les pucerons. Aucune des ouvrières, marquées en masse auparavant, qui s'occupaient des pucerons n'a pénétré dans le nid artificiel. Dans les cas où une ouvrière se heurtait directement au nid, elle démontrait une réaction fortement négative. Au bout d'un instant elle continuait sa route vers l'arbre avec les pucerons.

DESCRIPTION D'UNE ATTAQUE TYPIQUE DE *FORMICA SANGUINEA*

Au début ce sont des individus *F. sanguinea* isolés qui se heurtent à un nid d'une espèce esclave. Dans ce cas la réaction de ces individus n'est pas uniforme, certains d'eux dépassent tranquillement le nid, d'autres s'effarouchent et s'enfuient. Si le nid se trouve dans un lieu suffisamment fréquenté par *F. sanguinea* on peut y trouver au bout d'un certain temps une ou deux dizaines de fourmis. En fin de compte une ou quelques fourmis pénètrent à l'intérieur du nid en enlèvent des nymphes et les transportent dans leur propre fourmilière. Le nid commence à être envahi. Chaque ouvrière qui a déjà ravi une nymphe retourne au nid par le chemin le plus court et continue à prendre part au pillage. Je n'ai pas observé de cas, où une fourmi déposant sa nymphe dans le nid *F. sanguinea* entraîne d'autres dans la direction du nid envahi, comme cela a lieu par exemple chez *Myrmica* (Eidmann 1925, 1927, Dobrzańska 1958). Malgré cela les ouvrières

se trouvant sur le chemin entre le nid de *F. sanguinea* et le nid attaqué se dirigent en nombre croissant vers ce dernier. La quantité des fourmis qui prennent part à l'attaque augmente donc constamment, mais ce fait est dû non à la sortie de nouveaux individus du nid de *F. sanguinea* mais à la concentration des fourmis se trouvant déjà auparavant dans le voisinage et au retour des fourmis ayant déjà déposé leur butin.

Je souligne que pas toutes les fourmis qui circulent sur ce chemin ou qui passent près du nid attaqué prennent part au pillage.

Pendant toute l'attaque beaucoup de *F. sanguinea* circulent aussi bien dans le voisinage immédiat du nid attaqué, que sur la route par laquelle le transport des nymphes ravies s'effectue sans toutefois prendre part à l'attaque. Après avoir terminé le pillage les fourmis qui ont pris part à l'attaque, font comme celles qui n'y ont pas pris part, elles reviennent les jours suivants sur les mêmes lieux et continuent leur pénétration du terrain. Si, par contre, le nid de *F. fusca* est découvert par une ouvrière *F. sanguinea* isolée, l'attaque ne se produit pas. S'il est découvert par quelques individus, il arrive quelques fois qu'une ou quelques fourmis entrent dans le nid et en enlèvent des nymphes. Mais même dans ce cas si dans les environs il n'y a pas une quantité suffisante de fourmis l'attaque ne prend pas d'amplitude — on observe tout au plus l'enlèvement d'une quantité restreinte de nymphes esclaves.

Le transport des nymphes vers le nid de *F. sanguinea* par quelques ouvrières ne provoque pas l'arrivée de renforts près du nid pillé.

Comportement des *F. sanguinea* envers les nymphes de différentes espèces de fourmis

Expérience du type 8.

A 16 heures j'ai déposé sur un chemin de *F. sanguinea* côte à côte quelques nymphes des espèces: 1. *Tetramorium caespitum*, 2. *F. fusca*, 3. *F. rufa*, 4. *F. sanguinea* provenant du même nid, 5. *F. sanguinea* provenant d'un nid étranger.

Voici la description des événements:

16²⁰ Une *F. sanguinea* passa à une distance de 20 cm;

16⁴⁰ De même. Une esclave *F. fusca* examina toutes les nymphes déposées et suivit son chemin;

16⁵⁰ *F. sanguinea* N° 1 prit une nymphe de *F. rufa*;

17⁰⁰ *F. sanguinea* N° 2 passa sur les nymphes et prit une nymphe étrangère *F. sanguinea* tout en s'appuyant sur des nymphes *F. san-*

guinea de son propre nid. Le N° 1 revint du nid et prit une nymphe étrangère *F. sanguinea*;

17¹⁰ *F. fusca* N° 2 passa sur les nymphes *F. rufa*, *F. fusca* et prit une nymphe *T. caespitum*;

17¹² *F. sanguinea* N° 3 saisit la première nymphe qui se présentait — c'était *F. rufa*. En même temps *F. sanguinea* N° 4 prit une nymphe *F. fusca*;

17¹⁵ *F. fusca* prit sans hésiter une nymphe *F. rufa*, le N° 1 revint et prit nymphe *F. fusca*, le N° 5 prit une nymphe *F. Fusca*;

17²⁰ Le N° 5 revint et prit une nymphe *T. caespitum*;

17²² Le N° 6 prit une nymphe *F. fusca*;

17³⁰ Le N° 3 prit une nymphe étrangère *F. sanguinea* et la passa immédiatement à une autre ouvrière.

L'ordre dans lequel les nymphes étaient allongées aurait pu peut-être favoriser l'enlèvement de certaines d'elles.

Je dépose donc de nouvelles nymphes d'après l'ordre suivant:

1. *F. sanguinea* du même nid, 2. *F. rufa*, 3. *T. caespitum*, 4. *F. sanguinea* provenant d'un nid étranger, 5. *F. fusca*.

Grâce à cet ordre les nymphes qui semblaient être les plus attrayantes: les *F. sanguinea* provenant du même nid et celles de *F. fusca* étaient les plus accessibles.

Voici le résultat de l'expérience: les nymphes ont été déposées à 17²⁵ au même endroit que dans le cas précédent. C'est pourquoi on retrouve les ouvrières qui ont été marquées pendant l'expérience qui vient d'être décrite.

17²⁷ *F. sanguinea* N° 1 prit une nymphe étrangère *F. sanguinea*;

17³⁰ Le N° 6 tâta une nymphe *F. fusca*, prit ensuite une nymphe étrangère *F. sanguinea* écrasée et se mit à la manger en compagnie du N° 3.

17³² *F. fusca* (une esclave) prit une nymphe *F. fusca*;

17³⁵ Le N° 6 après avoir fini de manger prit une nymphe étrangère *F. sanguinea*, une *F. sanguinea* que je n'ai pas eu le temps de marquer prit une nymphe *F. sanguinea*, provenant de son nid natal;

17³⁷ Le N° 6 retourna, passa sur les nymphes et s'en alla sans rien prendre, *F. fusca* passa sur ses propres nymphes, *F. sanguinea* tira une nymphe *T. caespitum* de dessous un morceau d'écorce;

17⁴⁰ Le N° 6 passa encore une fois sur les nymphes, Le N° 7 prit une nymphe *F. sanguinea* provenant de son nid natal, le N° 8 prit une nymphe *F. rufa*;

17⁴⁵ Le N° 8 retourna et prit une nymphe étrangère *F. sanguinea*.

Je change encore une fois l'ordre des nymphes (peut-être est-il plus facile d'enlever les nymphes se trouvant au milieu du tas?): 1. *F. rufa*,

2. *F. sanguinea* provenant du nid natal, 3. *F. fusca*, 4. *F. sanguinea* de provenance étrangère. Le N° 8 passa sur ses propres nymphes et sur les nymphes *F. fusca* et enleva une nymphe étrangère *F. sanguinea*, le N° 9 passa par dessus toutes les nymphes et choisit une nymphe *F. sanguinea* de son propre nid; pendant que je la marquais elle laissa tomber sa trouvaille pour s'emparer ensuite d'une nymphe étrangère *F. sanguinea*.

Les n°s 5 et 7 ont été marqués encore auparavant en tant que participants des attaques naturelles.

Expérience du type 9 (5 expériences).

Ce genre d'expériences a été effectué pendant les attaques naturelles de *F. sanguinea*. J'ai déposé près d'un nid subissant actuellement l'attaque de *F. sanguinea* des nymphes provenant du nid attaqué, des nymphes de la même espèce mais provenant d'un autre nid et des nymphes d'une espèce non esclave (*F. rufa*). Avant de déposer les nymphes je marquais un grand nombre de fourmis *F. sanguinea* qui enlevaient les nymphes du nid attaqué.

Dans ce genre d'expériences j'ai également constaté que les ouvrières *F. sanguinea* ne démontrent aucune préférence entre les nymphes déposées. Voici les résultats d'une des expériences: sur 10 nymphes *F. rufa* et 16 *F. fusca*, déposées côte à côte, il n'en est resté après 10 minutes qu'une nymphe *F. rufa* et 2 *F. fusca*.

Expérience du type 10 (5 expériences).

Dans ce cas, à part les nymphes de différentes espèces, je déposais également près d'un nid attaqué des cadavres de différents insectes. Les ouvrières *F. sanguinea* prenant part au pillage n'ont démontré également aucune préférence, les insectes et les nymphes *F. rufa* furent ravis avec la même rapidité que les nymphes *F. fusca*.

Expérience du type 11 (2 expériences).

Pour obtenir un contrôle de résultats précédents, j'ai effectué deux expériences du type 8, mais en déposant non seulement des nymphes de fourmis, mais encore des cadavres d'insectes.

Voici le résultat d'une des expériences:

16¹⁰ Dépôt des nymphes et des cadavres d'insectes.

16¹³ *F. sanguinea* N° 1 prit une nymphe *F. fusca*.

16²⁰ Le N° 2 prit un cadavre d'insecte.

16²⁵ *F. sanguinea* passa sur les nymphes est s'en alla.

16²⁷ Le N° 3 prit une nymphe *F. rufa*.

16³⁵ Le N° 2 prit une nymphe *F. fusca*.

16³⁸ Le N° 3 prit une nymphe *F. fusca*, une ouvrière *F. sanguinea* passa indifféremment à côté.

16⁴² Le N° 4 prit un cadavre d'insecte.

16⁴⁵ Le N° 2 prit une nymphe *F. fusca*.

16⁵² Le N° 1 prit le cadavre d'un insecte.

16⁵³ Le N° 5 prit une nymphe de *F. rufa*.

On voit donc que les individus qui prennent les nymphes saisissent également les cadavres des insectes.

Expériences du type 12 (2 expériences avec chaque espèce).

Il restait donc à expliquer pourquoi *F. sanguinea* n'attaque pas en général les nids des espèces non-esclaves, en dépit du fait que les nymphes de ces espèces ne présentent pas pour elle moins d'intérêt que celles des espèces esclaves.

Dans ces expériences j'enterrais un nid artificiel de *T. caespitum* sur une route bien fréquentée par *F. sanguinea*. Après avoir enterré le nid, je déposais dessus un petit tas de nymphes provenant du même nid. Les ouvrières *F. sanguinea* qui trouvaient le nid prenaient en général immédiatement la fuite. Les quelques individus qui sont allés un peu trop loin ont été immédiatement attaqués par *T. caespitum* d'une manière qui leur est très caractéristique: toutes les extrémités des ouvrières *F. sanguinea* se sont trouvées saisies par quelques *T. caespitum* à la fois et les *F. sanguinea* ont été immédiatement obligées de s'enfuir. Pas une nymphe n'a été conquise, quoique le nid ait été enterré sur un chemin de *F. sanguinea* bien fréquenté. J'ai répété une expérience analogue avec un nid de *Lasius niger*. Dans ce cas dès l'apparition des fourmis étrangères, les ouvrières *L. niger* obstruent les orifices du nid et chaque ouvrière *F. sanguinea* se trouve attaquée par quelques *L. niger* à la fois. Grâce à une telle résistance, aucune ouvrière *F. sanguinea* n'a été capable de pénétrer à l'intérieur du nid.

L'emplacement d'un petit nid de *F. rufa* (dans un cristallisoir) donne le même résultat que dans le cas d'un nid naturel de *F. rufibarbis*.

L'attaque des *F. sanguinea* suscite une défense énergique des *F. rufa*, mais si le nid attaqué se trouve sur un lieu bien fréquenté par *F. sanguinea*, une partie des nymphes *F. rufa* est enlevée par les agresseurs et les fourmis attaquées, se mettent à évacuer leurs nymphes tout comme dans le cas d'une espèce esclave.

Mais en général les nids naturels de *F. rufa* sont plus grands, c'est pourquoi j'ai répété cette expérience en employant un grand nid placé dans un aquarium. Dans ce cas les ouvrières *F. sanguinea* qui réussirent à pénétrer à l'intérieur, furent tuées ou mises en fuite. Aucune nymphe ne fut enlevée.

DISCUSSION

Malgré les nombreuses expériences du type 1, 2, 3, 4, 6, 8 et 11 et l'observation des *F. sanguinea* je n'ai pas pu mettre en évidence un seul cas où une fourmi, ayant trouvé un nid d'une espèce esclave, en informe d'autres ouvrières et provoque une attaque. Tout au contraire, il semble que chaque ouvrière *F. sanguinea* essaie de pénétrer dans le nid étranger à son propre compte.

D'autre part, les expériences du type 1, 2, 3, 4 et 6 prouvent que si les fourmis mises en présence d'un nid étranger sont peu nombreuses, elles deviennent plus prudentes, moins sûres d'elles-mêmes et il est très rare qu'elles essaient de pénétrer à l'intérieur. Ce dernier fait est en accord avec l'opinion de Forel (1874) de Brun (1924) et de Ghigi (1951) que les fourmis sont plus agressives en grande masse et que les individus isolés sont en général beaucoup moins audacieux.

Il résulte de mes travaux que l'attaque d'un nid étranger ne se produit que dans le cas où le nid est trouvé par un grand nombre de fourmis à la fois, c'est-à-dire quand le nid se trouve sur un chemin bien fréquenté par *F. sanguinea*. Il en résulte également que la formation d'un tel chemin n'est pas le résultat d'une préparation à l'attaque.

Je n'ai jamais réussi à causer la formation d'un chemin de ce genre ni par la mise en contact d'ouvrières isolées avec un nid étranger naturel (expérience du type 1, 2 et 6), ni par l'emplacement sur l'itinéraire d'individus isolés de nids artificiels d'une espèce esclave (expériences du type 3 et 4). Je n'ai jamais remarqué qu'une colonne se forme sur un lieu où il n'y avait pas de chemin tout au moins depuis quelques jours. Sur ce genre de chemins les ouvrières sont très nombreuses et aussi sûres d'elles-mêmes que dans leur propre nid.

Dobrzańska (1958) affirme que *F. sanguinea* ne possède pas de chemins permanents, à part les chemins menant aux pucerons (ces chemins sont rares, Forel ne les a même jamais vu) et que les chemins existants sont passagers et ne durent en général pas plus qu'une journée.

Mes observations m'ont fait voir que les chemins de *F. sanguinea* peuvent durer quelques jours ou même quelques semaines. Cela dépend probablement de l'abondance en pâture d'une direction donnée. Un

chemin si durable se développe de jour en jour s'éloignant de plus en plus du nid natal. Sa largeur est très variable, elle peut atteindre même quelques mètres.

Ce n'est pas un chemin au sens strict du mot. Je l'appellerais plutôt une direction ou un terrain de pâturage intensif.

La composition personnelle de ces chemins semble être stable, les individus marqués s'y retrouvaient fréquemment, mais ce problème n'a pas été l'objet de recherches systématiques de ma part. Il semble que les ouvrières de *F. sanguinea* ont tendance à choisir la direction dans laquelle un certain nombre de fourmis s'était déjà auparavant engagé. Cela pourrait expliquer le fait, que la grande majorité des ouvrières choisit la même direction. Le pâturage individuel, si fréquent chez *F. rufa*, devient un phénomène rare chez *F. sanguinea*. Les nids des *F. sanguinea* qui possèdent un chemin permanent vers les pucerons nous fournissent un argument en faveur de ce raisonnement. Dans ces nids le chemin menant vers les pucerons détermine toujours l'orientation du terrain de pâturage intensif. C'est dans ce genre de nids qu'une fourmilière d'une espèce esclave, située très près du nid *F. sanguinea* mais dans une direction opposée au chemin menant vers le pucerons, peut exister en toute sécurité pendant des années entières. Ce phénomène a été observé 2 fois (expérience du type 1 et 2).

Donc dans ces nids le pâturage intensif est unilatéral: une direction de pâturage se trouve être développée aux dépens des autres.

Il me semble que ce que les auteurs prenaient pour être une armée, effectuant des opérations stratégiques, était tout simplement un chemin de pâturage. Ce chemin existe toujours avant l'attaque et subsiste souvent longtemps après cette dernière. Le marquage individuel d'un grand nombre de fourmis ainsi que les expériences du type 3, 4, 7, 8 et 11 ont démontré qu'une partie des individus circulant sur un tel chemin ne s'intéresse nullement ni à l'objet de l'attaque ni aux nymphes enlevées.

Je pense que c'est justement ces individus qui étaient pris par les auteurs pour des détachements effectuant de différentes manoeuvres d'encerclement, pour des agents de liaison, des émissaires etc.

Le comportement des fourmis envers un nid étranger, la façon décrite dans les expériences dont elles se concentrent autour de lui — tout cela rappelle tout à fait le comportement des espèces *Formica* envers n'importe quel appât (Reichle 1943, Dobrzański 1956, Dobrzańska 1958). Étant donné le fait établi (Dobrzańska 1958) que les ouvrières *F. sanguinea*, tout comme les ouvrières des autres espèces *Formica*, ne se signalent pas les sources de nourriture, il n'est pas étonnant de constater qu'elles ne se signalent également pas la

trouvaille d'un nid d'une espèce esclave. L'analogie s'accrue encore par le fait qu'en allongeant constamment leur chemin les ouvrières peuvent aussi bien se trouver en présence d'un nid étranger que de tout autre genre de trouvaille.

En aucun cas l'attaque de *F. sanguinea* ne peut être interprétée en tant que résultat d'une action dirigée vers un but précis. Ce n'est qu'après avoir trouvé accidentellement un nid étranger que les ouvrières commencent à se comporter d'une façon opportune: les individus qui ont déposé leur premier butin reviennent au nid attaqué vraiment directement, sans hésitations, sans arrêts en cours de route (c'est d'ailleurs alors, d'après Raignier, que leur vitesse devient maximale). Mais ce changement de comportement après la trouvaille d'un nid étranger n'a rien de spécifique: on peut aisément l'obtenir en exposant n'importe quel genre d'appât ou de nourriture.

D'ailleurs Brun (1924), dont les descriptions sont le plus dépourvues d'interprétation subjective, constate que l'armée connaît souvent un nid défini d'une espèce esclave; il ne trouve donc pas que cela soit une règle obligatoire.

Conclusion 1. *Formica sanguinea* ne possède pas d'éclaireurs qui signaleraient au reste des ouvrières les nids étrangers trouvés;

F. sanguinea n'organise pas d'expéditions ayant pour but la conquête d'un nid précis. Mes expériences démontrent que le pillage est accompli par les mêmes individus qui pâturent constamment et qui apportent au nid toute sorte de butin. Cela fait penser que les nymphes conquises sont considérées par *F. sanguinea* de la même façon que tout autre sorte de trouvaille.

Les expériences des types 8—11 confirment ce point de vue: les ouvrières ne font pas de choix entre les nymphes des espèces esclaves et des autres espèces, elles ne choisissent pas non plus — ce qui est très important — entre les nymphes et n'importe quel autre genre de trouvaille. Même pendant l'attaque d'un nid d'une espèce esclave les mêmes individus saisissent aussi bien les nymphes que les cadavres des insectes.

Cela oblige à analyser d'un nouveau point de vue les faits connus concernant le comportement de cette espèce.

Dans la plupart des cas, *F. sanguinea* possède en tant qu'esclaves des ouvrières de l'espèce *F. fusca*. Elle possède aussi, mais plus rarement, des ouvrières *F. rufibarbis* (8 nids sur 70 en possèdent — Wasmann 1915). On rencontre aussi très rarement, mais d'une manière suffisamment systématique pour que Wasmann puisse établir aussi un rapport constant des esclaves «anormales», c'est à dire pas classifiées en tant qu'espèces esclaves (1 nid sur 80 en possède). Il faut souligner le fait

que ce sont les nids grands et forts qui possèdent des esclaves « anormales » et qu'en général dans leurs environs se trouvent des nids faibles des espèces jouant le rôle d'esclaves « anormales » (Wasmann 1901, 1902, 1913, 1915; Brun 1913, Forel 1923). Viehmeyer (1908) trouve même que la présence des esclaves « anormales » est un phénomène tout à fait normal, puisque il écrit que « *F. sanguinea* pille le nid de *F. fusca*, *F. rufibarbis* et aussi d'autres espèces ». Forel (1874) a vu dans un nid de *F. sanguinea* des nymphes *L. niger* et *L. flavus*. Il fait souvent mention de luttes de *F. sanguinea* avec *F. pratensis* en rapportant que le résultat de ces luttes est variable. Wasmann (1915) affirme également que les *F. sanguinea* amassent et élèvent des nymphes de différentes espèces. Brun (1924) décrit une attaque *F. sanguinea* d'un nid *F. rufa*. Wilson est d'avis que les esclaves proviennent de l'espèce qui se trouve à la portée d'une colonie donnée.

Il semble donc être hors de doute que *F. sanguinea* attaque fréquemment les espèces non-esclaves, mais le résultat de ces attaques peut être variable.

Ces cas sont trop fréquents pour qu'on puisse les négliger, ou les attribuer à des « erreurs » des ouvrières, comme le fait Wasmann (1915).

Tous ces faits conduisent à la conclusion suivante:

Conclusion 2. *F. sanguinea* a tendance à attaquer les nids et à enlever les nymphes de toute espèce de fourmi, mais pas chaque espèce se laisse piller avec la même facilité.

Par exemple le nid de *Tetramorium caespitum* est imprenable pour *F. sanguinea*, cette espèce est redoutable pour des espèces plus puissantes, même pour une espèce aussi guerrière que *Polyergus rufescens*. Il est également difficile d'attaquer les nids de *Lasius niger* ou d'autres espèces du genre *Lasius* étant donnée, la quantité énorme de leurs habitants. L'espèce *F. exsecta* est très combattive, à la surface de leurs nids il y a toujours une grande quantité d'ouvrières très excitables ce qui rend la conquête de ces nids très difficile. Les ouvrières *F. rufa*, *F. pratensis* sont individuellement plus faibles que *F. sanguinea* et leurs nids, tant qu'ils sont petits peuvent être attaqués avec succès; mais en général ces nids surpassent considérablement en nombre d'habitants les nids de *F. sanguinea* et l'attaque de si grandes colonies devient impossible pour cette dernière espèce. *F. truncicola* est un adversaire égal pour *F. sanguinea*, qui devrait donc posséder une nette supériorité en nombre pour pouvoir pénétrer à l'intérieur de leurs nids. Il ne reste donc que quelques espèces, dont les colonies sont incapables de résister à *F. sanguinea*: ce sont justement les colonies des espèces qu'on appelle esclaves: *F. fusca*, *F. rufibarbis*, *F. glebaria*, *F. cinerea*. Comme *F. fusca* est

l'espèce la plus répandue il n'y a rien d'étonnant à ce qu'on la trouve le plus fréquemment dans les nids de *F. sanguinea* (ou également de *F. rufescens*).

Passons à présent à l'analyse de la littérature myrmécologique du point de vue du but dans lequel les ouvrières *F. sanguinea* enlèvent les nymphes des autres espèces. Escherich (1917) est d'avis que le trait caractéristique de l'esclavage est l'élevage d'une partie (mon soulignement — J. D.) des nymphes étrangères. Ghigi (1951) juge que *F. sanguinea* enlève les nymphes tout aussi bien pour en faire des esclaves qu'en tant que nourriture. La consommation des nymphes étrangères par *F. sanguinea* est mentionnée par Forel (1874) et aussi par Wasmann (1891-a) qui affirme que dans les nids faibles une partie des nymphes étrangères est élevée, tandis que dans les nids puissants ces nymphes sont mangées. Ce dernier phénomène s'explique d'après Wasmann, par le fait que les grandes colonies « n'ont plus besoin de main-d'oeuvre étrangère ». D'après Wheeler (1926) la majorité des nymphes est consommée et elles ne survivent que dans les colonies bien approvisionnées. Il me semble tout comme à Wheeler, que chez *F. sanguinea* ce ne sont que les nymphes que les ouvrières n'ont pas eu le besoin de manger, qui deviennent des esclaves. Cette interprétation est confirmée par le fait constaté par Wasmann que la quantité des esclaves dans les petits nids de *F. sanguinea* est toujours supérieure à celle des nids plus développés. Au fur et à mesure du développement du nid la quantité relative des esclaves y diminue (ce qui n'a pas lieu chez *Polyergus rufescens*). J'explique cela par le fait que même le plus faible des nids de *F. sanguinea* est toujours capable de piller n'importe quel nid de *F. fusca*, il enlève donc les nymphes de *F. fusca* en même quantité qu'une grande colonie; mais comme un petit nid a des besoins alimentaires inférieurs, une grande partie des nymphes enlevées peut éclore et augmenter ainsi le nombre des esclaves. Par contre une grande colonie demande plus de nourriture tandis que ces possibilités de ravitaillement en nymphes diminuent au fur et à mesure du pillage des nids avoisinants; la quantité des nymphes consommées augmente et la caste esclave devient de moins en moins nombreuse. Wasmann a remarqué que dans les taillis, où l'on rencontre la plus grande quantité de nids de *F. fusca*, les nids de *F. sanguinea* possèdent toujours une plus grande quantité de fourmis esclaves.

Forel mentionne que dans les nids de *F. sanguinea* privés d'esclaves, on trouve toujours un grand nombre de petites ouvrières. C'est très compréhensible: ces nids sont mal nourris ce qui limite la croissance des ouvrières et cause également la consommation de toutes les nymphes conquises.

D'après mes propres observations il me semble que *F. sanguinea* consacre nettement beaucoup plus d'efforts au pillage des nymphes étrangères que les autres espèces du genre *Formica*. Ces pillages s'accomplissent bien plus souvent qu'il n'est généralement admis. Il est possible que le ravissement des nymphes étrangères en tant que principale source d'aliment a été affermi chez cette espèce dans le processus de l'évolution.

Cet état de choses pourrait donner une réponse à la question, posée par Forel (1874): pourquoi *F. pratensis* et *F. exsecta*, qui ravissent également des nymphes étrangères, les consomment toujours?

Ces espèces ne ravissent des nymphes étrangères que très rarement, en nombre restreint c'est pourquoi ces dernières sont toujours ou presque toujours consommées.

Conclusion 3. *F. sanguinea* ne possède pas ou ne possède que pas toutes les nymphes soient mangées et qu'une partie éclore on obtient justement un des rares cas de nid hétérogène, qui servent aux myrmécologues comme preuve de l'existence du parasitisme temporaire chez beaucoup d'espèces de fourmis.

Conclusion 3. *F. sanguinea* ne possède pas ou ne possède que dans une forme très rudimentaire l'instinct d'élever des esclaves des nymphes ravies. Les nymphes sont enlevées pour être mangées et elles n'éclosent que dans le cas où le butin du nid est assez abondant pour qu'il ne puisse être entièrement consommé.

En conséquence une nouvelle question s'impose, pourquoi les esclaves appartiennent-elles toujours à des espèces définies et représentant uniquement le genre *Formica*? Pourquoi ne rencontre-t-on jamais dans les nids de *F. sanguinea*, *Lasius niger* en tant qu'esclave quoique il arrive que ses nymphes soient enlevées par *F. sanguinea*?

Les nymphes et les jeunes ouvrières après l'éclosion demandent des conditions strictement définies qu'elles ne trouvent pas chez les espèces éloignées du point de vue de la systématique et de leur biologie. Il me semble que c'est justement dans ce fait qu'il faut chercher la raison pour laquelle *F. sanguinea* n'élève que des espèces appartenant au genre *Formica*.

Il subsiste entre autres encore une question à laquelle je n'ai pas trouvé de réponse: pourquoi on ne trouve jamais dans les nids de *F. sanguinea* de mâles et de femelles des espèces esclaves?

Dans la littérature il y a très peu de mentions concernant ce sujet. Brun (1934) note que les nymphes des individus ailés « peuvent être enlevées dans le ferveur du combat » et sont alors dévorées sur place, mais ce ne sont probablement que des présomptions théoriques car ces travaux ne permettent pas de conclure que l'auteur a réellement observé

de tels cas. Wasmann (1915) écrit qu'il a observé quelques fois des cas où des individus ailés des espèces esclaves ont été enlevés dans des nids *F. sanguinea*; d'après l'auteur cela se produit très rarement car ces nymphes sont de plus attrayantes en tant que nourriture et elles sont mangées avant les autres.

Il est difficile d'établir si cette explication a été fondée sur des expériences ou seulement sur des suppositions.

Conclusion 4. Il serait intéressant de comparer mes résultats avec ceux de Dobrzańska et Dobrzański (1960) obtenus sur la tactique de lutte de *Polyergus rufescens*. Ces deux espèces, tout en tenant compte des grandes différences biologiques qui existent entre elles, possèdent un trait commun — elles possèdent des esclaves et attaquent des nids étrangers pour en conquérir des nymphes. Cette comparaison me permet de formuler les conclusions suivantes:

1. Les deux espèces — *Formica sanguinea* et *Polyergus rufescens* ne possèdent pas d'éclaireurs qui signaleraient au reste de l'armée la trouvaille d'un nid étranger.

2. Les nids étrangers sont trouvés par ces espèces d'une façon accidentelle. Chez *P. rufescens* néanmoins, les expéditions n'ont pour but que le pillage et l'armée marche tout droit jusqu'à ce qu'elle ne tombe sur un nid étranger, tandis que chez *F. sanguinea* il n'y a pas une telle concentration d'individus qu'on pourrait appeler l'armée. Les nids étranger sont trouvés pendant les recherches ordinaires dans le terrain et les nymphes ravies sont transportées dans le nid natal dans la même mesure que toute autre sorte de butin.

3. Il existe une certaine analogie entre la façon de pâturer chez *F. sanguinea* et la formation des expéditions chez *P. rufescens*. Chez les unes et les autres on remarque une tendance à choisir une direction déjà bien fréquentée. La différence consiste en ce que la masse des ouvrières *F. sanguinea* effectue ses recherches d'une manière individuelle. Les individus sont donc plus éparpillés, tandis que chez *P. rufescens*, les expéditions sont effectuées uniquement dans le but d'enlever des nymphes étrangères, c'est pourquoi les fourmis de cette espèce démarrent en grande masse en rangs bien serrés, et marchent rapidement dans la direction choisie; après avoir pillé le nid trouvé et atteint ainsi l'unique but de l'expédition, l'armée retourne immédiatement au nid natal.

Il semble que chez *F. sanguinea* la tendance à suivre une direction choisie est encore faiblement accentuée, car les recherches dans le terrain sont en général individuelles et une certaine concentration n'a lieu que dans le cas où un nid étranger est trouvé. Au fur et à mesure de

l'évolution de l'instinct de pillage, les attaques des nids étrangers deviennent de plus en plus fréquentes et le trait caractéristique de suivre la même direction en grande masse s'accroît de plus en plus. Chez *Polyergus rufescens* cette propriété atteint son point culminant, car les expéditions guerrières deviennent l'unique raison pour laquelle les fourmis quittent leur nid. C'est pourquoi l'armée prend ce caractère de formation de choc, aux mouvements décidés, rapides et possède cette précision d'action qui, d'une part donne à cette espèce une allure et une efficacité rare dans le monde animal et de l'autre les mène à ce manque bien connu de plasticité de l'instinct.

4. *F. sanguinea* ne possède pas ou ne possède que dans une forme très rudimentaire l'instinct d'élever des esclaves des nymphes conquises. Les nymphes sont enlevées en tant que nourriture et elles n'éclosent que dans le cas où le butin est assez abondant pour n'être pas entièrement consommé.

Mes observations permettent de juger que même chez *P. rufescens* une partie des nymphes conquises peut être aussi consommée.

J'ai observé une fois le pillage d'un grand nid de *F. rufibarbis* par *P. rufescens*. Malgré ce fait, il n'y a pas eu d'apparition d'esclaves *F. rubibarbis* dans ce nid. Il est fort possible que ces nymphes ont été consommées par *F. fusca* qui était l'espèce esclave de ce nid.

Si vraiment les nymphes conquises peuvent être consommées dans le nid de *P. rufescens*, cette consommation doit être bien inférieure à la consommation des nymphes dans un nid de *F. sanguinea*. Dans les nids de *P. rufescens* ce sont les esclaves qui jouent le rôle d'approvisionneurs et ce sont elles qui décident de la quantité de nymphes consommées, or l'espèce esclave la plus répandue — *F. fusca* est bien moins carnivore que *F. sanguinea* et les aliments de provenance animale ne jouent pas un rôle important dans sa nourriture. *F. sanguinea* par contre, est une espèce nettement carnassière, d'une façon encore plus prononcée que les autres espèces du genre *Formica* et, se nourrissant à son propre compte, elle mange évidemment bien plus de nymphes que *P. rufescens*.

5. Donc en somme, malgré les grandes différences entre le comportement des *Formica sanguinea* et de *Polyergus rufescens*, une analyse approfondie permet d'apercevoir certains traits communs qui pourraient être l'indice que *F. sanguinea* se trouve au début de cette voie d'évolution qui a été parcourue par *P. rufescens*, mais à un stade moins avancé qu'il n'est généralement admis. Il me semble que *F. sanguinea* se trouve à ce stade hypothétique, prévu par Darwin, où le pillage des nymphes est une des formes de pâturage et n'est pas encore devenu un instinct de conquête des esclaves.

Cette dernière conclusion doit être évidemment considérée avec toute prudence, comme d'ailleurs toutes les conclusions concernant l'évolution en général qui ne sont, après tout, que des suppositions plus ou moins fondées.

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CHEMOTAXIS IN *PARAMECIUM CAUDATUM* AS ADAPTIVE RESPONSE OF ORGANISM TO ITS ENVIRONMENT

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The environment of an organism is the sum of all physical, chemical and biotic factors under which it exists. There is much evidence that *Paramecium* can live and reproduce under variable conditions of external environment. It is found in streams, rivers, lakes, pools and ponds and bodies of stagnant water from every part of the world. The relatively rapid movement and ability to perceive the chemical stimuli from the environment ahead of it, ensure that *Paramecium* can find out the favourable conditions present in the medium. Among other factors chemotaxis proves to be the most important one, as it makes possible the choice of environment to a great extent. It is well known that *Paramecia* respond to various kinds of stimuli by means of the "avoiding" reaction described by Jennings (1905). The animals react positively to chemotactic stimuli which signalize the presence of bacteria or another food. Jennings believed that positive response to weak acid medium is adaptive, because under natural conditions it brings the animals to the area abundant in food and bacteria, which produce carbon dioxide in process of respiration and in this way they acidify the medium. It is also known that *Paramecia* show positive chemotactic response to oxygen, but only in the case of lack of oxygen in the medium (Verworn 1889).

The adaptive role of chemotaxis was also stressed by Lozina-Loziński (1930). He noticed that *Paramecium caudatum* shows higher rate of food vacuole formation in suspensions of certain dyes which cause positive chemotaxis than in suspensions of dyes showing negative chemotactic response or no response at all. Similar results were reported by the same author on correlation between chemotactic and phagotrophic response of *Paramecium* to suspensions of India

Ink, Carmine and *B. subtilis*. The highest rate of food vacuole formation was observed with suspension of *B. subtilis*, to which *Paramecia* showed positive chemotaxis, whereas India Ink and Carmine were ingested at a lower rate, the chemotactic response to India Ink being indifferent and to Carmine negative.

Jennings (1899) analyzed thoroughly the problem of possible correlation between chemotactic response and noxious effect of given substance. He came to conclusion that only in some cases the negative chemotactic response was connected with noxious effect of substance, but in general this principle proved to be not obligatory.

Author in an extensive series of experiments with ten lower alcohols (Dryl 1959) has shown that toxic properties of alcohols were strongly correlated with their negative chemotactic action and that both phenomena depended on some physical and chemical properties of alcohols, as the molecular weight and the position occupied by OH-groups in the carbon chain.

In the present study a new attempt is made to verify the views concerning the adaptive role of chemotaxis with special respect to the postulated correlation between the negative chemotactic action and toxic effects of the substance under examination.

MATERIAL AND METHODS

The experiments were carried out on *Paramecium caudatum* strain isolated in 1955 in Warsaw and subsequently grown in a mass culture on standard lettuce infusion inoculated with *Aerobacter aerogenes* (Sonneborn 1950). The average fission rate at room temperature ($21 \pm 1^\circ\text{C}$) was 2 f.p.d. A day before the experiment a dense culture of *Paramecia* was diluted in proportion 1:2 with the salt solution (Dryl 1959) of following composition: sod. citrate 0.1M-20 ml., sod. phosph. monobasic 0.1M-10 ml., sod. phosph. dibasic. 0.1M-10 ml., redistilled water 945 ml., calc. chloride 0.1 M-15 ml. The pH of solution was ca 7.1. In order to avoid precipitation of calcium salts, the CaCl_2 must be added last in preparing the salt solution. This solution proved to be not toxic for *P. caudatum*, *P. aurelia* and *P. multimicronucleatum*.

Determinations of pH were carried out with the aid of a Cambridge Instrument Co Potentiometer of an accuracy of up to 0.01 pH.

The culture fluid containing *Paramecia* was transferred into a flask with a long narrow neck. Usually, within 2—3 min. *Paramecia* produce a dense negatively geotactic aggregation in the upper section of the neck. This dense portion of Infusoria was decanted to another flask filled up with salt solution and subsequently the procedure was repeated 3 times in exactly the same manner as before, however with larger volume of salt solution. Finally, animals were allowed to starve during next 24 hrs. On the day of experiment, *Paramecia* were washed again in order to keep them in the possible purest salt solution.

Chemotactic response was studied with the aid of quantitative photomacro-

graphic method for determination of chemotaxis in Protozoa, as described previously by the author (Dryl 1959a).

Beams of light from two projectors were directed through heat filters from below and obliquely to the surface of glass plate and also obliquely to a camera optic axis. In this way a dark field illumination was obtained. A camera suitably adapted for taking macrophotographs was fixed vertically above the plate in a stand which makes possible regulation of distance between camera and glass plate. All the experiments were performed in dark-room.

When all the preliminary arrangements were made, a glass plate 10 by 15 cm. with a rough mated margin 1 cm wide was placed horizontally on the stage. Ca 7 ml. of salt solution (pH = 7.1) were spread uniformly over the surface of the glass plate until it reached the margin. Then to each of 4 corners of a glass plate, 2—3 drops of dense portions of *Paramecia* were transferred using micropipette. After 1 or 2 min. *Paramecia* were actively swimming into the area occupied by the salt solution and after next 1—2 min. the animals were uniformly dispersed in the medium. Five 1 cm. squares were drawn on the reverse side of glass plate, as indicated on the Phot. 1. When beginning the experiment, 8—10 drops of salt



Phot. 1. The chemotactic response of *Paramecium caudatum* to BaCl_2 solutions of different concentrations. The exposure time = 2 sec.

solution were added drop-wise in the central square area marked "control". Then the solutions under investigation were deposited with the aid of a micropipette in portions of 8—10 drops on the area outlined by the squares marked on the glass plate. This operation should be carried out within 30—40 sec., and should begin with higher concentrations and end with the lower ones. The Infusoria showed a chemotactic response, its intensity being in direct relation

to the concentration of the solution added. After an interval of 30 sec. since the last drops of solution were added, the first photograph was taken, with the shutter set at $\frac{1}{25}$ sec. Subsequent photographs were made at 5 sec. intervals. A total of 10 photographic afford a complete record of the experiment to be analyzed quantitatively later.

In the present study salt solution of pH 7.1 was used instead of tap-water which was applied in the previous experiments by the author. All substances used were also diluted with salt solution.

Besides chemotactic response, the rate of forward movement in negative chemotactic threshold concentrations of examined substances was determined using macro-photographic technique (Dryl 1958) with some improvement, as indicated in the more recent study (Dryl 1961). The registered paths covered by animals appeared on negatives as spiral lines of different length. The paths covered by animals showing avoiding reaction or cessation of movement were disregarded as belonging to another group of kinetic phenomena than these with which the present investigation is concerned. The exposure time was 2 sec. 100 measurements were made in each solution. In preliminary experiments it was established that average length of living *P. caudatum* specimens was $230 \pm 13\mu$ based on data obtained from microphotographs (exposure time = 0.01 sec.) of 100 living animals. The average length of registered path of *Paramecium* was diminished by the determined average length of animal. This correction was introduced before final calculations of velocity of ciliary movement were made. After making calculations, the data were expressed in micra per second as average swimming rate.

For determination of the survival rate, approximately 1000 (860—1210) animals taken from purified dense aggregations have been transferred to 10 ml. samples of solution under study for 24 hrs. The number of animals was determined in 1 ml. of solution on the day of experiment and after 24 hrs. the survival rate being calculated as the ratio between the first and the second estimation... taking into account the number of living and killed animals in given sample. The animals were counted after killing them with aceto-carmin solution in concentration ca 1—2% of that normally used for staining. The control experiments revealed that the fission rate during 24 hrs. of experimentation proved to be ca 2% (0.02), as estimated by macro-photographic recording of control sample of *Paramecia* at the beginning of experiment and after 24 hrs.

The osmotic pressure of urea, glicerol, d-glucose, CaCl_2 , MgCl_2 , NaCl and KCl solutions was examined by cryoscopic determinations and expressed in Δt^* .

RESULTS

The experiments concerning the chemotactic response were run with CaCl_2 , MgCl_2 , NaCl , KCl , BaCl_2 and quinine solutions. (Table I and II). Quinine and BaCl_2 showed very strong negative response, whereas in the group of remaining salts following sequence was established as regards the strength of negative chemotactic response: K^+ , $\text{Mg}^{++} > \text{Ca}^{++} > \text{Na}^+$.

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Table I

Chemotactic action of chlorides (CaCl_2 , MgCl_2 , NaCl , KCl) on *P. caudatum*

Concentration in m M	CaCl_2			MgCl_2			NaCl			KCl		
	m	s	%	m	s	%	m	s	%	m	s	%
30	—	—	—	—	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	3.9 ± 1.64	7.2	—	—	—	—
13.5	—	—	—	—	—	—	29.3 ± 3.29	59.2	—	—	—	—
9	3.1 ± 1.22	4.7	—	—	—	—	44.3 ± 4.90	89.5	—	—	—	—
6	7.0 ± 1.67	10.7	—	—	—	—	—	—	—	—	—	—
4	8.7 ± 3.95	10.2	—	—	—	—	—	—	—	—	—	—
2.7	42.4 ± 3.95	64.7	—	4.8 ± 0.98	8.1	—	—	—	0.6 ± 0.51	1	—	—
1.8	—	—	—	6.4 ± 0.92	10.8	—	—	—	7.1 ± 1.69	11.6	—	—
1.2	—	—	—	31.8 ± 3.99	53.9	—	—	—	75.0 ± 4.73	122.6	—	—
Control	65.5 ± 4.69	100	—	59.0 ± 3.77	100	—	49.5 ± 5.21	100	61.3 ± 3.43	100	—	—

m = average number of Infusoria calculated from 10 successive photographic recordings. s = standard deviation, % = percentage of Infusoria calculated with reference to control = 100. All concentrations of substances were prepared with phosphate-citrate salt-solution of pH = 7.1

Table II

Chemotactic effects of BaCl_2 and quinine solutions on *P. caudatum*

Conc. in m M	BaCl_2			Conc. in ‰	Quinine		
	m	s	%		m	s	%
0.15	—	—	—	0.045	—	—	—
0.1	33.8 ± 2.56	53.6	—	0.03	2.6 ± 0.92	3.9	—
0.067	96.3 ± 4.20	152.6	—	0.02	5.4 ± 2.37	8.1	—
0.045	78.3 ± 3.10	124.1	—	0.0135	18.6 ± 4.10	27.9	—
0.03	81.7 ± 2.90	129.5	—	0.009	41.1 ± 2.39	61.6	—
Control	63.1 ± 3.05	100	—	Control	66.7 ± 3.52	100	—

m = average number of animals calculated from 10 successive photographic recordings. s = standard deviation, % = percentage of Infusoria calculated with reference to control = 100. All concentrations of substances were prepared with phosphate-citrate salt-solution of pH = 7.1

The corresponding threshold concentrations proved to be: 4 mM CaCl_2 , 1.8 mM MgCl_2 , 20 mM NaCl , 1.8 mM KCl , 0.1 mM BaCl_2 and 0.0135‰ quinine.

The detailed analysis of the quantitative data revealed that in solutions of KCl and BaCl_2 of lower concentrations than threshold values, the number of recorded animals was higher than in corresponding control group, namely: 122.6% in 1.2 mM KCl and 124.1 — 152.6% in 0.03 — 0.0067 mM BaCl_2 in relation to control = 100%. The question arose, either these findings should be considered as the evidence of positive chemotactic response or if an other peculiar kinetic phenomenon takes place. To get the clear answer series of experiments were carried out with chemotactic response in *Paramecium* using the photomacrographic technique for recording the movement of Protozoa. However, no positive chemotactic response was found as regards the chemotactically active concentrations of KCl and BaCl_2 . Instead of that the slackening of the forward movement was noticed in all the concentrations

of salts showing the higher number of animals than in the control (Phot. 1). This rather unexpected discovery encouraged us to study more extensively the problem of possible correlation between chemotactic response and the velocity of the forward movement in *Paramecium*. The results of these experiments are given in Table III. The remarkable slackening of the velocity of the forward movement was established for negative chemotactic threshold concentrations of NaCl, KCl, BaCl₂ and quinine solutions. On the other hand there was no significant change in swimming rate as regards the chemotactic threshold concentrations of CaCl₂ and MgCl₂.

Table III
Effects of negatively chemotactic threshold- concentrations
of substances on the velocity of forward movement of
P. caudatum

Substance and its conc.	m s		Control	
	m	s	m	s
4 mM CaCl ₂	1265	± 124	1215	± 171
1. 8 mM MgCl ₂	1185	± 144	1203	± 158
20 mM NaCl	593	± 105	1161	± 150
1. 2 mM KCl	758	± 111	1113	± 136
0. 1 mM BaCl ₂	387	± 82	1044	± 271
0.02 ‰ Quinine	732	± 142	1194	± 162

m = average swimming rate in micra per sec. s — standard deviation. Temp. = 21–22°C.

Table IV
Effects of some organic substances and salts on survival rate of *P. caudatum*
after 24 hrs. of exposure

Concn. in mM	Urea	Glicerol	d-Glucose	CaCl ₂	MgCl ₂	NaCl	KCl
220	34	—	—	—	—	—	—
200	70	12	—	—	—	—	—
180	+	39	—	—	—	—	—
160	+	74	—	—	—	—	—
140	+	+	20	—	—	—	—
120	+	+	52	—	—	—	—
100	+	+	+	—	—	—	—
80	+	+	+	—	—	—	—
60	+	+	+	—	—	—	5
55	+	+	+	—	—	35	47
50	+	+	+	—	4	66	67
45	+	+	+	16	35	79	75
42.5	+	+	+	40	58	+	84
40	+	+	+	70	80	+	77
35	+	+	+	+	+	+	89
30	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+

The figures given in columns indicate the ratio of living Infusoria per cent. + = > 90% of survival. All concentrations of substances were prepared with phosphate-citrate salt solution of pH = 7.1

In the Table IV and V the data are included showing the effects of different substances on survival rate of *P. caudatum*, the all solutions being prepared on phosphate — citrate salt solution of pH = 7.1 formula of which was given before.

In lethal and sublethal concentrations of urea, glycerol, d-glucose, CaCl_2 , MgCl_2 , NaCl and KCl the animals become gradually thinner and less active. Similar results concerning the toxic effects of alkali chlorides were reported previously by Grębecki and Kuźnicki (1956).

Table V

Effects of BaCl_2 and quinine on survival rate of *P. caudatum* after 24 hrs. of exposure.

BaCl_2		Quinine	
Concn. in mM	Percentage of survival	Concn. in ‰	Percentage of survival
5	—	0,06	—
4	13	0,05	19
3	32	0,04	55
2	58	0,03	81
1	+	0,02	+
0.5	+	0,01	+

+ = > 90% of survival. All concentrations of substances were prepared with phosphate-citrate salt solution of pH = 7.1.

The results of the present study suggest that the lethal effects of these substances might be of pure osmotic nature. To verify the hypothesis cryoscopic measurements were performed on the osmotic pressure values of all the seven substances in LD₅₀ concentrations. Finally the following osmotic pressure values were found and expressed in Δt :

For urea (LD₅₀ concentration) — 0.440°C, for glycerol — 0.410°C, for d-glucose — 0.285°C, for CaCl_2 — 0.265°C, for MgCl_2 — 0.260°C, for NaCl — 0.220°C and for KCl — 0.230°C.

The results obtained are in favour of the hypothesis that lethal effects of alkali chlorides and of d-glucose depend on the osmotic pressure of solutions and not on specific properties of the substance.

DISCUSSION

The adaptive role of positive chemotactic response to the weak acid medium and to oxygen was suggested by Jennings (1899, 1905). He believed that the chemotactic effects of acids and salts are specific. However, Johnson (1929) found evidence that the positive chemotaxis to acid medium is due to a change of the pH only and does not depend on the specific structure of substance. These data were confirmed by the author in the previous studies (Dryl 1952, 1959). The optimum pH for chemotaxis proved to be at pH 5.2 — 6.2, whereas the extreme pH

values for survival (LD_{50}) were found at $pH = 4.65 - 4.75$ in acid range and at $pH = 9.05 - 9.15$ in alkaline range (Dryl 1961). Thus the optimum pH for chemotaxis falls in acid range and is close to the extreme pH value for survival. This observation is essential for understanding the mechanism of the toxic effects of acidifying factors present in the medium. It was also proved (Dryl 1952) that all the positive chemotactic substances possess the acidifying effect on the medium and their chemotactic effect depends on the induced pH of the medium showing no relation to the chemical structure of examined substance. Consequently, in this group of substances there is no reason for studying the correlation between the chemotactic and toxic effects as it was done previously by Jennings (1899, 1905).

In the present study we found the new evidence of protective role played by chemotaxis as regards $CaCl_2$, $MgCl_2$, $NaCl$, KCl , $BaCl_2$ and quinine solutions. In all the cases the LD_{50} was much higher than corresponding negative chemotactic threshold concentration.

There is a strong indication that the lethal effects of $CaCl_2$, $MgCl_2$, $NaCl$, KCl and d-glucose are pure osmotic. Consequently in the case of alkali chlorides the chemotactic response appeared to the substances devoid of specific toxic properties. However, even here the adaptive role of chemotactic response is clear, as it prevents the animals from entering the waters of higher salinity. In connection with this it is worth to mention that both *P. caudatum* and *P. aurelia* possess the capacity of adaptation to increased concentration of the sea water (Finley 1930, Yocom 1934, Frish 1939) or to increased salinity of the medium (Gause 1939, Gause and Smaragdowa 1939, Grębecki 1961). The data concerning the effects of adaptation to the environment on the chemotactic response in *P. caudatum* were published by the author elsewhere (Dryl 1959).

In literature there are very interesting data as regards the morphological adaptations of Infusoria to the parasitic life (Raabe 1947). Unfortunately, the data concerning the chemotactic response in parasitic Protozoa are rather scarce making impossible any comparative approach to the problem.

As yet, we possess the quantitative data on chemotaxis in *P. caudatum* only. Comparative studies in other Infusoria, both free-living and parasitic should be of great importance as well from pure physiological as from more general biological point of view.

SUMMARY

1. The chemotactic response in *P. caudatum* to $CaCl_2$, $MgCl_2$, $NaCl$, KCl , $BaCl_2$ and quinine solutions was studied using the photomacrographic method for quantitative determination of chemotaxis in Protozoa (Dryl 1959a) and the photomacrographic technique for recording the movement of Protozoa (Dryl 1958).

2. The slackening of the forward movement in the negative chemotactic threshold concentrations of $NaCl$, KCl , $BaCl_2$ and quinine solutions was noticed. However, no change in the swimming rate of Paramecia in $CaCl_2$ and $MgCl_2$ solutions was observed.

3. The toxic effects of $CaCl_2$, $MgCl_2$, $NaCl$, KCl and d-glucose proved to be pure osmotic.

4. The problem of adaptive role played by chemotaxis was discussed.

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ANTAGONISTIC-TASK LEARNING IN WHITE RATS

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In studies concerned with analysis of antagonistic-task learning various methods have been employed, which involved differentiation of visual and auditory stimuli as well as reversal learning of maze habits. Antagonistic tasks consisted in that, for instance, in a T-maze rats were trained to choose one path upon an auditory signal and the other when the auditory signal was absent, the path-signal combination being reversed in the next task after the first and been mastered to a certain criterion (Hunter and Yarbrough 1917). This type of learning proved very difficult for rats. To master the second, antagonistic, task it took them many more trials and errors than was necessary in learning the first task. Pearce (1917) investigated the same problem as Hunter and Yarbrough, and confirmed their results.

The negative effects of first — on second-task learning was in the case of antagonistic tasks further confirmed by Wylie (1919), Fritz (1930) and Munn (1932). Krechevsky (1932), on the other hand, did not confine his learning experiments to only two tasks but prolonged them to see what the effects of previous antagonistic tasks would be in multiple learning. He used ten albino rats, which had to learn 20 successive tasks each to a specified criterion. He found that the rate of learning, inversely proportionate to the number of trials, and efficiency, inversely proportionate to the number of errors, increased with successive tasks. The animals were able to master the last few tasks after a minimum of trials and errors. It follows that negative transfer occurs only in initial learning and is superseded by positive transfer as the number of tasks increases in multiple learning. The results obtained by the above authors have been con-

firmed by Kelleher (1956) and Hoffman, Perkins and Calvin (1956).

Multiple relearning has been studied in much detail by physiologists who investigated the motility of higher nervous activity in various animals. Fedorov (1951), Yakovleva (1944), Petrova (1945), Kamiński (1936 and 1949), Vacuro (1945 and 1947), Ponurova (1953), Tretyakova (1953) and others believed multiple transformation of signals meaning differentiation reversal concerning a pair of stimuli (learning of antagonistic tasks) to be one of the best tests in investigating the degree of development of higher nervous functions. These investigations have shown that animals with complex behaviour display a higher motility of nervous processes and should adapt themselves better to environmental conditions. However, the above authors have given little attention to the question of why there is a fundamental difference between reversal learning of compound maze habits and that of simple motor reflexes. In the former case, the first reversal learning is invariably more rapid than first-task learning, whereas in the latter case the reverse is true.

The subject of this work is to explain why, in the case of simple motor habits, the first transformation is more difficult than the first learning.

EXPERIMENTS IN LASHLEY'S APPARATUS

Method

The experiments were made in a slightly modified Lashley's apparatus. It consisted essentially of two parts: an immobile starting platform and an opposite mobile platform screened on the edge facing the starting platform with a wall with two hinged doors. The animal on the starting platform faced only this wall. On either side of the apparatus were out of the animal's reach two screens that obscured what was on the sides of the apparatus. The starting platform was crescent-shaped, with either end facing one of the two doors of the mobile platform. The latter rested on rails and, if it was brought close enough, the doors and the ends met, but when it was pushed away, the rats had to jump to reach the doors, which were flush with the walls not to allow the animals to hold on with their claws if the door was locked. If the animal jumped at the right door, this opened when lightly touched with the head gave the animal a foothold on the floor beyond, admitting it to the food inside

and closing immediately again (Fig. 1). But if the animal chose the wrong, i.e., locked door, it fell onto a net stretched at a certain level above the floor.

The animals made a total of six jumps a day, the criterion being six successful jumps with no errors between, though not on the same day.



Fig. 1. Lashley's Apparatus.

The albino rats used, aged 2.5 months at the beginning of the experiments, were 20 in number and kept fasting 22 hours a day. The first task was for any one rat to learn to chose the left door and to avoid the right one. In the next learning, this was reversed.

Results

The rats were required to relearn 20 times. The results are compiled in Table I.

To become established, the reflex needed an average of 9.5 runs, and the transformation 11.6 runs. As may be seen from the Table, most rats 16 in 20 needed fewer runs to elaborate the reflex than to transform it. But some behaved differently. One rat (No. 6) needed the same number of runs for reflex elaboration and transformation, and three (Nos. 11, 17 and 20) transformed the reflex more rapidly than they elaborated it. Animals which needed few runs to elaborate the reflex needed also few runs to transform it, whereas those that needed many runs to elaborate the reflex, needed many for transformation.

The data compiled in Table I have been analyzed statistically by the correlation method. A statistically very significant correlation was found to exist between the number of runs needed for reflex elaboration and that for transformation. This correlation proved linearly propor-

tional, which means that the more runs an animal needed for reflex elaboration, the more did it require for its transformation.

These results suggest that the longer an animal is trained in any one experimental pattern the more difficult it becomes to modify this pattern. All rats were trained to the same criterion, but the process of consolidation was more advanced in the rats which made more runs during elaboration of the reflex and they, therefore, needed more runs to transform it.

Table I

The average number of runs needed for the elaboration of the first reflex and transformation

Animal No.	1	2	3	4	5	6	7	8	9	10
Reflex	6	7	7	7	8	8	8	8	8	9
Transformation	8	9	8	11	9	8	10	12	7	11

Animal No.	11	12	13	14	15	16	17	18	19	20
Reflex	9	9	10	10	11	12	12	12	14	15
Transformation	8	12	12	13	17	14	11	20	18	14

However, why in this type of experiments elaboration of the reflex is more rapid than its transformation remained still an open question. To investigate this point, further experiments were made.

Experiments made by the Fedorov-Glebovski method.

The experiments described below were designed to determine the factors inhibiting both reflex elaboration and transformation. Two factors were considered: the stimulus that indicates the right way in a maze, and consolidation of the reflex elaborated. The experiments were made with twenty rats divided into four groups as follows:

group 1 was trained with a signal and reflex fixation,

group 2 was trained with a signal and without reflex fixation,

group 3 was trained without a signal and with reflex fixation, and,

group 4 was trained without either signal or reflex fixation.

The first reflex elaborated in any of the four groups was never consolidated but immediately, i.e., on the same and first day of the experiments reversed after the specified criterion of five consecutive error-less runs had been reached. In groups 1 and 3 — i.e., those trained with reflex fixation — this transformed reflex was consolidated on the second day, transformed again, the transformation consolidated on the

third day, and so forth. In groups 2 and 4 — trained without fixation — there were two transformations a day, each to the specified criterion and, obviously, with no reflex fixation between.

METHODS

The automatic Fedorov-Glebovskii apparatus (1954) used in these experiments consisted of a two-floored camera. The upper floor was the place the animal had to reach, with the lower floor as the starting platform, from which the rat had to run along an inclined

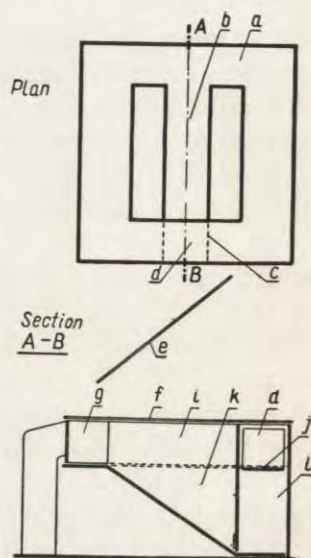


Fig. 2. Fedorov-Glebovskii's Apparatus.

Plan:

a — encompassing corridor, b — inclined corridor, c — door, d — chamber. Section A-B: d — chamber, e — mirror, f — glass lid, g — point of junction, i — encompassing corridor, j — removable floor, k — inclined corridor, l — bottom floor of the camera.

corridor to a point where it met an encompassing corridor. From this junction two paths led to the goal. The floor of the corridors was wired in such a way as to have currents of opposite signs conducted by alternating wires insulated from one another, the voltage being controlled at will. A small section of the encompassing corridor was separated from the rest by two side doors in either direction. It formed a separate chamber accessible through these doors from either of the two branches of the corridor and had a metal floor never charged electrically. This was the only place where the animal could escape the effects of the current. The apparatus was covered with a tilting glass lid. Above was a mirror inclined at an angle of 45° enabling the experimenter to watch the animal without approaching the apparatus (Fig. 2).

At the beginning of the experiment the animal is placed on the upper floor. After one minute, the floor is gradually withdrawn from under animal's feet (this lasts 30 sec.) and the animal thus forced to jump down onto the lower floor. The withdrawn floor, which is at the same time the ceiling of the lower compartment is swiftly slid back into position so that the animal is again walled-in from all sides. Next, the conditioned stimulus is switched on and at the same time opens the door leading into the inclined corridor and so does one of the encompassing corridor's terminal doors, which lead to the chamber which is never charged electrically. Low-voltage current is switched on seven minutes after the conditioned stimulus. It acts all along the wired surface, i.e., along the entire path the rat has to run.

The animal runs from the starting point along the inclined corridor till it meets the encompassing corridor where it has to choose the left or right direction. Animals with an established conditioned reflex start immediately when the conditioned stimulus is switched on and door of the end chamber opened. On arrival at the junction with the encompassing corridor they choose the direction with which the conditioned stimulus is associated, continue along the encompassing corridor and enter into the terminal chamber, where upon all the doors close. The entire run can be made by the rat before the current is switched on. If the conditioned stimulus is not yet well established, the animals starts running only after the unconditional stimulus, i.e., the electric current, begins to act. At the point of junction it may choose the wrong direction and remains under the effects of the current all the time till it eventually enters the door of the terminal chamber.

The apparatus works wholly automatically except that the animal is placed in it by hand. The animal's behaviour in the maze too is registered automatically. A detailed description of the apparatus will be found in the paper by Fedorov-Glebovskii (1954).

RESULTS

Initially, all animals were trained in the apparatus to choose the left direction at the point of junction. When this reflex became established, it was transformed by making the animals choose the right direction. This series of experiments was made with no preliminary training. In all the animals the conditioned reflex was elaborated and transformed on the first day (criterion, five consecutive error-less runs), and the entire subsequent pattern was as described for groups 1—4 in the previous experiments.

The results obtained in group 4 are compiled in Table II.

Table II

The number of runs required for successive transformations on group 4
(trained with neither signal nor consolidation)

Animal No.	Successive elaboration of transformations																			
	x)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	15	13	15	7	9	6	6	6	7	6	10	5	7	5	9	5	7	5	6	5
2	12	14	9	7	7	12	6	7	7	7	6	6	7	6	6	5	6	6	6	6
3	11	12	13	9	8	6	6	6	7	5	6	9	6	6	6	7	6	5	6	6
4	16	18	13	6	11	6	8	6	8	6	8	5	7	5	6	5	7	5	6	6
5	8	8	7	8	7	6	6	6	8	6	6	6	6	6	5	11	5	7	25	9

x) Number of runs to elaboration of first reflex.

As may be seen, one of the five animals needed for the first transformation fewer runs than for the elaboration of the original reflex (animal No. 1). The reverse was true of three rats (Nos. 2—4), whereas one animal (No. 5) needed the same number of runs for either elaboration or transformation. On the average, the first transformation needed more runs than the reflex elaboration, whereas in the second transformation, the number of runs needed to reach the specified criterion fell below the average needed for the latter. The number of runs gradually diminished with subsequent transformations to a level of about 6.5. The figures underscored in the table refer to experiments in which — on resumption on a given day — the rat chose, the door which had been the wrong one, i.e., locked, the day before. In principle, all the rats turned in the first run of any given day to the door that had been open the day before and found it invariably locked. Beginning with the eleventh transformation, two rats (Nos. 1 and 4) came to start every day with correct runs by turning from the beginning to the door that had been closed the day before instead of to that to which they had been trained. One did this five times and the other four times in succession. In other rats, this reaction was less frequent but did occasionally occur. In spite of this, it appears legitimate to assume that by continuous alternation of two paths, the animals acquire a general orientation in the experimental pattern, remember well either of the two paths possible in the maze and attempt to solve the task as a whole, i.e., to alternate the paths.

The source of these correct reactions, it appears, should be sought in the arrangement of the experiments. Each day the animals had to master two paths, either to the same criterion. The path that was the first to be mastered invariably entailed a left-turn at the junction, and the second, a right-turn. Thus, two explanations are possible for the behaviour of the animals. One is that in the course of continuous trans-

formations the animals mastered the principle that every new day begins with runs involving a right turn. The other explanation would be that the animals became accustomed to beginning the day with runs involving a turn opposite to that which they had made in the last runs of the day before. The experiments made afford no definitive clue as to which explanation is right. Possibly, both are. The second is favoured by the experiments with incomplete learning (Dąbrowska 1959), in which the rats came to avoid doors that had been open the day before, but it is not possible to claim with absolute certainty that the same phenomenon was involved in the experiments in question.

The first reflex transformation was for most of the animals in this group more difficult than elaboration of the original reflex, but with subsequent transformations this difference, expressed in the number of runs, gradually diminished and became almost nil.

Other animals (group 2) were trained with signal and without reflex fixation. The signal was a bell announced the opening of the door in the left or right section of the encompassing corridor, and was in either case invariably the same affording thus no more or less direct and immutable clue concerning the direction to be followed by the animal. It was switched off only between tests, i.e., when the animal was in the chamber of the upper floor. The results are recorded in Table III.

Table III

The number of runs required for successive reflex transformation in group 2 (trained with signal and without reflex fixation)

Animal No.	Successive elaboration of transformations																			
	x)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	18	17	5	8	10	7	8	7	5	6	7	13	12	6	6	6	7	13	10	6
2	16	13	8	8	6	7	6	6	6	6	6	10	5	6	11	10	5	6	12	6
3	8	13	17	15	17	8	8	6	20	8	6	6	6	6	5	6	6	6	8	6
4	8	16	10	8	7	7	7	6	6	5	8	7	6	5	7	8	6	6	6	5
5	7	16	12	10	7	9	8	7	5	6	5	8	6	9	5	6	6	11	5	6

x) Number of runs to elab. of first reflex

The results are similar to those recorded in Table I. On the average the animals needed more runs for transforming than for elaborating the first, original reflex. But individually, this again applied to only three animals (Nos. 3—5), while the remaining two needed fewer runs for transformation than for elaboration of the original reflex. Some of these animals too made occasionally error-less runs at the beginning of a new day, but in this the regularity recorded for the two animals in the previous group was absent. Indeed, one of the animals began four

days with five consecutive error-less runs each, but the days were not consecutive, and irregularly spaced (rat No. 5). This hardly warrants a claim that the rats were learning the succession of habit transformations and may — at best — be taken to suggest that such a process had only just begun in some animals and might have become manifest, but only after continued training.

However, interesting to note, no such behaviour was ever recorded in experiments with the remaining two groups of rats, which were trained with reflex fixation of the successive transformations. Tables IV and V show that the entire process of twenty consecutive transformations ran very much the same course as in the two previous groups, i.e., the number of runs needed to master a reflex — initially large — gradually diminished till it attained the minimum at which after one error all subsequent runs were error-less.

In group one (Table IV), one animal needed the same number of runs for reflex elaboration and first transformation (rat No. 1). Two (Nos. 2 and 3) needed fewer runs for elaboration than for transformation, while for the remaining two the reverse was true.

Table IV

The number of runs needed for consecutive reflex transformations in group 1
(trained with signal and reflex fixation)

Animal No.	Successive elaboration of transformations																			
	x)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	19	19	24	6	15	8	12	6	10	12	7	6	8	6	7	6	6	6	11	6
2	7	15	9	13	7	6	6	8	7	6	13	6	6	7	6	15	6	6	6	11
3	8	19	10	6	8	7	6	6	15	6	6	6	11	6	6	7	6	6	6	6
4	12	11	7	7	7	12	6	11	6	6	7	6	6	6	6	6	6	6	7	6
5	14	13	17	19	11	19	7	14	9	6	5	7	10	12	5	6	7	7	6	6

x) Number of runs to elab. of first reflex.

As may be seen from the four tables, the general pattern is similar for all the groups. However, when the numbers of runs needed to master consecutive transformations are surveyed, some differences may be noted between the particular groups. These were thought to be associated with the use, or omission, of signal and reflex fixation. Therefore, the relevant data were analyzed statistically. This analysis was applied, however, only to the first ten transformations in each group, as here the differences appeared most significant to the eye. The method applied to these data as shown in Table VI was that of differences.

Table V

The number of runs needed for consecutive reflex transformations in animal group 3 (trained without signal and with reflex fixation)

Animal No.	Successive elaboration of transformations																			
	x)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	15	37	25	10	24	10	7	13	7	9	6	8	6	17	6	12	8	7	8	11
2	13	11	11	11	9	8	7	7	8	7	13	9	17	6	7	6	6	6	6	6
3	6	9	19	6	7	6	6	7	6	9	6	6	10	6	6	6	6	6	5	9
4	7	8	8	6	7	7	6	6	6	5	6	6	7	6	6	6	6	11	6	6
5	8	7	26	8	7	6	8	9	8	6	6	6	6	6	6	10	7	6	8	14

x) Number of runs to elab. of first reflex.

Table VI

The number of runs needed to master the first ten consecutive transformation, compiled by groups

	with signal										Total	without signal										Total
with fixation	7	15	9	13	7	6	6	8	7	6		6	9	11	6	7	6	6	7	6	9	
	8	19	10	6	8	7	6	6	15	6		7	8	8	6	7	7	6	6	6	5	
	12	11	7	7	7	12	6	11	6	6		8	7	26	8	7	6	8	9	8	6	
	14	13	17	19	11	19	7	14	9	6		13	11	11	11	9	8	7	7	8	7	
	19	19	24	6	15	8	12	6	16	12		15	37	25	10	24	10	7	13	7	9	
Total	60	77	67	51	48	52	37	45	47	36	520	49	72	81	41	54	37	34	42	35	36	481
without fixation	7	16	12	10	7	9	8	7	5	6		8	8	7	8	7	6	6	6	8	6	
	8	13	17	15	17	8	8	6	20	8		11	12	13	9	8	6	6	6	7	5	
	8	16	10	8	7	7	7	6	6	5		12	14	9	7	7	12	6	5	7	7	
	16	13	8	8	6	7	6	6	6	6		15	13	15	7	9	6	6	6	7	5	
	18	17	5	8	10	7	8	7	5	6		16	18	13	6	11	6	8	6	8	6	
Total	57	75	52	49	47	38	37	32	42	31	460	62	65	57	37	42	36	32	29	37	30	427

Comparison of group totals reveals certain differences. Group (I) trained with signal and reflex fixation made a total of 520 runs in the course of ten transformations. Group (3) trained with reflex fixation but without signal, made a total of 481 runs. Group 2, trained with signal but without reflex fixation made only 460 runs, and group 4, trained with neither signal nor reflex fixation even 427 runs. Statistical analysis proved the number of runs necessary to master the first ten consecutive transformations to increase in relation to reflex fixation. The differences between the groups as trained with and without reflex fixation are statistically significant irrespective of whether the signal was or was not employed. Contrariwise, those between groups trained with and without the signal were so only when there was fixation of the transformations. When there was none the signal had no statistically significant influence on the number of runs made in the course of successive transformations.

The results obtained with the above four groups of animals show better inculcation of each successive reflex to retard its subsequent reversal. On the other hand, an additional stimulus — which in spite of immutably uniform features becomes associated with and indicate of alternately one and the other direction through mere experience and consequent association — also retard transformation. In effect, when these two factors — i.e., signal and reflex fixation — are both employed in the experiments, the mastery of consecutive habits is retarded most. It needs to be stressed, however, that reflex fixation has a more pronounced delaying effect than the signal. Another essential point is that the effect of either diminish with successive transformations. This is plain in the behaviour of the animals trained with the signal. With continued use, it merged gradually with the entire immutable experimental background and eventually ceased to interfere with further transformations.

In analyzing and comparing the progress of transformations in all four groups, attention should also be focussed on the underscored figures in the Tables, which indicate immediate correct responses after over-night changes of the path. As shown in Table II and III such immediate correct responses were recorded for all animals, although neither frequently nor regularly. In the remaining two groups, however, which were trained with reflex fixation (Tables IV and V), such responses were noted only four times and for only three animals in all. This suggests that the immediate correct responses were in the two groups trained without reflex fixation due not to chance but to a gradual mastering of either one or both of the principles referred to in the paragraph in which possible explanations of such behaviour are review-

ed. In the remaining two groups, trained with reflex fixation, this behaviour did not occur since reflex fixation on the day after made virtually impossible any relatively easy clues that would indicate to the animal a change in direction. The only clue were five consecutive successful runs made on the beginning of the day. Rats are known to be able to master simple alternations. After a suitable training they may also master every-second-trial alternations, but it appears completely impossible for them to learn to change directions every fifth run.

An interesting phenomenon was noted for almost all the rats (of all groups) beginning with about the eleventh transformation. Up to this point, the process of transformation involved first a few errors, next, several isolated error-less runs, again some errors, and finally a succession of runs with no errors. With about the eleventh transformation this behaviour begun to change. From that point on, transformation could take place after a single error. At the same time, though, the animal mastered the principle that the direction has to be changed every day after a few runs. This gradual mastery of this principle becomes manifest in the fact that after — in principle one error the rat ran to the right door, made three or four consecutive error-less runs, and then again an error. In subsequent transformations it frequently happened that four error-less runs were followed by an error. One of the rats made in the twelfth transformation an error in the first run, none in the subsequent three, one in the fifth, and none in subsequent five runs. Another rat made in the tenth transformation an error in the first run, none in the subsequent four runs, again an error, two error-less runs and then still another error. The same rat made in the twelfth transformation first an error, then four straight runs, an error in the sixth, another four straight runs, and again an error. Other rats too behaved similarly in further transformation. This warrants the surmise that the animals have learned the general pattern of the experiments, namely, that the two doors are locked alternately, but in spite of repeated attempts could not solve the problem as a whole since the system of five straight runs in succession proved too difficult for them. Compared with the early stage of the experiments, the character of the spoiled runs, made after a series of straight ones, was somewhat changed too. When the door to the inclined corridor opened in the first few transformations the rat ran rapidly up the corridor, slowed down a little before the junction, accelerated again and continued as rapidly as before to the terminal door. After a dozen or so of reversals, when the characteristic errors described before appeared, the runs became slower and intermittent, the animals reversed and hesitated similarly as described by Dąbrowska earlier (1959).

DISCUSSION

The experiments warrant the statement that the signal used in the trials matters only at the beginning, during elaboration of the original reflex and its first transformation, it slightly interferes with the latter, as becomes manifest in a larger number of errors. However, the significance of the signal becomes less with successive transformations. It appears to merge with the entire experimental background, immutable irrespective of changes in the direction of runs. Significance is acquired by the signal only through combination with conditioned-reflex fixation. If the latter is slight, so is the signal's significance, which in this case fails to become statistically significant. Reflex fixation, on the other hand, is of great importance in transformations irrespective of the signal. The signal, though, does emphasize the interfering effects of reflex fixation, and the differences between the results of the group trained with reflex fixation and signal and those of the other groups are all statistically highly significant.

The results as obtained by the methods of alimentary-and-defence reflexes in Lashley's apparatus and defence reflexes in the Fedorov-Glebovskii apparatus are essentially concordant. On the average, elaboration of the originated reflex was easier than the first transformation. This was not an absolute rule though, since some rats required for either the same number of runs, while others needed for the first transformation even fewer runs than for the elaboration of the original reflex. It cannot be inferred from an analysis of the behaviour of particular animals that training conditions were different for any. Consequently, different results must be supposed to be attributable to individual differences. In general, the first reversal is so much more difficult for most animals that the average number of runs it needs is plainly above the average required for the elaboration of the original reflex.

A most of authors, Fritz (1930), Munn (1932), Wylie (1919) and others, who studied mastery by animals of antagonistic habits invariably found negative transfer. The experiments described in this paper confirm these findings only in part, since not all animals behave exactly similarly, and it is only the general average that shows the second task to be more difficult than the first. These results would accord with the theory of stimulus and response, provided it is confined to the elaboration of the original reflex and first transformation. But they fail to explain the data here reported which confirm the results obtained by Krechevskii (1932), Kelleher (1956), and Hoffmann, Perkins and Calvin (1956), who found continued trans-

formations to reduce gradually the number of runs and cause positive transfer in the end. Fedorov (1951), Tretyakova (1953), Ponurova (1953) and others have found the nervous processes of stimulation and inhibition to be amenable to training, but they were not concerned with the point we are interested in, namely why the first transformation is more difficult than elaboration of the original reflex.

This subject was studied in much detail by Konorski and Szwejkowska (1950, 1952, 1953, 1956), but their approach was somewhat different. In their experiments they proved the principle of accumulation of neural connections proposed by Pavlov in connection with Fridmann's experiments (Pavlovian Wednesdays, 1949). My results appear to confirm indirectly Konorski's and Szwejkowska's results in that they show reflex fixation to affect relearning. When neural connections are firmly established, it becomes difficult to extinguish them and to elaborate new ones in the same points of the cortex. However, in virtue of multiple transformations, they both exist, and eventually the animal's first reinforced run determines which of them is to operate for some time. The conflict between stimulation and inhibition attends only the first transformation since one connection already exists, and has to be extinguished, while another has to be established. Hence, the first transformation requires the largest number of trials. In further transformation, when both connections exist, the animal has merely to determine by trial which is to operate, and relearning is rapid, involving eventually a minimum number of trials.

A further result of this work consists in that rats have been shown to treat the entire system of the tasks as a composite whole and to attempt to solve it without errors. The rats evidently perceived the periodicity of direction changes, but the principle of these proved too difficult for them to be solved to the end.

An analysis of the results as obtained in learning of maze habits (Dąbrowska 1959, 1959a) and in relearning of simple motor reflexes shows the essential difference between these two to concern elaboration of a second task. When the reaction of the animal is compound and requires it to react to several consecutive elements encountered in the experimental apparatus, first learning is more rapid than the original learning. The number of runs needed to master the second task drops to about one-half of those needed for the first task. In the case of a simple motor reflex, on the other hand, the second task, i.e., transformation is usually mastered after a larger number of runs than the first, i.e., elaboration of the original reflex. If the second task was ever

mastered more rapidly than the first, the difference did not exceed 1—3 runs. There was never such a large drop in the number of runs as that noted in maze habit reverse learning.

The question arises what does this difficulty in the transformation of simple motor reflexes consist in? The answer seems to be that it is rooted in the need for new connections to be established at points between which different connections, in a way opposed to that needed, already exist. These must temporarily be inhibited, and new ones produced. In the case of maze habit relearning, the situation — it seems — should be similar, but to say so with reasonable certainty calls for further experiments and investigations.

CONCLUSIONS

1. Multiple mastery of antagonistic tasks increases the rate at which successive transformations are learned.

2. In multiple learning, the differences between transformations of simple motor habits and of complex maze habits are confined to elaboration of the second task. The course of further transformations is similar in either of them.

3. In repeated transformations of simple motor habits, much depends on such factors as the degree of the reflex fixation and the signal employed.

Excessive reflex fixation retards the mastery of successive transformations. The signal alone does not conspicuously retard transformations, but used together with reflex fixation, it does very markedly.

4. Retarded, i.e., more difficult, mastery of the second task (first transformation of the original reflex) is due to complete reversal of the experimental situation at first associated by the animal with a well learned reflex.

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THE MORPHOLOGY AND TOPOGRAPHY OF THE THALAMIC NUCLEI OF THE DOG

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INTRODUCTION

The morphological studies on the thalamus of mammals concern mainly its topography and architectonics or the interthalamic and thalamo-cortical connections. The results of the morphological studies are presented usually as descriptions and atlases based on the histological sections of the brain. Therefore the localization of the particular nuclei is rather difficult and it proves necessary to compare the drawings in an atlas with a synthetic model.

In the literature available we have found a graphic reconstruction of the thalamus of the cat performed by Castellanos (1949), who applied the method used by Krieg. Krieg himself (1957) reconstructed the whole human brain taking into consideration all subcortical centers.

In the atlas of the nervous system (Ciba 1959) the model of the human thalamus is presented in the shape of a uniform lump on which some nuclei have been marked schematically with colours.

Ehmann and Winiwarter (1935—1936) and Klinger (1942) have presented models of the nuclei of the human telencephalon.

The aim of the present paper is to show the nuclei building up the thalamus of the dog in the form of a three-dimensional model. The author hopes that it may prove helpful in surgical and physiological work.

MATERIAL AND METHODS

The construction of the model of the thalamus is based on uninterrupted series of preparations stained in accordance with the methods of Weigert (modified by Wolters) and Nissl. The sections were 50μ thick, cut in three principal planes: frontal, sagittal and horizontal.

The model of the thalamus was made of a mixture of beeswax and paraffine of which a great number of plates about 4.8 mm. thick were prepared. The thickness of the plates (4.8 mm.) is the product of the thickness of the section (50μ) multiplied by the distance between the sections chosen for reconstruction (every eighth) and the scale of the magnification of the model (12 x). In this work only the nuclei of thalamus and metathalamus have been formed and therefore fiber bundles are seen as empty spaces between the nuclei (for example the empty space in the place of the external medullary lamina — Fig. 8, the optic tract — Figs. 1 and 2, and the occipital radiations — Figs. 1 and 2). The nuclei of the epithalamus and hypothalamus have not been taken into consideration in our model.

The descriptions and drawings concern the model of the right hemisphere.

DESCRIPTION OF THE MODEL

Looking at the model from the lateral side (Fig. 1), we see the division of the thalamencephalon into two parts:

- 1 — the thalamus proper covered by *n. reticularis* from the lateral side,
- 2 — the metathalamus with the geniculate bodies (medial and lateral).

The *nucleus reticularis* (Figs. 1 and 8) surrounds nearly all the thalamic nuclei on the lateral side except *n. lateralis* and *n. anterior dorsalis*. On the lateral side *n. reticularis* shows many concavities and grooves (Fig. 1) due to the pression of the bundles of the internal capsule on the surface of the nucleus. The intermediate thalamic radiations originating from the internal capsule to *n. medialis-dorsalis* and the *lamina medullaris interna* (Rioch 1931), pierce the middle part of *n. reticularis*, along the whole length of this nucleus. They are invisible on the model. Above the dorsal edge of *n. reticularis* there run the superior thalamic radiations (running from the striatum to thalamus — Rioch 1931).

N. reticularis is not in direct contact with the thalamic nuclei from which it is separated by external medullary lamina (Fig. 8). Laterally, *n. reticularis* borders on the internal capsule over its whole surface. The oral boundary of *n. reticularis* reaches the posterior part of commissura anterior, and in this region it bends medially surrounding the anterior group of the nuclei much like as it does in the cat and man (Ingram, Hannett and Ranson 1932, Dekaban 1953).

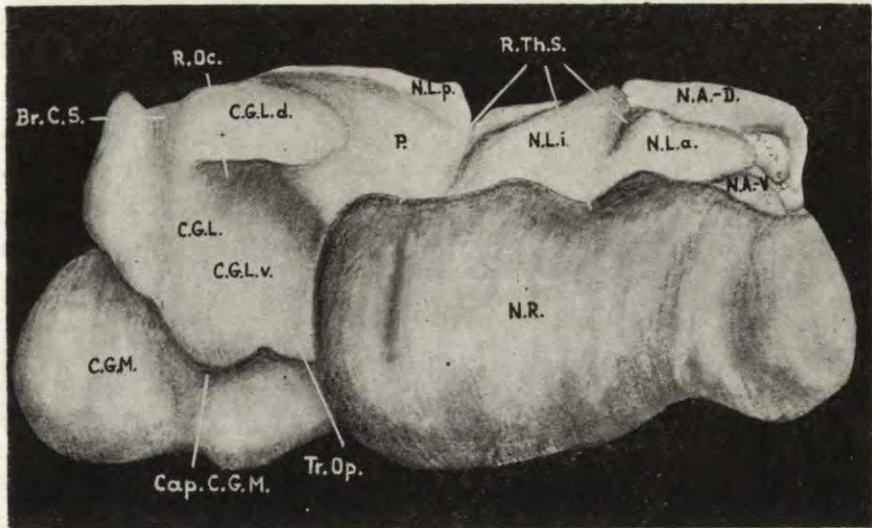


Fig. 1. Model of the thalamus seen from the lateral side

Caudally, *n. reticularis* touches the anterior parts of the geniculate bodies (Fig. 1). Basing upon the difference in the shapes of cells Rioch and Gurdjian have divided *n. reticularis* into two parts, dorsal and ventral. Our observations on the dog confirm these results. In the model the differentiation just mentioned is not visible.

When *n. reticularis* is removed from the model (Fig. 2), the deeper thalamic nuclei and the anterior part of the geniculate bodies with the oral-ventral part of the pulvinar are exposed. In this view (Fig. 2) the geniculate bodies and *n. ventralis* thalami draw our attention by their large size.

The lateral geniculate body is separated from the medial one by the bundles of fibers of the capsule of the medial geniculate body.

The lateral geniculate body (Figs. 1—7) is divided into dorsal and ventral parts by the optic and occipital radiations. The shape of the lateral geniculate body corresponds exactly to its name, because this nucleus being its caudal portion, shows a characteristic nodose flexion. The externally convex part of the diencephalon is the most caudally placed part of this flexion. In this region, on its lateral surface, a vertically running concavity is seen in which lies the brachium of the superior collicule (Figs. 1—7). The dorsal and ventral part of the lateral geniculate body are turned rostrally. In the dog the ventral part of the lateral geniculate body is distinctly larger and more rostrally protruded than the dorsal. An analogous situation was described in the

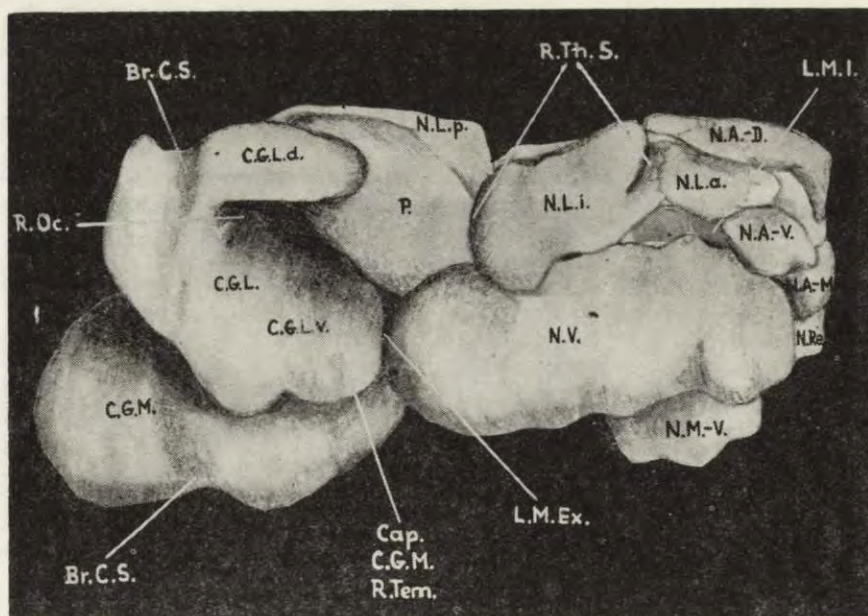


Fig. 2. As in Fig. 1 *nucleus reticularis* removed

rat by Gurdjian (1927). In the cat, on the contrary, the proportion between the two parts of the lateral geniculate body is reverse: the ventral part is smaller than the dorsal (Winkler and Potter 1914, Ingram, Hannett and Ranson 1932, Castellanos 1953, Jasper 1954). In the pig Solnitzky (1938) has found the similar relations. In the primates an increasing superiority of the dorsal part over the ventral is observed (Walker 1938) and in the human brain the geniculate body is cone-shaped (Dekaban 1953).

The medial geniculate body (Figs. 1—7) is placed medio-ventrally in relation to the lateral. It is a lengthened lump somewhat narrower orally. In its central part a little concavity is seen on the medial aspect. This concavity is filled with the ventral portion of the brachium of the superior collicule. This corresponds to Mettler's findings (1932). The part of the medial geniculate body, which lies more caudally than the concavity mentioned above, is like the lateral geniculate body the external convexity of diencephalon and is placed on the lateral side of the anterior region of mesencephalon. In the cat the external convexity of the medial geniculate body is similar to that mentioned above in the dog (Ingram, Hannett and Ranson

1932, Castellanos 1949). In the primates (Macacus, Man) it is the pulvinar (Walker 1938), which forms the most caudally and externally lying part of the diencephalon, and the medial geniculate body is placed somewhat rostrally. This shape, according to the opinion of Gurdjian (1927), results from the reduction of the optic tectum and the growth of the importance of cortex in the higher mammals.

The anterior part of the medial geniculate body is accompanied dorso-medially by *n. suprageniculatus* (Fig. 3). The shape of this nucleus resembles an irregular lump. Its frontal section is wedge-shaped, the sharp edge running along the dorsal border of *nucleus suprageniculatus*. This dorsal edge is turned towards the posterior commissure. The base of the wedge is placed on the dorso-medial border of the medial geniculate body. More caudally, the dorsal edge of this nucleus lowers ventrally. *N. suprageniculatus* is relatively distinct in the dog, cat and primates, but has not been described in the lower mammals.

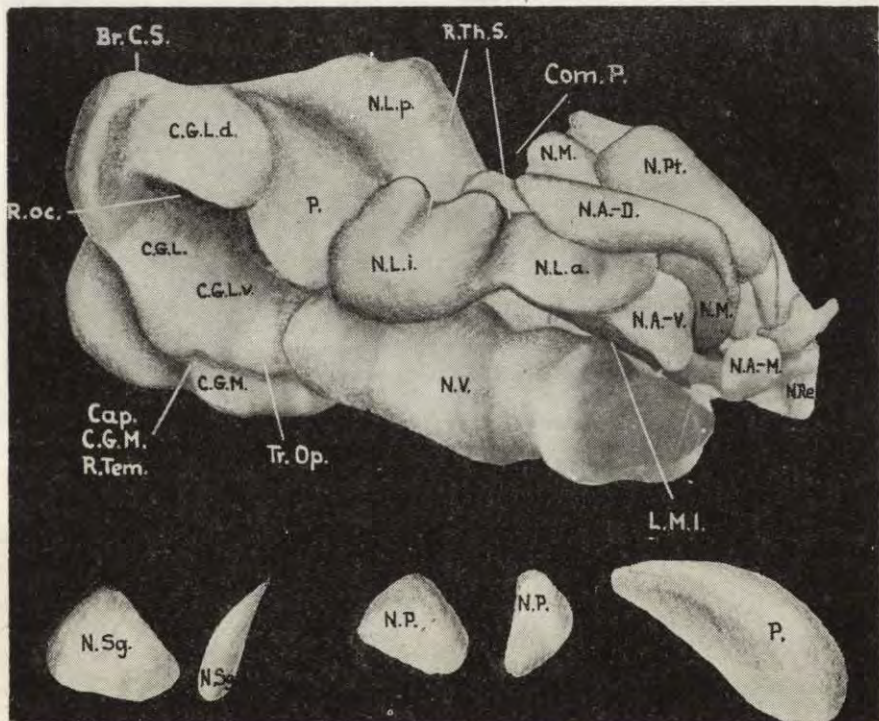


Fig. 3. Model of the thalamus seen partly from the lateral side, a little obliquely from the forepart and from above, after removing of *nucleus reticularis*. Separately *nucleus suprageniculatus*, *nucleus posterior* (lateral and oral view) and pulvinar (lateral view)

The *nucleus posterior* (Fig. 3) of the dog is placed between the ventral part of the lateral geniculate body and the medial geniculate body. It extends from the oral border of the two bodies to one half of the length of the medial geniculate body. It is a lengthened body extending oro-caudally, its thin edge penetrating between the geniculate bodies. This nucleus is a large mass in the lower mammals (Gurdjian 1927)

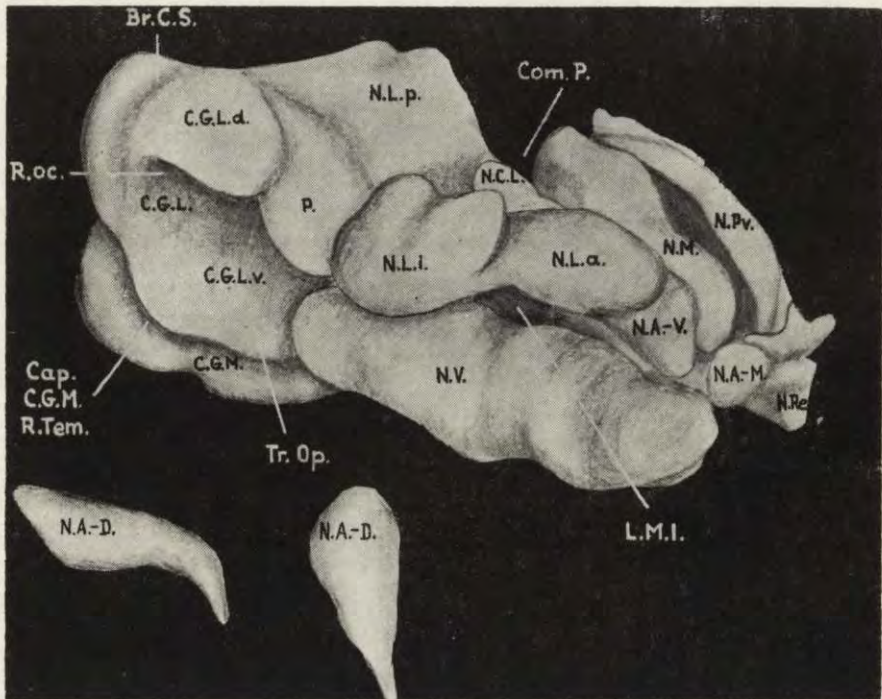


Fig. 4. As in Fig. 3 *nucleus anterior dorsalis* and *nucleus parataenialis* removed. Separately *nucleus anterior dorsalis* (lateral and oral view)

and carnivores. In the higher mammals *n. posterior* is homologized with the inferior part of the *n. pulvinaris* (Macacus) due to the connections with the geniculate bodies (Crouch 1934). In the primates and man *n. posterior* has not been described.

The pulvinar of the dog is a medium-sized nuclear mass of the shape of a thick wedge inclined medio-dorso-caudally and situated in the latero-dorso-caudal part of thalamus. In its posterior part the lateral side of the pulvinar neighbours upon the dorsal part of the lateral geniculate body and is separated from it by the lateral geniculate capsule. In the carnivores (Rioch 1929, 31, Ingram, Han-

net and Ranson 1932, Castellanos 1949, Jasper 1954) the pulvinar is a relatively small mass hardly divisible into parts, in the primates it is larger and differentiated into several parts. Unlike that of the primates, the pulvinar of the dog, as has been established in the reconstruction, does not extend far caudally but ends in the plane of the middle posterior half of the lateral geniculate body. In

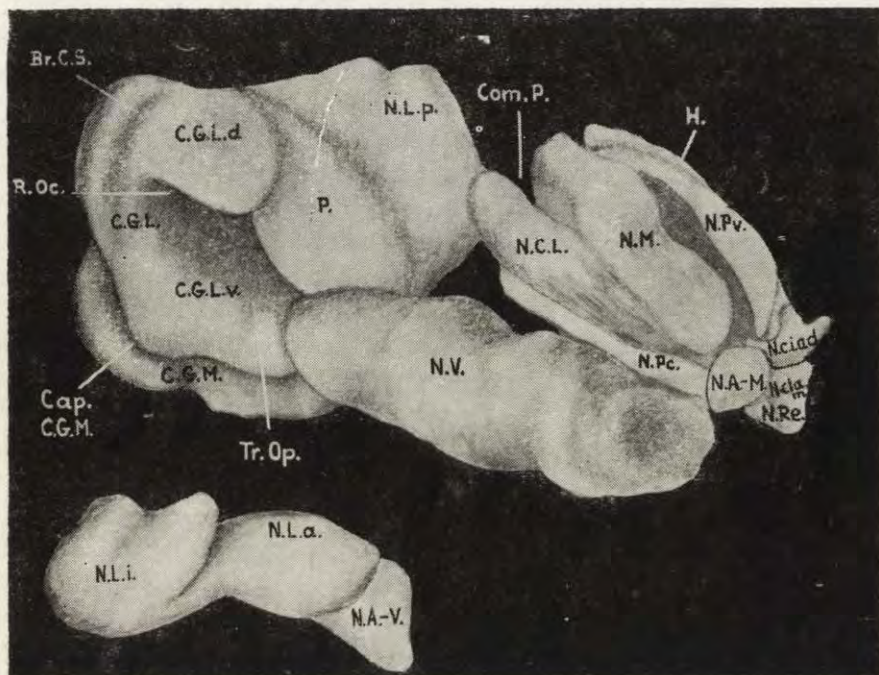


Fig. 5. As in Fig. 4 *nucleus lateralis pars anterior* and *pars intermedia* and *nucleus anterior ventralis* removed. Separately *nucleus lateralis pars anterior* and *intermedia* and *nucleus anterior dorsalis*

lower mammals (Insectivores and Rodents) it is not visible and is homologized with the pretectal area (Gurdjian 1927) and with the *n. posterior* (Crouch 1934).

N. ventralis (Figs. 2—8) resembles a flat roller in its form; it is parallel to *n. reticularis* and bent laterally along its dorsal border. The oral pole of *n. ventralis* lies behind the anterior edge of *n. reticularis*; the caudal pole is situated closely in front of the posterior edge of the latter. It borders upon the oral parts of the two geniculate bodies (Fig. 2), and is separated from them by the fibers of the external medullary lamina (Rioch 1931). The surface of *n. ventralis*

is dissected into numerous small concavities which on its lateral side depend on the bundles of the external medullary lamina and the inter-thalamic radiations, and on the medial side on the bundles of the internal medullary lamina. The microscopical structure allows to divide *n. ventralis* into 5 parts: pars anterior, pars medialis, pars externa, pars arcuata and pars commissuralis (*nucleus medio-ventralis*, Rioch 1929, 1931). Pars arcuata can be compared with pars postero-medialis of the primates. In our model *n. ventralis* with its parts is presented in one lump. The myeloarchitectonisc of this nucleus will be the problem of the next paper. The division mentioned above is different from that described in other animals and man. The opinions on the division of this nucleus into parts vary with different authors, sometimes even within the same species (Crouch 1934, Walker 1938).

Ventro-medially in relation to *n. ventralis*, near the nuclei of the midline, there lies the small *n. medio-ventralis*, the ventral part of which is seen in Fig. 2. In Fig. 8 is seen its commissural part determined by Rioch as *nucleus commissuralis inter-ventralis*. The shape of this nucleus resembles that of a lengthened cylinder lying laterally close to the medial aspect of the midline part of *nucleus ventralis*. In the caudal region of *nucleus medio-ventralis*, on its medial side, *nucleus commissuralis inter-ventralis* may be distinguished. According to the opinion of Rioch, Ingram, Hannet and Ranson, and Castellanos *nucleus medio-ventralis* of the dog and the cat is the fifth part of the *nucleus ventralis* (cf. above). On the other hand Solnitzky (1938) has included this nucleus in the pig to the group of the midline. A similar nucleus topographically was distinguished by Crouch (1934) in the maccacus, but more recent publications concerning the thalamus of this animal do not describe it (Walker 1938, Fulton 1943). In man, too, only nucleus submedius has been distinguished by Dekaban (1953).

Nucleus lateralis (Figs. 1—4) is a smaller mass than the complex of the *nucleus ventralis* discussed above. It resembles an irregular shape lump placed in the dorso-lateral part of thalamus, dorsally to *nucleus ventralis*. Orally this nucleus does not reach the rostral boundary of *nucleus ventralis*, caudally it extends like the pulvinar and disappears in the plane of the flexure of the lateral geniculate body. In the cat is disappears simultaneously with the lateral geniculate body and the pulvinar (Ingram, Hannet and Ranson 1932, Castellanos 1949, Jasper 1954). In the dog *nucleus lateralis* shows two distinct incisions on its surface: the dorsal one, at one third of its length from the forepart, and the lateral one, at two thirds of this length. The second incision causes a medial flexure of the posterior

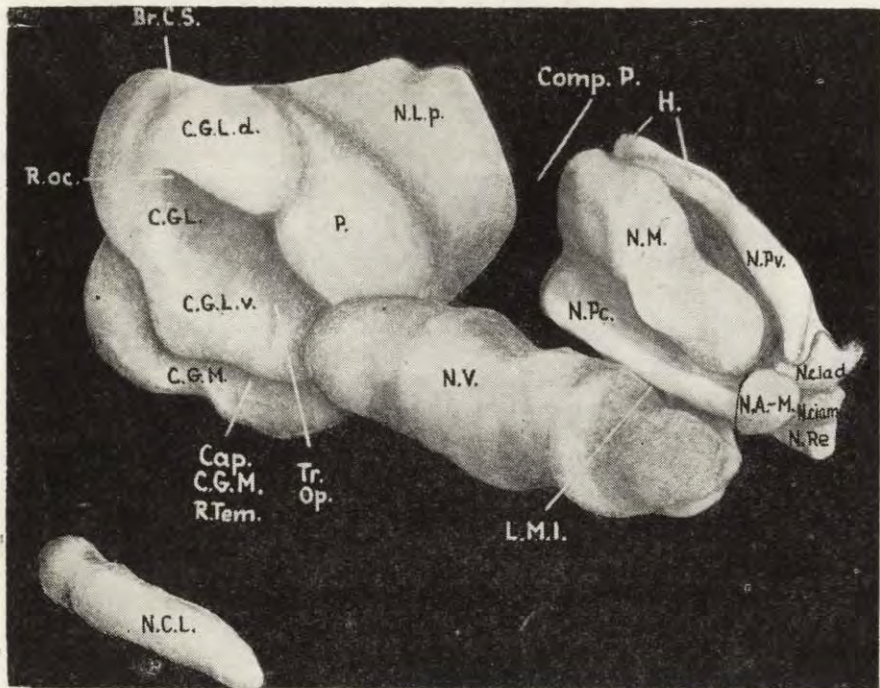


Fig. 6. As in Fig. 5 *nucleus centralis lateralis* removed. Separately *nucleus centralis lateralis* (oral view)

part which thus touches the medial side of the pulvinar. The anterior part of *nucleus lateralis* narrows a little in relation to the rest of the nucleus. More caudally, an incision on the surface of the nucleus is seen, across which the superior thalamic radiations run (Figs. 1—3).

In our model *nucleus lateralis* can be divided into three parts: the anterior, extending to the incision mentioned above, the inter-medial, reaching the oral border of pulvinar, and the posterior lying medially to the pulvinar. This division, according to observations of Rioch (1929, 1931) and Ingram, Hannet and Ranson (1932) occurs in the dog and the cat. On the other hand, Castellanos (1949), Jasper (1954), Solnitzky (1938) and Abe (1952) distinguish only two parts in this nucleus in the cat, pig and elephant. Crouch (1934) divided *nucleus lateralis* of the macacus into four parts but according to Walker (1938) two parts only should be distinguished, like in man (Dekaban 1953). It seems that the parts of this nucleus cannot be homologized in different animals.

The anterior group of the thalamic nuclei is represented in Fig. 2 only by *nucleus anterior dorsalis* and *nucleus anterior ventralis*. Both are placed in the neighbourhood of the anterior part of *nucleus lateralis*. *Nucleus anterior ventralis* is partly an oral continuation of the anterior part of *nucleus lateralis*; *nucleus anterior dorsalis* extends along its oro-medial border (Figs. 2, 3). On the model seen in the Fig. 3 obliquely from the forepart and a little from above the oral part of the thalamus together with its anterior nuclei is also visible.

Nucleus anterior dorsalis (Figs. 1—3 and 8) neighbours *nucleus lateralis* over its ventro-lateral surface. It is of the shape of a lamina

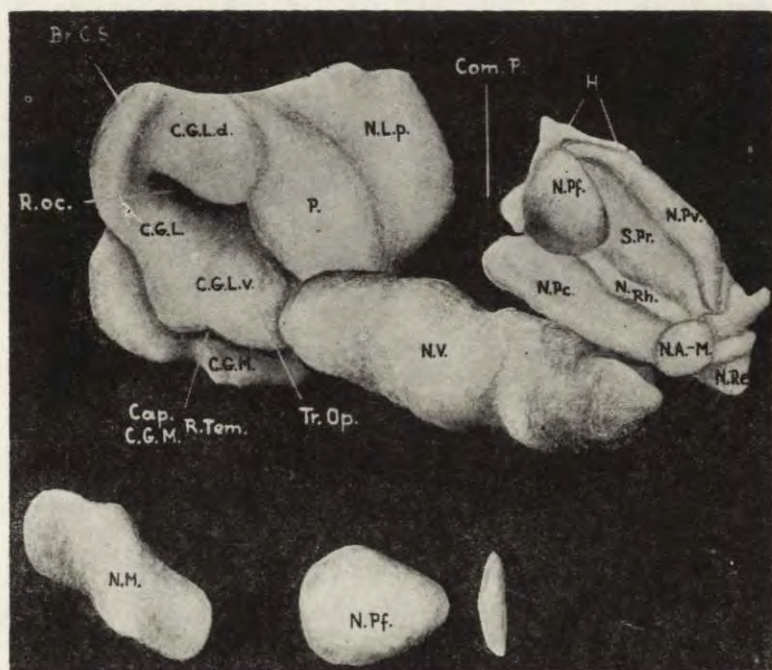


Fig. 7. As in Fig. 6 *nucleus medialis dorsalis* removed. Separately *nucleus medialis dorsalis* (lateral view) and *nucleus parafascicularis* (lateral and oral view)

narrowing in its oral part and bending medio-ventrally into a sharp-ended appendix turning to *nucleus commissuralis inter-antero-dorsalis* (cf. Fig. 8). The lamina of *nucleus anterior dorsalis* covers the anterior part of *nucleus lateralis* from the dorso-medial side and is placed a little obliquely, so that its lateral edge lies higher up than the medial (cf. Fig. 8).

Nucleus anterior ventralis (Figs. 2, 3, 8) is placed ventrally in relation to the anterior part of *nucleus anterior dorsalis* and dorsally with respect to *nucleus ventralis*. The shape of *nucleus anterior ventralis* is irregular. Orally, this nucleus narrows, caudally increases and gradually rises more dorsally. The anterior part of *nucleus anterior ventralis*

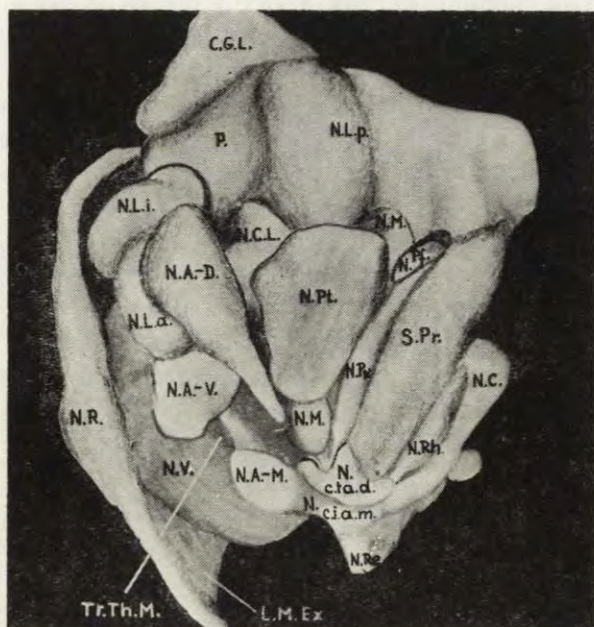


Fig. 8. Complete mode of the thalamus seen partly from the medial side, a little anteriorly and from above

is approached by the majority of the bundles of fibers belonging to the thalamo-mammilar tract of Vic d'Azyr (Fig. 8). This bundle fills up the gap between the discussed nucleus and ventro-medially lying *nucleus anterior medialis*.

Nucleus anterior medialis (Figs. 2—8) is situated most medially of all nuclei of the anterior group. It is a small mass (cf. Fig. 8), turned with its broader end towards *nucleus commissuralis inter-antero-medialis* belonging to the group of the intermedial mass.

Fig. 3 shows also *nucleus parataenialis* (Fig. 3, 8) which according to Ricch's opinion, belongs to the medial group of nuclei. It resembles a lamina in its shape and lies most dorsally in the anterior part of the thalamus very near the nuclei of the midline. *Nucleus para-*

taenialis extends along the stria medullaris which connects habenula with the rhinencephalon in the same hemisphere (Walker 1938). In the rostral part *nucleus parataenialis* is connected with its contralateral nucleus by the small *nucleus inter-antero-parataenialis* (not shown in the model because of its very small dimensions). Caudally, *nucleus parataenialis* extends to the anterior border of *nucleus parafascicularis* (cf. Fig. 8). Laterally it borders on *nucleus anterior dorsalis*, ventrally on *nucleus medialis dorsalis* (in Fig. 3 oral and caudal portions of *nucleus medialis dorsalis* are visible).

When *nucleus anterior dorsalis* and *nucleus parataenialis* are removed (Fig. 4), two nuclei can be seen, *nucleus paraventricularis* (Figs. 4–8) and *nucleus medialis dorsalis* (Figs. 5, 6). Particular attention is paid here (Fig. 4) to *nucleus paraventricularis* which is included into the group of the midline. This nucleus has the shape of a flattened cylinder placed in the parasagittal plane, laterally to the periventricular system. The anterior end of *nucleus paraventricularis* bends ventrally, the posterior end rises dorsally and narrows slightly. The most caudal part becomes thicker dorso-ventrally and a little laterally and in this region it borders on *nucleus parafascicularis* (Fig. 7). Some authors (Rioch 1929, Ingram, Hannet and Ranson 1932, Crouch 1934, Solnitzky 1938) divide this nucleus into two parts: the anterior and posterior, others (Castellanos 1949, Walker 1938, Jasper 1954, Dekaban 1953), on the contrary, consider it as a uniform mass. In our model this nucleus has also been constructed as a non-differentiated mass.

In Fig. 5 we see the aspect of nuclei after the removal of *nucleus lateralis* and *nucleus anterior ventralis*. Here a large space between *nucleus ventralis* and some of the posterior nuclei is seen. This gap is caused by the internal medullary lamina dividing the thalamus into three parts: lateral, medial and anterior (Walker 1938). Among the nuclei seen in Fig. 5 attention is drawn to two nuclear masses lying one beside the other, *nucleus centralis lateralis* and *nucleus medialis dorsalis*.

Nucleus centralis lateralis (Figs. 5, 8) is situated between *nucleus medialis dorsalis* and *nucleus lateralis* (cf. Fig. 4). Caudally it borders *nucleus lateralis posterior*, orally begins a little behind *nucleus medialis dorsalis*. It results from Rioch's observations that the oral part of *nucleus centralis lateralis* borders *nucleus anterior medialis*. Our observations do not fully confirm the data obtained by that author, because *nucleus centralis lateralis* does not neighbour *nucleus anterior medialis* directly, but begins more caudally. Rioch reports that both nuclei are separated by a kind of capsule built of fibers containing cells.

According to the opinion of the present author the existence of these cells renders difficult the establishment of an exact border between the nuclei in question. If undertaken the myeloarchitectonic observations of these two nuclei may help to solve this problem. *Nucleus centralis lateralis* is of elipsoidal shape (Fig. 5), narrow in the medio-ventral end. On the latero-ventral side it borders *nucleus paracentralis*. In its posterior part *nucleus centralis lateralis* shows a little narrowing which is traversed by one of the bundles of the superior thalamic radiations and by those of the internal medullary lamina (Fig. 6). This nucleus has not been distinguished in the rat (Gurdjian 1927), but Crouch (1934), Ingram, Hannet and Ranson (1932) and Solnitzky (1938) have described it in the monkey, cat and pig. Dekaban (1953) reports that the borders of this nucleus are difficult to establish in man. *Nucleus centralis lateralis* is accompanied by two neighbouring nuclei, *nucleus paracentralis* and *nucleus centralis* (Figs. 6, 7, 8).

Nucleus centralis, also called *nucleus centralis medialis* (Fig. 8), is an odd nuclear mass placed between *nucleus reuniens* and *rhomboidalis* (cf. Figs. 7, 8). Along the lateral edges of *nucleus centralis* there extends, in both hemispheres, *nucleus paracentralis* (Fig. 7) closely connected with the former. Both nuclei form the common laminar structure the lateral edges of which are inclined a little dorsally in relation to *nucleus centralis* (Fig. 7). The oral beginning of both these nuclei lies behind the posterior part of *nucleus commissuralis inter-antero-medialis*. Caudally, they end in the plane of the posterior pole of *nucleus centralis lateralis*.

Nucleus paracentralis is closely connected with the internal medullary lamina. On the dorsal side it borders *nucleus centralis lateralis* and *nucleus medialis dorsalis*, and on the ventral side (Figs. 5, 8) *nucleus ventralis* and *nucleus medialis dorsalis*. Our observations are in accordance with the descriptions submitted by Rioch (1929), Ingram, Hannet and Ranson (1932), Solnitzky (1938), Crouch (1934), Walker (1938), Castellanos (1949) and Jasper (1954).

After the removal of *nucleus centralis lateralis* (Fig. 6), *nucleus medialis dorsalis* the largest nuclear mass among the nuclei of the midline becomes revealed. It lies between *nucleus centralis lateralis* mentioned above and the remaining nuclei of the midline. Orally, it extends to the posterior border of the anterior group of nuclei, caudally to the posterior end of *nucleus ventralis*. It is cylindrical in shape, flattened laterally; in its oral part it narrows in the vertical dimension and bends ventrally (cf. Fig. 3, 8). More caudally, its dorsal

edge rises and reaches the ventral surface of the habenula (Fig. 6). In the posterior part the medial aspect of *nucleus medialis dorsalis* borders on *nucleus parafascicularis*. Between these two nuclei fasciculus retroflexus of Meynert is found originating from the habenula. The nuclei of the habenular complex have not been introduced in our model as they do not belong to the epithalamus. The ventral surface of *nucleus medialis dorsalis* borders on *nucleus paracentralis* from which it is separated by the internal medullary lamina.

In the cat, monkey and man a small nuclear mass has been described in the neighbourhood of *nucleus medialis dorsalis* lying ventrally in relation to the posterior part of *nucleus medialis dorsalis*. It is called "centrum medianum" and according to the opinion of Castellanos (1949), forms the ventro-caudal convexity of *nucleus medialis dorsalis*. Ingram, Hannet and Ranson (1932) consider the presence of centrum medianum in the cat as a disputable question. In the monkey and man centrum medianum is a relatively large mass situated ventro-medially in relation to nucleus medialis dorsalis. In the dog the existence of centrum medianum has not been confirmed by Rioch. Glorieux (1929) distinguished as centrum medianum the medio-caudal part of nucleus medialis dorsalis but he gave no details concerning this nucleus. Walker (1938), (after Monakow) divided nucleus medialis dorsalis on two parts, "a" and "b". Part "b" was homologized in carnivores by Walker with the presumable area of centrum medianum. Adrianow (1959) in the stereotactic atlas of the dog's brain (based on Nissl's sections) determined centrum medianum in the posterior part of nucleus medialis dorsalis. It probably is a posterior part of nucleus medialis dorsalis, difficult to distinguish. The detailed cytoarchitectonics of this nucleus will be the subject of our next investigations.

Nucleus parafascicularis (Figs. 7, 8) is the most caudal nucleus of the medial group. It is situated between the nuclei of midline and the caudal part of *nucleus medialis dorsalis*, separated from the latter by fasciculus retroflexus. This nucleus has the shape of the flat-convex lens turned to the nuclei of the midline by its flattened side (Fig. 7), and laterally to Meynert's tract by the convex side. The oral part of nucleus parafascicularis is slightly narrowed.

At the ventral side of *nucleus parafascicularis* there lies a small nuclear mass described by some authors as *nucleus subparafascicularis*. Its presence in the cat seems disputable. Castellanos (1949) does not mention its existence, Ingram, Hannet and Ranson (1932), on the contrary, distinguishes it in the form of a separate cellular mass. In the primates (Walker 1938, Crouch 1934) and also in man

(Dekaban 1953) *nucleus subparafascicularis* has not been distinguished. Rioch (1929) describes this nucleus in the dog on the ventral side of *nucleus parafascicularis*. Adrianow (1959) calls region of *nucleus parafascicularis* *nucleus tractus habenulae medialis* and *lateralis*, while the nucleus lying ventrally to it he calls *nucleus parafascicularis*. In our model *nucleus subparafascicularis* forms the ventro-caudal portion of *nucleus parafascicularis*.

The nuclei of the midline (intermediate mass) are represented in Figs. 7 and 8. On the whole they may be divided in two groups: the singular nuclei placed in the plane of the great fissure i.e. periventricular system, *nucleus rhomboidalis*, *nucleus centralis* and *nucleus reuniens* and the paired nuclei of *nucleus paraventricularis* discussed above. In order to make it clearer the nuclei of the midline, with exception of *nucleus paraventricularis* are shown with their symmetrical parts lying in the left hemisphere. The group of the nuclei of the midline shows a characteristic "stratified" configuration.

Nucleus reuniens lies most ventrally. In its oral part it is shaped like a cube with edges rounded and extends oro-caudally. Immediately of the dorsal surface of *nucleus reuniens* there is *nucleus centralis* (discussed above). The next nucleus in this stratified configuration, is visible in Fig. 8 *nucleus rhomboidalis* in its part belonging to the left hemisphere; The part belonging to the right is covered in that figure by the periventricular system. *Nucleus rhomboidalis* extends from the anterior border of the periventricular system; caudally it ends about the middle of this system. The periventricular system is the most dorsal among the nuclei of the midline. It is a thin lamina placed in the plane of *fissura magna*.

The problem of the nuclei of the midline (known also as the inter-medial mass or the interthalamic adhesion) forms the subject of an animated discussion especially as far as its homological aspect is concerned. Our observations of these nuclei are in accordance with Rioch's results. He considers the ventral part of *nucleus centralis* in the dog as homologous with the commissural part of *nucleus reuniens* in the rodents. According to the opinion of Winkler and Potter (1914) *nucleus reuniens* and *nucleus rhomboidalis* are equivalent. Ingram, Hannet and Ranson (1932) and Castellanos (1949) on the contrary, regard these nuclei as two different features. The nuclei of the midline in lower mammals (rodents, Gurdjian 1927) are well developed and form a relatively large part of thalamus easy to distinguish. In higher mammals they are developed less distinctly. In the pig, Solnitzky (1938) distinguished eight

nuclei in spite of a great reduction. In the primates Aronson and Papez (1934) have also distinguished eight nuclei, but Crouch (1934) and Walker (1938) mention only *nucleus reuniens*, *nucleus centralis*, and *massa grisea centralis*; in their opinion classification of the latter is very difficult. Both authors indicate that the nuclei of the midline

Table I

Percent ratio of particular nuclei in the volume of dog's thalamus

Name of nucleus	in mm ³	in %
<i>c.geniculatum laterale</i>	75.20	9.63
<i>c.geniculatum mediale</i>	106.50	13.63
<i>n.anterior dorsalis</i>	6.99	0.89
<i>n.anterior medialis</i>	3.99	0.51
<i>n.anterior ventralis</i>	7.32	0.94
<i>n.centralis lateralis</i>	15.30	1.96
<i>n.commissuralis inter-antero-dorsalis</i>	1.70	0.22
<i>n.commissuralis inter-antero-medialis</i>	1.70	0.22
<i>n.commissuralis inter-ventralis</i>	5.99	0.77
<i>n.lateralis pars anterior et intermedia</i>	37.62	4.82
<i>n.lateralis pars posterior et pulvinar</i>	106.54	13.64
<i>n.medialis dorsalis</i>	28.60	3.66
<i>n.medio-ventralis</i>	17.64	2.26
<i>n.parafascicularis</i>	12.98	1.66
<i>n.parataenialis</i>	9.31	1.19
<i>n.paraventricularis</i>	6.99	0.89
<i>n.posterior</i>	13.98	1.79
<i>n.reticularis</i>	99.89	12.79
<i>n.rhomboidalis</i>	10.30	1.32
<i>n.reuniens et n.paracentralis et centralis</i>	51.61	6.61
<i>n.suprageniculatus</i>	7.32	0.94
<i>n.ventralis</i>	141.17	18.07
<i>systema periventriculare</i>	12.60	1.61

are homologous with the similar nuclei of the lower mammals, but are less distinctly developed. In man Dekaban (1953) has established a further reduction of these nuclei in connection with the loss of their functional significance. These views on the homology of the nuclei of the midline cited above are in accordance with the opinions of Clark (1932) and Fulton (1943) who consider them to be the phylogenetically oldest part of the thalamus (*paleo-thalamus*).

The model proved also useful in the calculation of the volume of particular nuclei and next, their percent ratio to the whole volume of the thalamus in the brain. It was obtained from the following data: the density of beeswax, weight and volume of each nucleus in the model and the scale of enlargement of model in the relation to its real dimensions in the brain. The data concern only the brain modelled.

Volumen of particular components of thalamus in dog is shown in Table I.

SUMMARY

A model of the thalamic nuclei in the dog's brain has been constructed on the basis of its frontal, sagittal and horizontal sections prepared and stained according to the methods of Weigert and Nissl. The author has taken under consideration the configuration and relationship of the particular nuclei and their groups, i.e. the lateral, medial and a comparison between the thalamic nuclei of the dog and those of ventral thalamic nuclei and those of the midline. She has also drawn other mammals such as the insectivores, rodents, carnivores and primates. Owing to the model of the thalamus it was possible to carry out the measurements of the volume of the particular nuclei, and next to calculate the real volume of the thalamic nuclei in the brain of the dog.

The author is very obliged to Doc. Dr. J. Kreiner for his precious advices and critical notices concerning this paper.

ABBREVIATIONS

Br.C.S.	— <i>brachium colliculi superioris</i>	N.L.	— <i>nucleus lateralis</i>
C.G.L.	— <i>corpus geniculatum laterale</i>	N.L.a.	— <i>nucleus lateralis pars anterior</i>
C.G.L.d.	— <i>corpus geniculatum laterale pars dorsalis</i>	N.L.i.	— <i>nucleus lateralis pars intermedia</i>
C.G.L.v.	— <i>corpus geniculatum laterale pars ventralis</i>	N.L.p.	— <i>nucleus lateralis pars posterior</i>
C.G.M.	— <i>corpus geniculatum mediale</i>	N.M.	— <i>nucleus medialis dorsalis</i>
Cap.C.G.M.	— <i>capsula corporis geniculati medialis</i>	N.M.-V.	— <i>nucleus medio-ventralis</i>
Com.P.	— <i>commisura posterior</i>	N.P.	— <i>nucleus posterior</i>
H.	— <i>habenula</i>	N.Pc.	— <i>nucleus paracentralis</i>
L.M.Ex.	— <i>lamina medullaris externa</i>	N.Pf.	— <i>nucleus parafascicularis</i>
L.M.I.	— <i>lamina medullaris interna</i>	N.Pt.	— <i>nucleus parataenialis</i>
N.A.-D.	— <i>nucleus anterior dorsalis</i>	N.Pv.	— <i>nucleus paraventricularis</i>
N.A.-M.	— <i>nucleus anterior medialis</i>	N.R.	— <i>nucleus reticularis</i>
N.A.-V.	— <i>nucleus anterior ventralis</i>	N.Re.	— <i>nucleus reuniens</i>
N.C.	— <i>nucleus centralis</i>	N.Rh.	— <i>nucleus rhomboidalis</i>
N.C.L.	— <i>nucleus centralis lateralis</i>	N.Sg.	— <i>nucleus suprageniculatus</i>
N.c.i.a.d.	— <i>nucleus commissuralis inter-antero-dorsalis</i>	N.V.	— <i>nucleus ventralis</i>
N.c.i.a.m.	— <i>nucleus commissuralis inter-antero-medialis</i>	P.	— <i>pulvinar</i>
		R.Oc.	— <i>radiatio occipitalis</i>
		R.Tem.	— <i>radiatio temporalis</i>
		R.Th.S.	— <i>radiatio thalami superior</i>
		S.Pr.	— <i>sistema periventricularis</i>
		Tr.Op.	— <i>tractus opticus</i>
		Tr.Th.M.	— <i>tractus thalamo-mammillaris</i>

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THE EFFECTS OF UNILATERAL AND BILATERAL ABLATIONS
OF SENSORIMOTOR CORTEX ON THE INSTRUMENTAL
(TYPE II) ALIMENTARY CONDITIONED REFLEXES
IN DOGS

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One of the most important problems concerning the function of the so called sensorimotor area is connected with its functional organization. How much is this area functionally "equipotential", what is the significance of its particular parts, and what role it plays in the control of "voluntary" movements, these are the questions which have raised many discussions nearly from that time when this area was identified in Hitzig and Fritsch's experiments.

The problem may be subdivided into two different questions: 1° what is the organization and the functional role of the parts localized along the medio-lateral axis of the cortical surface, controlling movements of various parts of the body, and 2° what is the significance of particular transversal "strips" situated alongside the caudo-rostral axis.

In the present series of papers we are concerned only with the second of these questions, and try to answer it on the basis of experimental material brought out by the conditioned reflex (CR) method. In our previous papers (Stępień and Stępień 1958, Stępień, Stępień and Konorski 1959, a and b) we did find that these strips are not equipotential, that each of them has its own functional role, and its lesion produces a number of characteristic symptoms. Therefore the classical subdivision of the sensorimotor region into three areas, conventionally called sensory area, motor area and premotor area, which subdivision has been sometimes put in doubt by some authors (e.g. Dusser de Barenne 1941) seems to be well substantiated.

The aim of this paper is to elucidate what are the effects of ablations of sensorimotor area as a whole upon motor CR's and whether or not these effects may be understood as a combination of effects produced by removal of each part of it separately.

MATERIAL AND METHODS

Experiments were performed on 13 mongrel male dogs aged 2—4 years, in a soundproof CR chamber. In the preliminary training instrumental CR's were established consisting in raising of the right foreleg and putting it on the foodtray situated in front of the dog in response to various auditory or audio-visual stimuli (CS). The CR's were elaborated by the method of passive movements. 8 trials with intervals of 1—2 minutes were applied in each experiment. Alimentary reinforcement consisted in automatic putting into position the bowl with minced meat mixed with bread and moisted by broth.

When the animals were well trained, i.e. when they performed the learned movement to every CS with minimal latency, and intertrial movements, present at the beginning of training, disappeared, (this took at least 3 months) the animals were subjected to surgery. In aseptical conditions the scalp from the region to be ablated was removed, the dura matter incised and cortical tissue destroyed by subpial suction. Then the dura matter was sutured and the muscles, galea and skin were closed in layers. Several days after operation the CR experiments were resumed and conducted from 2 to 10 months.

Since our aim was to test the animals' CR's in the postoperative period without recurring to any training, it was important to apply a very cautious experimental procedure. When the animal did not perform the trained movement in response to the CS, care had to be taken neither to extinguish the reflex by non-reinforcement, nor to transform it into classical CR by reinforcement. Therefore it was necessary to take the middle course, namely: 1° to apply only a few trials in an experimental session; 2° to perform experiments not every day but only twice a week; 3° to reinforce irregularly the CS in spite of the fact that the animal did not perform the trained movement. Only when this movement reappeared in at least 50% of trials, was it possible to resume normal experiments. In some animals in which the motor CR did not return spontaneously after several months the retraining by recourse of passive movements was undertaken.

The chosen movement of putting the foreleg on the foodtray proved to be, unfortunately, not quite appropriate for this study for the following reasons. First the animals after sensorimotor ablations were very strongly incapacitated in all their motor performances. They had often great difficulties in standing on the stand, they tumbled down very easily and often could not perform the required movement for purely "technical" reasons. Therefore it was sometimes difficult to judge which was the source of the lack of the motor CR: whether it was abolished as such, or only could not be displayed because of the postural difficulties. Secondly, the sensorimotor animals manifested very often a more or less strong hyperkinesia of the forelegs, and consequently it was sometimes difficult to decide whether the given movement was a true instrumental response in its abortive form, or whether it was merely the result of hyperkinesia.

The sensorimotor ablations were performed either bilaterally in one stage, or first on one side and then on the other, or else in several stages, each involving

a particular transversal strip. The results of these last partial ablations were already described in previous papers of this series. Therefore, in this paper we shall deal only with: 1° sensorimotor unilateral ablations (contralateral to the trained movement), and 2° with bilateral sensorimotor ablations obtained after one or several operations.

RESULTS

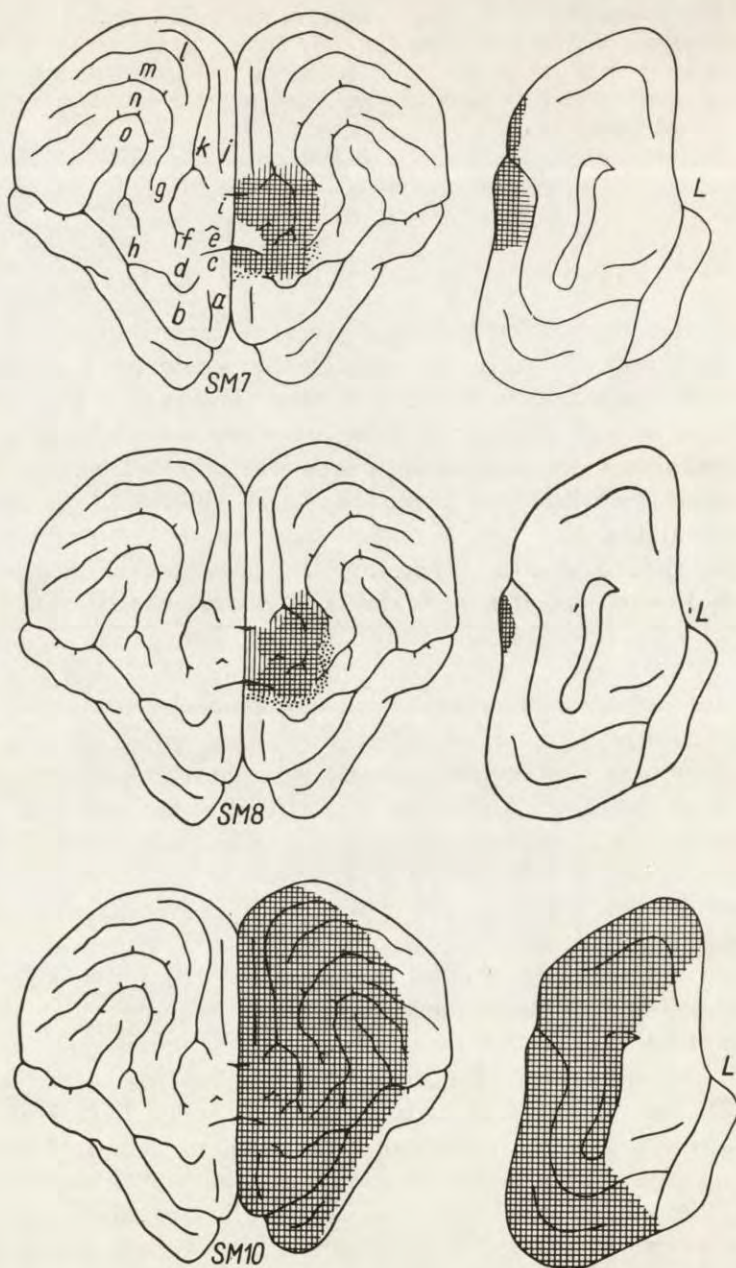
Unilateral sensorimotor ablations

The left sensorimotor ablations were performed in one stage in 2 dogs: SM 7 and SM 8. In dog SM 11 the whole left hemisphere was removed in one stage. In dog SM 12 first sensory and then motor-pre-motor areas as well as adjacent areas were removed, in dogs SM 10 and SM 13 first motor and then sensory area was removed, and in dog SM 10 additionally the whole left hemisphere was ablated in the third stage (Table I and Fig. 1).

In the first days after operation the dogs developed the ordinary and well known symptoms of ataxia, awkwardness of movements on the right side, etc. These symptoms were temporary and vanished usually within several weeks. Four dogs (SM 7, SM 11, SM 12 and SM 10 after third operation) displayed a strong tendency to turn left, which was particularly pronounced when the animal tried to run forward. These symptoms also became gradually almost imperceptible. Nearly all our dogs displayed a more or less strong hyperkinesia of the right foreleg, consisting either in many irregular movements of various amplitude, or in holding the leg permanently above the ground with small oscillations. This symptom was very persistent and only after a prolonged period subsided, but it reappeared whenever the animal was more excited. The general behaviour of the animals (even those with complete hemispherectomy) was fully adequate.

When first brought to the experimental situation and put on the stand the animals behaved normally, except for the effects mentioned above. The motor CR was present from the very beginning although the skillfulness of the movement was more or less impaired: either the animals were able to perform only the abortive movement, without putting the leg on the foodtray, or a full movement after several abortive attempts, or else a full, although awkward, movement from the very beginning. Never did the animals attempt to perform the movement with the unimpaired leg, however difficult it was for them to lift the right foreleg and put it on the foodtray.

The animals' motor performance improved rapidly, and some of them after several weeks became almost fully compensated.



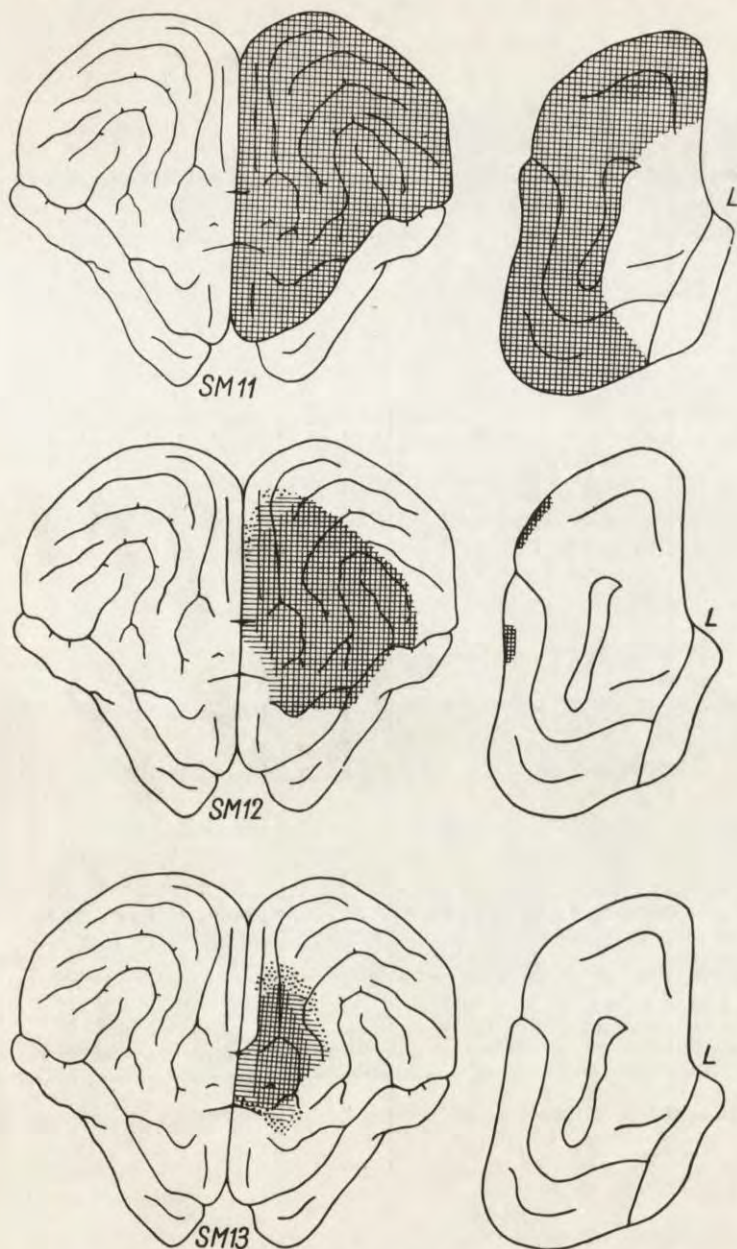


Fig. 1. Unilateral lesions of sensorimotor cortex. Flat projection of dog's cerebral cortex. Left — dorsal aspect. Right — medial aspect. Crosshatched — ablation of cortex with adjacent white matter. Hatched — ablation of cortex alone. Dotted — only superficial layers of the cortex removed.

Denotations: a — *g. proreus*, b — *g. orbitalis*, c — *g. precruciatu*s, d — *g. sigmoideus anterior*, e — *g. postcruciatu*s, f — *g. sigmoideus posterior*, g — *g. coronalis*, h — *g. compositus anterior*, i — *g. postcentralis*.

Synopsis of case histories

Dogs	Preoperative period in months	Extent of first lesion	Postoperative period in months	Extent of second lesion	Postoperative period in months
SM 1	7	Prefrontal (part), premotor (part), motor (part), sensory (part) bilateral	6		
SM 2	14	Prefrontal (part), premotor, sensory I — bl.	5		
SM 3	12	Prefrontal (part), premotor, motor, sensory I — bl.	6		
SM 4	3	Sensory I — bl.	1	Motor — bl.	2
SM 5	7	Motor — bl.	2	Premotor (part) — bl.	2
SM 6	12	Parietal cortex — bl.	1.5	Prefrontal (part) — bl.	8
SM 7	9	Premotor (part), motor (part), sensory I — left	4	Premotor, motor, sensory I — right	10
SM 8	3	Premotor (part), motor, sensory I (part), — left	0.75	Premotor (part) motor, sensory I — right	6
SM 9	3	Premotor, motor, sensory I and II — right	0.75	Premotor, motor, sensory I and II — left	2
SM 10	6	Motor (part) — left	1.5	Sensory I — left	5
SM 11	5	Hemidecortication — left	2	Prefrontal, premotor, motor, sensory I and II, — right	6
SM 12	3	Sensory I (part), sensory II, parietal, temporal (part)	0.25	Lateral part of premotor, motor and neighbouring areas — left	2
SM 13	7	Motor — left	0.75	Sensory I, parietal cortex — left	6

le I

of experimental dogs

Extent of third lesion	Postoperative period in months	Extent of fourth lesion	Postoperative period in months	E n d
				sacrificed
				sacrificed
				sacrificed
Premotor — bl.	2			sacrificed
Sensory I — bl.	5	<i>Gyrus entolateralis</i> — bl.	6	sacrificed
Premotor, motor, sensory I — bl.	9			sacrificed
				sacrificed
				sacrificed
				sacrificed
Remaining cortex of the left hemisphere	1	Premotor (part), motor (part), sensory (part) — right	2	died
				sacrificed
				sacrificed
				sacrificed

Bilateral sensorimotor lesions

Bilateral lesions in one or more stages were performed in 11 dogs. Postmortem examination of the brains was performed by Dr J. Kreiner. Its results are represented in Fig. 2; the order of the operations is given in Table I. As seen from the Table in 3 dogs (SM 1, SM 2 and SM 3) bilateral ablation was made in one stage, in 3 dogs (SM 4, SM 5 and SM 6) in three stages, in 5 dogs (SM 7, SM 8, SM 9, SM 10 and SM 11) first the operation on one side (in one or more stages) and then on the other side was performed. Fig. 2 shows that the extents of lesions were not quite identical, nevertheless the whole of our material seemed to be rather uniform; this proves that in all our dogs the essential part of the region studied was removed and that the larger extent of the lesion did not contribute considerably to the symptom described in this paper.

During at least 3 days after operation the dogs lay on their bedding and were usually not able to get up. Then they began to try to walk, but at first their attempts were unsuccessful and the dogs fell down. After a week or so walking was already possible. However, when the floor was smooth they easily fell down, sprawling and not able to get up. While walking they usually thrust their forelegs forward. Standing was generally more disabled than walking. The legs assumed abnormal positions and after some time the animals got tired and fell down. Some of them displayed a more or less pronounced hyperkinesia of the forelegs. This prevented to test their placing reaction. If hyperkinesia was not present, placing reaction appeared to be absent. When food was put on the table, climbing on it with the forelegs was impossible. The dogs were also not able to jump on the stand as they did before operation (except dog SM 1). They did not manage to draw food with the paw, if it had been located outside the cage, instead they tried unsuccessfully to grasp it by mouth.

Some of the dogs (SM 5, SM 8, SM 9, SM 10, SM 11) had great difficulties with the act of eating. They couldn't easily seize the food with their teeth or hold it in their mouth, and the movement of chewing and licking were clumsy.

The severity of all these defects was generally in accord with the extent and location of the lesion. For instance dog SM 1, in whom the medial parts of the right sensorimotor area were spared was much more skillful (especially on the left side of the body) than the other dogs. Eating movements were particularly impaired in those dogs in which coronal and anterior compositus gyri were bilaterally removed.

According to the extent of lesion some of the dogs were motorically incapacitated for the whole time of observation, while others improved gradually, although full compensation of the motor activity never occurred.

The general behaviour of the animals seemed to be more changed in those dogs in which the lesions involved also middle regions of the cortex (SM 5, SM 6, SM 7, SM 9, SM 11). The most severe symptoms were: disorientation in the familiar surrounding, lack of investigatory behaviour towards the environment, rushing forward with no heed of obstacles, and so on. In this respect particularly illuminating were experiments with dog SM 5, in which after the fourth operation involving medial parts of both hemispheres the enumerated symptoms, previously absent became manifest.

The instrumental CR activity was disturbed in all our dogs in a much higher degree than after any other lesions described in earlier papers of this series.

The experiments were resumed after operation as soon as the dog was able to stand and to walk. In response to the CS the animals at first displayed strong orientation reaction towards the source of the stimulus. This reaction sooner or later (sometimes already in the first trials) gave way to the alimentary reaction either abnormal (licking the empty bowl), or normal — with head directed towards the food-tray. When the bowl with food was put into position two of the dogs (SM 6 and SM 11) did not notice it at once and behaved as if the whole situation were quite new to them; others began to eat immediately. After several experimental sessions the general reaction to the CS'i became more regular, and in most cases it was an orienting-alimentary reaction, i.e. the animal first turned his head for a shorter or a longer time to the source of the stimulus and only then turned towards the foodtray.

In the first trials the instrumental response in all our dogs was absent. However, in most of the dogs it reappeared spontaneously, either already in the first experiment (SM 9), or in one of the next experiments (SM 3, SM 7, SM 8) or after a longer time (SM 11). In dog SM 1 the trained movement did not return spontaneously during four months in spite of his relatively good general skillfulness; this lack of spontaneous recovery might have been caused by inappropriate experimental procedure after operation (too many reinforced trials, cf. method and discussion). In dog SM 2 the trained movement was also permanently lost after operation, but instead the animal performed very many abortive movements (without putting the leg onto the foodtray) so that it was impossible to decide whether these movements

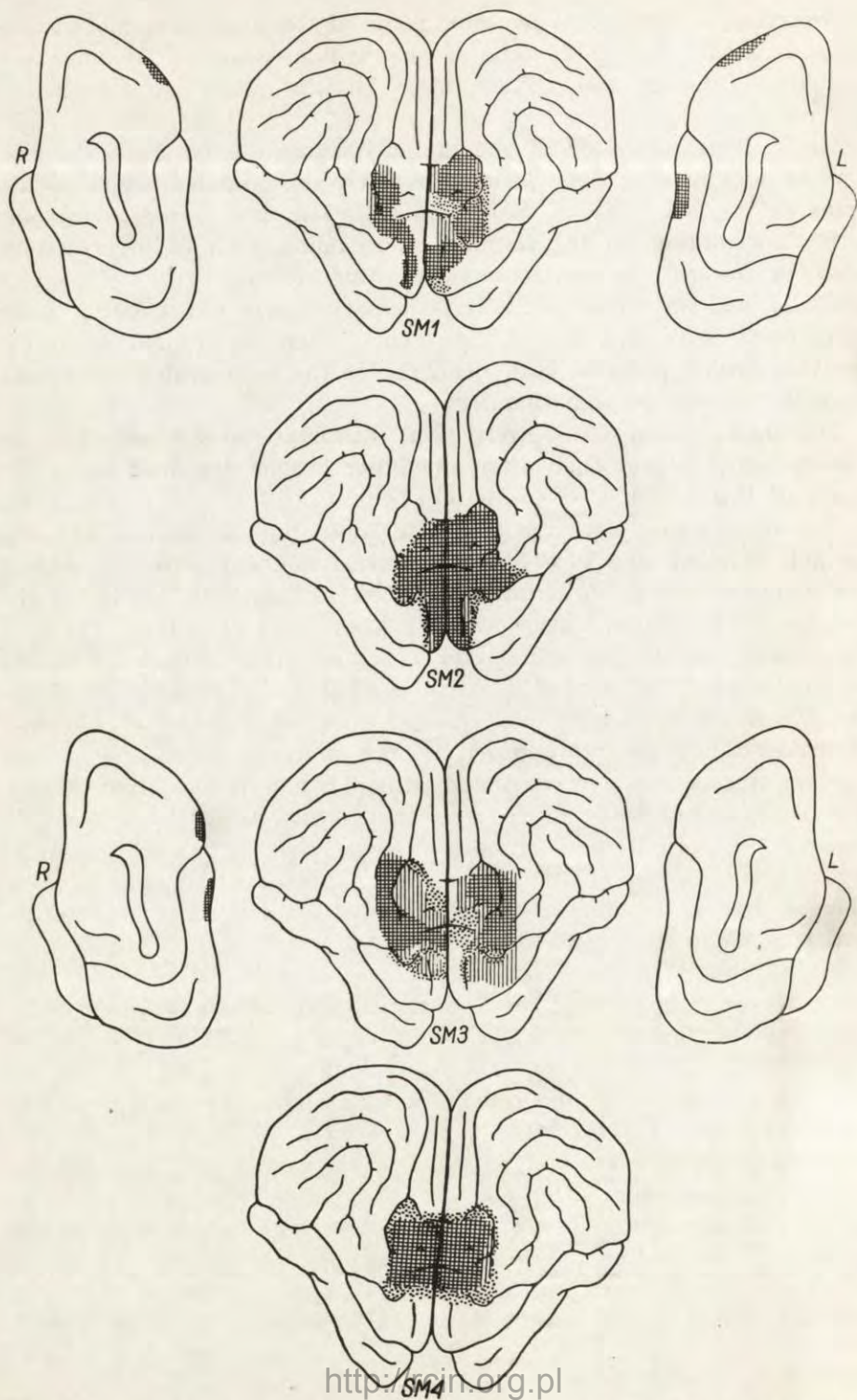
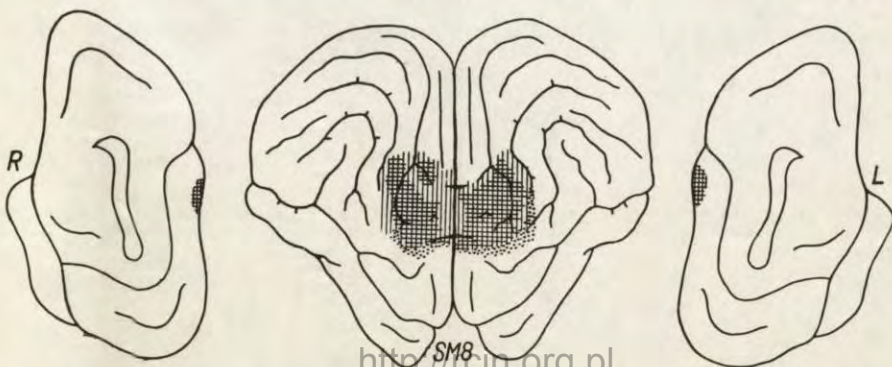
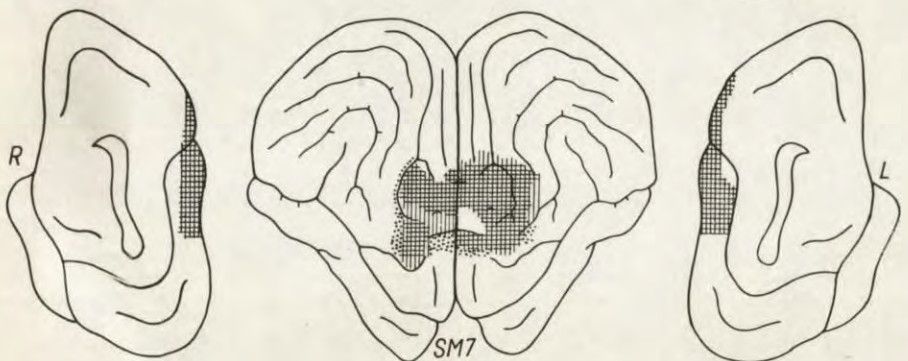
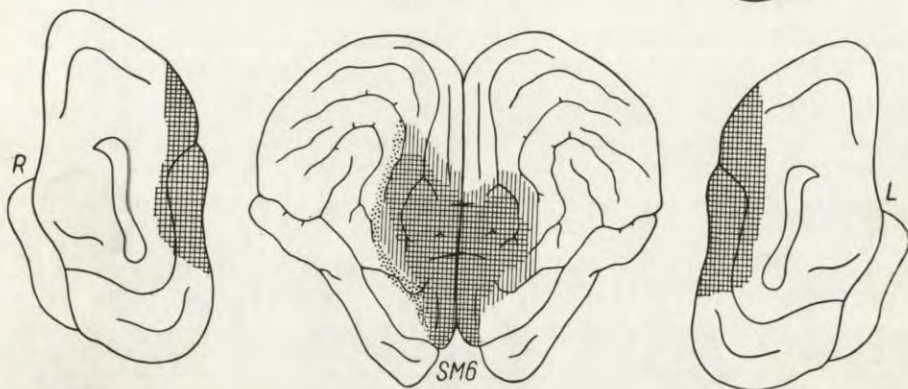
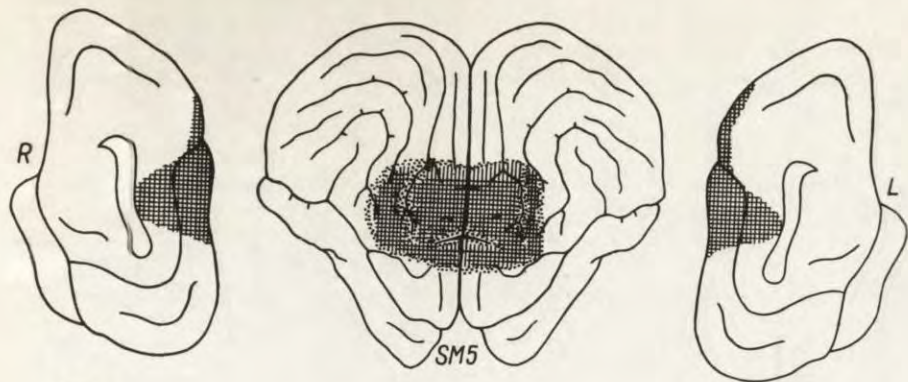


Fig. 2. Bilateral lesions of sensorimotor cortex. Flat projection of dog's cerebral



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Fig. 2 — continued

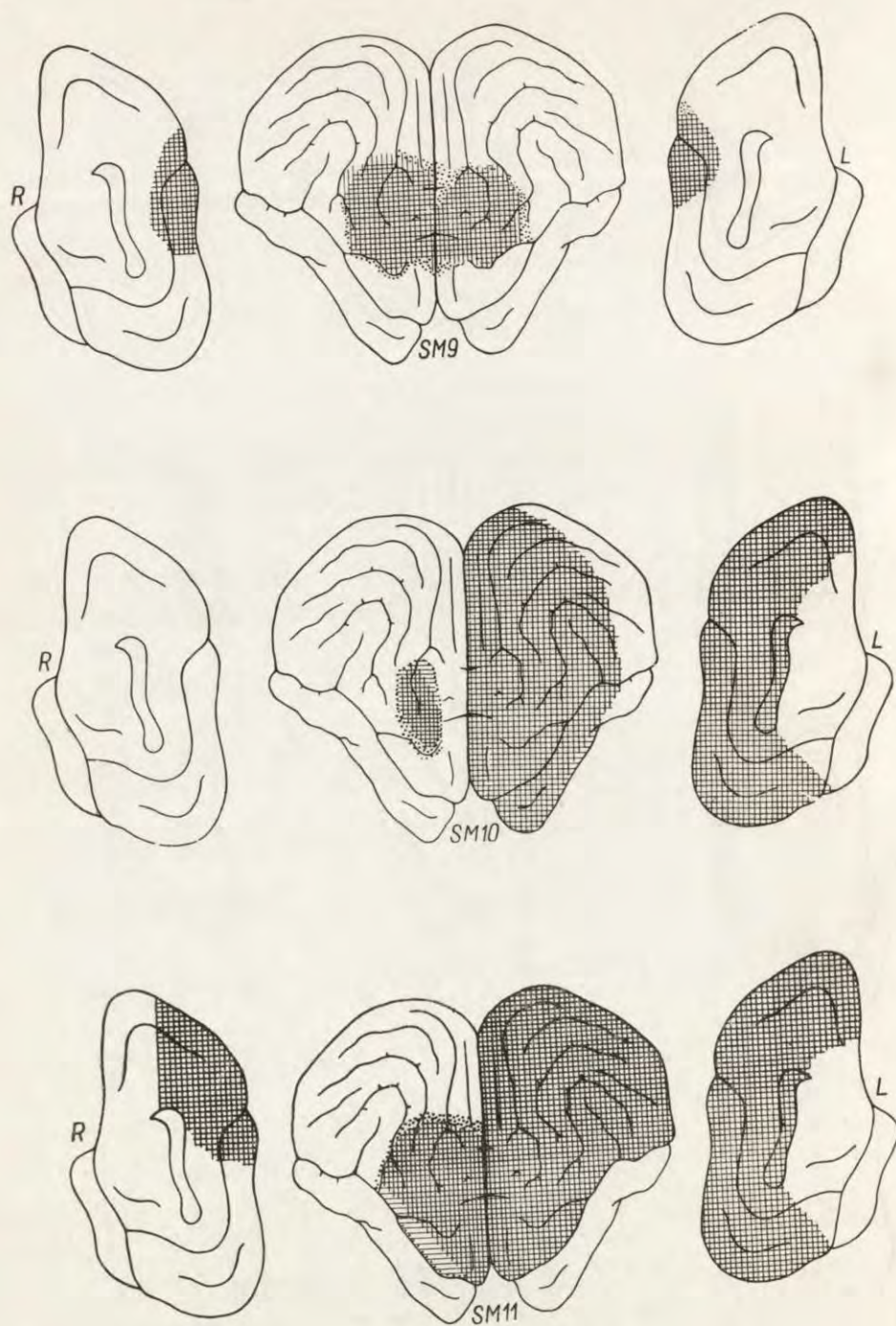


Fig. 2 — continued

were due to the preoperative training, or were simply a manifestation of hyperkinesia. The definite loss of the trained movement was also seen in dogs SM 5, after third operation, SM 6 and SM 10. Dogs SM 5 and SM 6 had very extensive lesions (cf. Fig. 2) and were very strongly impaired in their motor performances. The bad condition of SM 10 was due to epileptic seizures developed soon after last operation (see below).

In dogs SM 1, SM 5 and SM 6 after several months the retraining of the instrumental reflex was begun by recourse to passive movements. It appeared that the trained movement itself returned very quickly but its association with the CS was either lacking or very weak (see below).

Although the instrumental reaction was in all our dogs either preserved or easily retrained, the CR was severely deteriorated.

First, a great clumsiness of movements was in all animals (except SM 1 and SM 8) very pronounced. Often the proper movement was preceded by several abortive attempts to put the leg on the foodtray. The movement was unprecise, sometimes the dog put his leg into the bowl and was unable to change its position, so that the act of eating was much hindered. After eating the animals were usually not able to put the leg down actively on the floor, and could remain in such an awkward position for a long time till the leg was removed by the experimenter.

Another prominent symptom seen particularly in dogs SM 3, SM 4, SM 5, SM 6, SM 8, SM 9 and SM 11 was confusion of the legs. The animals put either left or right foreleg on the foodtray in a quite similar manner, as if not noticing any difference between these two movements. The preference of this or that leg depended on which leg was more free just before the performance of the movement. It is worth while to notice that in dog SM 6, which was retrained after operation by applying passive movements to the right foreleg, the movements of the left one started to appear as soon as the reflex was reestablished. In dog SM 3 an attempt was made to extinguish the movements of the left foreleg by not reinforcing them by food, but in spite of prolonged training of this sort, it proved to be unsuccessful (Fig. 3).

But perhaps the most pronounced symptom exhibited by our dogs was the utmost irregularity of the whole CR activity. The animals' performance was completely chaotic and unpredictable. First, it could be quite different on different days for no obvious reasons: in one session CR's seemed to be relatively good, and in the majority of trials the animal reacted correctly; in the next session, on the contrary, no

CR's appeared at all. Latent periods were greatly variable; while before operation the trained movements appeared always immediately after the onset of the CS, now the latencies lasted at least several seconds. Sometimes the animal did not perform the movement during the operation of the stimulus but did so after its cessation. The movements appeared also in a quite unpredictable way in intertrial intervals. In some cases the animals performed the movement in intervals rather than to the CS, but in most cases they either performed it both to CS'i and in intervals, or on the contrary neither to CS'i nor in

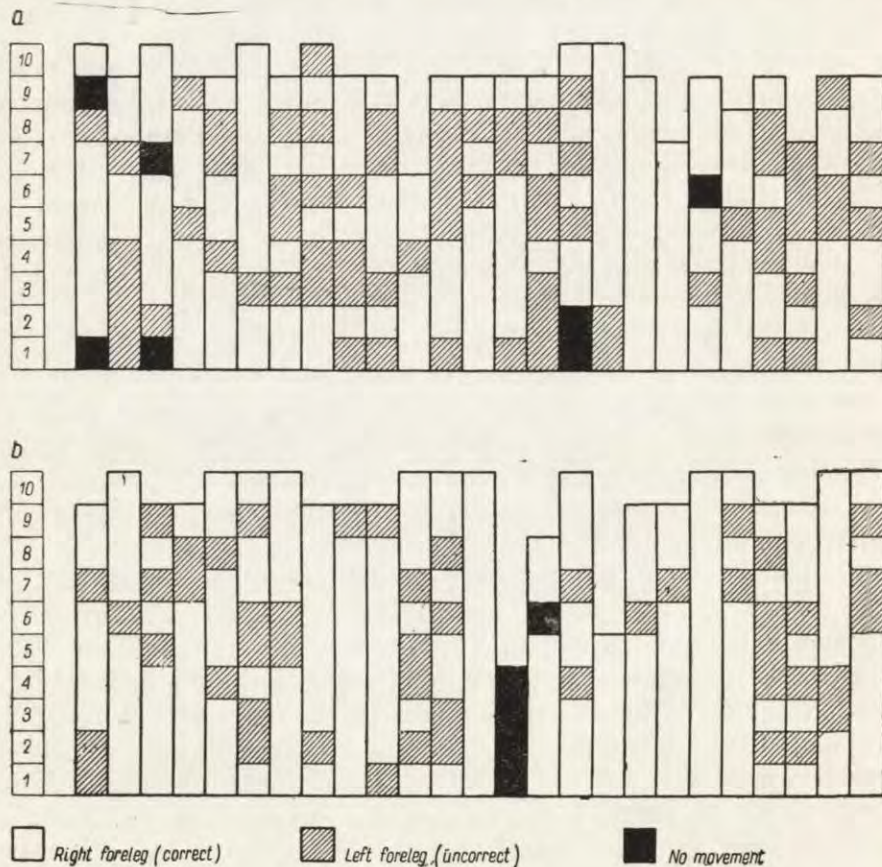


Fig. 3. Pattern of motor conditioned activity in dog SM 3 after operation, before and after training of extinction.

a — 25 experimental sessions preceding the series in which the extinction of movements of the left foreleg was trained. b — 25 successive experimental sessions during which extinction of movements of the left foreleg was trained. Ordinates: successive trials during an experimental session, 4 to 10 trials were made at each session. Abscissae: successive experimental sessions.

intervals. This depended on how much the animal was motorically excited in the given session. This state of affairs in some dogs did not improve at all (SM 5, SM 6, SM 11) while in others after several months the CR activity became more regular.

For illustration in Fig. 4 we present the course of experiments with dog SM 3 showing all types of irregularities described above. In Fig. 5

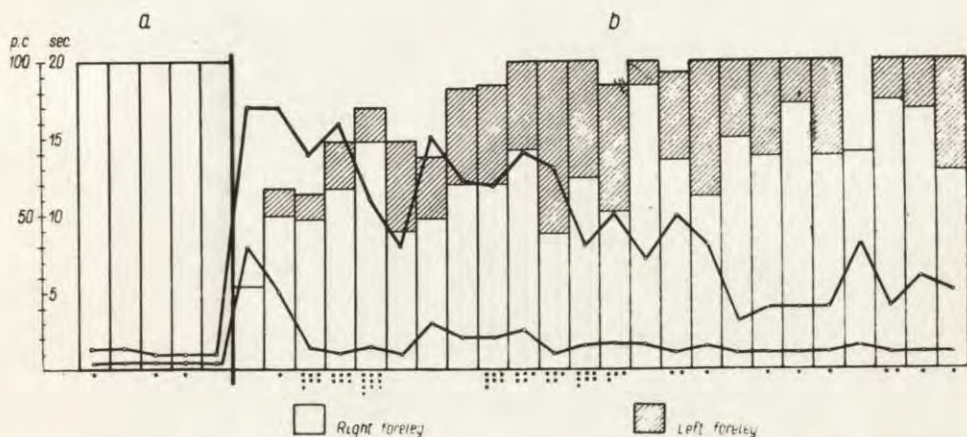


Fig. 4. Diagram showing the pre- and postoperative data of CR activity of dog SM 3. Averages of 3 successive experimental sessions are give Column — percentage of CRs (with either foreleg) Upper curve — longest latent period, lower curve — shortest latent period of CRs. Dots below show the numbers of intertrial movements.

Ordinates: percentage of performance of the trained movement in response to CS (with either right or left foreleg) and time in seconds. Abscissae: groups of 3 successive experimental sessions, a — preoperative period, b — postoperative period.

the comparison of the effects after unilateral and bilateral sensorimotor lesion is presented in dog SM 9. While after unilateral lesion the changes in animal's performances were insignificant, after the second operation his CR activity became strongly deteriorated.

In spite of this irregularity it was revealed that there are some factors which make the instrumental response more or less probable.

When the experimenter stood near the dog, even motionless, this often helped the animal to perform the movement. Other facilitating factors were: plaing with the dog, putting the hand on the foodtray („encouraging gestures”), slight touching the right foreleg, etc. On the contrary, when the dog stood alone and was not reinforced in some trials, this made the situation worse.

It was also discovered that the location of the stimulus played a very important role for the elicitation of the movement. If, for

instance, the metronome was located in front of the animal (behind the foodtray) so that the orienting reaction towards it was not antagonistic with putting the foreleg on the foodtray, the reflex was easier elicitable than when it was located in a corner of the chamber. Also, when the

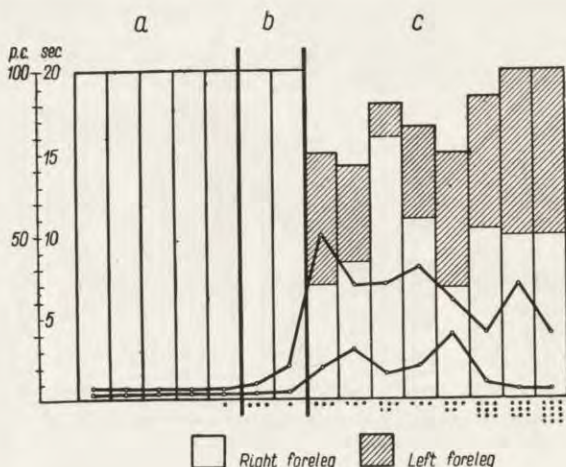


Fig. 5. Diagram showing pre- and postoperative data of CR activity of dog SM 9.

a — preoperative period, b — after unilateral ablation of sensorimotor cortex, c — after bilateral ablation of sensorimotor cortex. Other explanations as in Fig. 4.

apparatus provided not only auditory, but also visual sensations it acted even stronger. Therefore, it appeared that the noisily rotating toymill situated before the animal was the most „motogenic” stimulus. In some experiments it was proved that two CS'i acting together were also more „motogenic” than when acting separately.

The last point to be raised here is the effect of epileptic seizures which developed in some of our dogs sooner or later after operation. It was found that during the period in which seizures occurred the CR activity of the dog was strongly deteriorated, and all the symptoms observed in the first period after operation (full areflexia, strong irregularity of CR, etc.) reappeared. Also, after strong seizures the condition of the sensorimotor animals grew worse for several days and only slowly improved again. Therefore, the treatment with luminal which prevented the seizures had usually a beneficial effect on the sensorimotor animals.

Table 11

Dogs	SM 1	SM 2	SM 3	SM 4	SM 5	SM 6	SM 7		SM 8		SM 9		SM 10		SM 11		SM 12	SM 13
				after third oper.			after first (left) oper.	after second (right) oper.	after first (left) oper.	after second (right) oper.	after first (right) oper.	after second (left) oper.	after third (left) oper.	after fourth (right) oper.	after first (left) oper.	after second (right) oper.		
Disorders of movements																		
awkwardness	present absent	present absent	present absent	present	present absent	present absent	absent	present	absent	present absent	absent	present	absent	present	present absent	present	present absent	absent
sliding apart of legs	present absent	present absent	present absent	present	present absent	present absent	present in right legs	present	present absent	present	present in left legs absent	present	present in right legs	present	present in right legs	strong present	present in right legs absent	present in right legs
abnormal position of legs	present absent	present absent	present	present	present	present	present in right legs	present	present	present	present in left legs	present	present in right legs	present	present in right legs	present	present in right legs	present
hyperkinesia	slight	present	present	present	present	present	slight	present	slight	slight	absent	present	strong	strong	absent	absent	slight	strong present
hyperactivity	absent	absent	slight absent	present	absent	slight absent	absent	absent	absent	absent	absent	present absent	absent	slight absent	absent	present absent	present absent	absent
eating	not impaired	impaired normal	impaired	not impaired	impaired	impaired normal	not impaired	slightly impaired	not impaired	impaired	not impaired	much impaired	not impaired	impaired	slightly impaired, normal	much impaired	not impaired	not impaired
Conditioned activity general response to CS	alimentary: normal or abnormal	orientation orient-aliment.	orientation: orient-alimentary	indefinite or orientation, orient-aliment, alimentary	orient. or alimentary	orientation or orient-alimentary	normal alimentary	orientation orient-alimentary	alimentary	orientation orient-aliment or alimentary	alimentary or orient-alimentary	orientation or orient-alimentary	alimentary	indefinite	alimentary	orientation, orient-aliment, or abnormal alimentary	alimentary	alimentary
instrumental response to CS	absent chaotic after retraining	absent? (hyperkinesia)	chaotic(?)	absent chaotic	absent chaotic after retraining	absent chaotic after retraining	present	absent chaotic \pm regular	present	absent chaotic regular	present	chaotic	present	absent	present	absent chaotic absent	present	present
latency of CR	chaotic after retraining		chaotic	chaotic	chaotic after retraining	very prolonged chaotic after retraining	prolonged short	chaotic	short	chaotic short	short	chaotic	short only sometimes slightly prolonged		short only sometimes slightly prolonged	chaotic	prolonged short	short
confusion of legs	absent		present	present	present after retraining	present after retraining	absent	absent	absent	present	absent	present	absent		absent	present	absent	absent
intertrial movements	absent	very few	numerous present or absent	present	very few	present	very few	present	absent	present	very few	present	absent circles	absent	absent circles	present or absent	present circles	present

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DISCUSSION

The data presented in this paper show that extensive sensorimotor ablations produce heavy deterioration of all motor performances and a very severe and longlasting impairment of instrumental CR's: all movements of trunk, mouth and legs are defective, the proprioception is nearly abolished, and in addition the CR activity as a whole is strongly disturbed (Table II).

It may seem that this picture is the result of simply very extensive lesion in the sensorimotor area and cannot be subjected to any further analysis. However, if we compare the data obtained in this paper with the results described in the previous papers of this series, caused by partial ablations of sensorimotor cortex, we can easily discover that every symptom found in the present experiments may be also traced in particular partial lesions. And so, the syndrome consisting of irregular CR activity, the exaggerated orientation reaction to the stimuli, and the abnormal direct alimentary reaction is the effect of premotor lesions. Hyperkinesia and confusion of the legs in performing the trained movement are specific symptoms of motor lesions. The abnormal positions of the legs are chiefly due to the sensory lesions. Lastly, the general motor disability, also contributing to the impairment and irregularity of motor CR's, is the common result of both motor and sensory lesions. Therefore, the conclusion follows that the very complicated picture produced by sensorimotor ablations is nothing else than a combination of effects produced by definite partial ablations. In other words, the sensorimotor area must be considered not as an undifferentiated whole, but as a highly organized construction in which different parts play a different role.

The best illustration of this conclusion is provided in Fig. 6; it represents the experiments with dog SM 4 in which 3 bilateral ablations were performed successively: in the first only sensory areas were removed, in the second, motor areas, and in the third premotor areas were ablated. As seen in the Fig. 6 while after the first operation only the temporary disappearance of the CR was observed, after the second one we notice the symptom of confusing the legs, while after the third one the considerable irregularity of conditioned responses together with intertrial movements are added.

Such an approach allows us to understand also some seemingly significant differences in the symptoms found in the different dogs and not substantiated by anatomical findings. We have here in view the total abolition of instrumental reflexes in some animals and spontaneous recovery, even after a short time, in others. In previous papers

we found that both after premotor lesions and after sensory lesions there is usually a temporary abolition of the instrumental reflex, but this reflex returns spontaneously after a lapse of time. The cause of this abolition is different in each of these cases. While after sensory ablation it is due to the disorganization of proprioception, which in normal conditions facilitated the performance of the movement, after

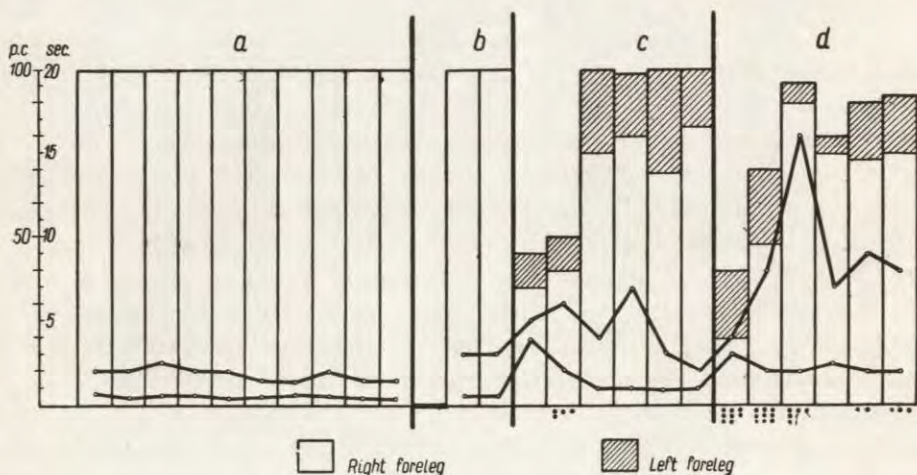


Fig. 6. Diagram showing pre- and postoperative data of CR activity of dog SM 4.

a — preoperative period, b — after bilateral ablation of sensory cortex, c — after bilateral ablation of motor cortex, d — after bilateral ablation of premotor cortex. Other explanations as in Fig. 4.

premotor ablation it is the effect of antagonism between the strongly disinhibited direct reactions to the stimulus (orientation and alimentary) and the instrumental act. After complete sensorimotor ablations these two mechanisms are jointly in operation, hence the probability of abolition of the trained movement is increased. In consequence the absence of this movement may be more prolonged. The longer is the period of the lack of movement — the more difficult is its recovery, since every testing experiment is inevitably connected with a counter training: the animal may learn to obtain food without performing the trained movement. Therefore, whether the trained movement is permanently or only temporarily abolished after sensorimotor lesions, may depend on some accidental factors, such as a greater or lesser agility of the animal, slight differences in postoperative training, etc. The proof of the correctness of this consideration is that the retraining of the instrumental reflex is very prompt but, when established, this reflex exhibits all the defective features and its irregularities charac-

teristic for this type of lesion. It should be emphasized that while the three transversal strips of the sensorimotor region contribute in different way to the performance of the movement, this is not so with the left or right parts of these strips. It is of course a well known fact that the contralateral side of the sensory and motor cortex contributes more to the dexterity of movements than does the ipsilateral side; but in dog the defects produced by unilateral lesions are easily compensated by the corresponding ipsilateral areas. In consequence, the bilateral lesions, even not very extensive, are in the long run more harmful than even very extensive unilateral lesions. The strong impairment of the mouth movements brought about by bilateral, but not by unilateral lesions, may provide a good example.

Now we pass to the most important problem, namely that of the role played by the sensorimotor cortex in instrumental conditioned reflexes. First, one should stress that while after sensorimotor lesions the instrumental reflexes are always severely impaired, it is not so with classical reflexes. Although salivation was not measured in our experiments, nevertheless the observation of animals shows that the alimentary CR to the stimulus is either preserved after operation or very quickly restored. Therefore, it seems that lesions in this part of the cerebral cortex affect chiefly, if not exclusively, the instrumental part of the CR, leaving the classical part more or less intact. However, the exact role played by the sensorimotor area in the performance of instrumental reflexes is far from being clear. The fact that practically we were not able to abolish totally and irreversibly (with no possibility of retraining) the instrumental reflex shows that, although the area in question plays a substantial role in these reflexes, this role is not so simple. May be that this problem will become more elucidated when the effects of sensorimotor ablations upon other motor acts than those used in the present series are investigated.

SUMMARY

1. The effects of unilateral and bilateral ablations of the sensorimotor cortex (including premotor, motor and sensory areas) upon motor type II CR's and general animal's behaviour were investigated in dogs. The motor conditioned reflex consisted in putting the right foreleg on the foodtray to auditory and visuo-auditory stimuli.

2. After unilateral ablation of this region the motor performances on the contralateral side are temporarily impaired, but after a lapse of time they are largely compensated. The general behaviour of the animal is unchanged. Motor conditioned reflexes involving movement

of the contralateral side may be absent after ablation, but they are later spontaneously restored, although for some time they may be awkward and atactic.

3. Bilateral ablations of the sensorimotor cortex, performed either in one or in several stages result in strong impairment of motor performances, general animal's behaviour and instrumental conditioned reflexes.

4. When the operation has been radical the animal's limbs are strongly paretic and atactic. This defect is compensated very slowly and incompletely.

5. In dogs with extensive lesions (especially including medial parts of the hemispheres) the general behaviour is chaotic and inadequate. This state does not alter much with the lapse of time.

Instrumental conditioned reflexes undergo severe disturbances. Absent at the beginning they may or may not reappear spontaneously. They may however be retrained without special difficulty. Recovered either spontaneously or by training they manifest a considerable irregularity and imperfection: the latent periods are variable, the movements tend to appear in intervals and the animal often uses left foreleg instead of right. The conditioned response seems to be much better to stimuli situated in front of the animal and having visual component than to those operating from other places.

6. The analysis of these symptoms reveals that they are a combination of symptoms produced by separate lesions of premotor, motor and sensory areas.

We are greatly indebted to Doc. Dr. J. Kreiner and Mgr. B. Sych for anatomical examination of the brains. We are also very grateful to the technical assistant Mr. Antoni Rosiak for his careful attention to the health and welfare of our animals.

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THE EFFECTS OF PREFRONTAL LOBECTOMIES
ON THE DELAYED RESPONSES IN CATS

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In one of the previous papers of this series (Ławicka and Konorski 1959) it was reported that ablations of prefrontal poles of the cerebral hemispheres in dogs rostral to the presylvian sulcus result in a severe and persistent impairment of delayed responses. While normal dogs are able to find their way to the signalled foodtray after the delay period independently of their bodily orientation, the prefrontal animals are able to perform this task only if throughout this period they remain oriented to the proper foodtray. Consequently, any distraction during the delay period which provokes the animal to change its orientation interferes with proper solution since the dog „follows its nose” and approaches the wrong foodtray. We have called the prefrontal animal's manner of solving the delay problem (i.e., by maintaining bodily orientation) a pseudo-delayed reaction and have attributed it to a lack of „recent memory”.

In a previous paper by one of us (Ławicka 1959) it was found that normal cats perform the delay response task in much the same way as do normal dogs (i.e. without preserving bodily orientation during the delay period), although their performance is on the average rather poorer than that of dogs. The inferiority of the cats is due chiefly to their tendency to approach not the signalled foodtray but rather the one which was last reinforced. We have called this type of error a “perseverative error”, but it might be more properly labelled a “one-trial-learning error”. It should be stressed that in the course of training this perseverative tendency was suppressed and often totally abolished.

The aim of the present paper was to elucidate the manner and the extent to which delayed responses in cats are impaired after prefrontal lesions similar to those performed in dogs. Since cats are in general less likely than dogs to preserve their bodily orientation during the delay period, it was expected that their delayed reactions might be even more severely impaired.

MATERIAL AND METHODS

The experiments were performed on 8 adult cats. The experimental setting was the same as that used in our previous studies. In a rectangular room 8×4 m. (Fig. 1) there were three foodtrays each in the middle of a different one of three walls, whereas in the middle of the fourth wall was the starting platform and the experimenter's seat. A buzzer was located on each foodtray. Before each trial

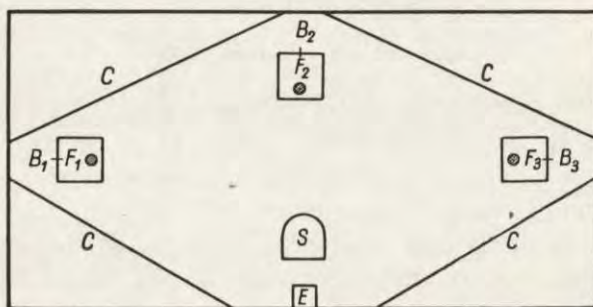


Fig. 1. Experimental setting used in our study.

F_1 , F_2 , F_3 — left, middle and right foodtray. The bowls are automatically moved into position by the experimenter using an electromagnetic device. E — table and seat for experimenter. S — starting platform. B_1 , B_2 , B_3 — buzzers.

the animal was placed in a small round cage on the starting platform and one of the buzzers (preparatory signal) was sounded for 3 sec. Then, either immediately, or after a lapse of time not exceeding a few minutes, the animal was released. If it went to the proper foodtray, the bowl, containing pieces of meat, was remotely moved into position by the experimenter; if the animal responded incorrectly, it did not obtain food even if afterwards it approached the proper foodtray. When the animal made an error the same signal was repeated on the next trial but a shorter delay was used.

In the preliminary experiments the animals were habituated to the experimental situation, and trained to find food in the foodtrays, to return to the platform after each trial, not to fear the cage, and to go to the signalled foodtray when released after various delay periods. Each experimental session consisted of 9–15 reinforced trials, in which every signal was applied for the same number of times in a random order.

In the preliminary training, which lasted from one to several months (some of the cats were used in the normative study of delayed responses), the delay

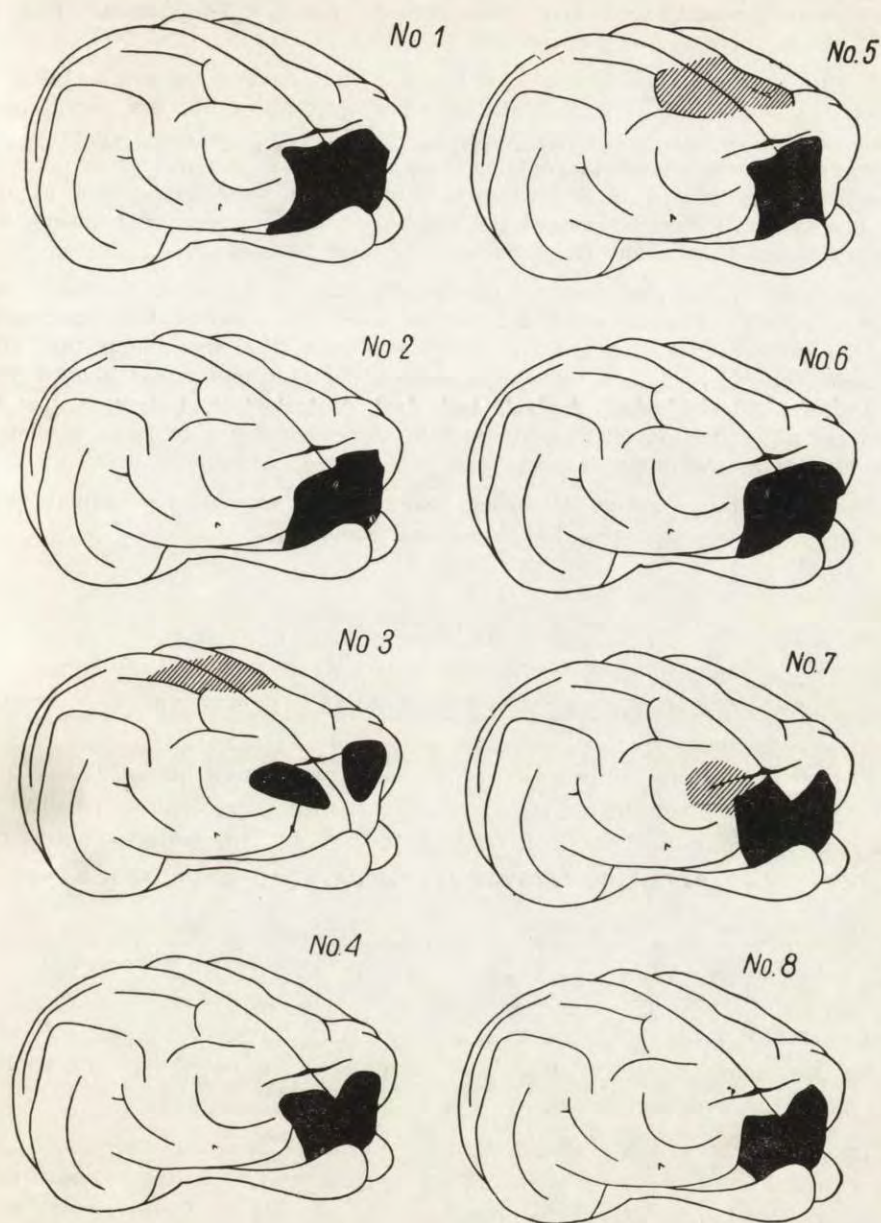


Fig. 2. The extent of ablations in experimental cats. On the right of each figure the Nos. of cats are indicated.

periods were gradually prolonged to one or more minutes. The number of errors in the preoperative period did not exceed 10%.

The surgical procedure consisted in removal by suction of the prefrontal poles (cortex and white matter) sparing the olfactory tract and bulb; the rostral parts of the precruciate gyri were also removed, but the cortex in the vicinity of the cruciate sulcus was always spared (Fig. 2). In 6 animals bilateral removals were performed in one stage; in 2 others unilateral lesions were made first. Finally, 2 of the cats also received control parietal ablations. Postoperative recovery was uneventful and after a few days the animals were retested.

The course of the postoperative experiments was the following. In those cases in which no clear change in the delayed reactions was observed the experiments took the same course as before operation. However, if it was found that after operation delayed responses were deteriorated, then retraining was started with the shortest (0 sec.) delay periods, and then progressively, depending on the improvement of the animals' reactions, with increased delays in steps similar to those applied in the initial training.

The postoperative period of testing lasted several months; the animals were then sacrificed, and the brain lesions reconstructed.

RESULTS

The effects of parietal lesions

Parietal ablations, shown in Fig. 2, were performed before (cat No. 5) and 6 months after (cat No. 3) the prefrontal operations. In neither case was there any detectable change either in the general behaviour of the animals or in the delayed response tests (cf. Fig. 4).

The effects of unilateral prefrontal lesions

In cat No. 7 the prefrontal region was first removed on the left side, in cat No. 8 on the right side. The animals were tested for one month, and then the second operation was performed.

In both cases very slight impairment of delayed responses was observed (Fig. 3). The probability of errors in the initial experiments after operation was slightly increased. Nearly all of these errors were of the "perseverative" type, i.e. the animals approached the last reinforced foodtray (cf. Fig. 9), and nearly always, after failing to obtain food there, ran immediately to the correct foodtray (perseverative error with correction). There was no detectable change in their general behaviour. In the final experiments in this period the level of errors fell again almost to 0.

The effects of bilateral prefrontal lesions

The general behaviour of the animals was the same as before operation, except for a slight locomotor hyperactivity in some cats (Nos. 3, 4, 5, 6). The hyperactivity was manifested during the intertrial intervals by increased wandering in the experimental room and more frequent approaches to the foodtrays. This disorder disappeared within several days.

Despite their excellent general condition, the delayed responses of all the animals were impaired, and the cats made errors even with very

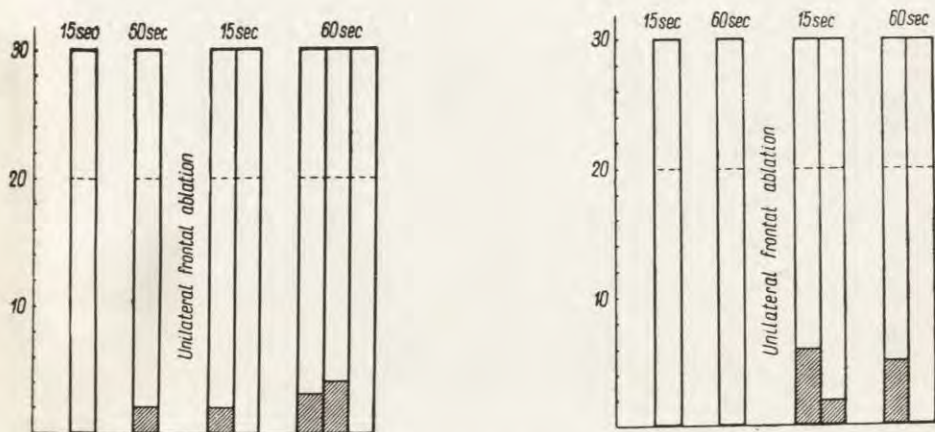


Fig. 3. Numbers of errors after unilateral frontal ablations in cat Nos. 7 and 8. Each column denotes a block of 30 trials. Striped part of the column denotes the number of errors. Above each column the respective delay period is indicated. The broken line denotes the chance level of errors. The number of errors which is nearly nil in the last blocks before operation, is slightly increased after unilateral prefrontal ablation.

short delay periods. However, their directional reactions to the sound of the preparatory stimulus itself were prompt and correct, sometimes even more pronounced than before operation, and, if released during the action of the signal, the animals rapidly ran to the proper foodtray.

The typical course of experiments after operation is presented in Fig. 4 for cat No. 5, and Fig. 5 for cat No. 6. It may be seen that initially the animals made errors even with 0 seconds delay, i.e. when they were released immediately after the cessation of the preparatory stimulus. Gradually their performance improved, but as soon as the delay was protracted the number of errors increased again. After several weeks the defect in most cases was nearly compensated within the delays used.

As seen in these Figs, even when the animals were performing most poorly their number of errors did not reach chance levels, suggesting that the animals were able to solve the problem of delayed response in principle, but that there were some factors which disturbed their performance.

This conclusion is strongly corroborated by the fact that in many



Fig. 4. Numbers of errors after bilateral prefrontal ablation in cat No. 5.

Explanations as in Fig. 3. Note that after perietal ablation no increase of errors occurred, but after prefrontal ablation they appear even within the delay of 0 sec.

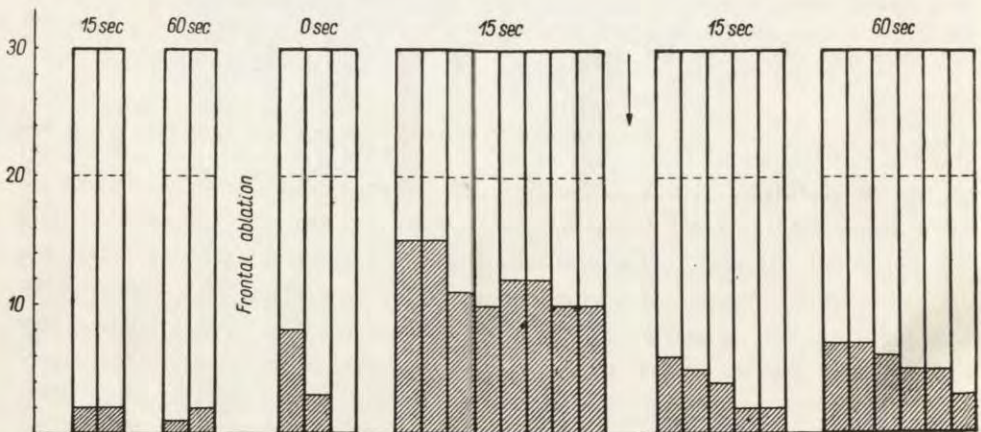


Fig. 5. Numbers of errors after bilateral prefrontal ablations in cat No. 6.

Explanations as in Fig. 3. By arrow is denoted the change in experimental procedure in two sessions represented in Table I.

cases, after having made errors, the animals corrected themselves and ran to the signalled foodtray, although, as indicated earlier, these runs were never reinforced by food. The percentages of all attempted corrections are presented in Fig. 6. It may be seen that in some cats

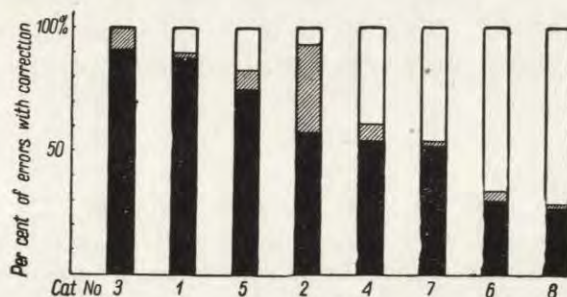


Fig. 6. The percentages of corrections after wrong runs.

Black parts of columns denote errors with „correct” corrections, striped parts denote errors with „wrong” corrections, white parts denote errors without corrections. Note that in all cats except No. 2 „correct” corrections significantly outnumber the „wrong” ones.

(Nos. 3, 1, 5 and 2) attempted corrections occurred after nearly every error; in others (Nos. 4, 7, 6 and 8) they were less frequent. It was noticed that in those cats which committed a great number of errors correction runs gradually extinguished. But as seen in Fig. 6 the “correct” corrections greatly outnumbered the “wrong”; this suggests that after having made errors the animals did remember which foodtray had been signalled.

The analysis of errors performed by the operated animals has shown that they may be divided into three groups, the errors of each group occurring in a different stage of the postoperative period. We shall describe each of these three groups separately.

1) Bodily orientation errors. As emphasized above normal cats do not, as a rule, preserve their bodily orientation during the delay period. On the contrary, they turn around in the cage many times and only very rarely glance at the signalled foodtray. Such behaviour does not prevent them from making a correct choice after being released. This was not so with some prefrontal animals immediately after operation. In the first postoperative sessions, some animals performed correctly only when they maintained their bodily orientation towards the proper foodtray. If they did reorient, they went to the incorrect foodtray to which they were turned even if the delay was 0 seconds.

Bodily orientation errors were not observed in all the cats, and, if present, they disappeared very soon.

2) Preference errors and omission errors. In the early stages of postoperative training another type of error was observed. The animals either had an increased tendency to approach a particular foodtray, and thus made errors when other foodtrays were signalled (preference errors), or they had an increased tendency to omit a particular foodtray and so made errors when this one was signalled (omission errors).

These tendencies were observed in nearly all the prefrontal cats to a greater or lesser extent, and were the sources of a considerable number of errors. Yet, they were not permanent. Often, for example, a preference to go to one particular foodtray was replaced in the further course of experiments by a preference to go to another.

We shall describe in detail the preference and omission tendencies in cats Nos. 5 and 6 (Figs. 7 and 8).

As seen in Fig. 7 cat No. 5 immediately after operation showed a preference for foodtray 3, which soon switched to a very strong preference for foodtray 1, with the omission of foodtray 2. To eliminate this preference, the signal for foodtray 1 was not applied at all in five successive experiments. This training measure succeeded, since the total number of errors decreased rapidly thereafter. In the further course of experiments there was no particular preference for any foodtray and there was only a slight tendency to omit foodtray 2.

Even more interesting was the preference-omission tendency in cat No. 6 (Fig. 8). This cat habitually turned its back to foodtray 2 during the delay period, though before operation this did not prevent it from selecting that foodtray when it was signalled. However, soon after operation cat No. 6 began to omit this foodtray completely, running instead either to foodtray 1 or to foodtray 3. When either of these foodtrays was signalled, however, the animal nearly always chose correctly.

In an attempt to retrain this animal the experimental procedure was changed in such a way that trials with foodtray 2 were repeated again and again with the same delay (15 sec.), until the animal made a correct choice. (An incorrect trial was usually repeated with 0 seconds delay.) The condensed protocols of two successive experiments are presented in Table I. It may be seen that of the erroneous runs to signal 2, 12 were to foodtray 1 and 10 were to foodtray 3. In response to signals 1 and 3 all runs were correct.

On the next day, unexpectedly, nearly all runs were correct. Thus,

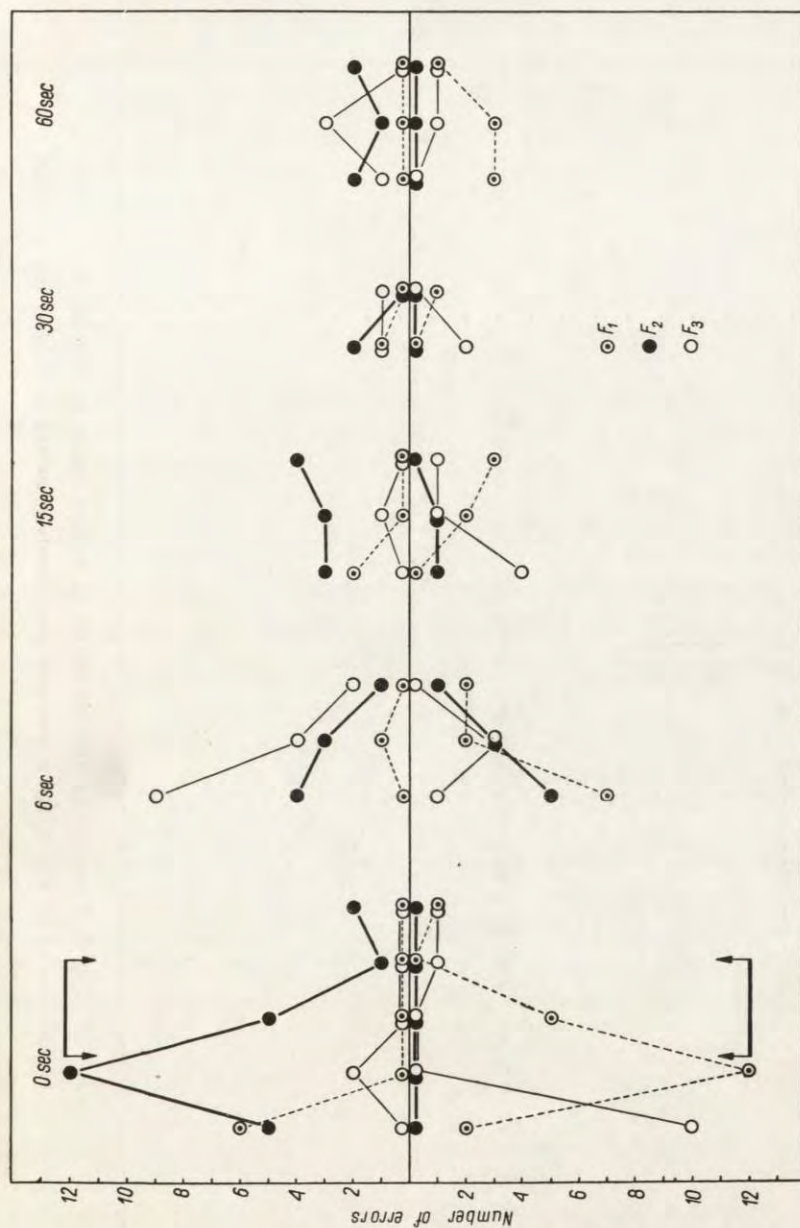


Fig. 7. Preferences and omissions in errors made by cat No. 5.

Abcissae: successive blocks of 30 trials each; ordinates: numbers of errors in each block. Up — the numbers of omissions of each foodtray, down — the numbers of preferences of each foodtray. Above the delay periods in seconds are indicated. The series of trials in which no signal 1 was applied is shown by horizontal lines with arrows.

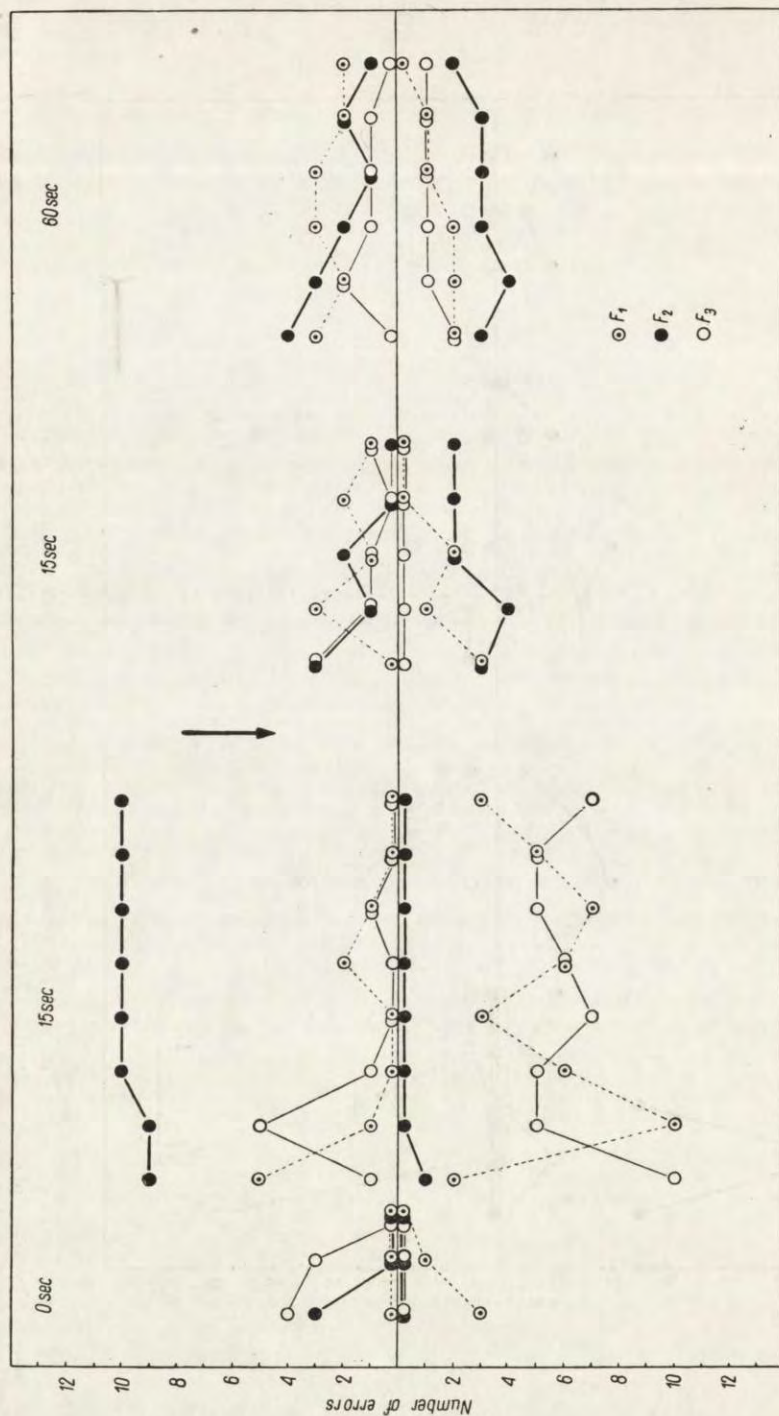


Fig. 8. Preferences and omissions in errors made by cat No. 6. Explanations as in Fig. 7. By arrows two experiments presented in Table I are indicated.

the short training period was quite sufficient to overcome the error-producing tendency in spite of the fact that during the delay period the cat continued to turn its back to foodtray 2.

It may be supposed that what the animal learned during this short training period was to inhibit runs to foodtray 1 and 3, and consequently to turn round to foodtray 2 when this foodtray had been signalled.

Table I

Training leading to extinction of erroneous runs in response to signal 2 in cat No. 6

1	2	3	1	2	3	1	2	3	1	2	3
Experiment 19.11.1959											
1	2→1	—	9	2→3	—	17	2→3	—	25	2→3	—
2	2→1	—	10	2→1	—	18	2→3	—	26	2→3	—
3	2→1	—	11	2→1	—	19	2→2	+	27	2→3	—
4	2→1	—	12	2→3	—	20	3→3	+	28	2→3	—
5	2→2	+	13	2→1	—	21	3→3	+	29	2→3	—
6	3→3	+	14	2→2	+	22	1→1	+	30	2→2	+
7	1→1	+	15	2→3	—	23	2→1	—	31	3→3	+
8	2→1	—	16	2→1	—	24	2→1	—	32	1→1	+
Experiment 20.11.1959											
1	2→2	+	5	2→2	+	9	3→3	+	13	2→2	+
2	3→3	+	6	2→1	—	10	3→3	+	14	1→1	+
3	1→1	+	7	2→2	+	11	1→1	+			
4	2→1	—	8	1→1	+	12	3→3	+			

First column — No of trial; second column — the signal presented and the foodtray visited; third column — reinforcement. All delay periods — 15 sec.

Preference-omission errors appeared in all the cats in the early postoperative stages and sooner or later they disappeared either with the help of special training (as shown in the above cited series of experiments) or in the normal course of testing.

3) Perseverative errors. As mentioned before, these errors were ones in which the animals approached the last reinforced foodtray instead of the one which was signalled.

As seen in Fig. 9, the perseverative tendency was not equal in all animals. In cats Nos. 7 and 8 it was very pronounced after unilateral but much less so after bilateral ablations (Fig. 9). It was not observed in cat No. 6 in the period in which this animal exhibited omission errors, but it appeared very distinctly when these errors were elimi-

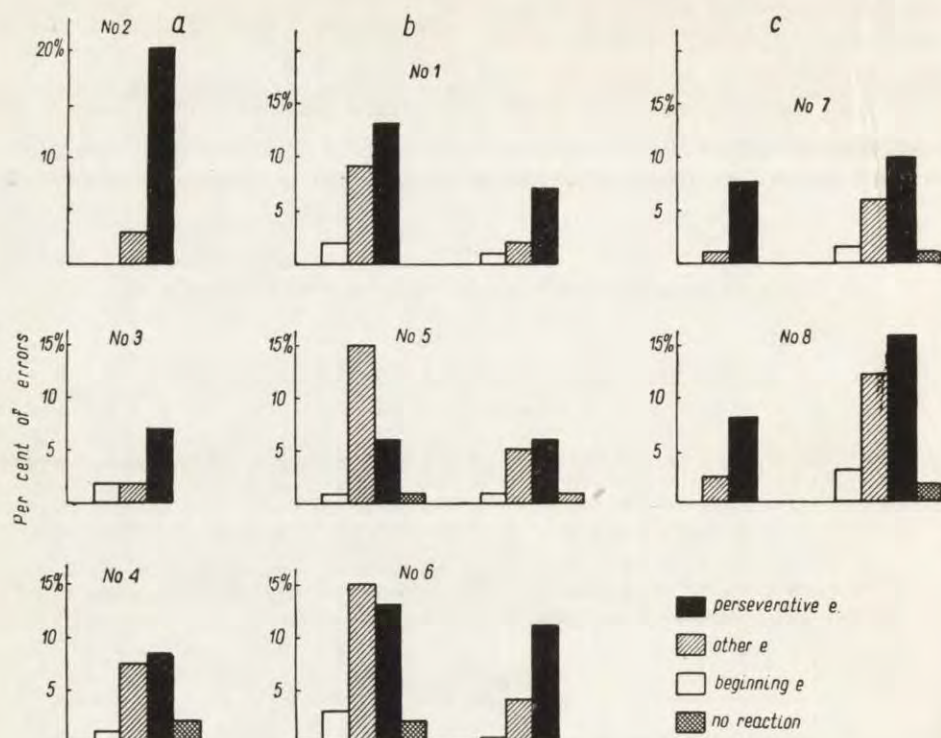


Fig. 9. Types of errors made by each cat.

Columns denote percentages of errors of each type in relation to the total number of trials. Black columns denote perseverative errors, striped columns — non-perseverative errors, white columns — errors in the first trial of experiment, checked columns — cat remains on the platform. a — percentages of errors in cats Nos. 2, 3 and 4, in which the relations remain stable throughout the series of experiments, b — percentages of errors in cats Nos. 1, 5 and 6 in which relations were different in early and later postoperative period, c — percentages of errors in cats Nos. 7 and 8 after unilateral and bilateral ablation.

nated. It was also not present in the early postoperative period in cat No. 1, but became very prominent in a later period. In general a relative predominance of perseverative errors appeared only when omission-preference errors were overcome.

DISCUSSION

Our results have shown that prefrontal lesions produce very distinct disorders of delayed responses in cats, which do not appear after control lesions in parietal region. These disorders, however, seem to be different from, and rather less severe than, those obtained after similar lesions in dogs in the same experimental situation. As described in

a previous paper (Ławicka and Konorski 1959), prefrontal dogs after having been released, run to that foodtray to which they were just turned, which was attributed to their lack of recent memory of directions. Although this symptom is also present in some cats immediately after operation, it very soon disappears and the animals' reaction is independent of their bodily orientation in the moment of release. The fact that after the overwhelming majority of errors cats correct themselves and proceed to the proper foodtray (even though such a run is never reinforced) indicates that their recent memory for directions is intact. Consequently, it would appear that the animal chooses the wrong foodtray after the delay not because of weak memory traces, but because there are some factors which divert its run. As found by an analysis of the errors, these factors are: the tendency to repeat the last reinforced run, and the preference for, or the neglect of, a particular foodtray. Let us analyse the errors caused by these factors separately.

The perseverative or "one trial learning" errors are encountered in normal cats and were discussed in detail in a previous paper (Ławicka 1959). We believe that the tendency to return to the place where food was last obtained is acquired early in ontogenesis and must be suppressed in experimental situations by special inhibitory training. The fact that these errors are much more frequent in prefrontal than in normal cats indicates that the compensatory mechanism leading to their suppression is impaired after this lesion.

Disinhibition of perseverative errors and their gradual compensation after retraining are analogous to disinhibition and compensation of inhibitory conditioned reflexes observed in dogs after prefrontal ablations (Brutkowski et al. 1957, Brutkowski 1958, Ławicka 1958). Therefore, the important question arises as to whether these two symptoms may be considered as a manifestation of one and the same functional impairment of the brain, or whether they are due to different pathophysiological mechanisms.

On the other hand, the preference and the omission errors were not encountered in normal cats and represent a new symptom appearing only after prefrontal ablations. For example, the persistent neglect of foodtray 2 found in cat No. 6 was not seen in the preoperative period. The fact that after special training the animal was able to overcome this difficulty shows again that there is no inability to solve this problem.

What physiological mechanism is impaired in these cases is unclear. Careful observation of the behaviour of animals when this type of error is committed reveals that the cat develops some stereotyped

reaction at the moment of release which leads it inevitably to a particular foodtray. E.g. the animal always turns clockwise or counter-clockwise so as to face foodtray 1 or 3, or it turns in a circle, etc. Therefore, the most probable source of this type of error would be abnormal fixations of particular motor acts. As shown in our experiments this fixation is subject to extinction when special training is applied. Such fixation of motor acts leading to preferential reaction has been observed by many authors concerned with behavioural changes following frontal lesions both in animals and man cf. Brush et al. 1961, Ettlinger a. Wegener 1958, Stępień 1956, Warren et al. 1957).

To end this analysis one should notice that the difference between the two types of errors observed in our animals may appear to be of minor importance and both of them may prove to be attributable to one and the same mechanism. After all both these types of errors consist in the tendency to repeat the same run, either many times in succession, as is the case in preference errors, or only once as is the case in "one trial learning errors". Therefore, it may be supposed that the appearance of preference errors is simply due to a stronger perseverative tendency of the animal than one trial learning errors. As observed in our experiments, preference-omission errors are rather encountered in the early postoperative stages, while the one trial learning errors appear in later stages, when the general animals' performance improves.

There remains a final problem to be discussed, i.e. that of bodily orientation errors which were sometimes observed in cats in the early postoperative period, and which are present as a rule in prefrontal dogs. On the one hand these errors might be interpreted as they were in a previous paper dealing with the impairment of delayed responses in dogs after prefrontal lesions, namely, as the manifestation of a recent memory deficit. On the other hand, they might also be interpreted in a manner similar to the interpretation given to the other types of errors dealt with in this paper. In other words, the question is whether the animal, when being released after the delay period, "follows its nose" only because it does not remember where to go (as suggested in the case of dogs), or, on the contrary, whether the strong and primitive tendency to run to the foodtray just seen prevents the animal from selecting the proper foodtray. For the present, the question must remain unanswered. When the cat commits an error the only evidence that it has preserved memory traces of the correct foodtray is its subsequent correction. Dogs, however, have very rarely displayed this correctional tendency. Therefore, the problem as to whether some

prefrontal lesions do produce a loss of recent memory of directions, as suggested in a previous paper, or whether all the symptoms observed in prefrontal animals in the delayed response situation result from the tendency to return to the more primitive reactions, thereby overshadowing reactions based on recent memory, still remains open.

SUMMARY

1. The effects of prefrontal ablations upon delayed reactions were investigated in 8 cats in a triple-choice experimental setting.

2. After bilateral prefrontal ablations the delayed responses of cats were impaired.

The following types of errors were observed, appearing in successive stages after operation: bodily orientation errors, preference-omission errors, and perseverative errors.

3. After having committed an error, the animals had a tendency to correct themselves, i.e. to go to the proper foodtray, although such a correction run was never reinforced.

4. The tendency to perform preference-omission errors and perseverative errors was gradually suppressed, either in the normal course of testing or by application of special inhibitory training. As a result, delayed responses were gradually restored within weeks or months, and in some cases were even fully compensated.

5. The nature of these errors, as well as the nature of the central disorder producing impairment of delayed responses are discussed.

The authors wish to thank Dr. M. Mishkin for his valuable help in preparing of English text of this paper.

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THE EFFECT OF PREFRONTAL LOBECTOMY ON WATER INSTRUMENTAL CONDITIONED REFLEXES IN DOGS

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In a number of papers from this laboratory (Brutkowski et al. 1956, Brutkowski 1957, Ławicka 1957) the effects of prefrontal ablations on food conditioned reflexes were investigated. After these lesions the excitatory conditioned reflexes remained practically unchanged while the inhibitory reflexes were disinhibited: the operated animals displayed a conditioned reaction (instrumental as well as classical) to inhibitory stimuli and also in the intertrial intervals. In new experiments performed by Auleytner and Brutkowski (1960) after prefrontal lesions a similar disinhibitory syndrome was observed in classical defensive reflexes.

In the experiments presented here the effect of prefrontal ablation on water conditioned reflexes was examined, in order to test whether or not the disinhibitory syndrome also appears when a positive agent other than food, viz. water is used as reinforcement of conditioned reaction. Since inhibition may be effected not only by the direct impairment of inhibitory processes, but also by increased excitability of the centre of an unconditioned reflex, the water consumption of the animals was studied at the same time.

METHODS AND EXPERIMENTAL PROCEDURE

Experiments were performed on 3 dogs in an ordinary conditioned-reflex chamber. In order to make the animals thirsty, two hours before the experiment water was removed from their boxes, and at the same time food mixed with a constant portion of sodium chloride was given. After each experiment the dogs were allowed to drink water ad libitum in the animal house.

Preoperative training was as follows. First, the positive water instrumental conditioned reflex (lifting of the right foreleg) was elaborated to the sound of

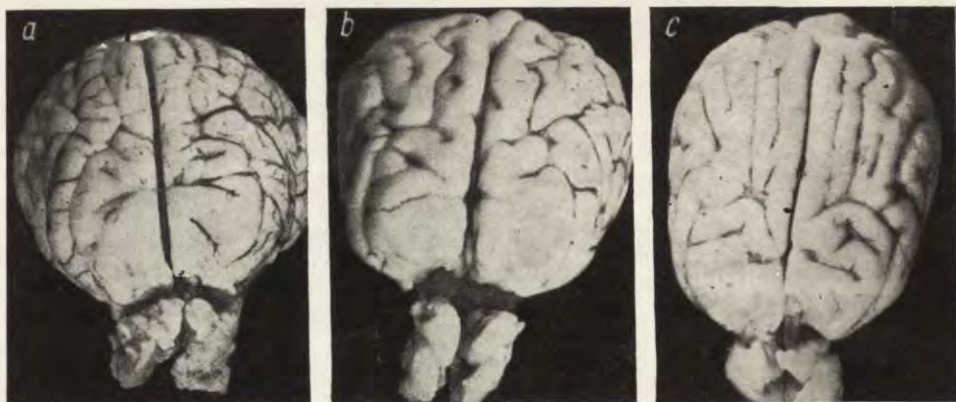


Fig. 1. Anatomical verification of the brains.

a — dog No. 1; b — dog No. 2; c — dog No. 3 (In dog No. 1 *gyrus praecruciatatus* is very large).

a metronome. The instrumental movement was reinforced by a constant portion of water presented in the bowls of the foodtray. Each experiment consisted of 7 trials. Intertrial intervals lasted about one minute. After about 1.5 months of daily training the conditioned reflex to the metronome was firmly established, and the dogs stopped to perform the trained movement and to look into the foodtray in the intervals. Then, conditioned inhibition was elaborated. The inhibitory compound consisted of a rattle (conditioned inhibitor) acting for 5 sec. and followed by the metronome lasting also 5 sec. and the latter not reinforced by water. The inhibitory compound was given twice daily among the excitatory stimuli. After some time a 5 sec. interval was introduced between rattle and metronome so as to make the inhibitory task more difficult. However, in one dog (No. 3) this prolongation produced neurotic symptoms, and we were compelled to return to the application of this compound without interval. Conditioned inhibition was established in all the dogs after about 2.5 months. The preoperative criterion was that in 10 successive experiments no inhibitory trial was disinhibited. At this time it was also discovered that suspension of experiments for one week did not produce any disinhibition.

The prefrontal ablation included *gyrus proreus* and *orbitalis* amputated by suction just rostrally to *sulcus praesylvicus*. Inferior-posterior parts of *gyrus orbitalis* as well as olfactory tracts and bulbs were spared (Fig. 1).

The conditioned-reflex experiments were re-started 7 days after operation.

RESULTS

Water excitability

As soon as the experiments were resumed it was found that although the dogs received the same portion of salt as previously they drank less water than before operation (Fig. 2a and b). Dog No. 1, drank gradually less and less water each day after the experiments

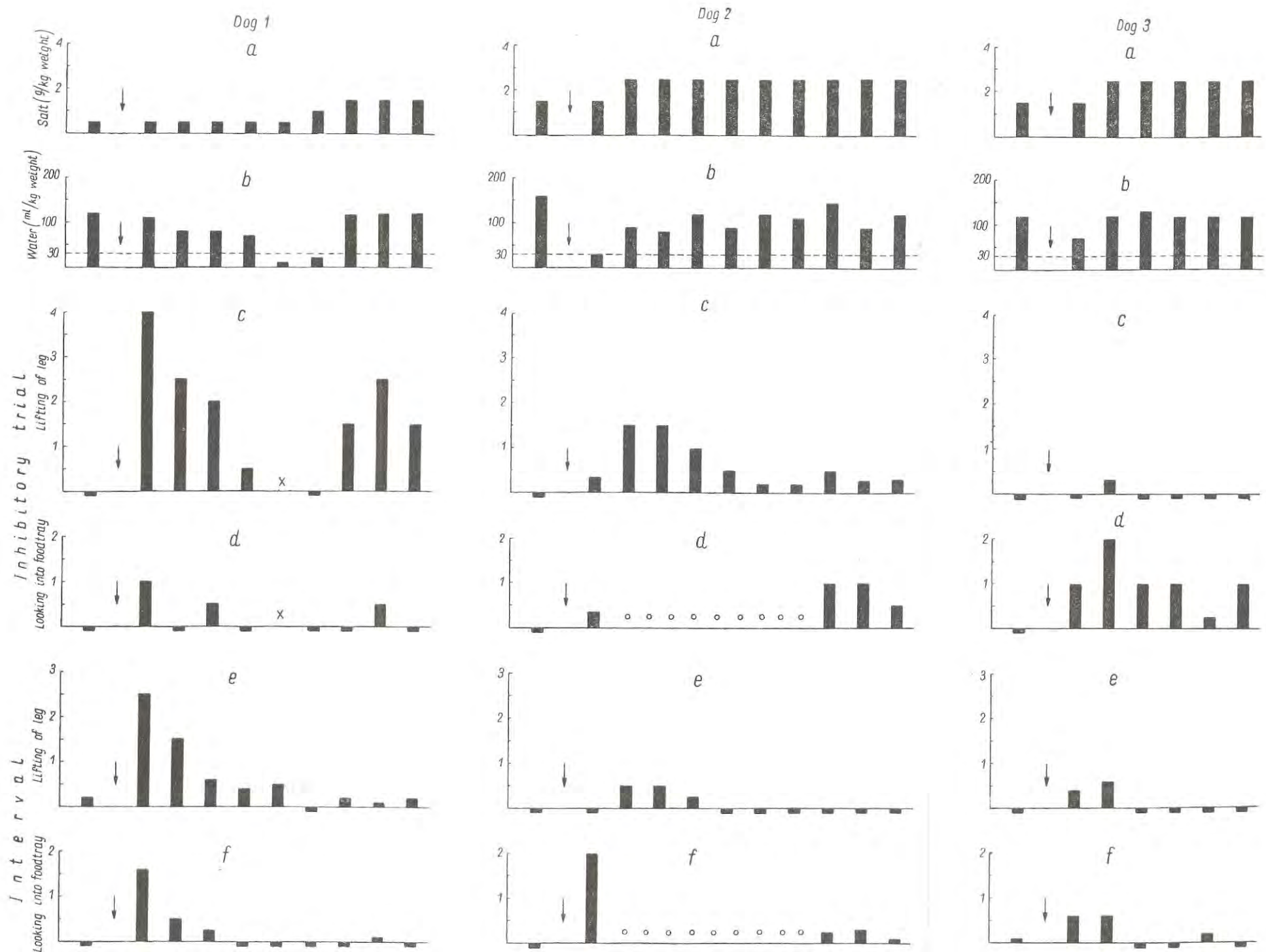


Fig. 2. Increase of water excitability and disinhibition after prefrontal ablations.

a. amount of salt added to food before the experiment, b. amount of water drunk during and immediately after the experiment. During the experiment the dogs drank 30 ml./kg. of weight of water, c. mean number of trained movements in one inhibitory trial, d. mean number of times dog looked into the bowl in one inhibitory trial, e. mean number of trained movements in one intertrial interval, f. mean number of times dog looked into the bowl in one intertrial interval. Arrows indicate the day of operation. Blocks before arrows represent mean value of last ten experiments. Down directed blocks indicate zero value, x. experiments consisted of a few trials only and inhibitory stimuli were not given, ooo. movements of the bowl were not recorded,

and finally stopped to drink it even during the experimental session. Only when his daily portion of salt was increased three-fold, after 3 weeks of resumed experiments was his normal water intake restored. In dogs No. 2 and 3 the water portions were increased much quicker, in order to keep the amount of water consumed on the preoperative level.

Conditioned-reflex activity

One week after operation when the experiments were resumed the general behavior of the dogs was found completely normal. The instrumental reaction to the metronome in the excitatory trials was also undisturbed (Fig. 3b). On the other hand the inhibitory reflexes were seriously affected (Fig. 3b and 2cdef). To the metronome preceded by the

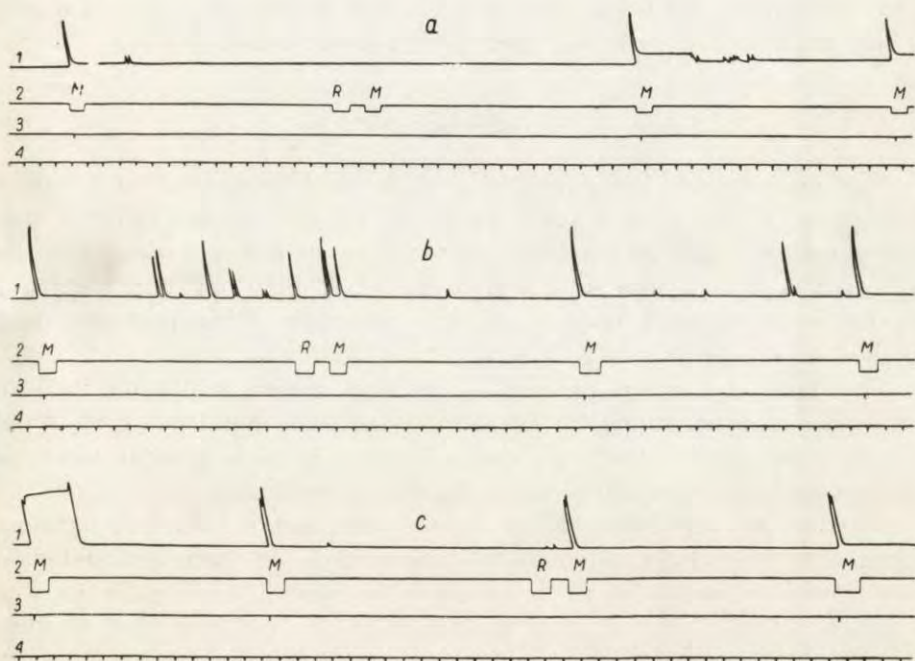


Fig. 3. Conditioned-reflex activity before (a), 10 days after (b) and 1 month after (c) prefrontal ablation in dog No. 2. Each record represents a part of one experiment. 1. lifting of the right foreleg. 2. conditioned stimuli (M-metronome, R-rattle). 3. unconditioned stimulus. 4. time (5 sec.). The smaller marks on line 1 are connected with step movements.

rattle the dogs performed the trained movement, looked into the bowl, and licked it. The same occurred in intertrial intervals.

Although the disinhibitory syndrome was present in all dogs its intensity and character was not the same (Fig. 2cdef). In dog No. 1 the instrumental reaction was strongly disinhibited both to the inhibitory stimulus and in the intervals in spite of the fact that his water excitability in the first three weeks was decreased. On the other hand in dog No. 3 the instrumental reaction to the inhibitory stimulus was almost normal which may be due to his much easier inhibitory task (no interval between rattle and metronome). Dog No. 2 performed the trained movement not only to the metronome preceded by the rattle but sometimes even to the rattle itself, i.e. to a purely inhibitory stimulus. The symptom of licking the empty bowl was more pronounced in dog No. 2 and 3, less in dog No. 1.

As seen in Fig. 2cdef the disinhibitory symptoms gradually decreased but full compensation was not achieved. About 3 weeks after operation inhibitory processes seemed to be unimpaired in some experiments while in others the trained movement and licking the bowl appeared to the inhibitory stimuli and in intervals (Figs. 2cdef and 3c).

DISCUSSION

The experiments presented here show that prefrontal ablations in dogs produce two strong and longlasting effects: impairment of inhibitory reflexes and increase of tolerance to sodium chloride. This last symptom indicates that the prefrontal lobes play an important role in the water-mineral balance of the organism. This problem needs further investigation.

The fact that after prefrontal ablation water inhibitory reflexes were also affected supports the supposition that impairment of inhibitory process produced by prefrontal lesions is of a general character and probably affects all sorts of inhibitory reflexes.

The water disinhibitory syndrome obtained in our experiments appears to be very similar to that described for food and defensive conditioned reflexes. In all cases inhibitory conditioned reflexes were seriously affected at the very beginning of the postoperative training, then the inhibitory processes rapidly improved but they did not become completely restored.

This similarity favours the supposition that for all those disinhibitory syndromes the same pathophysiological mechanism is responsible. As far as food and defensive conditioned reflexes are concerned the disinhibition could be not only due to the direct impairment of inhibitory

processes but also to an increased food and defensive excitability respectively. It is known that after prefrontal lesions animals manifest a more or less pronounced increase in food intake (Fulton, Jacobsen and Kennard 1932, Richter and Hawkes 1939), and also their general defensive behaviour may be augmented (Auleytner and Brutkowski 1960). However, in the water reflexes only the first possibility can be taken into account because the disinhibitory syndrome was independent of the level of excitability of the water unconditioned centre. It was clearly manifested not only in experiments in which normal water excitability was restored by an additional portion of salt but also in those cases in which the water intake was lowered. Therefore, it seems probable that for the disinhibitory syndrome in food and defensive reflexes also the direct impairment of the inhibitory processes is chiefly responsible.

SUMMARY

1. Water instrumental conditioned reflexes both excitatory and inhibitory were elaborated in dogs in order to examine the effects produced in them by prefrontal ablations.

2. After the operation the excitatory conditioned reflexes were unchanged but the inhibitory conditioned reflexes both to the inhibitory stimulus and to the experimental situation were disinhibited.

3. The process of inhibition improved rapidly in the first weeks after operation but later certain impairment of inhibition had a stable character.

4. The tolerance to sodium chloride was permanently diminished.

The author wishes to thank Prof. J. Konorski for his most valuable criticism and helpful advice. Also special appreciation is due to Dr S. Brutkowski for his help in the conducting of some experiments.

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THE EFFECTS OF ABLATIONS OF "ALIMENTARY AREA" OF THE CEREBRAL CORTEX ON SALIVARY CONDITIONED AND UNCONDITIONED REFLEXES IN DOGS

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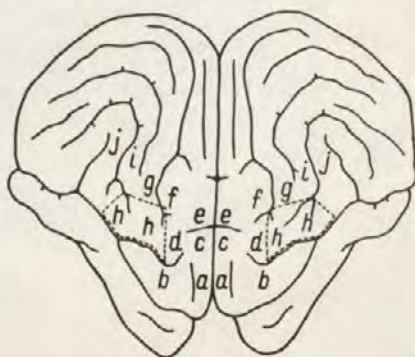
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There is some evidence that the area comprising anterior composite gyrus and the most anterior part of coronal gyrus in the cerebral cortex of dogs (cf. Fig. 1) which will be further called the alimentary area, plays an important role in their behaviour. On the one hand, electrical stimulation of anterior composite gyrus (excluding most posterior lateral

Fig. 1. Dorsal and lateral views of dog's brain shown on one plane.

Gyri are indicated as follows: a — pro-reus; b — orbitalis; c — precruciatatus; d — sigmoideus anterior; e — posterocruciatatus; f — sigmoideus posterior; g — coronalis; h — compositus anterior; i — ectosylvius; j — sylviacus; the boundaries of alimentary area marked by dashed lines.



part) produces many alimentary effects such as salivation, mastication, swallowing and inhibition of movements of pyloric antrum (Babkin and Buren 1951). On the other hand, stimulation of the chorda tympani nerve produces evoked potentials in the superior part of anterior composite gyrus (preliminary results of Santibanez-H. and others 1960), and stimulation of mouth produces those potentials in the most anterior part of the coronal gyrus and posterior-superior part of

the anterior composite gyrus (Pinto Hamuy, Bromiley and Woolsey 1956). Therefore, it seems worth while to examine the effect of partial or total removal of this area on alimentary reflexes. In the experiments presented here, food and acid salivary reflexes were submitted to such an analysis.

MATERIAL AND EXPERIMENTAL PROCEDURE

Experiments were performed on 14 dogs in a sound-proof conditioned-reflex chamber. All these dogs had a chronic parotid gland fistula performed by the Sołtysik and Zbrożyna method (Sołtysik and Zbrożyna 1957). The fistula was made in eleven dogs unilaterally and in three dogs bilaterally. Salivary secretion was recorded by Kozak's volumetric apparatus (Kozak 1950).

Excitatory conditioned reflexes were established to the sound and sight of a metronome. As reinforcement, food was used in 9 dogs and acid in 5 dogs. Food and acid reinforcement consisted of a constant portion of bread-powder moistened with broth, and 1% acetic acid (in different dogs from 5 to 15 ml.) respectively. Food was presented to the dog by means of a mechanical device, and acid was introduced by a small metal tube fixed to the mouth of the dog by Mendelev wax. In different dogs experiments consisted of 5—9 trials, intertrial intervals were 2—6 min., and the duration of the isolated period of conditioned stimulus was from 10 to 20 sec.

In 5 dogs besides excitatory conditioned reflexes, conditioned inhibition was also elaborated. The inhibitory compound consisted of the metronome preceded by a buzzer which acted for 10 sec. In two dogs, Nos. 5 and 10, so as to make their inhibitory task more difficult, an interval of 5 sec. was introduced between buzzer and metronome. The inhibitory compound was given twice daily among the excitatory stimuli.

When the full experimental training was completed and conditioned reflexes, both excitatory and inhibitory, were stable, an unilateral operation was first performed. The experiments were resumed usually one week after operation; only in two dogs, Nos. 6 and 10, they were resumed 11 days after operation and in dog No. 13 two weeks afterwards. 3 weeks to 5 months later, in different dogs, a similar operation in the second hemisphere was performed. The experiments were restarted one week after, but in dogs 10 and 13 two weeks after operation.

In some dogs, in which the parotid fistula was only unilaterally performed, either the homolateral or the heterolateral effect of ablation on salivary reflexes could be observed. However, in three dogs (Nos. 5, 6 and 9), in which salivation was recorded bilaterally, the homolateral and heterolateral effect was observed simultaneously.

The operations varied in extent (Fig. 2, abc). In some operations only the superior part of anterior composite gyrus was ablated i.e. projective area of chorda tympani (both operations in dogs 6, 10 and 12, first operation in dogs 11 and 14*, and second in dogs 5 and 9). In other operations the anterior composite gyrus, except its most posterior lateral part, i.e. area in which stimulation produces

* In these two dogs the second operation was not performed at all.

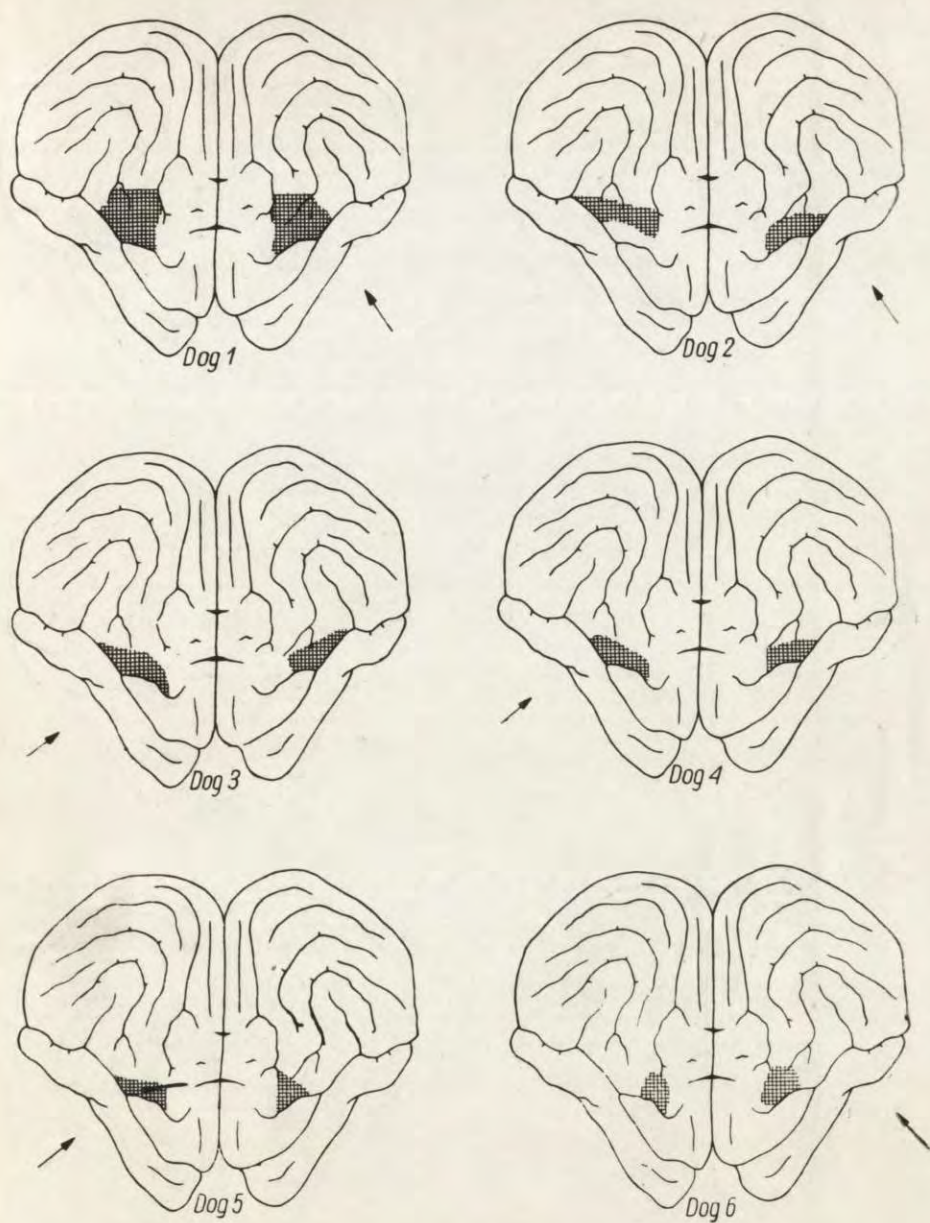


Fig. 2. Anatomical verification of the brains. Destroyed areas are checked. Arrows indicate the side of the first operation.

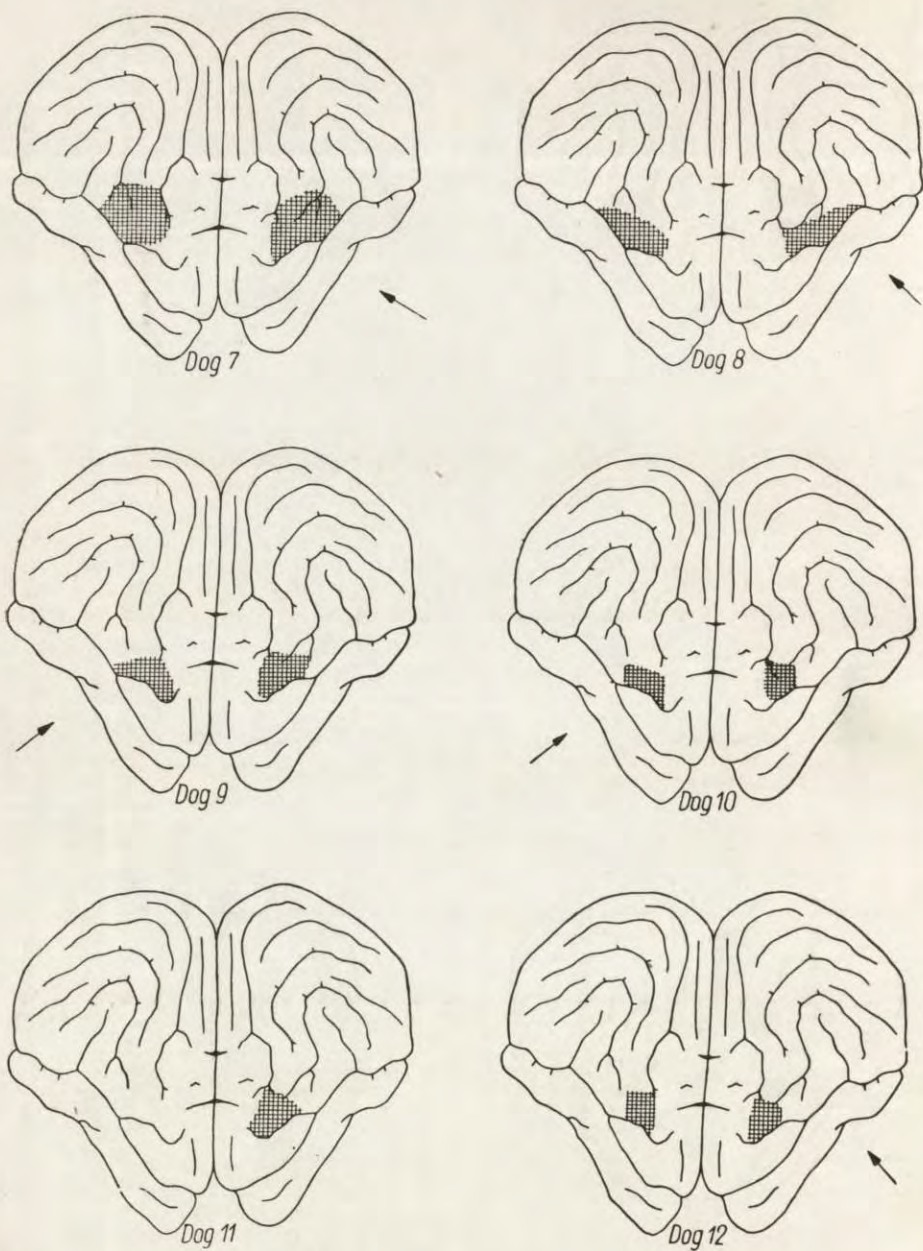


Fig. 2 — continued

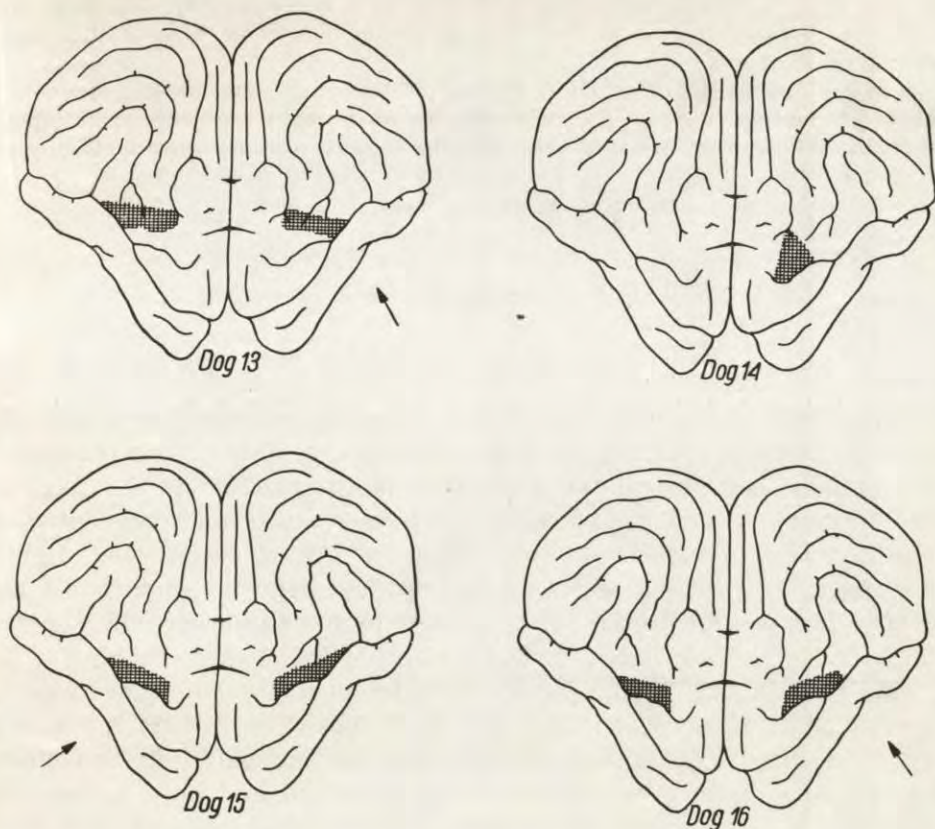


Fig. 2 — continued

alimentary effects, was removed (both operations in dog 8 and first operation in dogs 2, 3, 4, 5 and 9). In two dogs (Nos. 1 and 7) both ablations included anterior composite gyrus and most anterior part of coronal gyrus, i.e. whole alimentary area. In two dogs (Nos. 3 and 4) during second operation only the lower part of anterior composite gyrus was removed. Finally, in one dog (No. 2) in the second operation the lesion was limited chiefly to the anterior part of coronal gyrus and the lower part of anterior composite gyrus. In some dogs (Nos. 1, 2, 6, 7, 8, 11, 12, 13 and 14) the first operation was performed in the left hemisphere and in others (Nos. 3, 4, 5, 9, 10) in the right hemisphere.

Surgical procedures were done under aseptic conditions under Nembutal anaesthesia (35 mg./kg). After skin incision the temporal muscle was cut, bone trephined and dura matter divided. Small cortical vessels were coagulated and cerebral tissue removed by suction to about 3 mm. deep. The dura, muscles, subcutaneous tissue and skin were sutured in layers. During the first three postoperative days the dogs were given 300 thousands units Penicillin daily.

In two acid dogs (Nos. 7 and 8) food intake was measured. The dogs were allowed to eat ad libitum in the doghouse during the whole time of the experimental training.

In two additional dogs (Nos. 15 and 16) preference thresholds for quinine hydrochloride and sodium chloride were tested. In both dogs anterior composite gyrus was bilaterally removed (Fig. 2). The ablation was performed also in two stages.

To analyse the differences, t-test was used.

RESULTS

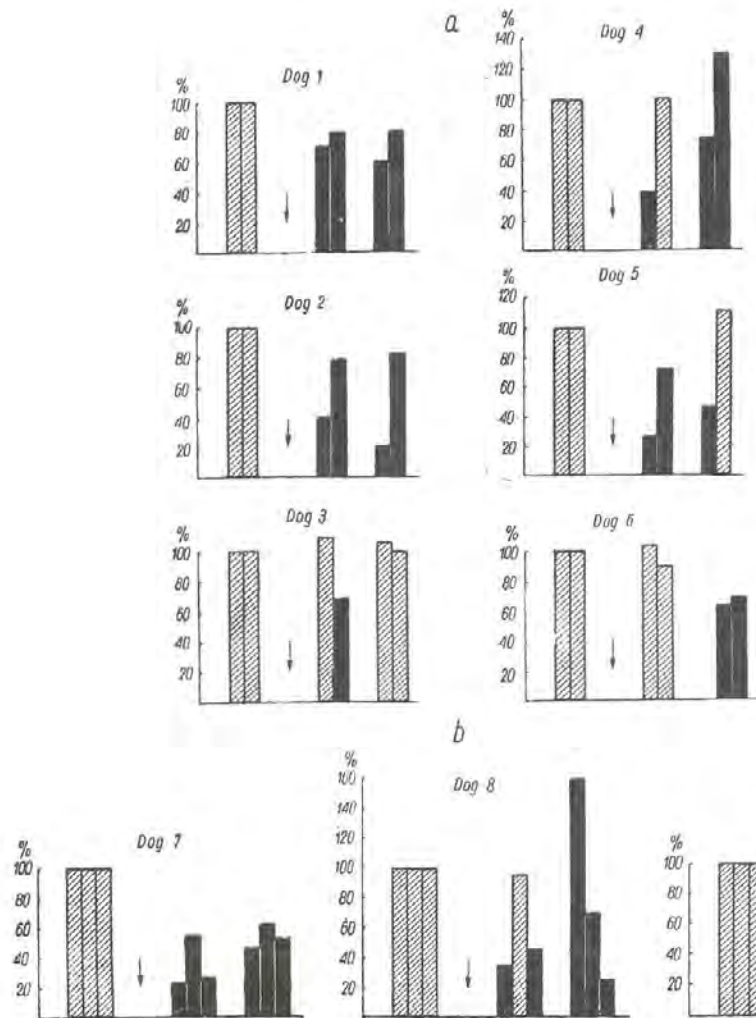
Excitatory reflexes after first operation

One week after operation, when experiments were resumed, the general behaviour of the dogs was completely normal. In response to food conditioned stimuli the directional motor reaction to the foodtray was preserved, and the dogs ate vigorously the food presented as reinforcement. However in some dogs the act of eating was slightly prolonged. The licking and masticatory movements to conditioned and unconditioned acid stimuli also seemed to be unchanged. In contrast the salivary reflexes were seriously affected in all our animals.

As seen in Fig. 3 after homolateral lesion a dramatical decrease of conditioned reflexes was manifested. In 6 dogs (Nos. 2, 4, 5, 7, 8 and 9) they amounted to less than 50% of their normal value, in two others (dogs 1 and 6) they were also visibly decreased and only in one dog (No. 3) did they remain unchanged. Unconditioned reflexes also were affected seriously but not so much as the conditioned reflexes. Intertrial salivation, which was measured only in acid dogs, was decreased very strongly. However in dogs No 4 and 8 hypernormal reflexes were also observed (in dog No 4 unconditioned reflex was hypernormal and in dog No 8 the conditioned reflex). It is interesting to note that during the first experiments the lowered conditioned reflexes sometimes increased (dogs 4, 5, 7 and 8) but it also happened that they sometimes decreased still further (dogs 1, 2, 6 and 9). Unconditioned reflexes usually increased. Finally it is worth mentioning that acid reflexes were more affected than food reflexes.

After heterolateral lesion (Fig. 2) conditioned reflexes were also decreased but much less than after the homolateral operation. They were strongly impaired only in four dogs (Nos. 10, 12, 6 and 14), in two further dogs (Nos. 5 and 9) they remained unchanged, and finally in two dogs (Nos. 11 and 13) they were increased. However the experiments with dog 13 were resumed later than with the others (after two weeks) and it could have been that earlier his conditioned reflexes were even

Homolateral operation



Heterolateral operation

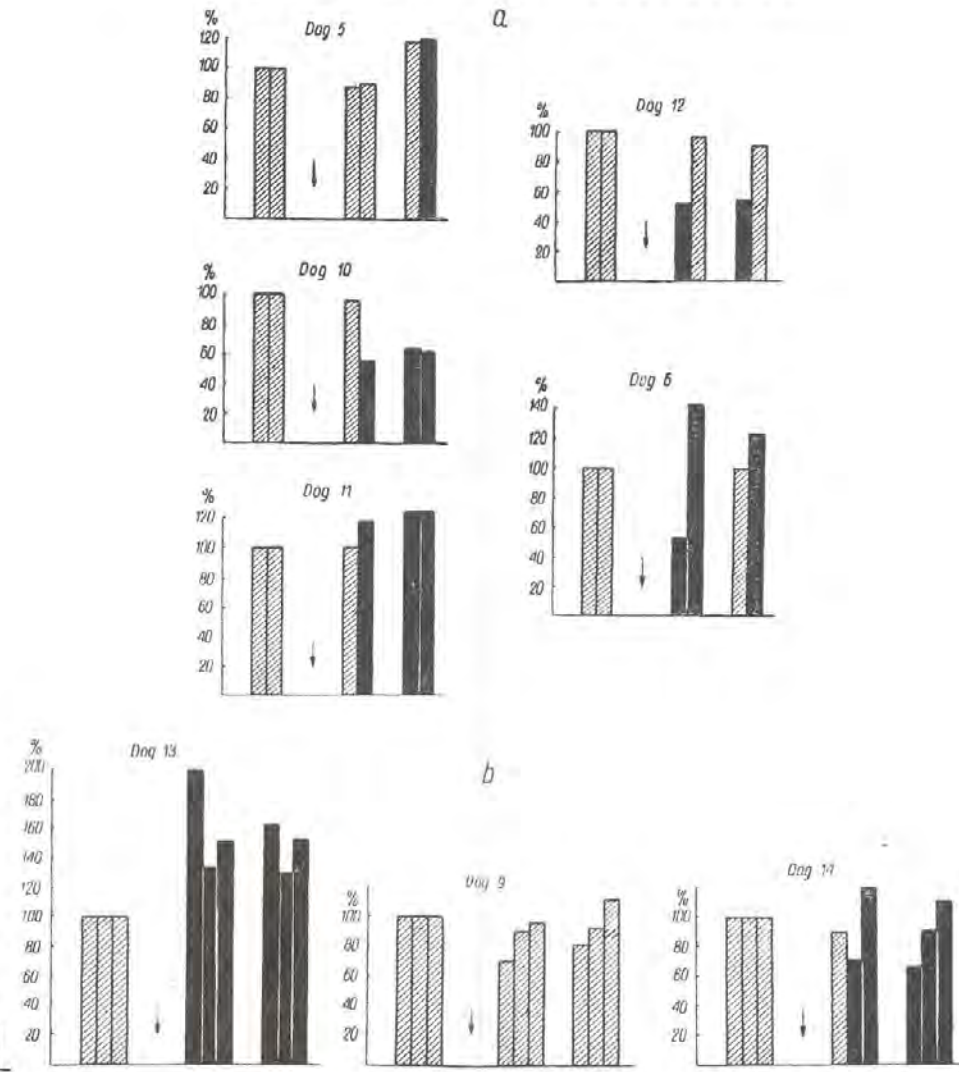


Fig. 3. The recent effect of first homolateral and heterolateral operation on food (a) and acid (b) salivary reflexes.

In every compound of blocks first block represents conditioned reflex, second unconditioned reflex, and third intertrial salivation which was measured only in acid dogs. As unconditioned reflex the salivation for 30 sec. after presenting food (the dogs usually ate it a little longer) or acid is regarded. Arrows indicate the day of operation. The blocks before arrows represent mean value of 10 last experiments before the operation which is denoted as 100%. Blocks after arrows represent mean value of three successive experiments after the operation. Black blocks represent statistically significant values (P. level less than 0.05).

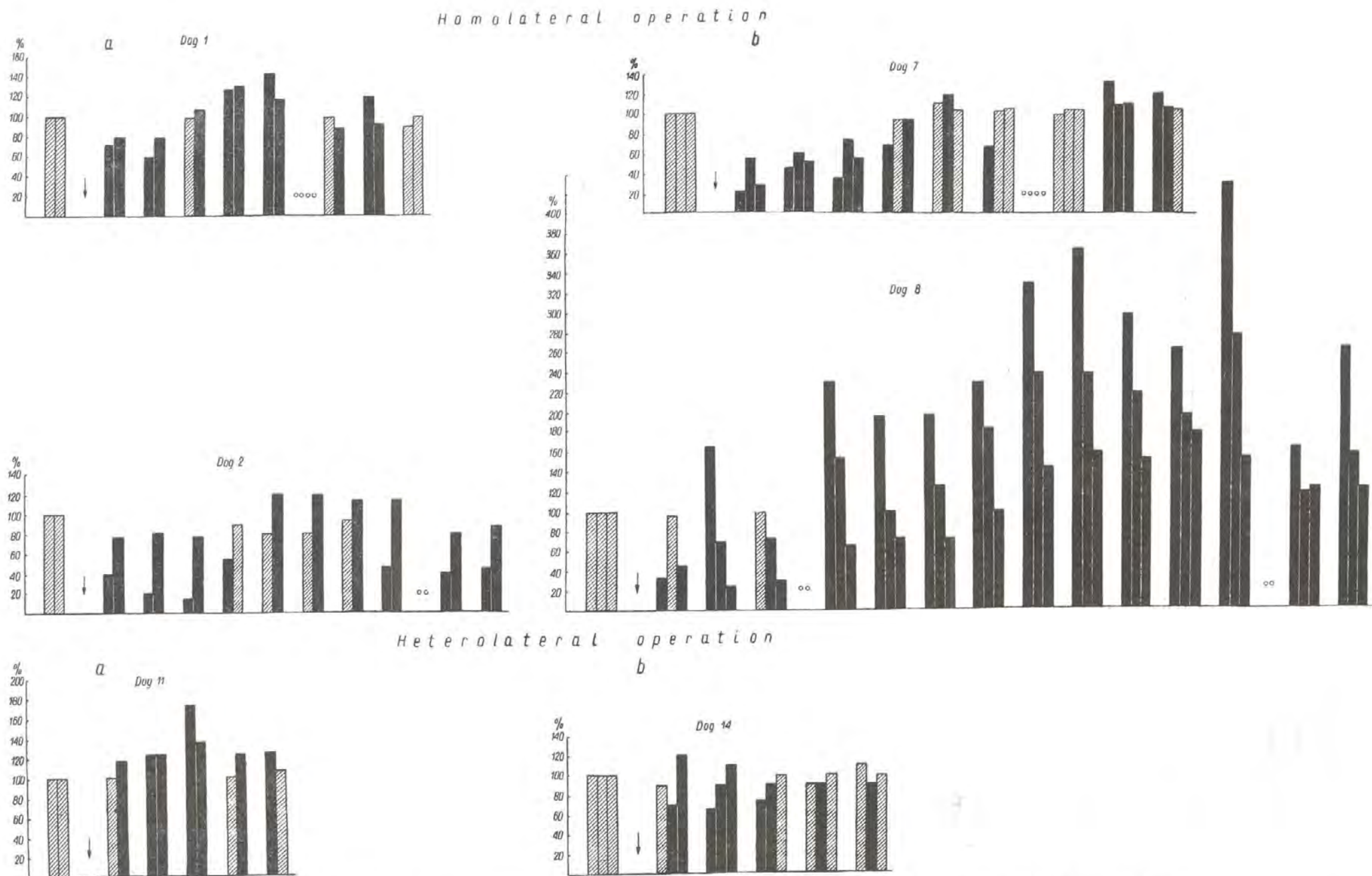
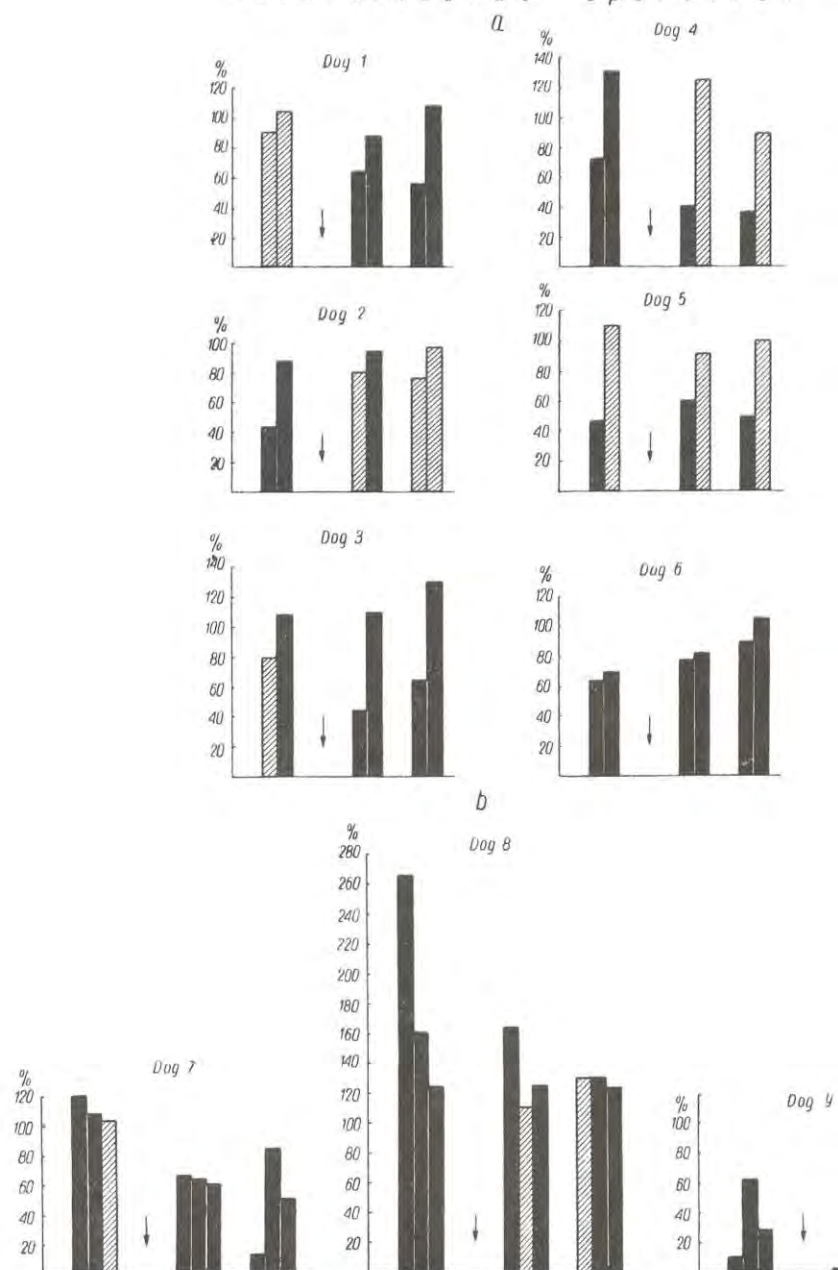


Fig. 4. The recent and later effect of first homolateral and heterolateral operation on food (a) and acid (b) salivary reflexes.

OO — break in the experiments of about 2 weeks; OOOO — break of about two months. Other explanations as in Fig. 2.

Heterolateral operation



Homolateral operation

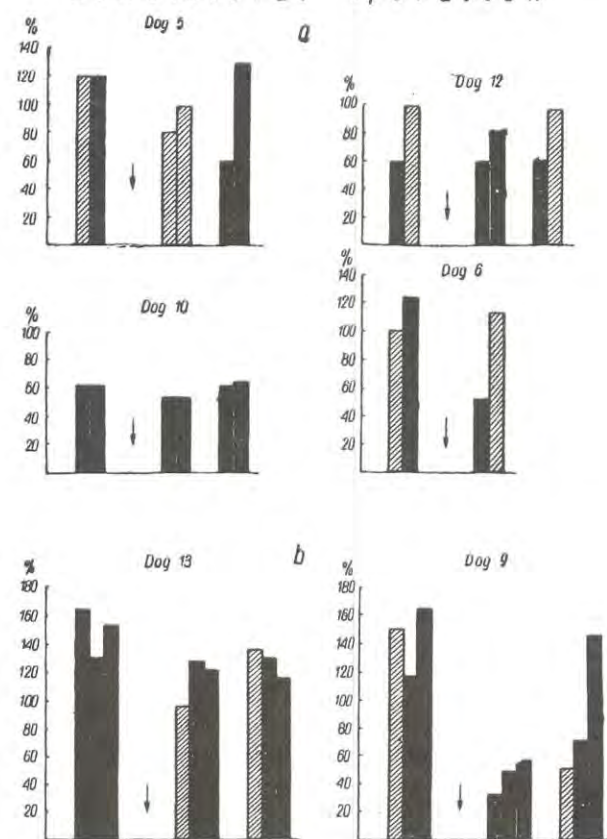


Fig. 5. The recent effect of second homolateral and heterolateral operation on food (a) and acid (b) salivary reflexes.

All values are given in comparison to the mean value obtained before first operation which was denoted as 100%. The blocks before arrows represent the mean value of the last three experiments before second operation. Other explanations as in Fig. 2.

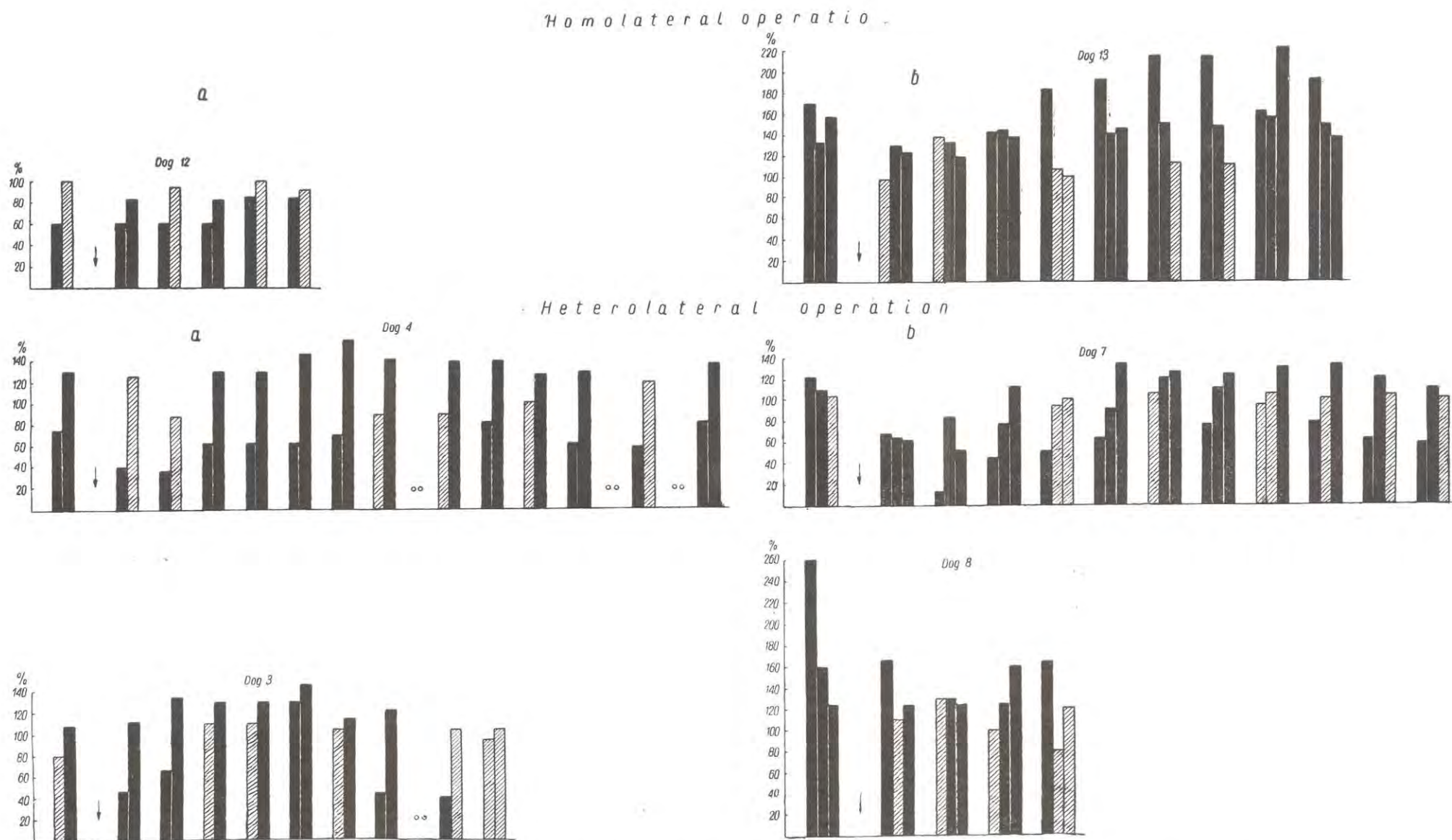


Fig. 6. The recent and later effect of second homolateral and heterolateral operation on food (a) and acid (b) salivary reflexes.
 Explanations as in Figs. 3 and 5.

diminished. The effect of heterolateral lesion on unconditioned reflexes varied in different dogs. In some dogs the unconditioned reflexes were decreased (dogs 10 and 14), in some dogs they remained unchanged (dogs 12 and 9) and finally in others were increased (dogs 5, 11, 6 and 13). Intertrial salivation (observed only in acid dogs) was increased or normal. Finally it should be noted that just as after homolateral lesion during first experiments the conditioned reflexes sometimes further decreased.

In some dogs salivary reflexes were tested for some months after operation (Fig. 4). After homolateral lesion conditioned and unconditioned reflexes gradually increased, achieved their normal value and then were hypernormal. The increase of conditioned reflexes (much more destroyed by operation) was usually quicker and so they became hypernormal in more or less the same time as the unconditioned ones. After heterolateral lesion which affected the reflexes less strongly, their hypernormal enhancement could be observed much quicker. This effect of hypernormal increase of reflexes was shown very clearly in all dogs and in one of them (No. 8) was extremely strong. In this dog, for some time the conditioned reflexes were 4 times bigger than before operation and the unconditioned reflexes 2.5 times. Usually the salivation achieved its maximum a few weeks after operation, and then decreased gradually back to more or less normal value. However in dog No. 8 three months after operation the conditioned reflexes were still very augmented but on the contrary in dog No. 2 both conditioned and unconditioned reflexes were lower than normal at this time.

It is interesting that all described effects (impairment of reflexes as well as their hypernormal enhancement) seemed to be independent of the kind of ablation and of whether the lesion was performed in the right or in the left hemisphere.

Excitatory reflexes after second operation

The second operation was performed in different dogs several weeks to a few months after the first one. Just prior to operation in some dogs the salivary reflexes were more or less on the preoperative level (Fig. 4, dogs 5, 6, 11, 3, 4 and 7), but in others they were either still lowered (dogs 10, 12, 2, 6 and 9) or hypernormal (dogs 13, 9 and 8). In general before the second operation the reflexes were rather unstable.

One week after the second operation when the experiments were resumed the general behaviour of the dogs was similar to that after the first lesion, i.e. completely normal, except that two of the dogs (Nos. 1

and 7) had become a little more aggressive. Also the act of eating was prolonged in some dogs.

As Fig. 5 indicates, after both homolateral and heterolateral operation the unconditioned reflexes were usually unchanged. On the contrary the conditioned reflexes were as a rule diminished after both kinds of lesions and usually they became hyponormal. The degree of their diminution seemed to be similar to that obtained after the first heterolateral operation. Intertrial salivation was also usually decreased. As far as acid reflexes are concerned they appeared to be more affected than the food reflexes.

In Fig. 6 are shown the later effects after second operation. As can be seen, the course of events was similar to that observed after first

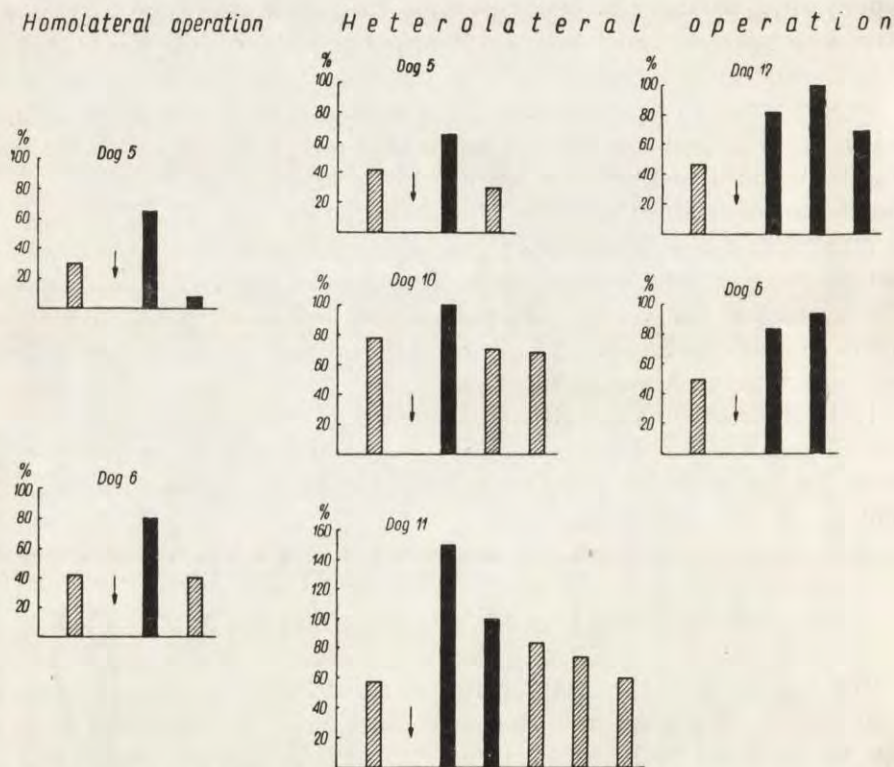


Fig. 7. The effect of first homolateral and heterolateral operation on conditioned inhibition in food dogs.

Blocks represent salivation to the metronome in inhibitory compound in comparison to the salivation to the metronome in excitatory trials in the same experiments which is always regarded as 100%. Arrows indicate the day of operation. Blocks before arrows represent mean value of 10 last experiments before the operation. Blocks after arrows represent mean value of three successive experiments after operation. Black blocks represent statistically significant values (P level less than 0.05).

lesion. First the reflexes (conditioned as well as unconditioned) became gradually hypernormal (only in dog No. 8 was this effect not observed) and then they showed a tendency to return to their more or less normal value.

Inhibitory reflexes

As seen in Fig. 7 after first operation (homolateral as well as heterolateral) conditioned inhibition was disinhibited in all dogs. The disinhibition was usually strongly evident in the first week of experi-

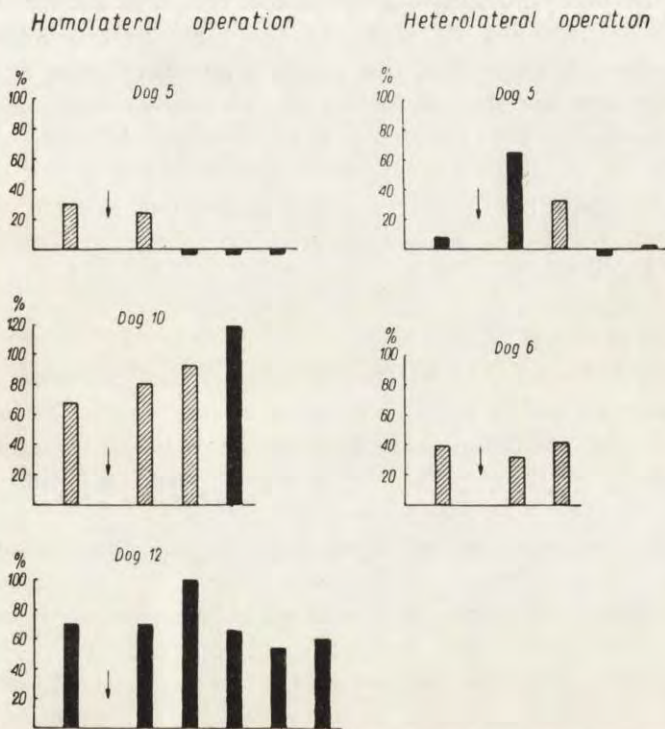


Fig. 8. The effect of second homolateral and heterolateral operation on conditioned inhibition in food dogs.

The blocks before arrows represent the mean value of the last three experiments before second operation. Down directed blocks represent zero value. Other explanations as in Fig. 7.

ments (second week after operation) and then it disappeared rapidly. After second operation (Fig. 8) the disinhibitory symptom was less accentuated. It appeared only in 3 dogs (Nos. 10, 12 and 5) while in two others it was absent.

Taste discrimination and food intake

The material referring to the taste discrimination and food intake is very scanty and permits us only to make the general statement that after unilateral as well as bilateral ablation of anterior composite gyrus the quinine chloride and sodium chloride preference thresholds are increased and food intake seems to be unchanged.

DISCUSSION

As seen from the presented data, after ablations of various extent, performed in the alimentary area of the cerebral cortex, the changes in the salivary reflexes were always the same in character. However, it is impossible to think that the whole alimentary area is functionally equipotential because the results of the aforementioned authors (Babkin and Buren 1951, Pinto Hamuy et al. 1956, and Santibanez-H. et al. 1960) have proved that it is not so. In consequence, it seems probable that the activity of individual parts of this area is closely related, and that they represent one functional entity, at least in respect to salivary reflexes. Therefore, the impairment of any one part of it could destroy its activity as a whole, and produce more or less the same effects.

However, it seems that in the cerebral cortex not only the alimentary area is responsible for the level of salivary reflexes because after total ablation of one hemisphere (Asratian 1955, Abuladze 1956b and Ordżonikidze 1958) or after a large ablation comprising ectosylvian, suprasylvian, and coronalis gyrus (Travina 1956), they were much more strongly affected than in our experiments. On the other hand, there is some evidence that several gyri surrounding the alimentary area are either not connected or connected very slightly with the salivary functions. According to experiments of Brutkowski (1957 and 1959) after removal of preoral gyrus and anterior part of orbital gyrus the salivary conditioned and unconditioned reflexes increased rather slightly. After ablation of sylvian gyrus the salivary reflexes were not affected (Travina 1956).

It is also interesting to compare the effect of lesions performed on homolateral side with those on the heterolateral side. However, first it is worth considering some facts obtained by other authors. According to the experiments of Abuladze (1956a) stimulation of one side of the tongue produces salivation only in the homolateral salivary gland, and in consequence the normal unconditioned salivary reflex may be considered as consisting of two separate reflexes which are responsible

for salivation of the right and left salivary glands respectively. According to Abuladze the same is true in relation to conditioned reflexes. Moreover, according to the experiments of the above mentioned authors after unilateral ablation of the cerebral cortex the impairment of salivary reflexes was manifested only homolaterally. From these results two conclusions can be derived. First, the unconditioned salivary reflex evoked by stimulation of one side of the mouth, and acting upon homolateral glands runs through the homolateral hemisphere. Secondly, a conditioned reflex arises only in that hemisphere by which the appropriate unconditioned reflex is mediated. However, those results are in contradiction to some histological experiments which indicate that the taste pathway is crossed (Allen 1923 and Gerebtzoff 1939) and to the results of Patton and Amassian (1952) and Benjamin and Pfaffmann (1955), which have proved that when the taste nerves are stimulated the evoked potentials appear bilaterally.

In our experiments the conditioned reflexes were impaired bilaterally although the homolateral decrease was much stronger. The unconditioned reflexes appeared to be affected only homolaterally after the first operation. However, since the compensation of these reflexes is very rapid, it is possible that after heterolateral operation the unconditioned reflexes are also decreased, but become compensated before the experiments are resumed. These results indicate that the alimentary area of the given hemisphere plays a predominant role for the homolateral salivary reflexes (unconditioned as well as conditioned), but for the conditioned reflexes, at least, the opposite hemisphere is also important.

As far as the postoperative decrease of conditioned reflexes is concerned, it can be partially understood as a secondary symptom connected with the impairment of unconditioned reflexes. On the one hand, since the efferent part of the conditioned reflex is the same as that of the unconditioned reflex, the destruction of the latter involves automatically the impairment of the first. On the other hand, when the unconditioned reflex is decreased, its reinforcing properties may be lowered, and consequently conditioned reflexes partially extinguished. This last mechanism may account for the fact that in some dogs conditioned reflexes reach their minimal values not immediately after operation but only after several experiments. (cf. Figs. 3 and 5). However, it seems that the impairment of salivary conditioned reflexes is partially independent from that of unconditioned reflexes. This is suggested by the fact that unconditioned reflexes were diminished only after the first homolateral lesion, while the conditioned reflexes were decreased after all kinds of operation, the first homolateral lesion causing their dramatic drop. Therefore the conclusion follows that by our ablations

the conditioned connections localized in alimentary area were also destroyed.

The hypernormal increase of salivary reflexes, which took place some time after operation, seems not to be connected with an increase of food excitability, since the food intake remained on more or less the same level. Rather it should be explained by sensitization of subcortical structures occurring in the course of compensation. It is worth noting that in one dog, in which the preoperative salivation was very small, the postoperative increase of reflexes was exceedingly great (400%). The reason of this unusual enhancement is not clear. It should be also noted that the hypernormal increase of conditioned reflexes may be partially due to the augmentation of unconditioned reflexes producing the increase of their reinforcing value.

The disinhibition of inhibitory conditioned reflexes obtained in our experiments appeared to be very transient and usually lasted no longer than one week (cf. Figs. 7 and 8). It can not be regarded as a symptom resulting from ablation of cerebral cortex itself since ablation performed in some other regions of the cortex do not produce disinhibition (Brutkowski 1957). This disinhibiting seemed to be independent of the hypernormal enhancement of salivary reflexes since it appeared immediately after operation, when salivary reflexes were as a rule still decreased. However, this group of dogs, in which the inhibitory reflexes were elaborated, was observed for only a short time after operation and they were killed before the full hypernormal enhancement of reflexes could be visible. Disinhibition obtained after our ablations was much less pronounced and lasted much shorter than that obtained after prefrontal ablations (Brutkowski 1957, Auleytner and Brutkowski 1960).

To end this discussion it is worth while to draw attention to the fact that acid conditioned and unconditioned reflexes were stronger impaired after operation than food reflexes. This may be due to the fact that in food reflexes perhaps not only taste of food but also reduction of hunger plays role of reinforcement.

SUMMARY

1. In 14 dogs the effect of partial or total ablation of alimentary area, i.e. area comprising anterior composite gyrus and most anterior part of coronal gyrus, on salivary conditioned and unconditioned reflexes was investigated.

2. The ablation was performed in two stages: first in one hemisphere and after some weeks or months in the other.

3. After the first homolateral operation (homolateral to the tested salivary gland) the conditioned and unconditioned reflexes were first decreased (the impairment of conditioned reflexes being very strong) and then hypernormal. After the first heterolateral operation and after both homolateral and heterolateral second operation the conditioned reflexes were at first decreased whereas unconditioned reflexes were unchanged; then both conditioned and unconditioned reflexes were hypernormal. The decrease of reflexes usually lasted several days or weeks, while the hypernormal enhancement lasted from several weeks to a few months.

4. After any operations the inhibitory conditioned reflexes were disinhibited for a short time (about one week).

5. The decrease of acid reflexes was stronger than that of food reflexes.

6. Quinine chloride and sodium chloride preference thresholds were increased; food intake seemed to be unchanged.

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IMPAIRMENT OF AUDITORY RECENT MEMORY PRODUCED BY CORTICAL LESIONS IN DOGS

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INTRODUCTION

The problem of recent memory was introduced to neurophysiology by Jacobsen (1936) who stated that after prefrontal ablations in monkeys delayed response tests are severely impaired. In spite of the importance of this study for our understanding of the functions of the prefrontal cortical areas, the problem of recent memory as such has not been subjected to further analysis.

As was pointed out recently by Konorski (1959, 1960), the problem of recent memory deserves careful attention by physiologists interested in the field of higher nervous activity and should be thoroughly investigated. According to his assumption the delayed response test does not evaluate recent memory in general, but only recent memory for directional cues, and therefore the prefrontal area cannot be considered as responsible for this function. Konorski suggested that recent memory for specific sensory modalities may be dependent on cortical areas situated in the vicinity of the corresponding primary projection areas and proposed a new test for recent memory investigation for any modalities of stimuli.

This paper is concerned with the problem of the localization of cortical areas responsible for recent memory of auditory stimuli.

METHODS

The experiments were performed on dogs placed in an ordinary soundproof conditioned-reflex chamber. For each testing session the animal was put on the stand and strapped by his hind legs. The foodtray was situated in front of the animal and the bowls containing food were put into position by an automatic

device. On the foodtray and to the right of the bowl, there was a small platform which recorded the movements of the right foreleg of the animal, when this was placed on it. The signalling apparatus was hidden behind a screen situated in front of the animal. The experimenter sat outside the chamber and could observe the animal through a one-way vision window. The auditory stimuli were produced by a variable frequency tone generator connected to a loudspeaker behind the screen. Tone signals differed only in their frequency, the intensity remaining the same at approximately 70 decibels.

In all our dogs instrumental (type II) alimentary conditioned reflexes were established using the test for recent memory proposed by Konorski in 1959. The conditioned stimuli were made up of two tone signals, each signal lasting for 2 sec. and the interval between them being 3 sec. Stimuli consisting of two tones of identical pitch were positive and were reinforced by food, if the animal in response to the second tone placed his right foreleg on the foodtray. On the other hand, when the second tone was of a different pitch from that of the first, the stimulus was negative and was not reinforced. The intertrial intervals varied from 1.5 to 2.5 min. Every experimental session consisted usually of 6—9 trials among which 1—2 inhibitory conditioned stimuli were applied in random order. The duration of the conditioned stimulus, the placing of the animal's foreleg on the foodtray and the moment of food presentation were recorded on a kymograph. Each portion of food contained about 10 small pieces of bread moistened with broth. The animal was allowed to drink as much liquid as he wanted before the experiment, and therefore thirst could not influence the alimentary excitability of the animal.

TRAINING PROCEDURE

Experiments were performed on 5 mongrel male dogs. Each animal was first habituated to the general experimental procedure and environment, and taught to stand quietly on the stand, to obtain food in small quantities from the foodtray. Then pairs of tones of identical pitch, 130 Hz in some dogs and 215 Hz in others, were introduced, and during the presentation of the second tone the right foreleg of the animal was placed on the foodtray by the experimenter. This passive movement was reinforced by food presentation. After several days the dog learned to perform the active movement after presentation of the positive conditioned compound stimulus.

Initially, the animal performed the movement during or immediately after the presentation of the first signal. Then, it sometimes took its leg off the foodtray and put it on again immediately after the second signal. At other times the animal kept its leg on the foodtray until the second tone and presentation of the food. In order to get rid of these untimely movements immediately after the first signal, the second signal was withheld and this was not reinforced by food. Such a training procedure helped the animal to hold back his response and to wait for the presentation of the second signal.

After 10—14 days of training with positive stimuli we proceeded to the formation of inhibitory reflexes. The inhibitory stimulus was made up of two different tones: the first tone was the same as hitherto applied, i.e. 130 Hz or 215 Hz respectively and the second one was 1670 Hz. This compound was not reinforced by food. Owing to generalization, this stimulus elicited the learnt

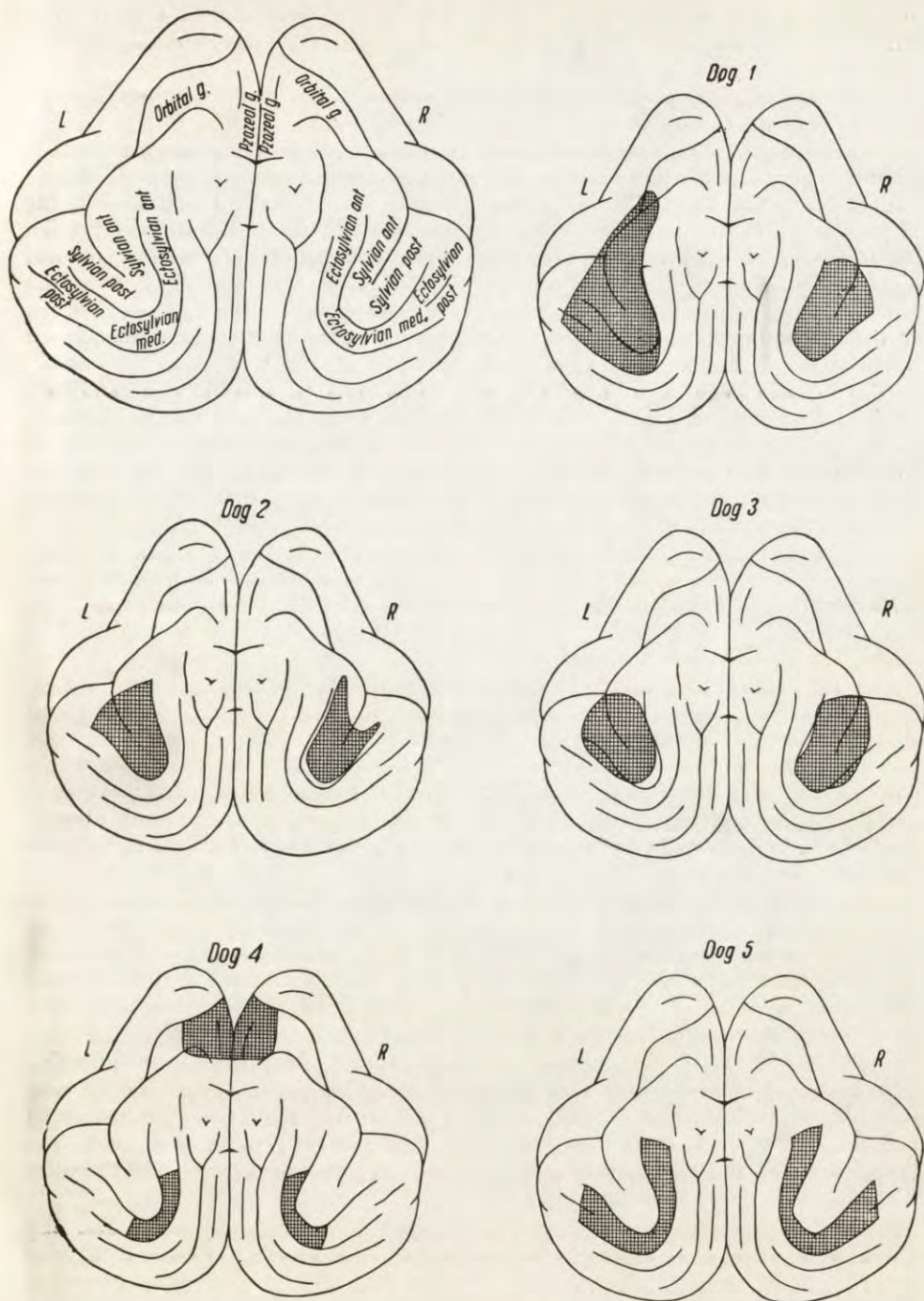


Fig. 1. The normal cerebral cortex of the dog flattened on the plain and extent of lesion in each dog.

movement at first, but after a number of unreinforced trials, i.e. in 4 to 14 days, differentiation developed and the conditioned response to this stimulus became inhibited.

Such training proceeded for 2—3 months during which the animals learned to react only to the second tone signal of each stimulus.

When these two reflexes were firmly established the task was gradually made more complex by the introduction of new positive stimuli made up of higher frequencies, while the inhibitory stimulus remained the same. Initially — owing to generalization — these new stimuli evoked positive responses. However, when the frequency of tones reached a level near the 1670 Hz of the negative stimulus some disinhibition of the inhibitory conditioned reflex was noticed, and at the same time the learnt movement began to appear again before the presentation of the second tone. After some weeks of further training these disturbances of conditioned reflex activity cleared completely.

In the last stage of training different frequencies of tones in the inhibitory compound stimuli were gradually introduced. Since our aim was not to elaborate discriminations between very similar tones, the difference in the frequencies of two tones in the inhibitory stimuli was usually from 500 Hz to 1000 Hz. The first tone was always lower than the second one, both of them changed in frequency from trial to trial.

In dog No. 4 after this differentiation having been established, a new differentiation of the reverse sequence of „high-low” tones was also undertaken. This was achieved after an additional 4 weeks' training (see Chorażyna 1959 for details).

In dog No. 2 a simple bell — buzzer sound differentiation was also established during the preoperative training.

In all animals the whole preoperative training lasted from 5 to 7 months.

Surgery was done under aseptic conditions; the cortical tissue was removed by subpial aspiration. Each animal was operated in two stages, first on one side and some 8 to 28 days later on the other. In three animals, Nos. 1, 2 and 3, the anterior and posterior sylvian gyri were removed. In dog No. 4, first the medial part of the ectosylvian gyrus and later the whole prefrontal area was ablated. In dog No. 5 the anterior, medial and posterior parts of the ectosylvian gyrus were destroyed (Fig. 1).

Following surgery there was no evident change in the general behaviour of the animals with ablations of the sylvian or the medial parts of the ectosylvian gyri, and testing could be resumed five days after surgery. Dog No. 5, however, did show some signs of general slowness and motor awkwardness after bilateral ablation of all parts of the ectosylvian gyrus. With this dog the experiments were resumed 2 weeks after operation.

All animals were then retested in preoperatively established differentiations. In dogs Nos. 1, 2 and 3 with bilateral ablations of the sylvian gyrus, simple bell-buzzer sound differentiation as well as a conditioned inhibition (the compound bubbling of water — bell being the inhibitory stimulus) were established. The three dogs with bilateral sylvian gyri ablations were observed during 7—10 months, the two other dogs, 2 months after operation.

When postoperative testing had been completed, the animals were killed and the lesions microscopically verified. The surface lesions were reconstructed and serial sections through the *thalamus* were studied for retrograde degeneration in the *corpus geniculatum medialis*. The analysis of these degenerations will be reported separately (Sych 1961).

RESULTS

Each dog was retested after unilateral ablation and no deficit found either in the response to the compound stimuli made up of two identical tones (positive stimulus) or to that following two different tones (inhibitory stimulus). Overall, there were 17 trials in which the inhibitory compound stimulus was presented to the animals, and in all of them the motor conditioned reaction was fully inhibited.

On the other hand, profound disorders in the performance of this task were encountered after bilateral ablations of the anterior and posterior sylvian gyri. Dog No. 1 did not perform the trained movement in the first four experiments after operation in response to the positive conditioned stimulus, although he displayed a strong alimentary reaction. Subsequently this behaviour improved gradually and the dog began to perform the trained movement to conditioned stimuli, but as a rule, the response appeared to the first as well as to the second tone. Dog No. 2 performed the learnt movement to the second signal only, while dog No. 3 in some periods displayed a proper reaction, and in other periods reacted to the first signal.

The most striking disorders were encountered when the inhibitory compound stimuli were applied. All three dogs displayed a long lasting motor reaction to the second tone: dog No. 1 placed its leg and scratched the bowl both during and for some seconds after presentation of the negative stimulus; dog No. 2 held its leg on the foodtray and looked for some 10 to 18 sec. after this stimulus into the bowl expectantly; dog No. 3 performed the learnt movement repeatedly. The motor reactions to the inhibitory stimuli during the whole postoperative period were similar to those of the animals in the early stages of the preoperative training when the inhibitory compound was first introduced. After some months of retraining the motor reaction to the second signal of the inhibitory compound stimuli became gradually weaker and lasted only during the time of the second tone presentation.

The correct responses to the inhibitory conditioned stimuli occurred very rarely and quite accidentally. Dog No. 1 made 82 errors in 85 inhibitory trials; dog No. 2 — 57 errors in 62 trials, and dog No. 3—48 errors in 51 trials. Of course, all these motor reactions which followed the inhibitory conditioned stimuli were not reinforced by food. This difficult experimental situation sometimes caused disturbances in the response to the positive stimulus presentation following the negative one. In these circumstances the trained movement would appear either after a long latent period, or else the movement would be executed before presentation of the second tone.

Fig. 2 shows the conditioned-reflex activity to the inhibitory compound stimuli before and after surgery. Each column represents 25 inhibitory trials: the first one — the last 25 trials before operation, and the others — the subsequent 25 trials after bilateral ablations.

In an effort to define more accurately the character of the post-operative deficit, dogs Nos. 1 and 3 were trained to perform simple bell — buzzer sound discrimination as well as conditioned inhibition. Both tasks were learned readily without any difficulty. As to conditioned inhibition, in the initial stage of training the interval between

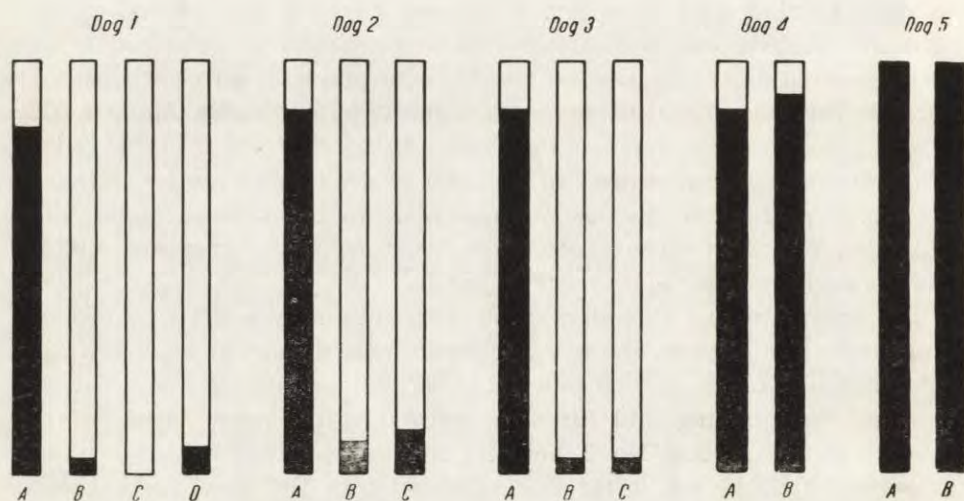


Fig. 2. The performance of recent memory test before and after bilateral operations in each dog.

Each column represents the average from 25 inhibitory trials. A — last stage of training before operation, B, C, D — successive blocks of trials after operation. Black parts of the columns denote correct responses to inhibitory compounds, white parts denote incorrect responses.

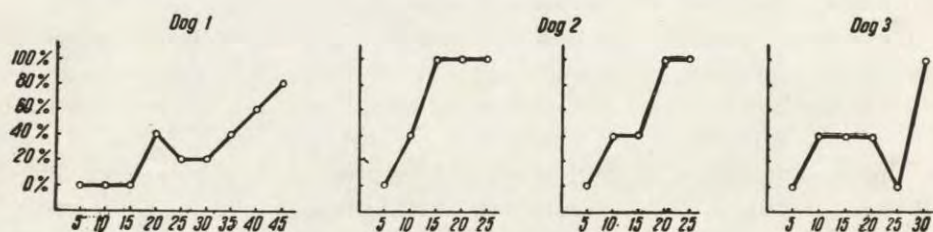


Fig. 3. The course of elaboration of simple auditory differentiation in each dog. Abscissae: successive inhibitory trials; each point represents the means of 5 trials. Ordinates: percentage of correct responses. Note, that in dog No. 2 both pre and postoperative training is presented.

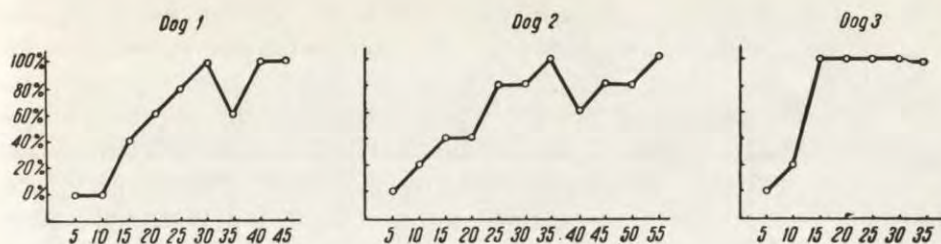
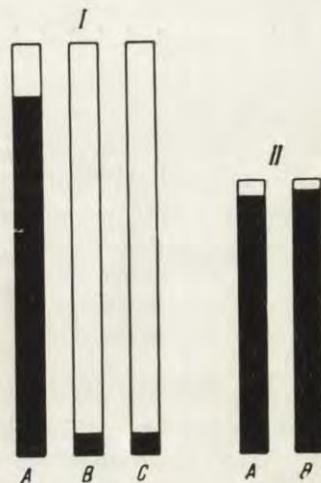


Fig. 4. The course of elaboration of conditioned inhibition in each dog.

Explanations as in Fig. 3.

Fig. 5. The performance of recent memory test before and after operation in sylvian dogs (I) and ectosylvian dogs (II)

Each column represents 75 inhibitory trials in I and 50 inhibitory trials in II, 25 trials for each animal. A — before operation, B, C — after operation. Black parts of the columns denote correct inhibitory responses, white parts — wrong responses. Note the nearly total abolition of inhibitory reactions in sylvian dogs and total preservation of these reactions in ectosylvian dogs.



the conditioned inhibitor and conditioned stimulus was about one second, then it was gradually lengthened to 14 sec.

In dog No. 2 simple bell — buzzer sound discrimination was established in the preoperative training. Immediately after operation there was some deficit in this task, but it was relearned very quickly.

Fig. 3 illustrates the data in postoperative learning of simple auditory discrimination, and Fig. 4 — in conditioned inhibition.

After bilateral ablations of the medial part or of the whole ectosylvian gyrus the animals displayed no deficit in the performance of auditory recent memory test. They performed the trained movement to the positive compound stimuli in proper time, i.e. always at the presentation of the second tone. The motor reaction was fully inhibited to the inhibitory compound stimuli. Dog No. 4 made two errors in 27 inhibitory trials and dog No. 5 — only one error in 29 trials (Fig. 2).

Fig. 5 shows the comparative pre- and postoperative performance

data in three dogs with bilateral ablations of the sylvian gyrus (I) and in two dogs with bilateral partial or total ablations of ectosylvian gyrus (II).

A bilateral prefrontal lobectomy was performed in dog No. 4 following bilateral ablation of the medial part of the ectosylvian gyrus. In the first two experiments carried out one week after surgery, the animal often performed the trained movement during intertrial intervals. Such behaviour had never occurred before operation. In the six subsequent experiments the movement appeared to the first as well as to the second tone signal of the positive compound stimuli. In the following experiments the motor conditioned reaction appeared usually only to second tone. Inhibitory conditioned reflexes were disinhibited in the first six experiments after operation. In the next two experiments the inhibitory capacity improved and the animal inhibited the motor reaction to one of two negative compound stimuli. Subsequently the differentiation between positive and negative compound stimuli was performed without errors. Overall the animal made 15 errors in 35 inhibitory trials out of these 14 had been made during the early post-operative period.

DISCUSSION

According to our findings one of the areas essential to recent memory for auditory stimuli in the dog would be situated in the ventral portion of the temporal region and would include the sylvian gyri, or at least some parts of them. As was shown in these experiments a unilateral lesion in this region does not impair this function in the least while bilateral lesions destroy it totally and irreversibly.

Similar experiments were performed recently by Stępień, Cordeau and Rasmussen (1960) in monkeys. These authors have shown that in this animal, the area functionally equivalent to the ventral temporal cortex in dogs, includes the anterior parts of the first and second temporal gyri. According to previous experiments by Neff and his associates (1957), bilateral lesions in the ventral parts of the temporal region in cats abolish their ability to respond correctly to the change of successive patterns of tones sounded throughout the experimental session. It could be argued that Neff's test also requires auditory recent memory for its performance, and his results could be considered as equivalent to the present ones.

The question could be asked if this loss of differentiation between pairs of signals made up of identical tones and those of different tones, unequivocally indicates an impairment of auditory recent memory. One

could argue that this sort of differentiation is simply a very difficult task for the animal, and therefore is particularly vulnerable to any cortical lesion. It is also possible that after ventro-temporal lesions the synthesis of auditory stimuli is impaired rather than recent memory itself.

The first possibility should be discarded on the grounds that other cortical lesions, even larger ones and situated in the close vicinity to the sylvian gyri, did not in the least impair this differentiation. As far as the second possibility is concerned it cannot be ruled out by our experimental data. However, Stępień et al. (1960) in their experiments on monkeys have shown that if two elements of the compounds followed immediately one after the other then differentiation could be established after bilateral superior-temporal lesions. This indicates that the animal is able to make a synthesis of compound auditory stimuli indispensable for their differentiation when this task does not require recent memory.

The experiments in which it was shown that conditioned inhibition is left intact after this operation deserves close attention. In this sort of training an inhibitory stimulus is applied, which tells the animal that the conditioned stimulus presented after it will not be reinforced. Therefore, it is not necessary for the animal to remember the stimulus itself, but only the general negative attitude which this stimulus has elicited. As was shown by our experiments, the animal is able to preserve this attitude even after a dozen seconds or so. Again this seems to show that the impairment brought out by the ventro-temporal lesion is specific for auditory recent memory.

Two types of control lesions throw more light on the specificity of this impairment. One is the destruction of prefrontal area in dog No. 4, the second is the lesion of the auditory projection area performed in dogs Nos. 4 and 5.

The lesion in the prefrontal area has produced a fully developed syndrome as described by Brutkowski et al. (1956). The marked impairment of inhibition following this operation was manifested by: 1. intertrial conditioned responses, 2. disinhibition of the conditioned reaction to the first element of the positive compound, and 3. disinhibition of the inhibitory compound. In agreement with the results of the above authors, all these symptoms disappeared gradually in the course of postoperative training.

The difference between the ventro-temporal and prefrontal syndromes is most instructive. In the first case we have a clearcut and permanent abolition of the differentiation between the two compound themselves. No intertrial responses were ever seen in any of our dogs,

and the only deficit observed was that the animals could not discriminate the two types of compound stimuli. On the other hand, after prefrontal ablation, the impairment of internal inhibition was observed with all its consequences.

The results of lesions in the auditory projection areas are much more difficult to explain. We had expected that after these lesions the full syndrome of abolition of auditory recent memory would be present, since it seemed reasonable to assume that the ventro-temporal area functions in close cooperation with the auditory area proper. However, this expectation has proved to be erroneous. Why it is that the removal of the whole area considered so far as the cortical auditory area does not affect our auditory recent memory test, while removal of the area situated beneath does so, is a problem for future investigation. It should be mentioned in this connection that according to the recent study of Desmedt and Mechelse (1959) the ventro-temporal area has to be considered as a part of the cortical auditory system since its ablation gives retrograde degeneration in the posterior part of the medial geniculate body and since this area sends corticofugal fibers which have an inhibitory effect on the activity of the cochlear nucleus. Thus the functional integration of the individual components of the auditory system proves to be a very intriguing problem.

SUMMARY

1. Unilateral ablations of the sylvian or ectosylvian gyri cause no deficit in the performance of preoperatively trained auditory recent memory test.

2. After bilateral removals of the sylvian gyri all three dogs lost their capacity to differentiate between positive and negative compound stimuli and no compensation was observed.

3. The animals showing this deficit in performance of recent memory test were still able to learn the simple auditory differentiation and the conditioned inhibition even when the interval between both components of the compound ranged to 14 sec.

4. Bilateral partial or total ablations of the ectosylvian gyri did not result in any impairment in the performance of the auditory recent memory test.

5. After bilateral prefrontal ablation transient disinhibition of the inhibitory conditioned reflexes was observed. The animal performed the trained movement in the intertrial intervals and to the presenta-

tion of the first tone of the positive compound stimulus. The inhibitory compound stimulus was disinhibited. The inhibitory capacity returned gradually to a normal state within two weeks after operation.

The authors are extremely indebted to Professor J. Konorski for his valuable advice in the performance of these experiments. We are grateful to Drs. J. Srebrzyńska and S. Brutkowski for performing operation on dog No. 4. We wish to express our thanks to the technical assistant Mr. A. Rościak for his careful attention in the postoperative care of our animals. Thanks are also due to Dr. J. P. Cordeau for his valuable suggestions during the preparation of this paper.

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THE EFFECT OF LESIONS OF THE MEDIAL HYPOTHALAMUS ON INTERNAL INHIBITION IN THE ALIMENTARY CONDITIONED REFLEXES TYPE II

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It has been established that lesions in the prefrontal areas cause a significant impairment of the inhibitory alimentary conditioned reflexes (Brutkowski et al. 1956, Brutkowski 1957, Ławicka 1957). As some anatomical connections between the prefrontal area and that of the ventro-medial nucleus of the hypothalamus were found (Le Gros Clark and Meyer 1950), the supposition was made that both areas may be a part of the same functional system and that the ventro-medial hypothalamus may play a role in internal inhibition (Konorski 1958). If it were so, the lesions of the ventro-medial hypothalamus should impair the inhibitory food conditioned reflexes.

The present paper deals with the results of lesions of the medial hypothalamus in rabbits, in which positive and inhibitory alimentary conditioned reflexes type II had been established.

MATERIAL AND METHODS

The experiments were performed on 38 adult rabbits, both males and females, weighing 2–3 kg. In all animals an instrumental conditioned reflex type II was elaborated to the experimental situation, i.e. without the use of any sporadic stimulus. The experimental compartment was a kind of a wooden box, 40×40 cm. with a low (5 cm.) food-tray through which the bowls could be pushed, at one of the walls. The conditioned reaction consisted in putting the right foreleg on the food-tray or in scraping with both forepaws on it. In 7 rabbits a movement of pulling a plastic ring with teeth was used as a conditioned reaction (Malinowski 1952). Every movement was immediately reinforced by food which consisted of potatoes, carrot or oats, depending on the particular animal. The

rabbit remained in the experimental chamber as long as it continued to perform the trained movements, so that the amount of food intake was regulated by the animal itself. After the experiment, the rabbits were given an additional portion of food, the same every day. When the conditioned reaction was firmly established, the sound of metronome, 200/min., was introduced for 1 min. in the course of the daily experiment; during the action of this stimulus the trained movements

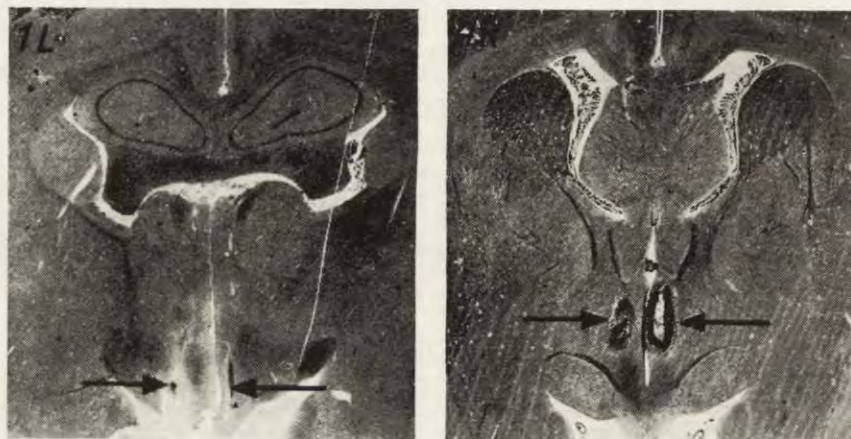


Fig. 1. Frontal sections through the brains of rabbits Nos. 1 L and 4 R, showing typical lesions made in the medial hypothalamus. The arrows indicate the sites of lesions.

were not reinforced by food. The metronome was applied between 5—12 min. after the beginning of the experiment. Sometimes the metronome was applied twice, e. g. in 7th and 15th min. after the beginning of the experiment. After 70—200 applications of the metronome, depending on individual capacity of each animal, an inhibitory conditioned reflex to this stimulus was firmly established, i.e. to the sound of the metronome the rabbit stopped to perform the conditioned movements and went away from the food-tray, returning only after the metronome had stopped. The criterium of the firmly established inhibitory reflex was the absence of trained movements during the action of metronome for 10 successive experiments.

In 9 rabbits an acute extinction of the conditioned reflex was performed. This was accomplished by the cessation of the food reinforcement. As a result the movements ceased to appear after several minutes (Fig. 5a); the course of extinction was very similar to that described for dogs (Konorski and Miller 1933).

Afterwards, an aseptic operation, under Nembutal anaesthesia, was performed, in which 2 unipolar electrodes were inserted in each side of the medial hypothalamus, with the use of an stereotaxic instrument adapted for rabbits by Sawyer, Everett and Green (1954) and according to the anatomical atlas of the same authors. Then coagulation was made with the use of direct current of 3 mA for 15 sec.

Several days after the operation the animal was taken again to the experi-

mental situation and the conditioned reflexes, both positive and inhibitory were tested in daily experiments. The acute extinction was also performed 2—3 weeks after operation in the same way and in the same rabbits as before operation.

About 2—3 months after operation the animals were killed and their brains removed for anatomical control. It was found that in most cases the lesions were made in the medial hypothalamus (Fig. 1).

RESULTS

In 29 of 38 rabbits some changes in behaviour were found after the operation. Most animals became more aggressive then before and some of them even scraped and bit the hand of the experimenter (Romanuk). The rabbits became voracious (Hetherington and Ranson 1942, Anand and Brobeck 1951) and they snatched greedily the food given to them in the home box. Being brought to the experimental box, they started at once to perform the trained movements which became more energetic and more numerous than before operation. (Figs. 2 and 3). The animals became „impatient” in eating and, frequently, having not finished one piece of food, they performed the next movement of putting the paw on the food-tray or pulling the ring with their teeth, and took a new piece of food. Some rabbits had a tendency to keep the paw on the food-tray and knocked strongly with it on the platform to get food. The rabbits remained in the experimental box longer than before, performing the trained movements all the time. The amount of food eaten during the experiment increased to 150—500% in particular animals. In one rabbit the increase of food intake was even 700% for some period (Balińska). When the rabbits were completely satiated in their home-box, just before the experiment, they could still eat in the experimental box, while before operation they did not want to eat more when they had been fed before experimental session.

All these changes developed gradually, so that in most cases, the maximum effect was reached 2—3 weeks after the operation. However, in some animals it was observed earlier. The changes in behaviour lasted usually 6—8 weeks, then the animals gradually returned to the preoperative state.

Several days after the operation, the inhibitory conditioned stimulus, the metronome, was again introduced in daily experiments. It was found that the inhibitory reaction to the metronome was disturbed after operation, i.e. during the action of this stimulus the rabbit performed some trained movements. This disinhibition was however only partial (Fig. 2) in most cases. In 6 rabbits the inhibitory reflex was

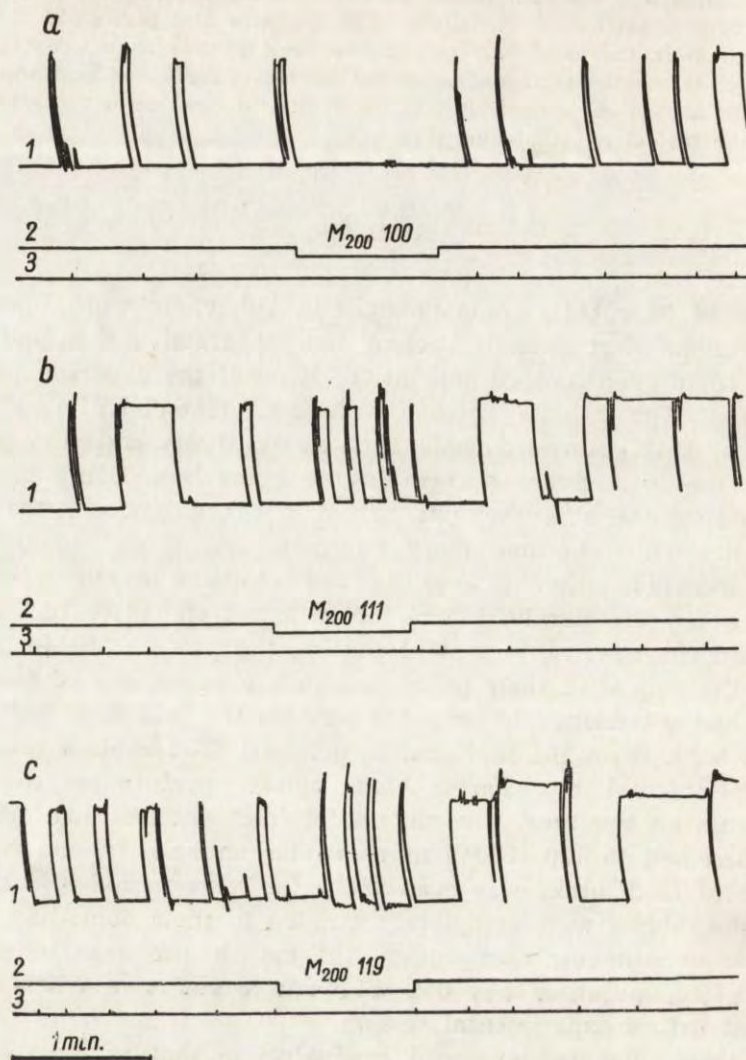


Fig. 2. Kymograms of experiments with rabbit No. 1 L.

A — before operation; B — 3 days after operation; C — 10 days after operation. 1 — trained movement; 2 — action of inhibitory stimulus, M_{200} , — metronome 200/min.; 100, 111, 119, — numbers of successive applications of metronome. As we can see the trained movement does not appear during the action of metronome before operation, while conditioned reaction is present to metronome in the post-operative period. The kymogram C represent the maximum disinhibitory effect. It should be noted that 170% increase of food intake was observed in that time.

fully retained (Fig. 3), i.e. no trained movements were present during the action of the metronome; however, the animals (in which food intake was increased to about 200%) moved restlessly near the food-tray, looked into the bowl, sometimes caught an edge of the food-tray with their teeth etc., which they did not before the operation. The behaviour of all rabbits was similar to that of the initial period of elaboration of the inhibitory reflex, when inhibition was not yet firmly established. However, now the restlessness of the animals was stronger than in the time of elaboration of inhibition.

The degree of inhibition was measured by the length of time in which the rabbit did not perform the trained movements during the action of the metronome. If we take the state of complete absence of the trained movements as 100% inhibition (as happened in the pre-operative period), we can consider the state after operation as on an average 50—75% inhibition, in limits 40—90% (Fig. 4). In most cases

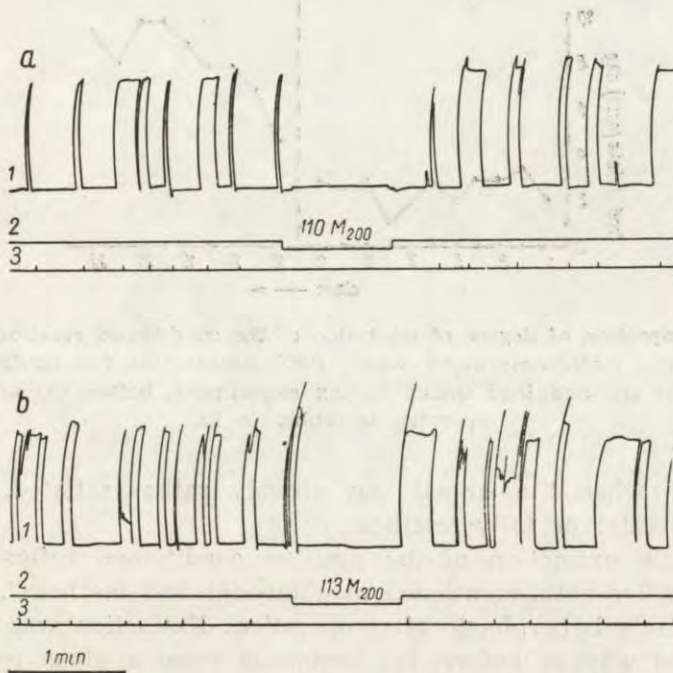


Fig. 3. Kymograms of experiments with rabbit No. 6 L.

A — before operation; B — after operation. Other explanations as for Fig. 2. The conditioned reaction does not appear during the action of the metronome. However, it can be seen in B (post-operative period) that the trained movements are somewhat more frequent and the inhibitory after-effect after cessation of metronome is considerably shorter than in A (pre-operative period). There was 200% increase of food intake in this rabbit after operation.

strong hyperphagia was parallell with rather strong disinhibition. When the animal was starved for 24—48 hours before the experiment, the degree of disinhibition was higher (Lewińska). On the other hand, if the inhibitory stimulus was applied towards the end of the daily

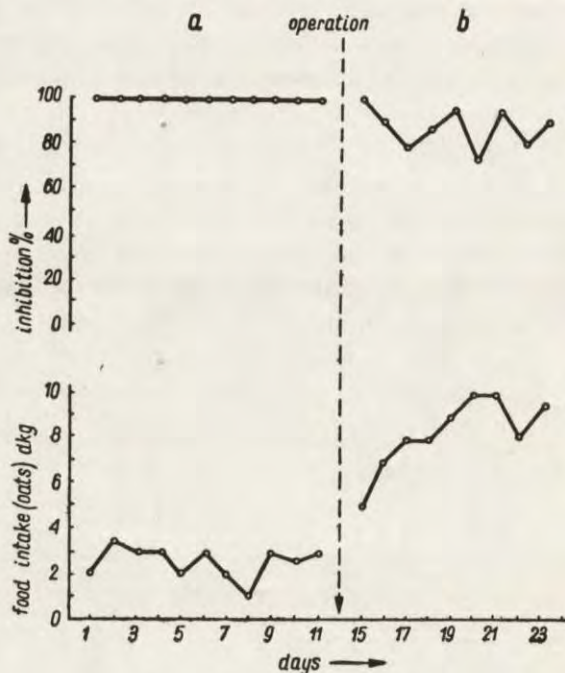


Fig. 4. A comparison of degree of inhibition of the conditioned reaction during the 1 min action of metronome in % (where 100% denotes the full inhibition, i.e. no movements at all) and food intake during experiment, before (A) and after (B) operation in rabbit No. 23.

experiment, when the animal was already rather satiated, inhibition was much better or fully retained.

The acute extinction of the positive conditioned reflex was also repeated in 9 rabbits mentioned in „Material and methods”. All these rabbits became hyperphagic after operation. Extinction was performed in the same way as before, i.e. beginning from a given moment the food reinforcement was no longer given. It was found that the course of extinction of the conditioned reflex was quite similar to that performed before operation; at first the movements became more energetic and more frequent, then they disappeared and the animal went away from the food-tray, returned again after several minutes, and at last after some minutes went away altogether (Fig. 5B). The time

necessary to extinguish the reaction was practically the same as before operation in 7 rabbits and longer in 2 others. In one of these rabbits (No. 34B) in which the food intake during the experiment was increased to 300% in the post-operative period, the time of extinction of the

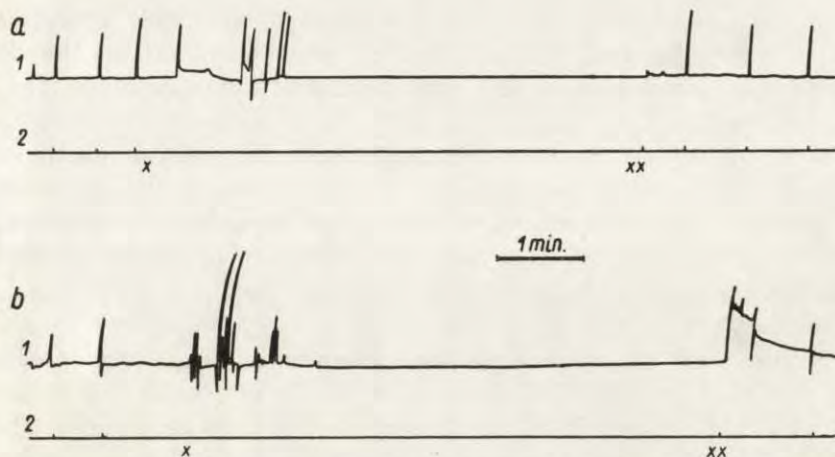


Fig. 5. Kymograms of experiments with acute extinction of the conditioned reaction in rabbit No. 23 B.

a — before operation; b — after operation; 1 — conditioned reaction; 2 — giving of food; x — moment in which the food reinforcement was stopped; xx — moment in which food was reintroduced. As can be seen, the course of extinction of the conditioned reaction is almost the same before and after operation. The rabbit showed on an average 300% increase of food intake in the post-operative period.

conditioned reaction took 5 times longer than before. The recovery of the reflex was accomplished in the usual way, i.e. the rabbits were brought to the food-tray and given the food; as a result, the trained movement reappeared immediately.

DISCUSSION

To understand the phenomenon of disinhibition of the inhibitory conditioned reflex after operation, we must take into account the following facts:

1) Disinhibition of the inhibitory reaction was observed only in those rabbits in which hyperphagia was observed. The inhibitory reflex was unchanged in those animals which showed no changes in food intake after the lesions of hypothalamus.

2) The stronger the increase of the positive conditioned reflex and food intake, the stronger the disinhibition.

3) Disinhibition was never full; the inhibitory reflex was always partly present.

4) In the transitory satiated hyperphagic animals the inhibitory conditioned reflex was fully maintained.

5) The course of acute extinction of the positive conditioned reflex was in most cases practically the same before and after operation.

As we see, the disinhibition obtained was dependent on the degree of alimentary excitation, which was increased after operation; the animals, however, did not lose their ability to stop the instrumental reaction to the inhibitory stimulus and to extinguish the positive reaction when the food reinforcement was not given in the course of the acute extinction. Taking this into account, we may conclude that the phenomenon of disinhibition, obtained in our experiments, is rather secondary, caused by the increased alimentary excitation resulting from the hypothalamic lesions.

On the other hand, disinhibition obtained in dogs after prefrontal lesions seems to be of a different character. It is usually not connected with hyperphagia and is often accompanied by hyperactivity (Brutkowski et al. 1956, Ławicka 1957). It would be interesting to compare the character of the impairment of inhibitory food conditioned reflexes, which was found in the present experiments after hypothalamic lesions, with that after prefrontal lesions in rabbits under the same experimental conditions. This could throw some light on the functional connections between these two structures.

SUMMARY

A positive food conditioned reflex type II to the experimental situation and an inhibitory conditioned reflex to the metronome were established in 38 rabbits. Afterwards, bilateral lesions in the medial hypothalamus of each animal were made by electrocoagulation. It was found that the trained movements became more energetic and more frequent and food intake during the experimental session considerably increased after operation in 27 rabbits. However, when the food reinforcement was not given, an extinction of the conditioned reflex took place as before operation. The inhibitory reflex was partly disinhibited in most animals; usually, the disinhibition more or less corresponded to the increase in food intake. The authors conclude that the impairment of internal inhibition is only secondary, caused by an increase of alimentary excitability resulting from the hypothalamic lesions.

The authors are greatly indebted to Prof. J. Konorski for his suggestions and valuable discussion. Thanks are also due to Dr. Cort for technical advice.

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ANGRY BEHAVIOUR IN DOGS FOLLOWING BILATERAL LESIONS IN THE GENUAL PORTION OF THE ROSTRAL CINGULATE GYRUS

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The past two decades have brought publication of a considerable number of papers on impairment in the previously developed habits and abilities as well as in emotional behaviour following cingulectomy (Smith 1944; Ward 1948; Glees, Cole, Whitty and Cairns 1950; Pribram and Fulton 1954; Rothfield and Harman 1954; Kennard 1955, 1955/56; Mirsky, Rosvold and Pribram 1957; Pechtel, McAvoy, Levitt, Kling and Masserman 1958). The interest in this field has been provoked by the Papez' theory (1937) which suggests that the cingulate cortex is involved in the „circuit” of central mechanism of emotion. Most observations on cats and monkeys have led to a rather consistent conclusion that lesions in this area produce tameness, and diminution of preoperative fear, rage and aggressiveness. These findings have been supported by clinical data that cingulectomy favourably affects „obsessive-compulsive” symptoms as well as irritability, aggressiveness, violence and agitation (Livingston 1953; Le Beau 1954). There are however some experimental indications that occasionally in cingulectomized animals more aggressiveness develops immediately after operation (Kennard 1955; Mirsky et al. 1957; Pechtel et al. 1958).

The purpose of our investigation is, first, to test the changes in general behaviour of dogs with lesions of the rostral cingulate cortex, and secondly to determine the effects of cingulectomy on conditioned reflexes. The present experiment has been undertaken to study the effects of this lesion on behaviour towards man.

MATERIAL AND METHODS

Animals. Observations were made on two groups of dogs comprising each two adult animals. In dogs of Group I, D-15 and D-16, cingulectomy was the first operation, while in dogs of Group II, D-20 and D-21, a bilateral lesion in the amygdaloid complex was placed prior to cingulectomy (Fonberg, Brutkowski and Mempel 1962).

Methods of observations. Observations were of qualitative character. In an attempt to evaluate the possible changes following surgery, each animal was seen nearly every day. He was observed in his cage and in the experimental room, both on the floor and in the Pavlovian frame. The response to stroking and rough handling was carefully noted. The dog was pulled or pinched on the skin of neck and back and bothered with a stick or threatening gestures.

Operations. All operations were carried out under Nembutal anaesthesia with sterile procedure. After skin incision approximately 5 cm. behind the rim of the orbit the temporal muscle attachments were cut in the midline and the

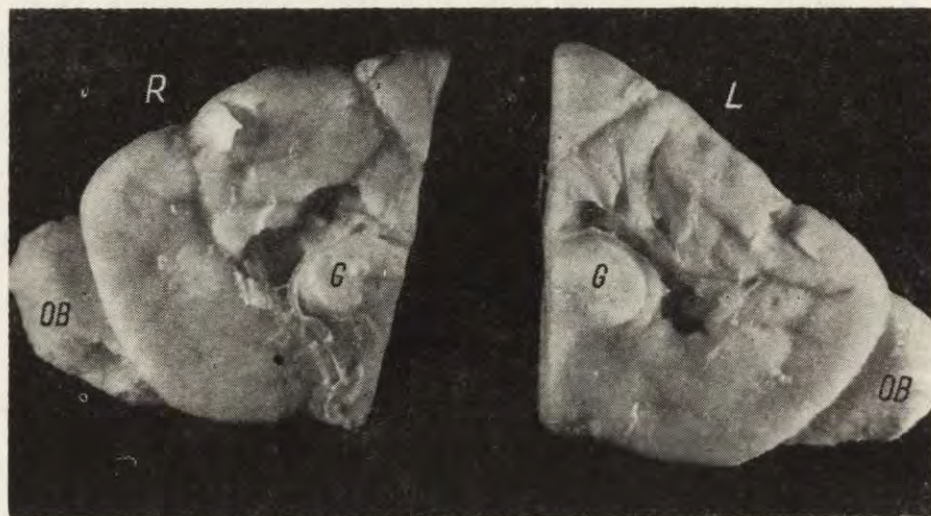


Fig. 1. Lesion of the genual area producing angry behaviour.

The picture shows the medial surfaces of the dog's frontal lobes. G — the genu of the corpus callosum; OB — the olfactory bulb.

muscles pushed aside. The frontal bone was rongeuired in order to gain access to the frontal sinuses. The mucosa was removed from there and the hiati to the sinuses were tightly packed with wax. Then the overlying bone close to the sagittal line was removed, more on the left and less on the right side. The dura mater was opened on the left side only. The medial surface of the left hemisphere was carefully retracted with a spatula in order to bring into the field the cingulate gyrus. The cortex above and in front of the genu of the corpus callosum (area genualis) was removed subpially by suction on both sides simultaneously under good visual control. The septal and subcallosal regions were left intact. Following this procedure the dura mater was closed, the muscles approximated, and the subcutaneous tissue and the skin sutured. The extent of typical lesion is shown in Fig. 1.

RESULTS

Before anterior cingulectomy the normal and amygdalectomized animals were rather timid, running away when approached or disturbed. They also frequently exposed themselves to petting or stroking. Subjected to rough handling, they tried to escape showing neither



Fig. 2. Excerpt from a moving picture showing the behaviour of D-21 in response to a threatening object before (a) and after (b,c) ablation of the genual region.



Fig. 3. D-20. The same as in Fig. 2.

struggling nor biting (Figs. 2a and 3a). Even severely noxious stimuli did not produce any rage responses.

After simultaneous bilateral removal of the rostral cingulate gyrus the behaviour of all the four animals changed remarkably.

In the immediate postoperative period of about 10 days the dogs were hypomotile. Left alone, they usually rested on the floor or — less frequently — they slowly circled around the room perseveratively, head and tail having drooped. Very often they approached the corners

of the room or walked into narrow passages between the furniture and kept standing for hours there. Walking was clumsy although there were no apparent motor disorders. They preferred dark spots in the room and used to hide under the chairs and other small pieces of furniture. However, they omitted obstacles and ate spontaneously.

In this time, and also later, they showed apparent savageness and violent aggressiveness in the presence of man. When approached, they growled and grimaced. They did not attack actively but they bared their teeth and attempted to bite, showing increasing anger even if the experimenter or the animal's caretaker tried to stroke them or exhibited other friendly gestures (Figs. 2b and 2c, 3b and 3c, and 4a and 4b).

They seemed to be afraid of being touched. They struck at the offending objects, attempted to bite investigator's hand and struggled



Fig. 4. Excerpt from a moving picture showing the behaviour of D-15 in response to a threatening object after ablation of the genital area (a,b.), and after superimposed amygdalectomy (c).

vigorously when placed in the harness (what never happened before operation). When the skin was lightly pulled or pinched, the angry behaviour became even more marked and the observer was in obvious danger. Two or three people had to cooperate to restrain a genital dog or to put him in the Pavlovian frame. Briefly, when before surgery the animals responded to the rough handling by only trying to escape, they later reacted to even mild stimuli with progressively severe and immediate rage. Such behaviour continued for three months of post-operative observation although it tended to diminish with time.

Three months after the removal of the genital areas, bilateral amygdalectomy was performed in D-15. This operation resulted in changes which did not differ in any way from those described by many other authors previously (Cf. Fonberg, Brutkowski and Mempel 1961). This dog became fearful of man and tame. The aggressivity disappeared almost completely (Fig. 4c).

DISCUSSION

From the above description appears that angry behaviour occurred in all the genual cingulate dogs apart from the fact whether they had been subjected to amygdectomy or not operated before. The effect of lesion was clear-cut, although the changes were most severe in the immediate postoperative period and then slowly subsided.

These data are contrary to many findings obtained by previous investigators on cats and monkeys, indicating that anterior cingulate animals lose their preoperative shyness but they do not become aggressive. The temporary increase in aggressive behaviour in cingulectomized monkeys was reported only by Mirsky et al. (1957) and Pechtel et al. (1958). Kennard's (1955) cingulate cats were not aggressive but rage reactions were more easily elicited in them.

The different effects may be due to species differences or degree of domestication. It is more likely though that the structures removed were not exactly comparable in all investigations. As far as the Kennard's cats are concerned, it appears from her illustration (Fig. 1) that the lesion was mainly placed in the splenial gyrus and slightly destroyed the posterior cingulate gyrus. It seems that the anterior division of the cingulate gyrus was left intact. This might explain the behaviour of her animals which was chiefly characterized by "a plastic tendency whereby the animal may be posed for long periods of time in bizarre positions". On the other hand, the lesions made by Mirsky et al. did not confine to the rostral cingulate gyrus but involved also medial frontal and polar cortex. In addition, the corpus callosum and the orbital cortex were invaded in all animals. All these ablations largely differ from those of our dogs which were small and limited to the region dorsally and anteriorly of the genu of the corpus callosum (area genualis). They are rather somewhat similar to the lesions made in monkeys by Glees and his associates (1950) in spite of different effects.

Thus the function of the rostral cingulate cortex in the angry and aggressive behaviour of various species remains contradictory and requires further investigations.

Another question is that of the nature of aggressiveness of genual dogs.

Smith (1944) describes a "goose flesh" appearance of the skin in his cingulectomized monkeys and also piloerection. "Blowing on the erect hair -he says- causes a vigorous startle reaction as if there is an increased cutaneous sensibility to this type of stimulus".

We found that our dogs occurred aggressive when approached, and we have had the impression that one of the reasons of such behaviour was that the animals were afraid of being touched. Therefore, even stroking evoked anger and violent rage. This would suggest that aggressiveness in genual dogs might result from an increase in sensibility to the tactile stimuli. This increase was appreciable since not only touching but approaching the animal elicited a marked rage. This indicates that even conditional tactile stimuli produce aggressiveness in genual dogs.

These observations closely resemble those made long ago by Babkin (1909) who performed frontal lobectomies in a few dogs in Pavlov's laboratory. The lesions in Babkin's dogs were extensive and it is quite possible that they included the rostral portion of the cingulate cortex *regio genualis*. Following this operation a dramatic excess in sensibility of the skin occurred (Pavlov 1940). This result was confirmed by other Pavlov's co-workers (Demidov 1909; Saturnov 1911; Kuraev 1912) who also extensively removed the frontal lobes in their dogs. It is worth while to note that similar observations have been made on a good number of frontal dogs in our laboratory when the lesions extended posteriorly of the presylvian sulcus and into the depth of the prefrontal lobes.

Thus the aggressiveness in genual dogs and -occasionally observed- in other species is not necessarily of emotional origin but may result from changes in some sensory functions.

SUMMARY

1. In two normal and two amygdalectomized dogs, in which genual portion of the rostral cingulate area was removed, angry behaviour occurred postoperatively. This symptom persited for three months of observation although it tended to diminish with time.

2. In one genual dog amygdalectomy was superimposed. This operation abolished the previous rage responses almost completely.

3. Assumption has been made that the angry behaviour in genual dogs may result from an increase in sensibility to tactile stimuli.

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THE EFFECT OF DEAFFERENTATION ON INSTRUMENTAL (TYPE II) CLEANING REFLEX IN CATS

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There is a discrepancy in the literature concerning the effects of deafferentation on instrumental reflexes. First Mott and Sherrington (1895), on the basis of their experiments on apes, came to the conclusion that deafferentation practically abolishes voluntary movements in the deafferented limb. Their finding seemed to be supported by other authors (Kopczyński 1907; Lassek 1953; Twitchell 1954 and others).

In recent experiments, however (Knapp, Taub and Berman 1958; Jankowska 1957, 1959; Górska and Jankowska 1959) it appeared that deafferentation of a limb does not prevent the animal from performing with this limb the instrumental movements established prior to deafferentation. Knapp et al., in their experiments used the avoidance reflex of the upper limb, established by means of nociceptive stimulation of the contralateral limb. They found that after deafferentation on the "avoidance side" the reflex disappeared for some time but then would reappear gradually. The authors suggest that the animals were retrained. In our experiments in which the instrumental scratching of the ear region was used, deafferentation of the hindleg which was performing the movements did not abolish this reflex. As shown by Sherrington (1906) the unconditioned scratch reflex is not affected by deafferentation of a limb and is evoked by appropriate stimulation of the skin. It is possible that in the instrumental scratch reflex the information from the limb likewise plays no essential part and that the conditioned movements are evoked merely by some sort of excitation or sensitization of the ear region (Jankowska and Sołtysik 1958). Such a sensitization could be established during the training experiments

in which the scratch reflex is specially evoked in the experimental situation and then reinforced by food. This could explain why deafferentation of a limb, leaving intact the receptive field of the unconditioned scratch reflex does not abolish the conditioned scratching.

If so, the denervation or local anaesthesia of the ear region should abolish the instrumental scratch reflex. It was done by Kozak and Norsell (1959) in normal cats. In their experiments they anaesthetized the ear so that the unconditioned scratching on stimulation of this ear was abolished. The instrumental scratch reflex on the same side, however, did not disappear under this condition.

In the present experiments we tried to verify the above hypothesis by denervation of the receptive field of the unconditioned reflex in normal as well as in animals with deafferentation of the appropriate limb. As a test we used the instrumental reflex of cleaning the anal region. This reaction has much in common with the instrumental scratch reflex but denervation of the receptive field is much easier to accomplish.

MATERIAL AND METHODS

Experiments were performed on 9 cats. In each animal an alimentary instrumental reflex of lifting one of the hindlimbs as for cleaning the anal area was established by evoking and then reinforcing the appropriate movements. The

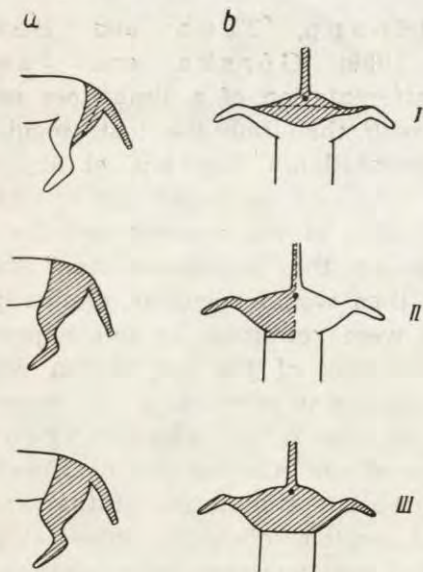


Fig. 1. Area anaesthetized after section of dorsal roots in cats in different experimental groups

a — lateral view, b — ventro-medial view; I, II, III — experimental groups (in group I the interrupted line denotes the rostral limit of the anaesthetized area in cat No. 85).

preoperative training included 13–22 experimental days. Since each experimental session consisted of about 20 trials, the total number of reinforced movements ranged from 220–440. Conditioning was carried out in a great glass cage, in which the animal was allowed to move freely. Small bits of boiled meat were

used as reinforcement, and the experimenter offered them to the cat through a hole in the front wall of the cage.

Three variants of operation, each of them on three cats, were performed. In the first experimental group the anal region and partially both hindlimbs were deafferented. In the second one, deafferentation of the limb taking part in the movements and unilateral denervation of the anal area were performed. In the third group both hindlimbs, as well as the whole anal area were deafferented.

In the first group all the dorsal roots which innervate the anus and the areas either closely adjacent to it (L_7 —Coc₁) or even more remote (L_4 , L_5) were sectioned. In the second group the deafferentation involved all the dorsal roots caudal to L_2 . In the third group the cats were deafferented bilaterally from L_2 or L_3 . As the sacral and coccygeal roots were sectioned at the level L_6 — L_7 or some millimeters below, where no precise identification of respective dorsal roots is possible, deafferentation involved nearly all coccygeal roots, since we cut all the visible dorsal roots at this level. The extent of deafferentation in different experimental animals is shown in Table I, while in Fig. 1 the resulting

Table I

The extent of deafferentation in different experimental groups

Experimental group	Cat No.	Kind of deafferentation	Extent of deafferentation
I	85	bilateral	L_7 —Coc ₃
	92		L_4 , L_5 , L_7 —Coc ₃
	93		L_4 , L_5 , L_7 —Coc ₃
II	89	unilateral	L_2 —Coc ₃
	91		L_2 —Coc ₃
	100		L_2 —Coc ₃
III	90	bilateral	L_2 —Coc ₃
	95		L_3 —Coc ₃
	102		L_3 —Coc ₃

anaesthetized areas (determined by pricking) in the various groups are presented.

The operation was performed under aseptic conditions with nembutal anaesthesia (40—45 mg./kg. of body weight). The dorsal roots were cut intradurally, proximal to the spinal ganglions. The operative technique has been described by Jankowska (1959).

The testing of conditioned reflexes started 6—15 days after operation, depending on the general state of the animal. It was followed up by standard experiments during the following 4—12 weeks (11—18 experimental sessions). In the majority of animals the number of reinforced movements was more or less the same as during preoperative training and amounted to at least 200 trials*.

* Except for two cats, Nos. 85 and 90, which had to be sacrificed before the end of experiments.

RESULTS

I. Elaboration of the instrumental cleaning reflex in normal animals

The unconditioned reflex of cleaning the anal area consists of the following successive reactions: 1° the cat sits down on one side of its rump; 2° it lifts up, almost vertically, the opposite hindlimb; 3° it turns the head, neck and rostral part of the body backwards; 4° it starts, in this position, to lick the anus, buttocks, internal parts of the thigh etc. The unconditioned stimulus of this reflex consists in the excitation of skin receptors of those areas involved in the act of licking. The process of conditioning this reflex occurs in a way similar to conditioning the scratch reflex.

At the beginning of training (first two experimental days) the unconditioned reactions were provoked by wetting the cat's hair around the anus*. Each such reaction was immediately reinforced by the presentation of food. After several repetitions of this procedure, usually by the third experimental session, the animals began to display these reactions in the experimental situation without previous moistening of the anal area. Gradually the instrumental reactions became more and more frequent, and after some time the animals performed the movement almost immediately after they had finished eating the food ration reinforcing the preceding reaction.

Parallel to the increase in frequency of instrumental reactions, the pattern of the conditioned reflex became more and more simplified when compared with that of the unconditioned reflex. In the first period of training already the animals desisted from a protracted act of licking the moistened anal area and after passing the tongue over it once, immediately turned towards the experimenter and waited for food with a raised limb. In further experiments the act of licking completely disappeared and eventually the conditioned reflex became reduced to the cat's sitting down on one side of its rump and lifting up the opposite hindlimb with flexion in all joints. Examples of movements performed at the beginning and end of training are shown in Fig. 2**.

* The wetting of the adjacent parts of the body, e.g., more lateral parts of rump and thighs also evoked the unconditioned reflex of licking the soaked areas, but without lifting the hindlimb.

** In this series of experiments the lifting of the hindlimb, without licking the anal area, was reinforced. The process of conditioning of this reflex, when only its full form is reinforced, will be described in another paper.

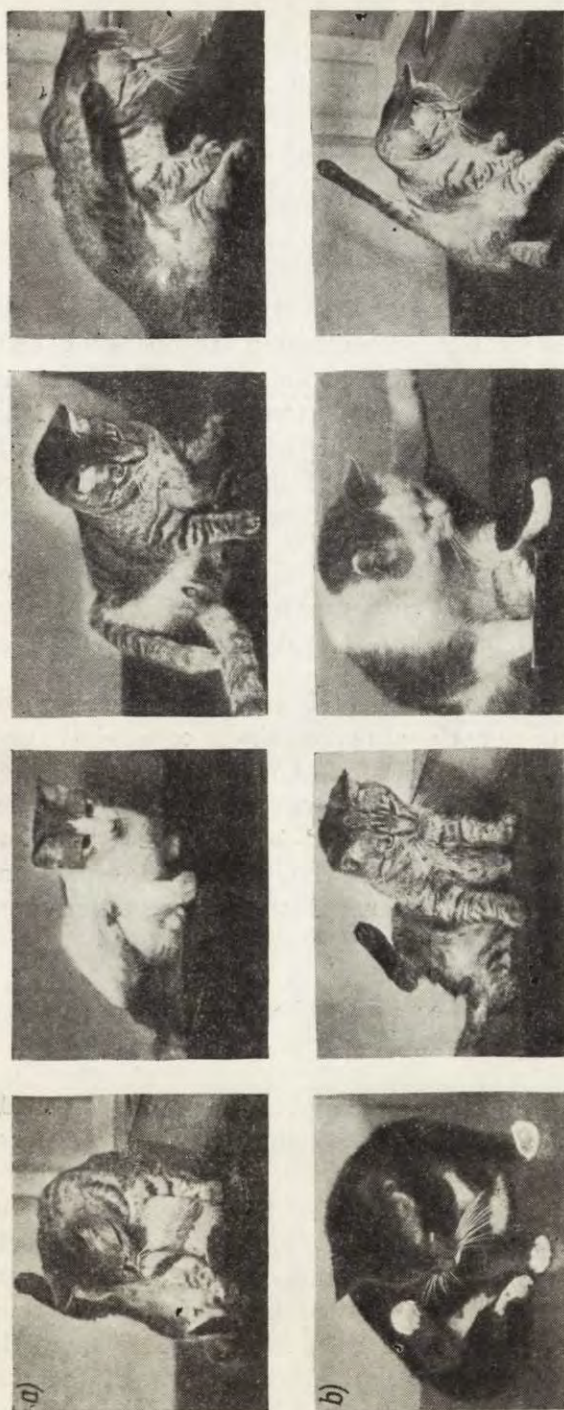


Fig. 2. Examples of motor conditioned reactions before and after various kinds of deafferentation.

A — normal animals, I, II, III — cats from respective experimental groups; I — movements at the beginning (a) and end (b) of training; I — both movements (a) and (b) are identical as in normal animals; II — movement of 1st (a) and 3rd (b) categories (in b cat with a conditioned reaction of the hindlimb); III — movements of 2-nd (a) and 3rd (b) categories.

The acute extinction of this reflex led, in normal animals, first to reappearance of turning the head and neck towards the anus and later also to sporadically observed acts of licking the anal area. This proves that the movements of lifting the hindlimb really derived from the unconditioned reflex of cleaning the anus.

II. Postoperative state of reflexes

In all the animals the instrumental reactions established during preoperative training were also present after the operation and during the entire period of postoperative examination they remained at a relatively constant level. However, since the quality of the performed movements was somewhat different in the various experimental groups, the more detailed analysis of the effects of deafferentation of the limbs and anal region will be described separately.

1. Denervation of the receptive field of the unconditioned reflex of cleaning the anal area.

In all cats of the first experimental group the conditioned reflex of lifting the hindlimb for cleaning remained completely unchanged after operation (Table II and III, Fig. 2). On the other hand the unconditioned reflex of cleaning the anal area disappeared completely as shown in special tests and as indicated by the fact that the rumps of the operated cats were very dirty. The limits of the uncleared hair precisely corresponded to the extent of the anaesthetized area.

2. Unilateral deafferentation of the hindlimb and unilateral denervation of the anal area.

In the second group of experimental animals the learned movements performed with the deafferented limb markedly differed from those executed in the preoperative period. This was probably due to the strong curving and twisting of the spine towards the normal limb, which made it difficult to the animals to assume the correct sitting posture and consequently to perform the conditioned movements.

The instrumental reactions performed by cats of this group could be divided into the following three categories: 1° Flexion in the thigh and knee joints, often connected with an abduction of the thigh, but without lifting the foot off the floor; 2° movements similar to the

proper reactions, consisting of flexion in all the joints and connected with raising of the foot. They were of lesser extent than before operation, and without maintaining the leg in the air (in this position the animals lost their equilibrium); 3° movements analogous to the proper reactions, consisting of a relatively high lifting of the affected limb, but performed chiefly in the thigh joint, whereas the remaining part of the leg was in extension. Examples of movements of the first and third categories are shown in Fig. 2, while in Table II the percentages of all these categories are computed.

Table II

The quality of conditioned movements after various kinds of operation

Categories of movements	Percentage of different categories of movements											
	Group I				Group II				Group III			
	Cat 85	Cat 92	Cat 93	Mean	Cat 89	Cat 91	Cat 100	Mean	Cat 90	Cat 95	Cat 102	Mean
1° Flexion in thigh and knee joints, without lifting the foot off the floor.	0	0	0	0	28	72	41	47	37	8	32	25
2° Movements similar to proper reactions, but small and with an immediate lowering of the limb.	0	0	0	0	65	28	52	48	21	12	23	19
3° Movements analogous to proper reactions, relatively high, but with a marked extension in distal joints	0	0	0	0	7	0	7	5	42	80	45	56
4° Movements identical to proper reactions	100	100	100	100	0	0	0	0	0	0	0	0

The comparison of the frequency of instrumental reactions before and after operation (Table III) in cats with unilateral deafferentation shows a marked diminution in the first period after operation. This was connected with the fact that in this stage the worst movements, i.e. those classified in the first category were often unreinforced, in order to provoke the animals to execute movements more similar to the proper ones. This procedure, however, was unsuccessful for it led

to a partial extinction of instrumental reactions, as a result of their irregular reinforcement. In view of this fact this method was rejected in further experiments.

Table III

Frequency of conditioned movements before and after operation in different experimental groups*

Experimental group	Cat No.	Before operation (mean from last 100 movements)	After operation	
			Mean for first 50 movements	Mean for last 50 movements
I	85	11.4	13.7	—
	92	7.5	5.3	5.3
	93	6.9	7.6	6.4
	Mean	8.6	8.9	5.9
II	89	7.5	14.0	8.3
	91	5.4	16.1	7.8
	100	8.1	18.1	6.5
	Mean	7.0	16.1	7.5
III	90	8.2	7.3	—
	95	10.0	9.9	5.7
	102	5.4	30.1	3.1
	Mean	7.9	15.8	4.4

*Mean interval in secs between the end of eating the food ration which had reinforced the last movement, and the next reaction.

In all animals of this group wetting of the anal area outside the experimental cage provoked the unconditioned cleaning reflex involving, however, only the normal limb.

3. Bilateral deafferentation of hindlimbs and denervation of the anal area.

The instrumental reactions performed by cats in this group could be divided approximately into the same categories as the movements performed by cats of the second group. The comparison of the percentage of movements, belonging to the above categories shows, however, that the animals of the third group executed many more reactions similar to the proper ones, than did cats from the second group, and the quality of these movements was much better (Table II, Fig. 2).

This was probably due to the fact that bilaterally deafferented animals had no difficulties in assuming the proper posture, since they were placed in the required position in the experimental cage. The frequency of instrumental reactions after operation (Table III) was generally unchanged (except cat No. 102).

The unconditioned reflex of cleaning the anal area disappeared in all animals of this group as it did in the cats of the first group.

DISCUSSION

The results described above show that deafferentation does not abolish the instrumental reflex of lifting the hindlimb as for cleaning the anal area, established during preoperative training. These results are in conformity with our previous findings concerning the effect of deafferentation on the instrumental scratch reflex. They also seem to prove that the performance of instrumental reflexes does not depend on the receptive field of the unconditioned reflex, since the denervation of this receptive field does not abolish the respective instrumental conditioned reflexes. The above results are in agreement with those on the instrumental scratch reflex in normal cats (Kozak and Norsell 1959). They contradict the hypothesis according to which the process of instrumentalization of the unconditioned reflexes consists of a gradual sensitization of their receptive fields.

We now have to consider the question of the cause of the discrepancy between the results obtained by us and Knapp et al., and those obtained by other authors. In our opinion these contradictory results are due to the differences between the reflexes under investigation.

The motor conditioned reflexes, like the classical ones, can be divided into "natural" reflexes, i.e. those which are established in the course of the animal normal life, and "artificial" reflexes, i.e. those which are elaborated by the experimenter under laboratory conditions. For example, all movements of the animal occurring in its ordinary behaviour, such as stretching out a limb for food which is directly unavailable, grasping of objects or removing a source of nociceptive stimulation, could be classified as "natural" conditioned reflexes. On the other side, instrumental scratching and cleaning of the anal area belong to the category of "artificial" conditioned reflexes since they have nothing in common with getting food. The different ways in which these two kinds of reflexes are formed may be the cause for the differences after deafferentation. In natural instrumental reflexes there exists a strong tendency to replace a movement which in a given

situation is ineffective either by other movements performed with the same extremity, or, more often, by an analogous reaction of the symmetrical limb. On the contrary, in artificial instrumental reflexes this latter tendency does not exist, since in the course of training only a definite movement of a given extremity, and never the analogous movement of the symmetrical limb, was reinforced. It seems that this difference might be considered as a decisive factor in regard to the effect of deafferentation on the instrumental conditioned reflexes.

The disappearance of the "natural" conditioned reflexes after deafferentation is probably due to the change of balance between the analogous reflexes of symmetrical limbs, like in the case of the unilateral ablations of the sensori-motor cortex (Jankowska and Górská 1960) where the movements of the unaffected extremity became predominant. Therefore the disappearance of the "natural" movements after deafferentation seems to be brought about not so much by an absolute inability to perform voluntary movements with the affected limb, as by their replacement by movements of the symmetrical limb. On the other hand, in the case of "artificial" conditioned reflexes the motor centre for movements of only one limb should be excited as a result of the preoperative training. After deafferentation this reaction, despite its impairment, is still reinforced. In consequence, the movements of the affected limb are neither replaced by those of the normal leg nor extinguished. Incidental observations concerning the ability to perform with the deafferented limb even some complicated natural movements, like grasping, in a state of emotional excitement (Mott and Sherrington 1895; Lassek 1953; Lassek and Moyer 1953) support our hypothesis concerning the relation between the execution of a voluntary movement and sufficient excitation of the respective motor centres.

SUMMARY

The aim of the present paper was to reexamine the effect of deafferentation on the instrumental (type II) conditioned reflexes. It has been found that in cats neither deafferentation of the limb engaged in the movement, nor denervation of the anal area, abolish the instrumental cleaning reflex established by preoperative training. These results have confirmed our previous findings on the possibility of execution of conditioned scratch movements with a deafferented limb. They have shown, moreover, that this fact could not be interpreted as due to the sensitization of the receptive field of a given unconditioned reflex. The different effects of deafferentation on "natural"

and "artificial" motor conditioned reflexes are discussed. A hypothesis has been put forward according to which the disappearance of "natural" conditioned reflexes after deafferentation is caused by their extinction and substitution by more effective movements of the symmetrical limb, whereas in "artificial" instrumental reflexes this process could not take place and therefore these reflexes are, after deafferentation, still present.

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THE EFFECT OF DEAFFERENTATION ON INSTRUMENTAL (TYPE II) CONDITIONED REFLEXES IN DOGS

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In our previous experiments on cats and rats (Jankowska 1957, 1959; Górska and Jankowska 1961) it was found that the animals are able to perform some learned movements with a deafferented as limb in order to obtain food. These results concerned the instrumental scratch reflex and the instrumental reaction of lifting the hindlimb in the cleaning reflex. Both reflexes were established before the operation by food reinforcement of the respective unconditioned reflexes. The mechanism of instrumental reflexes derived from some complex unconditioned reflexes is, in comparison with other simpler conditioned motor reactions, not well understood. The question therefore arose of the effects of deafferentation on instrumental reflexes consisting of a single movement and established by routine methods (e.g., reinforcement of passive movements). Moreover, in our previous experiments the quality of instrumental reactions of the deafferented limb could not be precisely analysed, owing to the difficulties in recording (the animals moved freely in the experimental cage). In the present series of experiments the effects of deafferentation were studied in dogs, in which well defined, simple motor conditioned reactions have been established and analyzed in a proper way.

MATERIAL AND METHODS

Experiments were performed on 12 dogs. In each animal an instrumental conditioned reflex to an auditory stimulus was established. The instrumental reaction consisted either of high flexion of the hindlimb, or of lifting the foreleg and putting it on a high platform. In 4 dogs the presentation of food was used as reinforcement, in 8 other animals, an electric shock, 50 c/s, applied either to the ear or to one of the legs. In these 8 dogs the instrumental avoidance reflexes

were established, i.e. if the dog performed the instrumental reaction to the conditioned stimulus, the nociceptive stimulus was not given, but if the dog did not execute the proper movement during a definite time of conditioned stimulus exposure (5—7 sec.), the electric shock was applied. It was switched off only when the dog performed the learned reaction (so-called escape reaction).

The instrumental reactions were established either by reinforcing the passive movements or the unconditioned defensive flexion reflex. In two of 4 dogs with alimentary reflexes, passive movements were used, in the other two the reinforced motor reaction was provoked by application of the electric shock to the foot at the beginning of training. Among 8 dogs with avoidance reflexes, passive movements were used in 5 (in this case the electrodes were fixed either to the animal's ear or to one of his paw, but not to the limb whose movements were trained), and in 3 other dogs, the avoidance reflexes were elaborated from the classical defensive reflexes, i.e., the electric shock was applied to the same limb which was engaged in the execution of the movement. The training of instrumental reflexes was carried on according to the normal procedure of elaboration of type II conditioned reflexes. Experiments were conducted in a typical sound-proof conditioned reflex chamber.

The preoperative training lasted for 3—6 months, up to the moment when the instrumental reflexes were firmly established. It consisted in different animals of 35—101 experimental sessions, with 6—8 trials per session, i.e. 235—540 trials with the positive conditioned stimulus. In two dogs (Nos. 16 and 17) the instrumental reflexes had already been previously established*. In the final preoperative period (100 trials preceding the operation) the instrumental reactions to the positive conditioned stimulus were present in all the dogs in 97—100 percent of trials.

In the course of training special attention was paid to the amplitude of instrumental movements. To the conditioned stimulus the animals had to react with fairly high flexion and only such movements, and not those of lesser amplitude, were in principle considered as a correct reaction. This was achieved in the majority of dogs by using a special device which automatically either presented the bowl with food or, in avoidance reflexes, switched off the stimulus only when the animal had raised its limb sufficiently high.

In 10 out of 12 dogs, in addition to the positive conditioned reflex, an inhibitory reflex was established to another auditory stimulus much different from the positive one. During the entire training the inhibitory stimulus was applied 35—70 times. In the last 20 trials before operation the number of correct reactions to the inhibitory stimulus amounted, in all the animals but one, to 90—100 percent.

Surgical procedure

In all the animals deafferentation of the limb engaged in the movement was performed. For the hindlimb, the extent of deafferentation varied from L₁ or L₃ to S₄ or Coc₁ (9—12 roots), when the foreleg was deafferented all the dorsal roots from C₅ to Th₃ or Th₄ (7—8 roots) were sectioned. The dorsal roots

* These dogs had been trained by Dr. A. Zbrożyna, and in both animals the prefrontal area of the cortex had been ablated about 2 years before deafferentation. This operation did not affect the positive conditioned reflexes.

were transected extradurally, proximal to the spinal ganglions. In longer roots a piece of several millimeters was excised and the ganglion was, if possible, crushed. The shorter roots were simply sectioned.

In all animals some ventral roots were also severed in addition to the sectioned dorsal roots. This had to be done with all ventral roots which strongly adhered to the dorsal roots, such as the upper lumbar (L_1 , L_2) and lowest sacral (S_3 , S_4) anterior roots for the hindlimb, and ventral root C_5 for the forelimb. The number of transected ventral roots varied in different animals from 1 to 4. The extent of deafferentation and the ventral roots additionally cut are shown in Table I.

The operation was performed under aseptic conditions with nembutal anaesthesia (40—45 mg/kg of body weight). The operative technique did not differ from the analogous operation in cats (Jankowska 1959), except for the fact that in dogs the dorsal roots were cut extradurally, while in cats intradurally.

Macroscopic verification of the sectioned roots indicated that deafferentation within the required limits was complete, and sensory tests (pricking) likewise indicated complete anaesthesia.

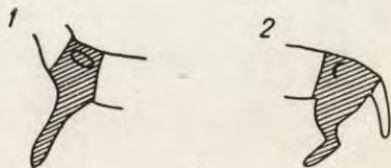
THE GENERAL STATE OF THE ANIMALS AFTER DEAFFERENTATION

Deafferentation in dogs was followed by a number of symptoms identical with those found in other animals. These symptoms are described here in order to give a better picture of the experimental animals.

1. Cutaneous sensation

In all dogs section of the dorsal roots, within the limits described above, resulted in a complete loss of cutaneous sensitivity (reaction to pricking) over the entire surface of the limb. When the hindlimb was deafferented, the limits of the

Fig. 1. Area anaesthetized after section of respective dorsal roots in dogs.



anaesthetized area varied, according to the extent of deafferentation, rostrally from 2—6 cm. in front of the pelvis and caudally 3—10 cm. beyond the base of the tail. For the forelimb, the anaesthetized area extended from about 3 cm. in front of the anterior border of the shoulder-blade, to about 3—4 cm. to the back of the posterior border of this bone. The typical extent of the anaesthetized area following deafferentation is shown in Fig. 1.

Dog No.	Kind of reflex	Movement of leg	Kind of movement	Kind of training	Electrode position
2	Avoidance	Left hindleg	Moderate flexion	Classical defensive reflex	Left hind-leg
3	Avoidance	Left hindleg	Moderate flexion	Classical defensive reflex	Left hind-leg
4	Alimentary	Left hindleg	Moderate flexion	Classical defensive reflex	Left hind-leg
5	Alimentary	Right hindleg	High flexion	Passive movements	—
8	Avoidance	Left hindleg	High flexion	Passive movements	Right hind-leg
9	Avoidance	Left hindleg	High flexion	Passive movements	Left ear
12	Alimentary	Right hindleg	High flexion	Classical defensive reflex	Right hind-leg
13	Avoidance	Left hindleg	High flexion	Classical defensive reflex	Left hind-leg
14	Avoidance	Left hindleg	High flexion	Passive movements	Left ear
15	Alimentary	Right hindleg	High flexion	Passive movements	—
16	Avoidance	Right foreleg	Putting on platform, or high flexion	Passive movements	Right hind-leg
17	Avoidance	Right foreleg	Putting on platform or high flexion	Passive movements	Left hind-leg

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Extent of deafferentation	Ventral roots additionally cut	Postoperative character of movements	Remarks
$L_1 - S_3$	L_1, S_3	Unchanged during the whole period; marked extension of a limb	
$L_1 - Coc_1$	L_1, L_4, S_4, Coc_1	Unchanged for first 20 trials, experiments then interrupted	
$L_2 - S_3$	L_4, S_2	Unchanged during the whole period; marked extension of a limb	
$L_1 - S_3$	S_2, S_3	Unchanged for first 24 trials, experiments then interrupted	
$L_2 - S_4$	L_2, S_3, S_4	Somewhat irregular and lower than before, chiefly in the 1st period marked extension of a limb	Strong neurotic symptoms, which diminished in the 2nd period
$L_3 - S_4$	S_3, S_4	Unchanged during the whole period; marked extension of a limb	
$L_3 - Coc_1$	S_3, S_4, Coc_1	Unchanged during the whole period; marked extension of a limb	
$L_2 - S_4$	L_2, L_3, S_3, S_4	Unchanged during the whole period	
$L_2 - Coc_1$	L_2, S_3, S_4, Coc_1	Unchanged during the whole period	Slight neurotic state in 2nd period
$L_2 - S_4$	S_2, S_3, S_4	Unchanged during the whole period	
$C_5 - Th_3$	C_5	First unchanged, then gradual diminution of amplitude and increase in latency of movements	Chronic inflammation of the limb beginning 4 weeks after operation
$C_5 - Th_4$	C_5	Unchanged during the whole period	

2. Position of the deafferented limb and walking

The deafferented dogs began to get up and tried to walk within 2–5 days after operation. At the beginning, while standing, they kept the affected extremity contracted and, while walking, they hopped on three legs. After some days, when in an upright position, they put the operated limb on the floor, often on the dorsal part of the toes, and tried to support themselves on it. The normal pattern of movements during walking, i.e. rhythmic and alternative flexions of the deafferented extremity reappeared in the majority of animals 2–3 weeks after operation.

In 5 dogs with a deafferented hindlimb (out of 10) a regression in the process of walking was observed 3–4 weeks after operation. This was due to a strong curving and twisting of the spine towards the normal limb which, in the worst cases, prevented the animals from putting the affected limb on the floor and even from maintaining an upright position (e.g., dogs Nos. 3 and 5). This curving of the spine was the more distinct the more extensive was the deafferentation. In order to partly counteract this process we took special care not to let the animals sit or lie on the deafferented side.

In half of the animals with a deafferented hindleg a marked tendency to keep the affected extremity in extension was observed 4–6 weeks after operation. The deafferented limb, completely flaccid directly after operation, became gradually more rigid and resistive to passive flexion. The extension of the deafferented limb was more marked in the distal than in the proximal joints (cf. Bickel 1897; Ranson 1928; Ranson, Hinsey and Taylor 1929; Hnik 1956; Jankowska 1959).

The tendency to extend the affected limb caused a change in its position during walking and standing. While standing the dogs kept the affected extremity stretched forward and when walking, movements were usually limited to the thigh joint. In the case of deafferentation of the foreleg no tendency to keep the operated limb in extension was noticed, but rather, a tendency to flexion could be observed.

In 4 dogs oscillating movements of the affected leg synchronous with respiration were observed (Orbeli and Kunstman 1929; Jankowska 1959). They appeared in one or a few experiments and only in a state of strong emotional excitement (dogs with avoidance reflexes).

3. Other anomalies

Trophic skin changes, ulcers, etc. were noticed in 4 dogs. As in cats, they resulted chiefly from mechanical damage, such as abrasion of the leg during walking. In order to protect the animals, the dogs were kept on a rubber floor and wore leather shoes on the affected limb. Nevertheless, in two cases it was necessary to amputate the digits of the deafferented hindlimb.

RESULTS

The types of reflexes established in each dog and their postoperative state is presented in Table I. Below is a more detailed description of the results of deafferentation.

1. In all the dogs the instrumental reflexes established before the operation were also present after deafferentation. During the period of postoperative observation they did not reveal any tendency to disappear.

In 10 out of 12 deafferented dogs postoperative testing continued to at least 100 trials with the positive conditioned stimulus. Since in several animals the experimental sessions were performed only once or twice a week, or were discontinued for some weeks, the period of postoperative observation varied in different dogs from one and a half to three and a half months. On two dogs (Nos. 3 and 5) the instrumental reflexes of the deafferented limb were examined for a very short time only (20 and 24 trials respectively), because the general state of these animals did not permit further observation.

Table II

Percentage of instrumental reactions to the positive stimulus before and after deafferentation

Kind of reflex	Dog No	Before operation (last 100 trials)			After deafferentation					
					1-50 trials			51-100 trials		
Alimentary	4	99			100			100		
	5	97			95			—		
	12	99			100			100		
	15	98			100			100		
		Av.	Esc.	Sum	Av.	Esc.	Sum	Av.	Esc.	Sum
Avoidance	2	100	0	100	100	0	100	100	0	100
	3	99	1	100	100	0	100	—	—	—
	8	100	0	100	70	22	92	92	8	100
	9	98	2	100	100	0	100	100	0	100
	13	100	0	100	100	0	100	100	0	100
	14	100	0	100	100	0	100	94	6	100
	15	100	0	100	100	0	100	100	0	100
	16	100	0	100	100	0	100	100	0	100
	17	100	0	100	100	0	100	100	0	100

Av. — avoidance reaction; Esc. — escape reaction; Sum — sum of conditioned reactions

Comparison of the number of instrumental reactions to the positive stimulus before and after operation (Table II) shows that in 8 out of 10 dogs observed for a long time, the instrumental reactions were present in all the trials (3 dogs with alimentary reflexes and 5 with avoidance reflexes). In 2 other animals (Nos. 8 and 14) the instrumental avoidance reactions were present in 81 and 97 percent of trials.

If trials with escape reactions (see under Methods) are also counted, the number of conditioned movements amounts to 96 and 100% respectively.

Examples of typical conditioned movements performed by the operated animals are shown in Fig. 2.

2. The mean latent period of instrumental reflexes remained almost unchanged in all operated animals when compared with the last



Fig. 2. Examples of conditioned movements performed with a deafferented limb. a, c, d — dogs with avoidance reflex: (Nos. 9, 14 and 17); b — dog with alimentary reflex (No. 12).

preoperative period, nor did it show any prolongation during the period of postoperative examination (Table III). The differences were in general less than 1 sec., and only in one dog (No. 16) was a twofold increase observed in the latency of movements after operation.

3. The amplitude of instrumental reactions after deafferentation also remained practically unchanged in the majority of animals (Table IV). In some animals the percentage of relatively high movements

Table III

Mean latent period of instrumental reactions before and after operation

Dog No.	Before operation (last 100 trials)	After deafferentation		
		1-50 trials	51-100 trials	Mean
2	1.2	1.0	1.3	1.1
3	1.9	1.5	—	—
4	1.3	1.8	2.7	2.2
5	1.0	1.1	—	—
8	2.2	4.2	2.0	3.0
9	2.0	1.3	2.0	1.7
12	1.7	1.8	1.7	1.7
13	2.0	1.6	2.6	2.1
14	1.6	1.5	1.6	1.5
15	1.0	1.0	1.0	1.0
16	1.6	2.4	3.8	3.1
17	1.1	1.0	1.0	1.0
Mean	1.5	1.7	2.0	1.8

Table IV

Amplitude of instrumental reactions before and after deafferentation
(percentage of trials in which high flexion was performed)

Dog No.	Before operation (last 100 trials)	After deafferentation		
		1-50 trials	51-100 trials	Mean
5	96	87	—	—
8	92	32	72	54
9	63	78	98	88
12	98	92	86	89
13	74	74	40	57
14	95	96	96	96
15	100	100	100	100
16	100	86	32	59
17	100	100	100	100
Mean	91	83	78	80

In dogs. Nos. 2, 3 and 4 the amplitude of movements was not precisely recorded.

even increased after operation, while in others a small decrease was observed. Only in 2 dogs (Nos. 8 and 16) was deafferentation followed by a marked reduction in the total number of high flexions.

4. The instrumental reactions of the deafferented limb, although in principle unaffected, were performed in a somewhat changed manner. They were rather awkward and ataxic and sometimes seemed to be executed by the animals with a greater "effort" than normally. These features were constant and during the period of postoperative observation no changes in the skill of the performed movements were noticed.

In some cases the reaction of the deafferented limb had a more tonic character, i.e. the movement, despite its unchanged latency, was performed more slowly, and, or the animals continued for a while to hold the affected limb in the air after the conditioned stimulus had been switched off, which had never occurred before operation (cf. Teasdall and Stavsky 1953).

Table V

Percentage of trials with no instrumental reaction to the inhibitory stimulus before and after deafferentation

Dog No.	Before operation (last 20 trials)	After deafferentation (first 20 trials)
8	90	90
9	50	100
12	95	85
13	100	100
14	100	85
15	100	85
16	100	100
17	100	100
Mean	92	92

In dogs Nos. 3 and 5 the inhibitory stimuli were not applied after the operation.

In dogs which kept the deafferented limb in marked extension the flexion movement was largely limited to the thigh joint.

5. Deafferentation had no effect on the inhibitory reflexes established in the animals (Table V).

6. Acute extinction of the conditioned reflexes of the deafferented limb was performed in 3 dogs. The criterion was 5 successive trials with no conditioned reaction to the positive stimulus. In 2 dogs with alimentary reflexes (Nos. 12 and 15) this criterion was reached after 18 and 7 application of the unreinforced stimulus respectively. Extinction of the avoidance reflex (dog No. 8) took place after 18 trials*. In all these animals a single application of the unconditioned stimulus (food or electric shock) led to an immediate restoration of the extinguished conditioned reflex.

7. The role of vision in the execution of movements with a deafferented limb was investigated in 4 animals. Exclusion of vision did not affect at all the instrumental reactions performed with the hindlimb (dogs Nos. 9, 13 and 14). In the dog which performed the movement with the foreleg (No. 17), movements of high flexion were maintained but the paw was no longer placed on the platform. This fact, however, might have been due to factors other than the exclusion of vision, since the blindfolded animal kept its head very low and probably could not put the foot into the platform in this position.

DISCUSSION

The results described above show that firmly established instrumental reflexes consisting of a relatively simple movement are regularly performed with a deafferented limb and do not show any tendency to disappear. Deafferentation produces only a slight impairment in the skill of such movements but does not abolish the capacity to execute them with the same regularity, latency and amplitude as under normal conditions. Results similar to ours were obtained in animals only by Knapp, Taub and Berman (1958)**.

In the light of our data, the part played by proprioception in the performance of voluntary movements has to be revised. The proprioceptive impulses are generally considered as an indispensable factor in the execution of even the simplest voluntary movements, as well

* The extinction of avoidance reflexes was carried out by prolongation of the conditioned stimulus to 10 sec., according to the method of Fonberg—Bregadze (cf. Soltysik 1960).

** These authors established in monkeys an avoidance reflex of the foreleg to the criterion of 80% positive reactions, and then performed deafferentation. They found that in the first period after operation the learned movements did not appear, or were present only in some trials. The reconditioning of these reflexes was, however, possible. As it seems, the temporary loss of reflexes might be due to the shorter preoperative training and, or their earlier postoperative examination, as compared with our experiments.

as in the establishment of new instrumental reflexes. For instance, according to the theory of Konorski and Miller (1933, 1936) the active reproduction of passive movements during the training of motor conditioned reflexes occurs on the basis of connections between the pattern of proprioceptive impulses, generated during the execution of a given motor act and proper states of excitation and inhibition of the centre of the unconditioned stimulus. According to this theory no voluntary act can be established nor occur without proprioception.

In contrast with most experimenters (except Knapp et al. 1958) who came to the same conclusion about the effects of deafferentation, our results have shown that the proprioceptive feedback enhances only the skill and precision of instrumental reactions but is not necessary for execution of a simple motor reaction. A question then arises, as to how the animal can perform a "voluntary" motor act without knowing which movement is required and whether or not it has been accomplished. It seems that the following explanations of this fact are possible:

1. The deafferentation of one leg does not abolish the proprioceptive feed-back from other extremities and the body. Flexion of one leg is accompanied by a simultaneous shift of the body's centre of gravity and subsequently by a change in muscle tonus in the other extremities. The pattern of proprioceptive impulses from the unaffected legs when the animal stands quietly on the floor differs from that arising when the deafferented leg is flexed. Therefore, it might be possible that the unaffected proprioception of the body sufficiently informs the animal what the starting position of the deafferented limb is, and which movement has been executed. In the light of this hypothesis the essential part played by proprioceptive feed-back in voluntary movements should not be rejected, provided that afferent impulses of the deafferented leg are replaced by the proprioceptive message obtained from other parts of the body.

Some experimental data, however, seem to contradict this interpretation. First, according to this hypothesis, the deafferented animals should, after operation, relearn to perform the trained movement on the basis of proprioception from other extremities. It seems, that in normal animals the afferent impulses from the limb engaged in a definite movement should be more important than those from other extremities. Consequently, the impairment of instrumental reactions should be especially marked in the first period after operation, and then, in the course of postoperative training, a gradual improvement of this movement should be observed, as the animal learns to discriminate the position of the affected limb on the basis of proprioception of the

rest of the body. However, our experiments show that the quality of motor reactions was, during all the time of postoperative observation, quite constant, if not best in the first experimental sessions.

It should also be recalled that, as was found in our previous experiments on cats, the deafferentation of both hindlimbs does not produce a greater deterioration of the instrumental reaction than unilateral deafferentation.

2. It may be possible that the motor centre activity associated with the execution of the leg movement is also signalled to other brain centers by an intercentral feed-back mechanism other than the one arising in the limb. The recently described (Magni et al. 1959; Jabbur and Towe 1960; Kuypers 1960) direct nervous connections between the pyramidal tract and the cuneate and gracilis nuclei, as well as the nucleus proprius in the spinal cord, might support this hypothesis.

3. It may be assumed that all concepts emphasizing the essential part played by proprioceptive afferentation in voluntary movements should be restricted to other kinds of motor activity than relatively simple motor acts. According to this view the execution of a simple voluntary movement may not need any proprioceptive afferentation, since the reflex arc for such a reaction does not pass through the appropriate afferent centres. This hypothesis could explain the facts observed in our experiments.

Let us consider the reflex arc of a learned simple motor act. According to the schema of conditioned motor reflexes (Wyrwicka 1952; Soltysik and Kowalska 1960) the performance of a learned movement to a given stimulus is due to nervous connections established between the centre of the conditioned stimulus and the centre of a definite motor reaction. As a result of these connections (both direct, and indirect through the centre of the corresponding drive) the application of a conditioned stimulus causes an excitation of the appropriate motor centres and consequently elicit the instrumental reaction. Not only flexion of the leg, but also putting it back on the floor may be attributed to the function of these centres. While the application of the conditioned stimulus activates the definite motor centres, the discontinuation of this stimulus in avoidance reflexes, or the presentation of food in alimentary reflexes, cuts short their excitation. This is followed by a return of the leg to a normal position*. Furthermore, the

* This interpretation seems to contradict the hypothesis according to which the proprioceptive stimuli generated by the instrumental avoidance response play the role of conditioned inhibitors of classical defensive conditioned reflexes (Soltysik and Kowalska 1960).

greater the excitation, the stronger be the reaction performed. Hence prolongation of the conditioned stimulus leads to increased flexions. This is comparable to the effects of direct stimulation of the motor cortex, which also results in some simple movements, like flexion or extension, varying in amplitude and latency according to the parameters of the electric current. Experiments with stimulation of the motor cortex (Lassek 1953), pyramidal tract (Teasdall and Stav-raky 1953) and ventral roots (Lassek 1953) in normal and de-afferented animals have shown that the excitability of the efferent parts of the reflex arc are not diminished after deafferentation. This explains why the learned movements were performed by our operated animals with the same regularity, latency and amplitude as before the operation. It may then be assumed that afferent influx plays only a supplementary function in simple voluntary movement, modulating its character and increasing its precision.

These considerations have only dealt with the role of afferent information in the performance of those voluntary movements which consist of a single and simple motor act, such as movements elicited by direct stimulation of the motor cortex. However, regardless which of the above hypotheses proves to be true, there is no doubt whatsoever that proprioception plays an indispensable part in many kinds of motor activity. The motor behaviour of animals usually consists of more complex reactions than those found in laboratory conditions; they are either composed of several successive simple movements, as for example in walking, or need a very subtle analysis of muscle tonus, as in the case of precise movements. In all these chain-like reactions proprioception would be an indispensable factor, since the afferent impulses set up during execution of the first movement should form a conditioned stimulus for the next movement. The reflex arc of such complex motor reflexes would then involve, besides the motor centres for the successive movements, also the sensory centres involved in their performance. Therefore, it is to be expected that deafferentation will completely abolish the possibility of performing such chain-like voluntary movements.

SUMMARY

It was found that deafferented dogs are able to perform some simple instrumental movements with the affected limb, established by pre-operative training. Deafferentation of a limb does not abolish the ability to execute them with the same regularity, latency and amplitude as under normal conditions. These results are interpreted

according to the following three hypotheses: 1. Execution of a voluntary movement with a deafferented limb occurs on the basis of the unaffected afferent influx from the rest of the body; 2. deafferentation does not abolish the afferent feed-back based on intercentral connections; 3. performance of a simple voluntary movement requires no afferent information since this feed-back is not involved in the reflex arc of type II conditioned reflexes. In view of the last hypothesis proprioceptive information plays only a secondary role in the mechanism of such instrumental reflexes, modulating their character, but not necessary for their execution.

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CARDIAC RESPONSES TO SIMPLE ACOUSTIC STIMULI IN DOGS

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A. INTRODUCTION

Changes in the frequency of the heart beats, elicited by the so-called "indifferent" or "neutral" exteroceptive stimuli are usually considered as cardiac components of the orienting reflexes to these stimuli (Robinson and Gantt 1946, 1947; Anokhin 1958, Biriukov 1958, Karamian 1958, Sokolov 1958, 1959; Petelina 1958 and others). However, in the literature on this subject, there is no agreement as to the direction or the size of these cardiac responses; there are also different opinions on the rate and the course of their extinction. Thus, for instance Robinson and Gantt (1947) described cardiac acceleratory responses to acoustic stimuli and the lack of response or weak deceleratory responses to visual stimuli (flashing lamp) in dogs. For the complete extinction of these responses to acoustic stimuli, about 150 (Gakenheimer 1960) or 250 to 440 (Dykman and Gantt 1956) trials were necessary.

On the contrary, Petelina (1958) observed in dogs cardiac deceleratory responses to acoustic stimuli and cardiac acceleration to light. Extinction of cardiac responses to acoustic stimuli occurred in her experiments after only 20 to 30 trials. This extinction had a two-stage course: in the beginning the cardiodeceleratory responses were replaced by acceleratory ones and then they disappeared altogether.

Unfortunately it is impossible to compare and to evaluate the significance of these conflicting observations, because they were not treated statistically.

The aim of this paper is to demonstrate orderly the cardiac res-

ponses to simple acoustic stimuli and to establish the effect of intensity, duration and the way of exposition of the stimuli on these responses. Moreover, the extinction of these responses was studied. Besides this, an attempt was made to solve the problem of whether or not the cardiac response to acoustic stimulus represents a "cardiac component of the orienting reflex". Therefore, together with responses to sporadically presented stimuli, the responses to cessation of a continuous sound and responses to a sound slowly increasing in intensity were studied. Thus, both sudden and slow changes in acoustic situation were produced.

In all instances special attention was given to the "orienting reflexes"; by this term the motor reaction consisting of turning the tele-receptive surfaces toward the stimulus (e.g. turning the eye-balls, pinnae and the head) is understood (Pavlov 1951, Bekhterev 1928, Kvasov 1958).

B. MATERIAL, APPARATUS AND PROCEDURE

Material. Experiments were performed on 12 adult male mongrel dogs, ranging in age from 1½ to 6 years, and in weight from 12 to 21 kg. All dogs were kept in the Institute at least several months prior to the experiments and a friendly relationship between them and experimenters was established. They were well habituated to the experimental situation and recording devices. During the habituation period, carotid loops according to van Leersum's method were prepared in all dogs in order to facilitate the registration of heart beats.

Apparatus. In general the apparatus consisted of a conditioned-reflex chamber, a source of sound and a sphygmograph.

More specifically, the piezoelectric device transforming the pulse waves in carotid artery into electric pulses, A.C. — amplifier, kymograph and the Electronic Diagnostic Stimulator (Newton Victor LTD) connected to a loud-speaker as a source of sound, were used. The emitted buzzer-like sound of 50 c.p.s. had a range of intensities from 0 to 80 decibels.

In part of the experiments, pneumatic registration of pulse waves through Marey's tambours was also used (cf. Fig. 2). Along with the pulse rate, movements of forelegs and breathing were pneumatically recorded on a kymograph.

The acoustic isolation of the experimental room was not complete but the continuous noise of the electrical appliances (rectifier, transformer etc.) on the wall of this room provided an effective sound screen for any sound from outside. This noise had an intensity of 40 db. measured at the place where the animal stood.

Procedure: The schedule of each experimental session will be described later together with results for each series of experiments.

Sphygmographic records were taken in each trial from about 12 secs. before the stimulus to 12 sec. after. The examples of resulting sphygmograms are represented in Figs. 1 and 2. On both figures the way of transforming the records into numerical data is shown in some particular experimental situations, described in detail in the proper sections of this paper.

In Fig. 1 the intervals between pulse waves were transformed into frequencies

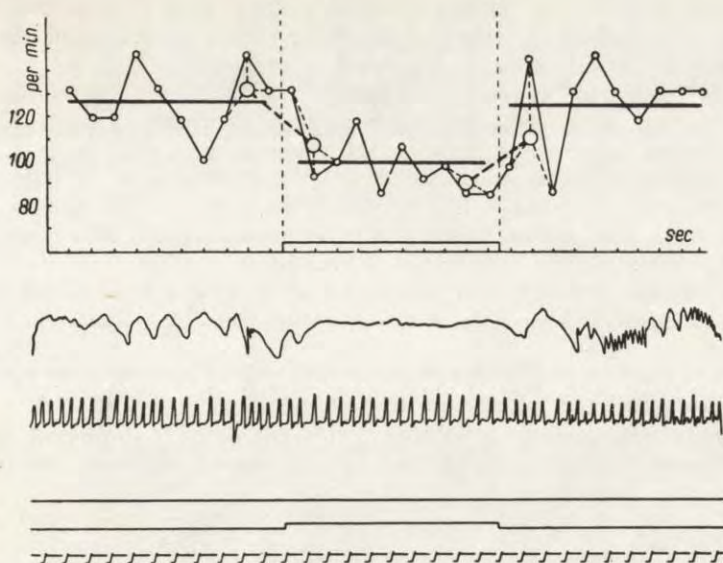
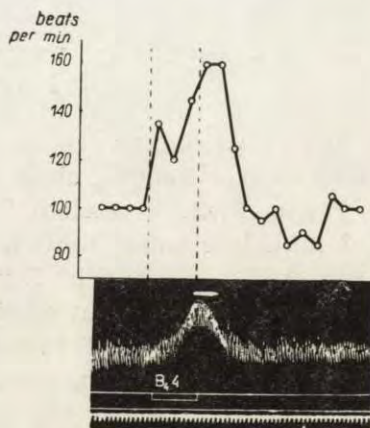


Fig. 1. Cardiac response to the turning-off of the buzzer. Second-by-second method.

Below: record of heart beats and breathing; one trial in one dog. Above: graphic presentation of changes in heart rate of this record in one-second intervals. Small circles: cardiac frequency in 1-sec.-intervals. Big circles: cardiac frequency in 3-sec.-intervals preceding and following off-set and on-set of the buzzer. Solid horizontal lines: cardiac frequency in 10 sec.-intervals. Interrupted lines connecting white circles: slopes of the off-response and the on-response.

Fig. 2. Cardiac response to 10-sec. action of the buzzer. 3-sec.by-3-sec. method.

Below: record of heart beats; one trial in one dog. Above: graphic presentation of changes in heart rate of the record in 3-sec. — intervals. The upward deflection of the record corresponds to the diminution of the diameter of the carotid artery (probably the constriction of the artery).



per minute for every second of the record, to show the form of the response. In Fig. 2 heart rate in 3-sec. intervals is shown. Of course, for statistical purposes the data from a group of animals and a number of trials were taken. Besides the form of response some parameters of response were used:

as "10-sec.-response" (when the stimulus lasted 10 sec.) we accepted the difference between mean pulse frequency during the action of the stimulus and during the 10 sec. preceding the onset of stimulus;

as "on-response" we accepted (arbitrarily) the difference in pulse rate in the 3 sec. preceding and the 3 sec. following the onset of the stimulus; and as "off-response" we took the difference in pulse rate in the 3 sec. preceding and in the 3 sec. following the termination of stimulus.

All these parameters are exemplified in Fig. 1 for one trial in one dog.

Cardioacceleratory responses are described arbitrarily as positive and deceleratory as negative ones.

The significance of differences in heart rate was evaluated using the "t" test. In order to establish and to measure the extinction in series of trials or experiments, the data are presented graphically with the curve of regression calculated by the least squares method. Usually formula for linear regression was computed

$$S_{xy}$$

and the significance of the coefficient of regression $b = \frac{S_{xy}}{Sx^2}$ was found according to the formula:

$$t_b = \frac{b}{\sqrt{\frac{Sy^2 - \frac{(S_{xy})^2}{Sx^2}}{Sx^2 (N - 2)}}$$

where b is the coefficient of regression in the equation: $Y = a + bX$; $x = X - \bar{x}$; $y = Y - \bar{y}$; (Arkin and Colton 1959; Ruszczyc 1955).

In some instances, besides the linear regression, the best fitted formula for non-linear regression was found and presented graphically.

C. RESULTS

1. Cardiac response to a 65 db. buzzer of 10 sec. duration

In 12 dogs cardiac responses to a buzzer (65 db.) transmitted through a loud-speaker hanging about 1 m. in front of the animal were recorded in 1 experimental session. The stimulus was presented 10 times in 1—2 min. intervals; duration of the stimulus was 10 sec. Results are shown in Table I and in Figs. 3 and 4.

In 11 dogs the onset of the stimulus produced a rise of the heart rate ranging (means of 10 measurements) from 3.90 beats per minute (dog Tep) to 69.00 b.p.m. (dog Nor). In the individual trials the maximal rate during the action of the buzzer exceeded the pre-stimulus level

by more than 100 b.p.m. The vast majority of 10-sec.-responses were acceleratory responses: 100 cases in 120 trials. Cardiac deceleratory responses occurred less frequently (13 in 120 trials) and in none of the dogs did they exceed 30 per cent (i.e. 3 cases in 10 repetitions). Even in Max, the only dog that had a negative mean response (see Table I), there were 4 positive responses, three times no response and only 3 negative responses; the high value of the latter ones (e.g. -17 and -20 b.p.m.) caused the shift of the mean toward the negative values.

A similar distribution of values was found in the on-responses: in only 17 out of 120 trials (10 trials in 12 dogs) did we record a drop of pulse frequency and in only 1 out of 12 dogs did the mean of 10 measurements get the minus sign.

Table I
Cardiac response to a buzzer in 12 dogs

DOG	Response (10 sec.)	On-response (3 sec.)	Off-response (3 sec.)
Ami	6.80	20.40	— 16.20
Bar	42.00	30.00	— 8.70
Bob	8.00	5.00	— 7.00
Cyg	38.30	18.00	5.00
Lal	11.10	16.50	— 13.00
Max	— 1.80	— 4.50	— 15.00
Nor	69.00	55.00	— 83.00
Pus	13.40	15.20	— 9.50
Rud	13.00	13.30	— 12.80
Spu	25.60	19.00	— 16.00
Tch	13.60	20.50	— 4.30
Tep	3.90	3.90	— 5.70
\bar{X}	20.24	17.69	— 15.52
SD	24.30	22.75	27.49
SE	2.22	2.08	2.51

10-sec.-response refers to the heart rate (mean of 10 trials in each dog) during 10 sec. of a stimulus minus the rate in the corresponding pre-stimulus interval. On-response refers to the heart rate during 3 sec. after the onset of a stimulus minus the rate during 3 sec. before the onset (cf. Fig. 1). Off-response refers to the heart rate during 3 sec. after the termination of a stimulus minus the rate during 3 sec. before the termination. \bar{x} — arithmetic mean of responses in all dogs; SD — standard deviation; SE — standard error of the mean.

Quite similar though reversed proportions were found in the off-response: in only 24 out of 120 trials were there acceleratory responses and in only one dog had the mean of 10 trials a positive value; however in this dog the number of positive responses did not exceed 50 per cent.

In this experiment we observed also an extinction of the cardiac response. Even in the course of the first 10 trials all parameters

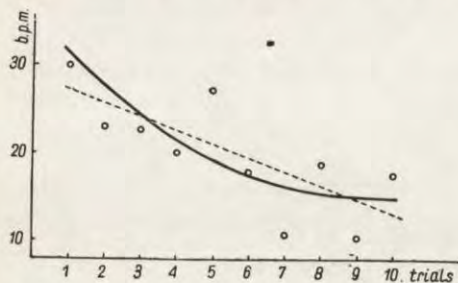


Fig. 3. Partial extinction of cardiac 10-sec.-response in the first 10 repetitions. The data are obtained from 12 dogs.

Coeff. of linear regression (see slope of the interrupted line): $b = -1.55$; $t_b = 3.2419$; $p < 0.02$; Solid line corresponds to the best fitted equation obtained by the least squares method. The ordinate shows heart rate during 10 secs. of a stimulus minus the rate in the corresponding prestimulus interval.

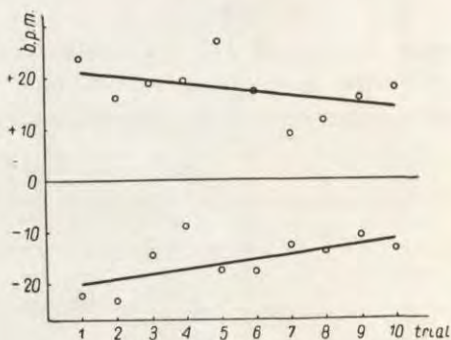


Fig. 4. Extinction of the on-response (above) and the off-response (below) during the first 10 repetitions of a buzzer. The data are obtained from 12 dogs

Coeff. of regression for the on-response: $b = -0.805$; $t_b = 1.5093$; $p < 0.2$; Coeff. of regression for the off-response: $b = (-) -0.95$; $t_b = 2.3091$; $p < 0.05$.

of response, i.e. 10-sec.-response, on-response and off-response, decreased in amplitude (Figs. 3 and 4), and this regression is significant both for the 10-sec.-response (coeff. of regression $b = -1.55$; $t_b = 3.2419$) and for the off-response ($b = -0.95$; $t_b = 2.3091$). In the case of the on-response the significance did not reach the 0.05 level, ($b = -0.805$; $t_b = 1.5093$).

2. Chronic extinction of the cardiac response to the buzzer

The buzzer (65 db.; 10 sec. duration) was presented 100 times in 10 experimental sessions (10 trials in each session) in 10 dogs. Cardiac response to this stimulus decreased consistently during this series of trials; the off-response decreased quicker than the 10-sec.-response and this latter in turn decreased quicker than the on-response: Figs. 5 and 6. The coeff. of regression amounted respectively:

$t_b = -1.71$ for the on-response ($t_b = 8.6103$); $b = -1.99$ for the 10-sec.-response ($t_b = 6.2805$); and $b = -3.70$ for the off-response ($t_b = 3.4207$) in the first 50 trials where the regression of the off-response was nearly linear.

These differences in the rate of extinction might be explained by the quicker extinction of cardiac response in the last seconds of the

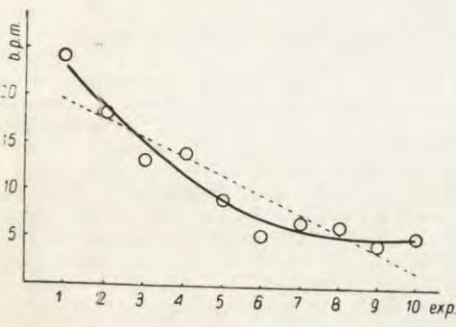


Fig. 5. Extinction of cardiac 10-sec.-response to buzzer in 10 experimental sessions (each one consisting of 10 trials). The data are obtained from 10 dogs.

The ordinate shows heart rate during 10 sec. of a stimulus minus the rate in the corresponding prestimulus interval. Coeff. of regression (slope of interrupted line): $b = -1.994$; $t_b = 6.2805$; $p < 0.001$;

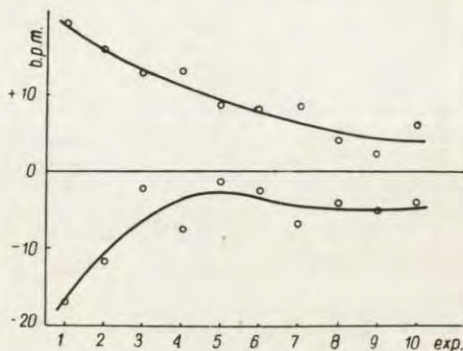


Fig. 6. Extinction of the on-response (upper curve) and the off-response (lower curve) to 10-sec. buzzer in 10 experimental sessions (each consisting of 10 trials). The data are obtained from 10 dogs.

action of the stimulus as compared with the response in the first seconds after the onset of the stimulus. In order to check this supposition we recalculated the data in such a way that cardiac responses were shown separately in seconds 1—4, 4—7 and 7—10 of the duration of the stimulus. Fig. 7 illustrates the obtained result. It was shown that in fact, the highest rate of extinction was found during the last four seconds of the stimulus (small white circles, continuous thin line in the Fig. 7), and the lowest rate of extinction was found in the first seconds (big white circles, solid line in this figure). Coefficient of regression in the first 50 trials, where the regression was approximately linear, was found for the response in the first 4 sec. of the stimulus: $b = -2.05$; in the seconds 4—7: $b = -2.45$; and for the response in the last four seconds: $b = -4.25$. The difference between the two former and the latter is statistically significant ($p < 0.05$).

The rate of extinction of the 10-sec.-response may be characterized by the number of 10-trial daily sessions necessary for obtaining a drop of response to a value amounting to a definite fraction of the response in the first session. For example, a 50 per cent decrease in response

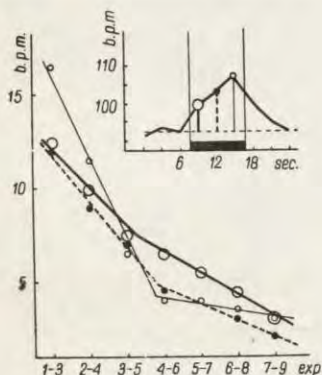


Fig. 7. Extinction of the 10-sec.-response to buzzer (shown in Fig. 5) shown separately for each third of the duration of the stimulus.

Big white circles (see also upper right inset): cardiac response in the first four seconds of the buzzer; Small black circles: response in sec. 4-7; Small white circles: response in sec. 7-10; The rate of extinction is the highest in the last third of the whole 10-sec. — response: small white circles, thin continuous line; the lowest rate of extinction was found in the first seconds of response: big white circles, solid line. The rate of extinction is much higher in the first 5 exp. sessions (i. e. 50 trials) than in the next 4 experiments. For this reason equations for linear regression are separately calculated and graphically presented for the first 5 and the last 4 experiments. Data are presented in moving averages of 30 trials.

was obtained in the 4th experimental session, and the drop to 36.8 per cent („time constant” of this approximately exponential curve) occurred in 5.2 sessions (i.e. about 52 trials).

Such indices of extinction should be easier comparable than for example the number of trials necessary for the complete extinction. Dykman and Gantt (1956) found that complete extinction of the cardiac response to tone required from 250 to 440 repetitions of that stimulus, but as it is shown in Figs. 5, 6 and 7, this extinction occurred at highest rate (in our dogs) in the first 50-60 trials and then only slowly approximated to the zero level.

3. Cardiac response to a brief acoustic stimulus

In order to explain the interrelations between the on and the off-response, a brief (0.4 sec.) and rather loud (80 db.) buzzer was presented 100 times (10 sessions of 10 trials) in 5 dogs. The data obtained are shown in Table II and in Figs. 8 and 9. It should be mentioned that these dogs were used in the preceding series of experiments with 10-sec. buzzer of 65 db.

In this series of experiments, the stimulus differed from the buzzer used previously in duration, intensity, pitch and in localisation: the loud-speaker was lowered about 1 m.

Table II

Cardiac response to brief acoustic stimulus (80 db., 0.4 sec. duration)

	Pre-stim.	sec. 1	sec. 2	sec. 3	sec. 4	sec. 5
\bar{X}	102.49	106.20	106.09	101.66	99.87	100.11
SD	24.68	25.12	26.74	25.64	22.86	23.26
SE	0.35	1.12	1.20	1.15	1.02	1.04
Response		+ 3.71	+ 3.60	- 0.83	- 2.62	- 2.38
Significance of response		$p < 0.01$	$p < 0.01$	$p < 0.6$	$p < 0.05$	$p < 0.05$

Mean cardiac rate (100 trials x 5 dogs) is shown during 10 sec. before the stimulus (pre-stim.) and in the 5 following seconds. As response — the difference between the rate in a given second and the pre-stimulus rate is shown. See also Fig. 8.

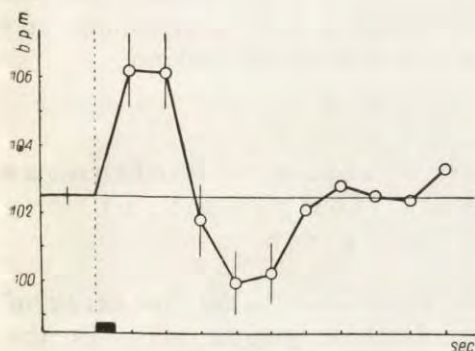


Fig. 8. Cardiac response to a brief sound (80 db. buzzer of 0.4. sec. duration)

The data are obtained from 5 dogs and 10 experimental sessions i.e. 100 trials. Pre-stimulus level is measured in the 10 secs. preceding the onset of the stimulus. White circles correspond to cardiac frequencies in the 10 successive seconds following the onset of the buzzer. Each circle is an arithmetic mean of 500 measurements. Vertical lines show the standard errors of these means.

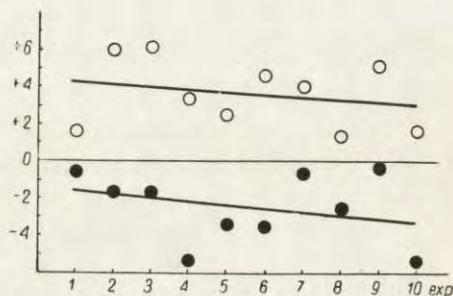


Fig. 9. Positive and negative cardiac response to the 0.4 sec. buzzer (cf. Fig. 8). in 10 consecutive experimental sessions.

White circles: differences between cardiac rate in the first two seconds following the onset of the 0.4 sec.-buzzer and the prestimulus level. Black circles: differences between cardiac frequency in the fourth and fifth seconds following the stimulus and the pre-stimulus level in this series of experiments. There is no definite trend in either case. Coeff. of regression for the positive responses in 1st and 2nd seconds: $b = -0.139$; $t_D = 0.7439$; $p < 0.5$; Coeff. of regression for the negative responses in the 4th and fifth seconds:

$$b = (-) 0.176; t_D = 0.6893; p < 0.6;$$

Fig. 8 shows both form and size of the response to this stimulus: data from all the dogs and all trials are presented in means of the heart rate for each of 10 sec. following the onset of the stimulus. The response had biphasic form: in the first two seconds there was an acceleration of the heart rhythm amounting to about 3.5 b.p.m., which was statistically significant as compared with the pre-stimulus level ($p. < 0.01$). Then, a deceleration occurred with its maximum (c. 2.5 b.p.m.) in the 4th and 5th seconds; this drop of frequency was also statistically different from the pre-stimulus level ($p. < 0.05$).

When compared with the on- and the off-response in the previous series of experiments (see Table I), this acceleration in the first two seconds and the deceleration in seconds 4th and 5th (after the onset of the stimulus) are rather small. Moreover, as shown in Fig. 9 these responses (positive in secs. 1—2, and negative in secs. 4—5) did not undergo any consistent change during 10 consecutive sessions. On the other hand, this stimulus elicited a vivid orienting reflex, sometimes even a startle response, and this motor reaction clearly decreased during this series of daily sessions. So it is quite probable, that the biphasic response to a brief stimulus resulted from overlapping and partial interference of positive on- and negative off-response.

4. Cardiac response to interruption of a continuous sound constituting a stable component of the experimental situation

In 9 dogs we observed changes in heart rate during the break of a continuous buzzing. The resulting drop of general noise in the experimental room amounted about 40 decibels: from 80 db. to 40 db. After 10 sec. the buzzer was turned on again. Dogs were brought to the experimental room and placed in the Pavlovian frame only after turning on the buzzer which was acting during all the session. The turning-off of the buzzer for 10 sec. occurred in 1 to 2 min. intervals. The source of sound (a loud-speaker) was placed on the ground aside the frame, so that any orienting reflex (turning the head to the right and looking down) was easily observed.

In spite of the fact that all the dogs were used in the previous series of experiments, the orienting reflexes to the termination of sound arising from a new place were present in them. Changes in the heart rate accompanying the interruption of buzzer are shown in an individual trial in Fig. 1, and averaged from all our dogs (10 trials in each dog) in Fig. 10 and Table III.

Table III

Cardiac response during the 10 secs. break in stimulus. Data obtained in 5 dogs; 100 repetitions in each dog.

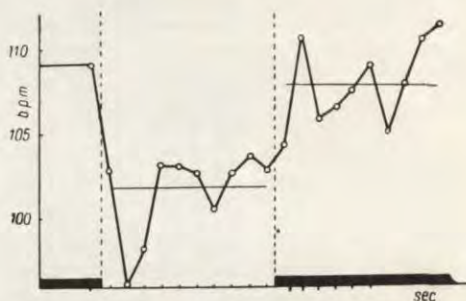
	Stimul. acting	Stimulus turned off for 10 sec.										Stimul. recomm.
		1	2	3	4	5	6	7	8	9	10	
Heart rate	112.82	106.14	100.34	103.22	107.99	106.04	103.02	103.86	107.42	107.84	105.90	112.21
S. E.	± 0.946	± 3.00	± 3.09	± 2.89	± 3.21	± 3.93	± 2.75	± 2.73	± 3.16	± 2.27	± 2.91	± 0.959
Differ. from initial level		-6.68	-12.48	-9.60	-4.83	-6.78	-9.80	-8.96	-5.40	-4.98	-6.92	-0.61
t		2.1276	3.9651	3.0672	1.5280	2.1567	3.1416	2.8736	1.8107	1.5864	2.1939	0.4523
p		0.05	0.001	0.01	0.2	0.05	0.01	0.01	0.1	0.2	0.05	0.6

First column: cardiac frequency in the 10 secs. preceding break in stimulus. The next 10 columns: cardiac frequency in each successive second during break. The last column: cardiac frequency in the first 10 secs. after stimulus was recommenced. 1st row: mean cardiac frequencies; 2nd row: standard errors of the corresponding means; 3rd row: heart rate in a given second minus heart rate preceding termination of stimulus; 4th and 5th rows: significance level of differences.

It was found that the cessation of the buzzer elicited a typical off-response, i.e. a drop of heart rate. This drop of heart rate reached its maximum in 2nd second after the termination of the stimulus

Fig. 10. Cardiac response during the turning-off of the buzzer. The data are obtained from 9 dogs: 10 trials in each dog.

Black dots represent cardiac frequency in one-second intervals. Horizontal lines represent mean heart rate in 10 sec. intervals.



(-12.99 b.p.m.; $p < 0.001$) and then, during following 8 sec. the rate remained statistically lower (-7.16 ; $p < 0.001$). After recurrence of the sound also the heart rate returned to the initial level (-0.61 ; $p < 0.7$).

5. Cardiac response to a sound slowly increasing in intensity

In the following experimental session in 9 dogs a buzzer of increasing intensity was presented 10 times. In contradistinction to the preceding experiments, as well as to the experiments of other authors (Robinson and Gantt 1947, Petelina 1958, Jaworska 1958, Zagorul'ko, Sollertinskaia and Jaworska 1959), where the acoustic stimuli had a sudden onset and a steady intensity, here the buzzer increased in intensity from 0 to 65 decibels (the noise level was about 40 db.). The intensity of the stimulus was increasing during 120 sec. and then it ceased suddenly. After some 15–20 sec. the trial was repeated. While orienting reflexes were seen at the moment of termination of the buzzer, they were not observable during the period of slow increase of intensity of this stimulus.

The records of heart rate obtained during the whole period of presentation of the stimulus (i.e. 120 sec.) was divided into eight 15-sec. intervals and the cardiac rate for each interval is presented in Fig. 11. It is clearly seen that the increase of intensity of the buzzer is accompanied by an increase of the heart rate from 90.44 b.p.m. to about 100 b.p.m. As the intensity of sound increased from 40 db. to 65 db., the increase of heart rate amounted to about 3.73 b.p.m. per 10 db. This, approximately linear rise of cardiac frequency is highly significant ($p < 0.001$).

The termination of the buzzer caused a drop in the heart rate from 97 b.p.m. in the last 10 sec. preceding the termination, to 91 b.p.m. in the 10 sec. after termination: Fig. 12. The difference between these two levels of heart rate is highly significant ($p < 0.001$).

Fig. 11. Cardiac response to the buzzer slowly increasing in intensity

Intensity of the buzzer increased during 120 sec., so that the noise in the experimental room increased from about 40 to about 65 decibels. Pulse rate was measured in 15 sec. intervals (white circles) during the presentation of the stimulus and in the 15 sec. after its sudden termination. Data are obtained from 9 dogs: 10 trials in each dog. Vertical lines show standard errors of mean heart rate in 15 sec. before and in 15 sec. after the termination of the buzzer. Coeff. of regression: $b = 1.135$; $t_b = 6.1374$; $p < 0.001$;

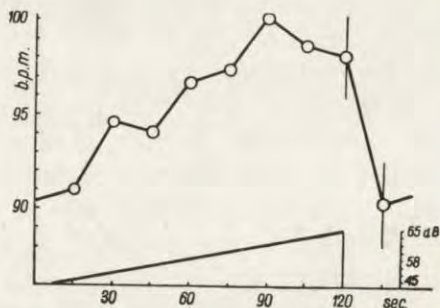
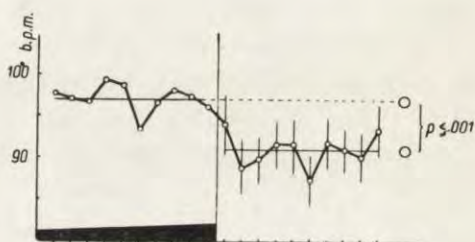


Fig. 12. Cardiac response to the termination of the buzzer slowly increasing in intensity

Only the last 10 sec. of the operation of the stimulus are shown. Black dots represent cardiac rate in 1-sec. intervals; Vertical lines show standard errors. Horizontal lines correspond to the cardiac frequency in 10 sec. intervals.



Similarly as in the previous experiment (cf. Fig. 10) the drop in the heart rate occurred in the first 2 sec. and afterwards the level of the heart rate did not change very much.

6. Extinction of orienting reflexes and sleep

According to observations made in Pavlov's laboratories (Pavlov 1951, III, 1, p. 377) extinction of orienting reflexes leads to falling asleep. We observed that occasionally some of our dogs fell asleep during experimental sessions where neutral stimuli were presented. However, we did not perform any systematic study on this problem and therefore we are not in a position to give a definite answer.

DISCUSSION

The results of our experiments allow us to confirm some of the previously known facts and to add some new ones.

First of all, we have found that the heart rate increases after the onset of an acoustic stimulus and decreases after the termination of that stimulus.

Secondly, the extinction of cardiac response to acoustic stimulus was found to occur both in the series of daily sessions and in the first 10 trials during the first session. This extinction occurs at higher rate in the last few seconds of the action of the stimulus and at a lower rate in the first seconds of its duration.

The third fact we want to stress is the close correlation between the level of the heart rate and the intensity of sound.

Our last observation is that there is no evident correlation between orienting reflexes and cardiac responses.

All these facts we shall discuss separately.

An increase of the heart rate in response to acoustic stimuli was found in all our dogs, though occasionally some few deceleratory responses occurred too. This confirms the results of Gantt's Laboratory and is in obvious contradiction to the observations of Petelina (1958). The latter author found in 6 young dogs a deceleratory response to a 60 db. tone. It is likely that two factors were responsible for the differences in results: first, contrary to our procedure, Petelina compared the pulse rate in rather long intervals (up to 25 sec.); secondly, probably her dogs were much younger.

It is interesting to note, that cessation of a stimulus produced an opposite response, i.e. a drop in the pulse rate. This happened not only when the stimulus acted for 10 sec. and the off-response could be considered as a simple return to the initial pre-stimulus level, but it also occurred in the experiment in which the stimulus was a stable component of acoustic situation or its intensity increased slowly from 0 to 65 decibels. Moreover, it seems obvious that this off-response has two components: a phasic steep drop in heart rate immediately after the cessation of the stimulus, and a tonic, longlasting decrease in the heart rate: see e.g. Figs. 10 and 12.

It is known that one of the effects of sensory stimulation is an arousal response due to collateral activation of reticular formation. It is reasonable to suppose that one of the peripheral effects of this arousal reaction is the increase in heart rate due to inhibition of the nuclei of the vagus nerve and the resulting release of the cardiac pacemaker. Krayer and Benforado (1955) have shown that in dogs the

intrinsic pacemaker rhythm does not depend on race, age, weight or sex, and amounts to about 140 b.p.m. On the other hand, Bond (1943), Murphy (1942), Rohse and Randall (1955) and Dykman and Gantt (1959) supported the evidence that sympathetic innervation plays little or no role in inborn and acquired cardiac responses to external stimuli. If so, most of the responses to acoustic stimuli in our dogs might be effected by the inhibition of vagal centers. However, in some responses of our dogs cardiac frequencies of more than 200 b.p.m. were observed. They were usually accompanied by startle responses, panting and general restlessness — and on sphygmograms — a constriction of the carotid artery was seen (cf. Fig. 2). In these individual cases, an adrenergic response should be assumed; the maximum of cardiac frequency coincided with the maximum of vasomotor response and occurred in the 12th second after the onset of the stimulus. The same latency for sympathetic response in dogs was noted by Bond (1943).

Contrary to the effect of the onset of the stimulus, a sudden break in the stream of impulses from the acoustic nerve might release the tonically functioning vagal nuclei and inhibit the cardiac pace-maker.

In agreement with the hypothesis that cardiac response to an acoustic stimulus is a component of reticular activation is the observation of one of the authors of this paper (Jaworska 1958, Zagorul'ko, Sollertinskaia and Jaworska 1959) who observed an apparent correlation between cardiac responses to exteroceptive stimuli and suppression of slow waves in EEG-records of pigeons.

The extinction of the cardiac response was observed by other authors (Dykman and Gantt 1956, Petelina 1958, Gakenheimer 1960) and usually related to the extinction of the orienting reflex. The extinction of this response in our dogs had a typical exponential course. Since this curve declines to the zero level (see Fig. 5) with the decreasing rate, we suggested as a measure of the rate of extinction, a number of daily session necessary to decrease the response to 50 or 36.8 per cent of its initial size. The lower values of partially extinguished response lie on the flattened "tail" of the curve and could not be determined with sufficient accuracy.

The extinction of the cardiac response was found also in the first 10 repetitions during the first experimental session. In this case, however, the asymptote of extinction curve lies on a higher level (about 15 b.p.m.) than that of chronic extinction. On the next day partial recovery occurred because the mean value of the responses

in this session was about 18 b.p.m. Thus, a chronic extinction of cardiac response resulted from the accumulation of residua of acute extinctions in each session. Another interesting property of the extinguishing response is that it decreases with repetitions quicker in its later part (i.e. during last seconds of the stimulus) than in the beginning of the stimulus: see Fig. 7. It is possible that here the effects of acute extinction during one trial ("acute continuous extinction" in Pavlovian terminology) accumulate in some way in series of trials and sessions. A similar effect in cardiac food reflexes was found by Aleksanian (1958).

As it was stated before, besides an immediate "phasic" on- and off-responses, a tonic relationship between the intensity of continuous sound and heart rate level was found. This phenomenon clearly illustrates the supposition that the level of heart rate during acoustic stimuli cannot be attributed to phasic orienting reflexes, and that it is a more or less independent effect of the intensity of afferent impulsion in the acoustic nerve. Slow increment in the intensity of sound produced in our dogs a parallel increase of heart rate without observable orienting reflexes. The cessation of sound elicited an orienting reflex but simultaneously a drop in the heart rate occurred. Visual stimuli elicit in dogs orienting reflexes accompanied by weak cardiac deceleratory, or no responses (Robinson and Gantt 1947, also our unpublished data).

All these facts seem to contradict the conception that the cardiac response to „neutral” stimuli is a component of the orienting reflex. The latter view was expressed first by Robinson and Gantt (1946) and then adopted by other authors (Biriukov 1958, Karamian 1958, Petelina 1958, Sokolov 1958, 1959, and others). However, even the pioneer of this view Gantt stresses that "there is not a strict parallel among them — the heart rate sometimes being markedly increased when there is little movement and vice versa" (Gantt 1960, p. 274). The classical formulations of Pavlov (1951, p. 132—133) and Bekhterev (1928, p. 112—113) emphasized the motor nature of the orienting reflex. Also Kvasov (1958) recently stressed the motor or „propriomuscular” nature of the orienting reflex.

Therefore, we suggest calling the cardiac response to acoustic stimuli an „acoustico-cardiac reflex”. This term best applies to the response which, according to the discussion presented above, is elicited by acoustic stimuli but is not closely related to the other overt behaviours, which are also elicited by these stimuli.

SUMMARY

1. Cardiac responses to acoustic stimuli were observed in 12 dogs. A detailed description of a form, size and extinction of these reflexes is given. Besides this, an attempt was made to find the relationship between cardiac responses and orienting reflexes.

2. It was shown that the onset of an acoustic stimulus produced regularly an increase of heart rate; the termination of the stimulus, no matter how long it had been acting before (0.4 sec., 10 sec., 2 or more minutes), produced regularly a drop in the heart rate.

3. Orienting reflexes were elicited by any sudden change in the acoustic situation; no difference between these reflexes to the onset of the stimulus or its cessation was detected. Slow changes in sound intensity did not elicit any clear orienting reaction. On the contrary, cardiac responses reflected precisely both the direction and the rate of changes in the stimulus intensity.

4. The possibility that the cardiac response to an acoustic stimulus is not a component of the orienting reflex, but that it is a relatively independent „acoustico-cardiac reflex” is discussed.

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A STUDY OF RETURNING BEHAVIOUR OF WHITE RAT ON ELEVATED MAZE

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The maze-method, which has been widely used in zoopsychology has given rise to many studies on the behaviour of rat. One of the essential advantages of this method consists in testing the animals on solving problems in space. This evoked a good deal of investigations related to learning and retention of a path. In spite of many reports on threading the maze, however, returning to the starting point remains still to explore.

Although the rat, living under natural or laboratory conditions is not a classical returning animal, as bees or ants are, he has certainly developed an ability to return home or to some other places. The maze appears to be an appropriate method to investigate this ability.

The problem of returning may be studied from two different aspects. First, as a general spatial orientation, when the animal returns to the starting point using any of the accessible ways, and secondly, as an ability of finding the particular path he went to the goal.

This paper deals with the latter problem. Experiments were carried out under the conditions of two different paths leading to the goal from two starting places. On the way back to the starting place the rat faced only one point of choice. The ability to return to the starting place using the same way the animal went for food we have called the return reaction.

INTRODUCTORY OBSERVATIONS

Before these experiments had been begun some observations were made on rat returning to the cage placed on the floor of an empty room. The rat went out to fetch a piece of biscuit put in a cup in front

of the cage. Initially, the cup was so close that the rat had but to lean out to catch the biscuit. As the cup was shifted further away the rat put his fore legs out and outstretched his body more and more attempting to reach the food with his mouth. If, however the distance to the food exceeded the length of his body he took a few steps forwards and backwards. Finally, as the cup was pushed even more, he took no longer steps backwards but turned round (180°) and went directly to the cage.

It seemed that the behaviour of rat was determined not only by the distance, which the animal had to cover but also by his timidity. At first, some rats put out their heads stretching their bodies maximally and it was not until they failed to catch the piece of biscuit; then, they came out and went hesitantly forwards. Some other animals ran out immediately when the cup was placed far away. Stepping backwards from long distances also resulted from shyness. When the rat was leaving the cage he faced an unknown way which could be dangerous, and thus when he came back he preferred to maintain the same position.

In most instances, however, having ventured a long way off the rats turned round (180°) and going straight ahead they reached the cage. In the same way the rats hit the cage even then when the cup was placed not in front of the cage but at some angle, and the cage was screened (Fig 1). In the successive runs it was possible to shift the cage

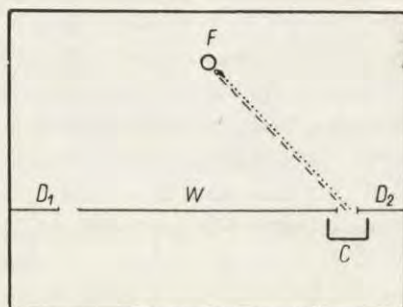


Fig. 1. The route of rat between the cage and the food in introductory experiments
 F — cup with food C — the cage, W — wooden screen D₁, D₂ — the doors in the screen. The broken line represents the route of rat for food, dotted line — the route to the cage.

from one door of the screen to the other and the rat was not disturbed in his return. He found his way to the cage regardless of where he came from. It was so because to run for food and to return with it was a single chain reaction. Having left the cage the rat ran towards the cup and while catching the food he kept the direction by the position

of his body. Turning back and running ahead determined his correct return*. This situation that made the automatic reaction of the rat possible did not allow to draw a conclusion if the animal is able to find the path he went to cup. It was necessary to establish such conditions in which the rat would not be able to maintain his gross bodily orientation in the direction of the cage.

MATERIAL AND METHOD

Experiments were carried out with the experimentally naive white rats aged from 3 to 6 months. Four different kinds of an elevated maze have been used as shown in Fig. 2.

The mazes were made of boards 14 cm. wide and placed on poles 70 cm high. The dimensions of mazes have been given in Fig. 2. The mazes A, B and C had

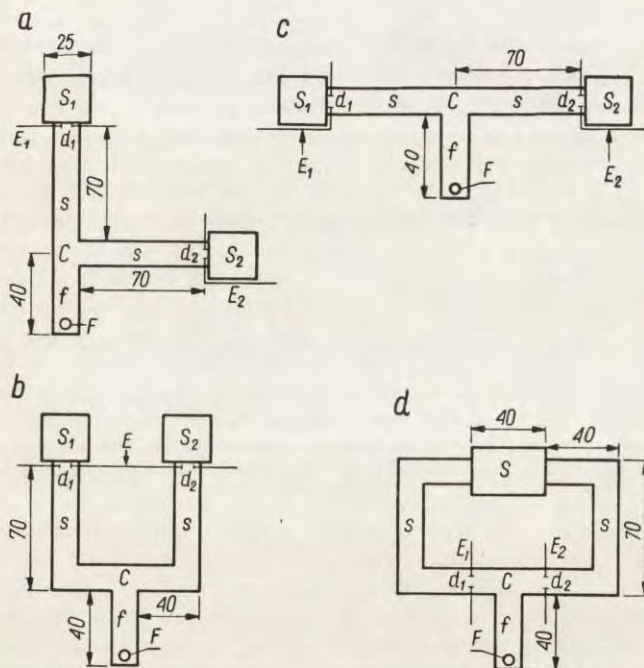


Fig. 2. Mazes A, B, C, D used in experiments

S, S_1, S_2 — starting platforms, s — starting path, F — cup with food, f — food path, C — choice-point of return route, E, E_1, E_2 — wooden screens, d_1, d_2 — two way doors in the screen.

* In the above experiments the cup was placed at a distance of less than 2 metres from the cage; it is hard to know whether, if the distance had been greater, the rat would have been able to keep the correct direction.

two starting platforms S_1 and S_2 ; each was covered with the wooden screen E_1 , E_2 . A cage (22 cm \times 25 cm \times 22 cm) was put on one of the platforms. The cage covered the whole platform and fitted tightly into the screen with its front part that was left open towards the starting path. A rat came out through a two way door in the screen. The door in the screen covering the opposite starting platform was locked with a small hasp. The maze D had only one uncovered starting platform. The cage used in this maze was open on the two opposite sides. The maze had been fitted with two screens E_1 , E_2 , being at a certain distance from the choice-point C. In the given experiment the path to the cup was determined by leaving open the proper door in the cage and the two way door of the proper screen. The door in the opposite screen was locked.

The rat was required to come out of the cage, reach the cup on the food path (40 cm. from the choice-point) catch a piece of the biscuit and return the same way to the cage, where he was allowed to eat the biscuit. The rat came out of the cage spontaneously, no signal was used. There was only one piece of biscuit in the cup, the next pieces were put there while the rat was eating in the cage.

The rat was not allowed to eat the food just after he had caught it. Thus, to make him return to the starting platform with the food special conditions had to be established. For this purpose the cage placed on the starting platform was small and darkened (since the rats like cosy places), whereas the cup with food was in an open place of the maze. It was very important to use the elevated maze because while having an enclosed maze the rats kept staying at the cup and they could hardly be provoked to eat in the cage.

It was also important to apply a special preliminary training. During the first few days of the training the rats were taught to eat in the cage for 10–15 min. Next, biscuits were placed outside the cage at the beginning of the starting path. The rat was afraid of the open and unknown space and he returned to the cage immediately after catching the biscuit. The main point was to develop in the rat a habit to return to the cage; however, it was also necessary to familiarize the rat with the maze at least in order to provoke him to go towards food. This was achieved by pushing the biscuits further and further away. Every time the rat returned to the cage after he had caught the food. The training was finished when the rats were fully habituated to the maze and did not hesitate to approach the cup. For the whole group of rats the training usually lasted about the fortnight. Some rats were not afraid any more to leave the cage on the second or third day, some others, however, if the pieces of biscuits were somewhat further, turned round and ran away to the cage. Under such circumstances the biscuits were gradually shifted away and a great care was taken not to discourage the rat to come out. Some rats happened to take a few steps away from the cage, or just to lean out and returned many times and then they sat motionless in the cage and did not dare to leave it. It was of no use to cut down the daily ration of food or to starve them to provoke them to leave the cage. In order not to prolong the training these rats were taken away. The number of the removed rats varied considerably in various groups and amounted up to 30%.

The same 10-days series of experimental sessions were carried out on every maze. In every series another group of rats was used. During every experimental session three or four trials were given. The cage was placed in each trial on the same starting platform and its position was changed every day in such a manner that the rat had to run to the cup one or the other way alternately. In some series on the following day after finishing the 10-days series the cage was placed

on the same starting platform as previously. This was made in order to know whether the rat did not learn to change his way every day. When the animal in his return way entered the incorrect path as far as the length of his body such a run was counted as erroneous. The correction method was used.

The mazes used in this study differed from each other and these differences could have some influence on the choice of the proper path. In maze A both paths differed more than in the other mazes. From one of the starting platforms the road led to the cup along a straight line whereas from the other it turned at an angle of 90° . On maze B the rat had to turn twice on his way to the food. The same turns were required on the way back in the same succession. When the rat started from platform S_1 he had to turn first to the right and then to the left. On his way back to the cage he also had to turn first to the right and then to the left.

Thus the way back on the maze B was kinestetically a repetition of the way to the cup. In maze C, on the other hand, the way back was its reverse: here the returning rat was required to turn in the opposite direction to that he had done on the way to the cup. In maze D, contrary to the previous mazes, the cage was constantly at the same position what could have a certain significance for the problem of returning.

The mazes were placed in the visually heterogenous room with two windows. The experiments were carried out in the morning. Every maze was oriented in the room so that opposite arms were symmetrically placed to the light. The experimenter sat at a distance of two to three metres from the side of the cup or that of the choice-point. After every experiment the rats returned to their cages in the breeding room where they received their regular food for a period of three to four hours.

RESULTS

1. The general behaviour of the rat on his way to the cup and on his way back

At the beginning of every experiment the rat was put into the cage on one of the starting platform. The rat ran out almost immediately. At times however, he sniffed the walls and floor. In the next trials the rat usually went out as soon as he had eaten the biscuit. It took the rat about 5—6 sec. to reach the cup and to return to the cage. The intervals between the runs depending on how fast the rat ate and how he behaved, were half a minute to two minutes long.

On leaving the cage the rat went directly to the cup, though sometimes he happened to run to the door of the opposite screen instead to the cup, tried to open it and returned to the cage. Occasionally, he ran up to the opposite door and only then to the cup. On returning to the cage the rat did not usually hesitate to find his way at the choice-point. If there was a moment of uncertainty it was so as a rule in the first trial of the experiment. The rat stopped at the choice-point then, and

turned his head right and left. At times, before he made the final choice he took a few steps one way or the other, after which he turned round and changed his direction. In most cases, however, the hesitation was slight and it could be estimated by some minimal changes in his run only. Usually the rat took the turning smoothly rounding the angle between the boards of the maze and taking a little shortcut — as though leaping from the food path to the starting path; while hesitating he took the turning at right angle.

If the rat chose the incorrect path he knocked into the locked door in the screen covering the platform. Initially rats kept knocking at them for quite a time. Some of them took a few steps away and returned again before they made for the proper platform, or ran back to the cup and only then to the cage. Later on, having knocked into the locked door the rats ran directly to the other platform.

2. The ability to choose the proper return path

Correct choices were estimated separately for the successive trials of each experimental session as a whole for all the experimental sessions and for all the rats in the given group. The results have been given in Table I.

Table I

The correct return reactions on maze A, B, C and D
(100% — all return runs of all rats in the respective trial)

Maze	No of rats	I trial in %	II trial in %	III trial in %	IV trial in %
A	6	93	97	95	98
B	7	87	98	100	100
C	13	96	100	100	100
D	15	88	95	98	—

In the first trial of every experimental session the rats found the way to the cage in about 90% of cases. In the successive trials the number of correct returns increased up to 100% in mazes B and C. Since the cage was on the same starting platform during every experiment the return run in the successive trials took place in the same direction as that of the first trial. The rat that made errors in the first trial could correct his choice in the next one owing to one trial learning. Thus, the return runs of the first trials were most essential, for just in them the rats chose the return route on the basis of retention of the

way to the cup. The high percentage of correct reactions shows that the rats are able to choose the way they had gone previously in the opposite direction.

The control trials with runs from the same starting platform as that of the preceding day showed no increase in errors. Thus, the rats did not follow the principle of an alternating choice of the return route in the successive experimental sessions.

The purpose for using four mazes was that the difference in the shape of traversed paths might influence remembering them. As it may be seen, however, from Table I the results obtained in these mazes have shown no marked difference. Hence, for the returning rat the features of the choice-point are important and not those which are related to the whole path. The choice-point on the other hand is identical in mazes B, C and D (Fig. 2). At this point both starting paths turn at right angles to the food-path. On each of these three mazes the returning rat had to turn in the opposite direction to that it had followed at this point to the cup. Thus, the mazes B and D were in some way analogous to maze C. Maze A had also a similar point of choice since one of its starting paths was at right angle to the food-path.

3. Do rats learn to choose the traversed path?

From Table II one may read how many errors the rats made in the first trials during the successive days of one experimental series carried

Table II

The number of correct and incorrect return reactions in the first trials of successive experimental sessions on maze D

No. of exp. session	1*	2	3	4	5	6	7	8	9	10
Path	right	left	right	left	right	left	right	left	right	left
Correct reactions	10	15	10	12	15	13	15	13	15	12
Incorrect reactions	2	0	5	3	0	2	0	2	0	3

* On that day 3 rats refused leaving the cage.

out on maze D. As we see at the beginning of the series there are nearly as many errors as on the last days. The results did not improve as the experiments went on. The experiments on the other mazes were very similar and no learning took place there either. On maze C all the rats made a correct choice of return way on the first day already. All this suggests that the rats chose the correct returning route in our experiments without learning.

4. Sources of errors in the first trials

Table III shows the number of errors made by various rats in various experimental series. Most rats solved the problem of return to the starting platform errorless, some of them made two to three errors. Only two rats made five errors. One of the sources of errors in the first trials is due to preference to the given turning. Thus some rats made errors of one kind only, e.g. when the correct path turned left.

Table III

The number of rats which made errors in first trials on maze A, B, C and D

Maze	Errors					
	0	1	2	3	4	5
A	3	2	1	0	0	0
B	3	2	2	0	0	0
C	9	3	1	0	0	0
D	8	2	3	0	0	2
Total	23	9	7	0	0	2

The error might have also resulted from retention of return route of the preceding day. Thus the rat took a turning to the right instead of one to the left, since he still remembered that he returned to the cage along the right path on the preceding day. To elucidate this, experiments have been carried out on maze C on another group of rats; the cage was on the same starting platform as on the preceding day, and the rat was not placed into it but close to the cup. Thus on the given day the rat did not run to the cup, but they ran from the cup to the cage. The choice of the path, if it was not an accidental one, was due to the retention of the return runs of the preceding day. Each rat ran back only once on the given day. Four experiments of this kind have been carried out on 11 rats. In 34 runs out of 44 i.e. in 77% the rats went to that starting platform to which they had returned on the preceding day. 4 rats chose the return way of the preceding day in all 4 cases, 4 rats — in 3 cases, and 3 rats — in 2 cases. These results show that the rats do remember the return way from the preceding day, and thus the assumption that this memory may produce errors in the first trials appears to be correct.

5. Lengthening of the food-path

The problem which rats had to solve in this investigation is related in some way to the delayed response. When choosing the return way to the cage, the rat cannot be oriented by the actual stimuli, since the cage has been screened. The choice is presumably due to the retention of some elements of the run to the cup. In our experiments the delay, i.e. the time in which the rat went from the choice-point to the cup and back again, was about 2 sec. It is well known that in delayed responses the time factor is highly important and one of the purposes of this study was to check whether a cup while considerably placed ahead would affect the choice of the return way.

Table IV

The influence of the distance between the food and the choice-point on the return reaction

Series	Correct reactions	Incorrect reactions
I. The distance about 40 cm	55	15
II. The distance about 100 cm.	61	9

Experiments have been carried out on maze C on the group of 7 rats. After the series I with a usual distance of the cup (40 cm.) had been performed, the cup was gradually shifted to a distance of 1 m. (series II). No negative influence of the extension of the food-path was noticed. The number of errors in the first trials was even lower in series II than in series I. (Table IV). All rats but one made less mistakes then. It is possible that this improvement was associated with the improvement of inhibition of remembering the return way on the preceding day.

DISCUSSION

The introductory experiments which preceded testing the rats in the choice of the correct return way yielded information on more primitive ways of return. Under certain circumstances the rat did not face the necessity of choice. The first tendency of the rat — if the circumstances allowed him for this — was to keep the direction between the starting

place and the goal with the position of his body and this showed him automatically the return way. The rats returned to the cage taking steps back at times from quite a distance till they accustomed to the experimental situation. Later on, after they had reached the food they turned round (180°) and ran straight ahead. In both cases a successful return depends on whether the axis of the body on a straight line connecting the starting place and the goal has been kept. Such a primitive way of return has made it possible for the rat to enter the covered cage after he has left it for any direction, but is no longer successful in case the conditions impell the rat to change his position. The latter takes place in experiments carried out on mazes. Here a new problem arises — the choice of the correct way. The task the rat has to face now consists of two parts: 1. to run to the cup for food, 2. to return to the cage with the food. To run to the cup involves no difficulty, for it is visible from every point of the maze. The actual task is to return to the cage. The rat does not see the cage, but is able to reach it by remembering the run towards food.

In our experiments no learning of the choice of the correct return way has been noticed: by means of the maze we simply discover the already existing ability. One may believe that this ability has been either inborn, or acquired by the rats in their pre-experimental period of life. We do not know whether rats kept since their birth under restriction of locomotion would be able to find the way to the cage in experiments similar to our own. Wyrwicka (1959) put new-born puppies into boxes and after a few weeks she tested whether they could ommit an obstacle. The puppies appeared to be unable to perform such simple action and they had to learn ommiting. It is possible that the life in breeding cage despite its small size, gives the rats an opportunity to learn to return to a given place in the same way. Until however the control experiments have been carried out one may not exclude the possibility that during the preliminary training the animals learn the relation between the stimuli operating on their way in both directions and take the advantage of this ability under the experimental conditions. This problem could be perhaps explained by a suitable modification if the preliminary training that will be carried out in other experiments.

The correct return of the rat was not influenced by an increase of the distance between the cup and the choice-point. This might have been expected taking into account that the additional delay provided by the prolongation of the food-path protracted the delay period for 1—2 sec. In the experiments of Mc Allister (1932) which were in some way similar to ours the maximal delay available for rats was 11,5 sec. The prolongation of the food-path in our experiments was

made in order to see whether the rat are able to find the proper return way in a case in which the choice of this way does not follow immediately the corresponding turn in the way to food. The answer to this question was affirmative.

Although the rat's return to the starting place has not been investigated so far, we do find in the papers of Dorcus (1932) and Bunch and Lund (1932) some information on relation between learning of some definite path on the maze in two direction. These authors were chiefly concerned with a determination of backward conditioning; their results however, show that rats are able to take advantage of cues concerning the given path when learning the same path in the opposite direction. In the experiments of Dorcus the rats while learning the same path in the opposite direction needed less trials to master the task and made less errors. Bunch and Lund found that several runs guided in one direction had resulted in an improvement of learning in the opposite direction.

The authors working with a maze on insects, on the other hand, find that the way to the food is quite different for the animals than the return route. The returning bees and wasps do not use the orientation cues which they acquainted with on the way to the food (Weiss 1954). The two directions of the maze represent separate portions of the way which are mastered by ants quite apart from each other. To master the path in one direction did not make it easier for the ants to master it in the opposite direction (Schneirla 1929, 1934). When the bees had mastered the path to the goal and the return path, paths were exchanged: then the animals showed a complete disorientation (Kalmus 1937).

The identification of the way in two opposite directions is a more difficult problem than it may seem at first. It ought to be realized that among the cues which an animal may follow some are reversed on the way back, whereas others occur at the opposite side and still others are completely changed. An object seen from one side looks differently when looked at from the other, whereas the turnings change into the opposite ones; if the path turned right, when going back the turning had to be taken to the left. We do know from our own experience how difficult it is for us at time to return the same way in a forest or in an unknown town and what an important part is played then by verbalization.

In the previous paper (Łukaszevska 1959) the return reaction on the perpendicular maze was investigated: the rats had to make a choice between a path leading up and that leading down. The animals proved unable to solve the problem under such circumstances. The path

to the food on the perpendicular maze apparently provided the rat with no cues due to which it could identify it on the way back to the cage. At present, it is difficult to determine what is the difference between a choice of the path on a perpendicular maze and the horizontal mazes used in this investigation. In order to learn the mechanism which enables a rat to find the proper return way it is necessary to establish what kind of cues he uses when choosing this way. The experiments described in this paper provide no information on this problem, since the experimental conditions allow a rat to use either kinesthetic, or intra- and extra maze cues. This will be the subject of our further investigation.

SUMMARY

The ability to return to the starting place has been investigated in white rats on four different mazes. A correct return required a choice of the same way the animals went for food. The choice could be made on the basis of the previously traversed path.

1. The rats found the proper return way almost in all trials, about 90% of correct reactions were obtained.

2. The main source of a small number of errors was the memory of the return runs from the preceding day.

3. The correct choice was not influenced by 2,5- fold increase of the distance between the food and the choice-point.

4. The number of correct choices does not change in the course of experiments. This suggests that the ability to find the proper return way is inborn, or acquired by rats in the pre-experimental period of life.

5. Under certain circumstances rats may use a more primitive way of returning to the starting place: it consists in keeping the proper direction by means of a position of the body.

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