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THE RESPONSE OF THE CILIATE DILEPTUS AND ITS FRAGMENTS TO THE WATER SHAKE

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(Received June 15, 1962)

The occurrence of the ciliary reversion as a response to the bisection of the ciliate cell and its return to normal movement was described in the previous paper (Doroszewski 1962). The reactions of the *Dileptus* fragments to some of the environmental factors were the next to be studied. Water shake was chosen as the stimulus because it is simple to produce, and occurs in nature.

As in the former experiments the reaction of the ciliary reversion was chosen as the subject of investigation, but other responses were also observed.

The main problem was to discover which fragments of the ciliate were capable to respond and to what extent the regeneration of the cellular structure was necessary for the restoration of the reactivity.

MATERIAL AND METHODS

The general methods of cultivation and feeding of the ciliate *Dileptus anser* O.F.M. were similar to those described in the previous paper (Doroszewski 1962) except that the axenic cultures of *Tetrahymena pyriformis* were used as the main source of food to standardize the experimental conditions. This kind of food proved, however, to be insufficient, and therefore, once in two weeks normally cultivated *Colpidia* were added to the standard food. The day before the experiments the ciliates were placed in Pringsheim solution or Dryl's buffer solution (Dryl 1959).

The bisection of the ciliate was performed in the way described previously. After the operation, the *Dileptus* fragment and an intact individual were placed on a slide. In preliminary observations, the water vibration was produced by shaking the slide, or with a needle. In the main series of experiments a different

method was applied. The drop containing the intact ciliate and the ciliate fragment was placed in a specially constructed chamber between a slide and a big cover glass with a small droplet of water surrounded by paraffin oil (Figs. 1 and 3). The whole construction resembles the paraffin oil chamber of de Fonbrune

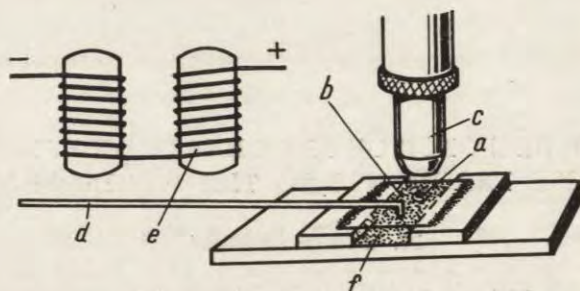


Fig. 1. The paraffin oil chamber for stimulating the ciliates

- a) The droplet of fluid containing the ciliates, b) the paraffin oil stabilizing the droplet of fluid and preventing drying up, c) the objective of the microscope, d) the rod producing the stroke, e) the electromagnet, f) the chamber made of a slide with a fixed cover glass

(1949). The size of the drop was adjusted to the field of vision of the microscope. Phase contrast equipment was used to make observations on the ciliary movement.

To produce the stimulation, the cover glass was gently touched with a rod fixed to an instrument functioning like an electric bell*. A single stroke was normally used, although continuous vibrations could also be obtained.

Photomicrographs were taken to record the shape of the fragments immediately after the operation and in the course of regeneration. In order to register the reactions motion pictures were taken. To avoid unintentional effects of a longer series of stimuli, the stimulation given was only about 20 shakes within 5 minutes once an hour, or in some instances, once per half an hour. In the main experiments the stimulation was applied only while the ciliates were moving forward.

Only those results from ciliates whose shape and behaviour appeared normal on the next day, were considered.

RESULTS

Stimulation by shaking the water in a Petri dish causes a reversion in almost all the ciliates swimming forward, and the individuals attached to the bottom of the dish start to swim upward displaying a negative geotactic reaction in response to the water movement. When

* This device was designed by K. Golińska.

stimulated in the paraffin oil chamber conditions, the ciliate reacts by a regular avoiding reactions which starts with the ciliary reversion. By increasing the force of the stroke nearly 100% response is obtained, but as such strong water disturbance causes the passive transportation of the ciliate, in the main experiments a level of about 90% response in the normal ciliates with weaker stimulation was considered adequate.

While making these observations other reactions were also noticed. The ciliate may move very slowly or stay immobile but with weak movements of the cilia; upon stimulation the ciliate can either accelerate its movement or start and rush forward. In the flattened drop there was of course no room for the geotactic reaction as in the Petri dish.

Posterior fragments were produced by the cutting the ciliate in the middle of the cell body (transection 4 Fig. 2). Observations immediately

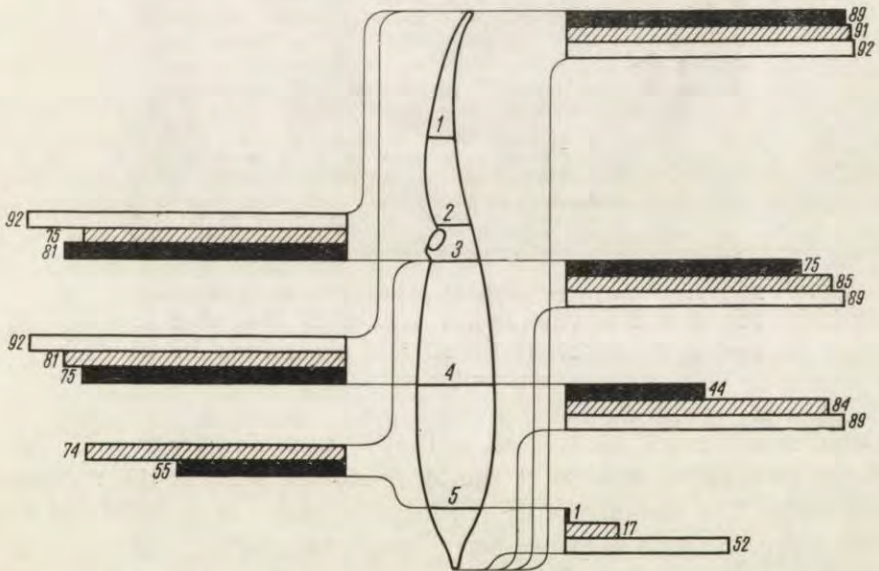


Fig. 2. Diagram showing the percentage of reactions in normal ciliate and in its various fragments

The black columns: the percentage of reactions in the initial period after operation; the striped columns: 60 to 65 min. after operation; the white columns: 180 min. after the operation. Numbers 1, 2, 3, 4 and 5 indicate the levels of transection. Further explanations in text

after the transection indicate that the reactivity of the fragments is rather low. Between 5 and 15 min. after the transection the percentage of the reactions is 44%. During the next hour it increases to 84%, and to 89% in 2 hours. The durations of the periods of ciliary reversion were remarkably shorter than in the intact ciliate so that the distance

covered in the withdrawal phase was 2 to 4 times shorter also. The next phase of avoiding reaction, however, took place normally i.e. the fragment turned its anterior end, and started forward in a new direction.

Although the resting stage during regeneration was not observed, the movement of the fragments was markedly slow in the period of 20 to 70 minutes after the transection. In this period the reaction of starting or accelerating was observed.

The reactivity of the anterior fragments produced by transection 4 is not so disturbed as that of the posterior ones. The periods of ciliary



Fig. 3. Photomicrograph showing a normal individual of *Dileptus* serving as the control together with one having the proboscis cut in half (transection 1). Magnification ca. 50x

reversions are much shorter than in the posterior fragment, and are difficult to observe because of the locomotory effects of the movements of proboscis. The distance of the withdrawal phase is about of the length of the fragment. The response occurs more regularly.

The special attention was paid to the most posterior fragment produced by transection 5 (Fig. 4). As was previously indicated such a fragment is the only one which regularly does not respond to cutting by the reversion. This behaviour was used as the criterion for further observation. Only the fragments which do not withdraw were used in this series. The fragments not containing nuclei died within a few hours and the records from these were discarded. The percentage of reactions of the posterior fragments No. 5 is outstandingly low. After 5 to 15 minutes the fragment responds only in 1%. The restoration of reactivity is very slow: after an hour 17%, after 2 hours 52%, and after



Fig. 4. The posterior fragment (transection 5) after operation

3 hours 68%. In Fig. 5 may be seen the regeneration of the fragment after 2 hours. Although the regeneration of proboscis is well advanced, the size of the fragment is very small as compared to the control ciliate.



Fig. 5. The regenerating fragment (transection 5). 2 hours after transection

The anterior fragment resulting from transection 5, i. e. the normal *Dileptus* when its posterior tip is cut off, does not differ in its capacity for response to being shaken from the intact control individuals.

As a control to the above experiments dealing with transection 5, those fragments remaining after cutting off of the anterior and posterior regions (transections 3 and 5) were also stimulated. The results obtained

were very similar to those of the posterior fragment from transection 4. During the first 5 to 15 minutes the percentage of response was 55% and after an hour 74%. The regeneration of both ends was then already in course.

Very small fragments (with a length equal to the width of the normal ciliate individuals) were also produced. Observations on the reactivity of these fragments were rather difficult to perform because of their rotating movements, however, it seems that the responses were rather rare.

The percentage response of individuals having half of their proboscis cut off (Fig. 3) was only slightly lower than that of normal individuals (75%) but it did not recover in the next two hours (81%).

The posterior fragments obtained by transection 2 has a rather similar reactivity to that of the control ciliates, but in the posterior fragments from transection 3 it is lower.

As all the experiments were made in the field of vision of the phase contrast microscope, supplementary observations of the ciliary movement were carried out. It was observed that during stimulation great activity of the ciliary waves appeared on proboscis. In the fragments moving forward the ciliary waves were propagated backwards. In the course of these experiments a total number of 120 transections was made. The percentage of reactions was calculated from 1300 single stimulations of normal individuals and about 300 for the various posterior fragments.

DISCUSSION

Under natural conditions the response to the water shake by backward movement may be profitable for the animal in that it can avoid some approaching danger, e. g. a predator. The general pattern of the avoiding reaction described by Jennings (1902) may be applied in general to this reaction to shaken water. On the other hand, the recording of other reactions confirm the statements of Alverdes (1922) and Dryl (1952) that the avoiding reaction is not the only motor reaction in ciliates. The acceleration of the ciliary movement when the water is shaken has already been reported (Vervorn 1897, Shmagina 1947). Another response to disturbed water, namely contraction, was investigated in *Spirostomum* by Kinastowski (1962).

In the various fragments of *Dileptus*, the percentage of reactions is markedly low, although the reversion can occur. It appears that the presence of the anterior portion with the proboscis is not a necessary condition for the reaction, i. e. the location of reactivity is not absolutely

determined. The significance of the role of neuromotorium (Vissner 1927) is not excluded. The receptive role of proboscis seems more certain, if we take into account the paper of Dumont (1961). The traumatic effect of injury may certainly decrease the reactivity, and the different reactivity of the anterior and posterior fragments may mark the existence of more and less susceptible areas. Regular but weak reactions of the anterior portions, and the strong but irregular ones of posterior portions, were revealed and may be considered as evidence of some differentiations of the receptory and effector functions in the different parts of the cell. The role of the gradient may be the important factor. As to the quite specific behaviour of the most posterior fragment which does not in general respond to shaking there is the parallel behaviour in the lack of the reaction to cutting. In both cases this may depend on the relatively small size of the fragment or may be due to the absence of some essential elements. The complete regeneration of the shape of the ciliate is not necessary for the restoration of the reactivity, yet the appearance of the small proboscis occurs in the same period as restoration of reactivity.

The similarity of the reaction to cutting and to water-shake can be compared to the analogous reactions to touch and irradiation by UV (Doroszewski 1961). In all these cases each fragment of *Dileptus* above a minimum size can react. This may be proof rather against than for the existence of specific senses in ciliates (Gelei 1934), and of their sharply determined localization. We are dealing rather with great autonomy and plasticity of reactivity.

The author wishes to express his gratitude to Docent Dr. S. Dryl for reading the manuscript, and to Miss K. Golińska for the technical assistance.

SUMMARY

The reactions to the water shake of the normal individual of *Dileptus anser* O.F.M. and its fragments after the transection were investigated.

The results obtained were the following:

1. The ciliary reversion as the response to the water shake occurs regularly in normal *Dileptus* individuals.
2. The acceleration or the starting of movement are other types of response to the shake.
3. The posterior half of the individual may respond to the shake, but relatively more rarely.
4. The most posterior fragment is almost deprived of the capacity to react to water-shake, as well as to the cutting.

5. The capacity of response may be localized in the surface layer and cilia of the cell. However, normally the anterior end with the proboscis plays an important role in initiating the reaction.

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AN ANALYSIS OF REVERSAL LEARNING IN RELATION TO THE PATTERN OF REVERSAL IN RATS

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(Received July 1, 1962)

In the first paper of this series (Dąbrowska 1959), it has been demonstrated that repeated reversal learning in the multiple unit discrimination apparatus becomes increasingly easier for the animal, so that after a number of reversals the rat solves the problem almost immediately. In a later paper (Dąbrowska 1962), it has been shown that, if only one reversal learning is conducted, the savings in comparison with the original learning are greater in a multiple unit than in a single unit differentiation apparatus.

The present paper is concerned with the further elucidation of this problem. If we have at our disposal the four unit quadruple choice differentiation apparatus we can alter pathways either in a single partition or in a few partitions in various combinations.

The problem is to discover what is the rate of reversal learning in relation to the various patterns of such alterations.

MATERIAL AND METHOD

Apparatus. A four unit quadruple choice apparatus was used. The apparatus was 4 m. long, divided by four partitions. Each partition was fitted with four doors opening only in one direction (Fig. 1). An animal was always placed on the starting platform just in front of the first partition, and when it had passed through the maze to the end platform it entered the cage, where it was usually kept and fed.

Subjects. 60 white rats, 3 months old, were used in experiments. During the training period the animals were deprived of food for 22 hours before testing.

Preliminary training. During the preliminary training the animals

became adapted to the experimental procedure and apparatus. All doors in each partition were unlocked. This training lasted 6 days.

Main training. During regular training only one of the four doors in each partition was left unlocked, thereby determining the route the animal had to follow. The other doors, three in each partition were locked. There were 256 different combinations of routes within the maze. When the rat had mastered one task, the doors, which had been open before, were locked, and others were opened,

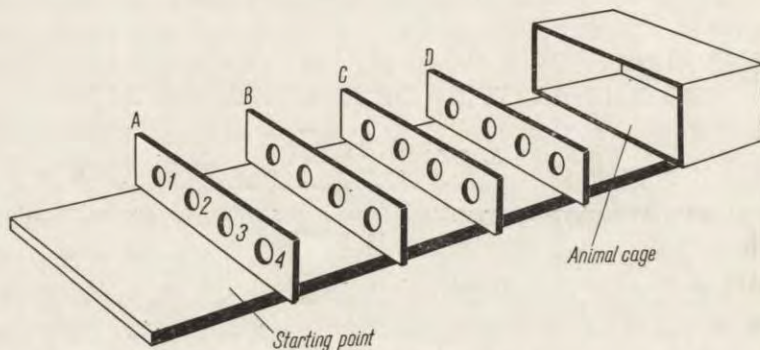


Fig. 1. Four unit quadruple choice apparatus
A, B, C, D — partitions; 1, 2, 3, 4 — doors

and the animal faced a new task to solve. A task was considered as mastered, when the animal made 6 consecutive runs (not necessarily within one day) without errors and hesitations. Touching a locked door was defined as a full error. Running up to a door, no matter whether it was locked or not, without attempting to open it was defined as hesitation behaviour.

Table I

Alterations in reversal learning in successive experimental series for different animal groups

Series	Reversal learning			
	group	group	group	group
I	ABCD	BCD	CD	D
II	A	AB	ABC	ABCD
III	A	B	C	D

Each partition in the maze was marked in the following way: partition A was the one from which the animal began its run, it was followed by partitions B, C and D. The cage with food was placed behind partition D.

The doors in each partition were marked from left to right, with the numbers 1, 2, 3 and 4.

To show which task the animals had to learn, the numbers of successive unlocked doors were given. For example, the task 3244 means that in partition A

the third door, in partition B the second door, in partition C the fourth door, and in partition D again the fourth door were unlocked.

Experiments were divided into three series. In each series 20 animals were used, divided into four groups, each consisting of five specimens.

The first task for animals of all groups in all series was the same. The differences in experimental procedure were in reversal learning. The alterations in reversal learning are shown in Table I. The letters of different partitions given in Table I signify the partition in which the pathway was changed. For example, in Group ABCD all pathways were changed, in Group AB, pathways in partition A and B were altered, in Group D, only pathway in partition D was changed, etc.

RESULTS

Series I. This series included groups ABCD, BCD, CD and D.

Results of these experiments are presented in Table II. As seen from this table all animals mastered the first task in about the same number of runs. On the other hand, there were great differences between the groups in the reversal learning. The reversal learning was mastered most quickly in Group ABCD in which the task was altered for all pathways. The reversal learning in Group BCD and CD took longer, and the task took longest to master in Group D in which only the last pathway was changed. It appears that the fewer the pathways changed, the longer was the reversal learning.

Table II

Number of runs needed to master the tasks in learning and reversal learning for animal groups in Series I

Groups	ABCD		BCD		CD		D	
	L	RL	L	RL	L	RL	L	RL
Tasks	3241	4324	3241	3123	3241	3212	3241	3244
Number of runs for each animal	50	28	48	35	46	38	53	61
	48	21	59	32	47	33	53	56
	52	30	53	37	60	47	44	59
	46	27	46	41	55	35	50	46
	48	25	51	38	42	30	46	57
Mean	48.8	26.2	51.1	36.6	50	36.6	49.2	55.8

L — learning, LR — reversal learning

Table III

Number of errors in each run and at each partition of maze in reversal learning. Individual data for rat No. 1 from Group D

Runs	Partitions			
	A	B	C	D
1				3
2			2	1
3				
4				2
5			1	
6			1	1
7			3	1
8	2	3	1	
9				
10		2		
11	1	1		
12		2		
13		2		
14		1	1	
15	1			
16	1			1
17				3
18			3	
19			1	
20				
21	1			
22				
23				
24				
25				
26	1			
27	2			
28	1	1		
29				
30	1			

Runs	Partitions			
	A	B	C	D
31				
32	1		1	
33				
34	1		1	
35				
36	1			
37				
38	1	2		
39	1			
40		3		
41				1
42				
43	1			
44	1			
45				
46				
47				
48				
49				
50	1			
51				
52				
53				
54	1			
55		2		
56				
57				
58				
59				
60				

Table IV

Number of errors appearing at each partition of the maze in reversal learning for Group D

Partitions \ Runs	Runs									
	1-6	7-12	13-18	19-24	25-30	31-36	37-42	43-48	49-54	55-60
A	8	10	8	6	14	14	10	9	5	0
B	4	13	6	3	5	13	6	5	1	0
C	5	15	24	10	6	9	3	3	1	0
D	44	12	14	6	1	3	2	1	0	0

The experimental data presented in Table III for a single rat and in Table IV for all Group D explain the difficulty in reversal learning when the task is changed only in partition D. It may be observed that in the first six runs most errors were made at this very partition. Subsequently, the greatest number of errors was switched to partition C up to about the twenty fourth run. Afterwards, the majority of errors appeared at partition A, while the errors at other partitions gradually decreased. The number of errors at the partition B was always the least (Fig. 2).

Table V

Number of errors made at each partition of the maze during reversal learning in Group CD

Partitions \ Runs	Runs							
	1-6	7-12	13-18	19-24	25-30	31-36	37-42	43-48
A	7	9	5	5	5	1	1	0
B	8	1	4	6	6	5	1	0
C	52	10	14	0	1	0	0	0
D	21	2	3	0	0	0	0	0

Similarly although less pronounced, difficulties were observed in Group CD (Table V). In the first six runs most errors were at partitions C and D, and much less at partitions A and B. After 18 runs errors at partitions C and D dropped to zero, while those at partitions A and B continued to appear.

Series II. This series included Groups A, AB, ABC and ABCD (Table VI).

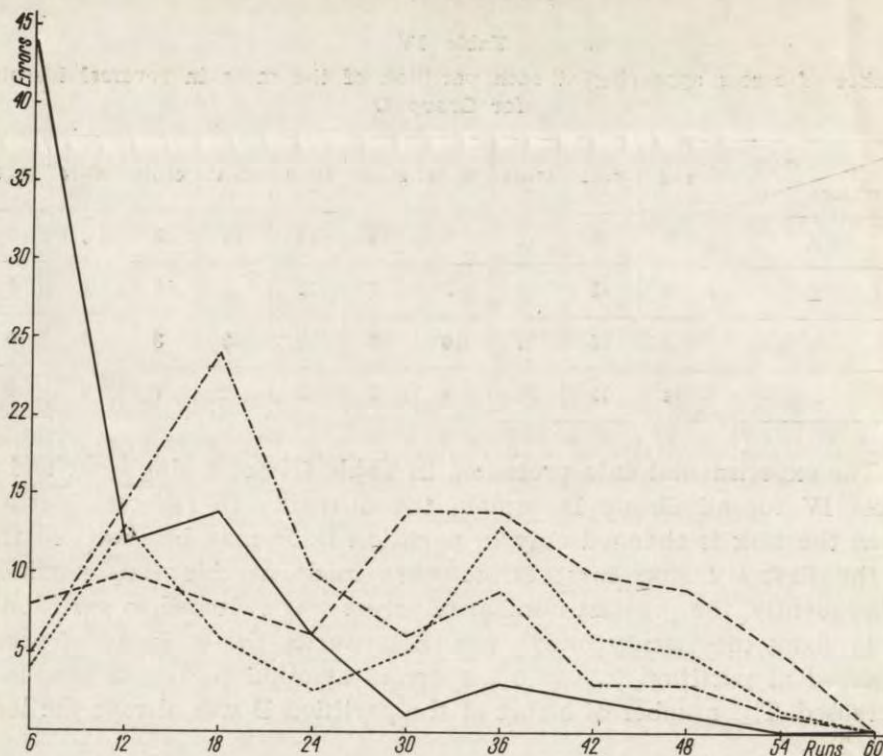


Fig. 2. Number of errors appearing in the consecutive runs at each partition in Group D

— errors at partition D - - - - errors at partition B
 - · - · errors at partition C - - - - errors at partition A

Table VI

Number of runs needed to master the task in learning and reversal learning for animal groups in Series II

Groups	A		AB		ABC		ABCD	
	L	RL	L	RL	L	RL	L	RL
Tasks	3241	1241	3241	1341	3241	1321	3241	1324
Number of runs for each animal	54	23	55	25	54	25	52	21
	54	24	50	25	52	23	53	25
	54	25	51	21	53	24	55	20
	52	27	49	25	52	26	54	25
	53	26	53	24	54	23	51	27
Mean	53.4	25	51.6	24	53	24.8	53.6	24.4

L — learning, LR — reversal learning

As seen from this table, irrespective of number of altered pathways, the rapidity of reversal learning in this series does not differ within the experimental groups. The reversal learning for all groups of animals was as quick as for reversal learning in which all pathways were changed.

Table VII
Number of errors at each partition of the maze
in reversal learning in Group A

Partitions \ Runs	Runs				
	1-6	7-12	13-18	19-24	25-30
A	77	77	11	5	0
B	26	23	18	4	0
C	10	12	7	1	0
D	2	0	2	0	0

Table VIII
Number of errors at each partition of the maze during
reversal learning in Group AB

Partitions \ Runs	Runs				
	1-6	7-12	13-18	19-24	25-30
A	79	21	16	1	0
B	52	11	2	5	0
C	16	13	6	0	0
D	5	2	6	0	0

When the task was changed solely in partition A, the errors in reversal learning were not limited only to that partition, but they appeared from the very beginning at all partitions, the further from the altered pathway, the smaller the number of errors (Table VII, Fig. 3). In the other Groups of this series the distribution of errors was similar (Tables VIII and IX).

Series III. In this series only the pathway in one partition was altered in each group. Accordingly, we have here to do with Groups A, B, C and D.

The results of experiments are presented in Table X. It was found that in this series the number of runs to master the task in reversal

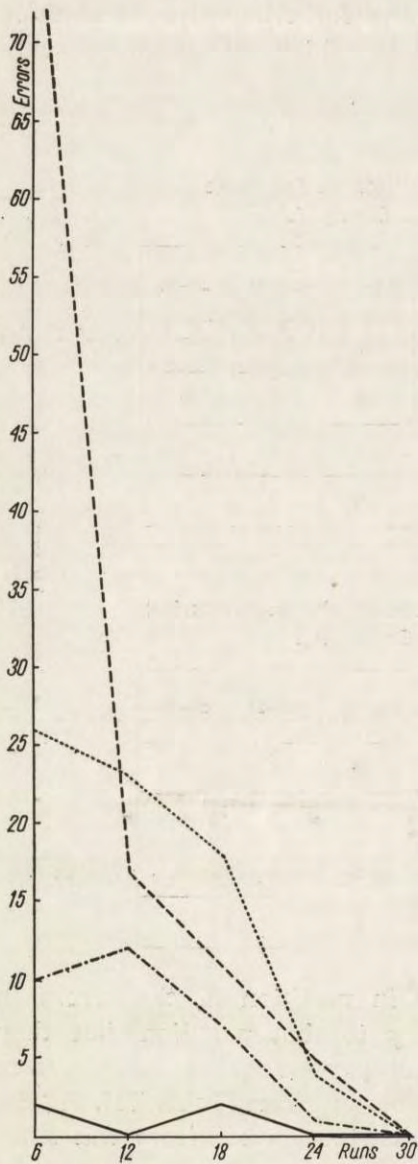


Fig. 3. Number of errors appearing in the consecutive runs at each partition in Group A

Partitions are marked as in Fig. 2.

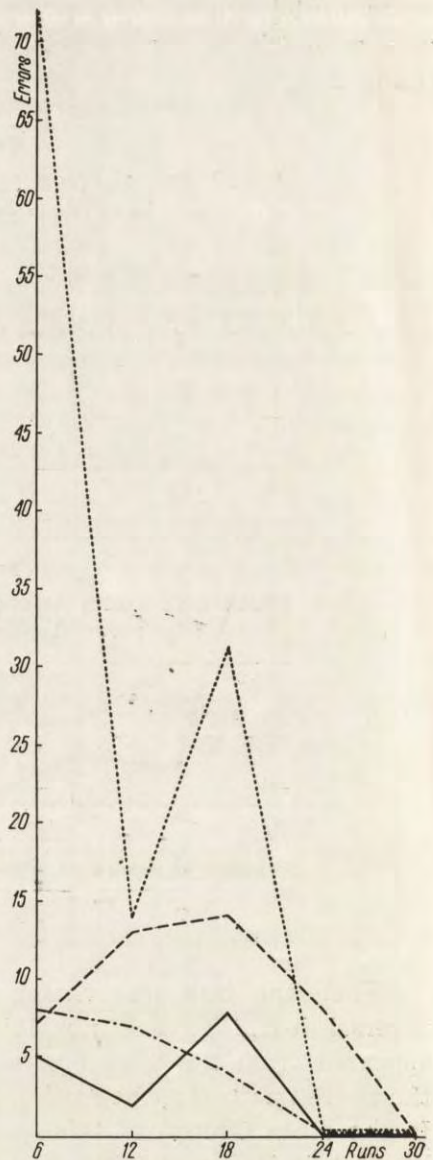


Fig. 4. Number of errors appearing in consecutive runs at each partition in Group B

Partitions are marked as in Fig. 2.

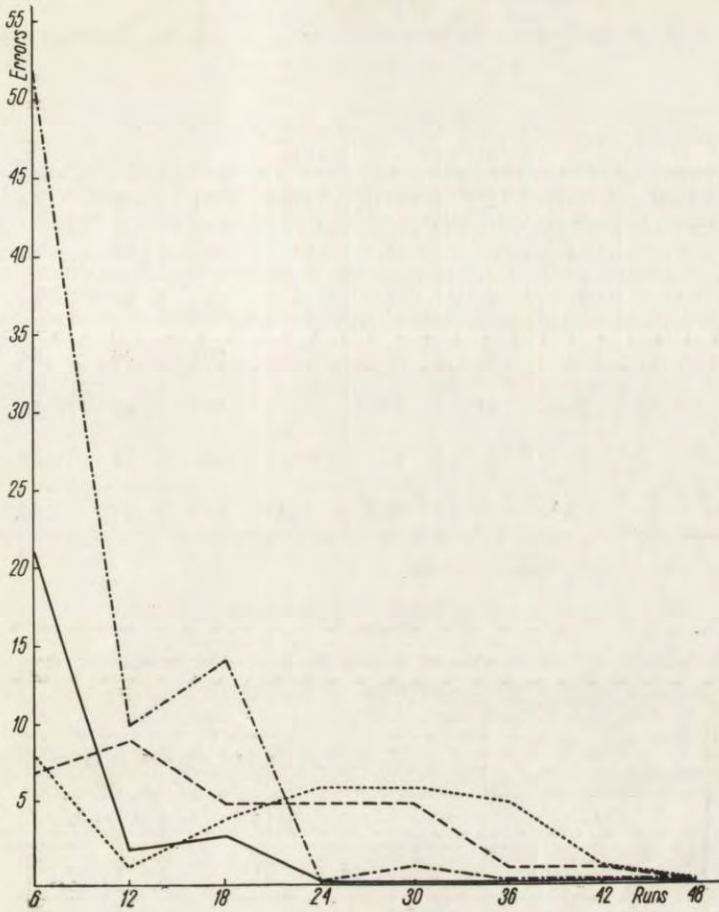


Fig. 5. Number of errors in consecutive runs at each partition in Group C
Partitions are marked as in Fig. 2

Table IX

Number of errors at each partition of the maze during reversal learning of Group ABC

Partitions \ Runs	Runs				
	1-6	7-12	13-18	19-24	25-30
A	61	25	17	0	0
B	31	12	13	3	0
C	43	12	14	3	0
D	7	3	9	0	0

Table X

Number of runs needed to master the tasks in learning and reversal learning for animal groups in Series III

Groups	A		B		C		D	
	L	RL	L	RL	L	RL	L	RL
Tasks	3241	1241	3241	3141	3241	3231	3241	3244
Number of runs for each animal	54	23	54	26	49	39	53	61
	54	24	54	29	51	30	53	51
	54	25	54	27	50	36	44	59
	52	27	53	25	54	42	50	46
	53	26	48	29	54	38	46	56
Mean	53.4	25	52.6	27.2	51.6	37	49.2	54.6

L — learning, L — reversal learning

Table XI

Number of errors at each partition of the maze in reversal learning of Group B

Partitions \ Runs	Runs				
	1-6	7-12	13-18	19-24	25-30
A	7	13	14	8	0
B	72	14	31	0	0
C	8	6	4	0	0
D	5	2	8	0	0

Table XII

Number of errors at each partition of the maze in reversal learning of Group C

Partitions \ Runs	Runs						
	1-6	7-12	13-18	19-24	21-30	31-36	37-42
A	18	6	9	10	6	10	0
B	18	3	6	1	1	2	0
C	85	10	5	15	0	6	0
D	1	0	0	1	0	0	0

learning depended on the place in which the pathways were changed. The increase in the number of runs in reversal learning for each successive group shows that, the further the altered pathway from the starting point in the maze, the more runs are needed in reversal learning. In consequence, the results of this series are very much similar to the results of series I.

Distribution of errors for Groups B and C is shown in Tables XI and XII, and in Figs. 4 and 5.

DISCUSSION

According to the results obtained in the first series of experiments (Groups ABCD, BCD, CD and D) the reversal learning becomes progressively difficult with the decrease of number of alterations in the pathways. Thus while in Group ABCD the reversal learning needs only 50% of the trials used in the original learning, in Group D this number raises to 110 per cent. However, if we consider the second series of experiments (Groups A, AB, ABC and ABCD), we see that in all groups the rate of reversal learning is identical, amounting up to 50 percent of the original training. This seeming contradiction between series I and series II is solved by series III (Groups A, B, C and D). In this series the alteration of the pathway in the first partition produces the same results as alteration in pathways at all four partitions (i.e. 50% savings), while the alteration of the pathway in the fourth partition prolonged the reversal learning by 10 percent in comparison to the original learning.

The problem arises, why the reversal learning after alteration of the pathway in the last partition becomes much more difficult than that following alteration of the pathway in the first partition.

One possible explanation could be based on the evidence that in the multiple unit discrimination apparatus the last discrimination unit is mastered most rapidly (Husband 1929, Spence 1932, Hull 1932, and others) and, therefore, it may be very well fixed. However, this explanation is easily discarded by our finding showing that, when the pathways in all four partitions are altered, the reversal task becomes just as easy as in the case of the change of the pathway in the first partition only.

Explanation of differences of reversal learning in relation to the task presented to the animal may be found, if we make an analysis of errors in Groups A, B, C and D (cf. Figs. 2, 3, 4 and 5). As seen in Fig. 2,

the behaviour of animals of Group D (alteration of the pathway in the fourth partition only) was peculiar. When the animals succeeded in reversal of the pathway in the fourth partition (which occurred after about 12 runs, and was completed after about 30 runs), they began to commit errors in the third partition, and, thereafter, in the second and in the first one. On the other hand, when the animal was confronted with an alteration of the pathway in the first partition, the greatest number of errors was made at that partition, but, in addition, from the first run a considerable number of errors was made at other partitions (Fig. 3). Since the decrease of errors was the steeper, the greater was their original number, all errors vanished in approximately the same time (in the 25th run). A quite similar situation was encountered when the pathways in all four partitions were altered.

It seems that our results throw some light on the mechanism of acquisition of a complex habit in the multiple unit discrimination apparatus. If we accept that learning and reversal learning of each unit occur quite separately, the easiest task would be the alteration of the pathway in the single partition, while the most difficult task would be that provided by the change in all four partitions. Our results contradict, however, this assumption. They point out that the multiple unit discrimination apparatus represents one single task which is changed as a whole even when the alteration of the pathway concerns only a part of it. The reversal learning under conditions of alteration of the pathways in all four partitions is rapid because errors at all partitions are eliminated more or less simultaneously. The same holds true for the situation in which only the pathway in partition A is changed because the reorganization of the whole habit occurs from the very beginning of the reversal learning. However, the situation is quite different when the change of the pathway is made in the fourth partition only. In this instance, the reversal learning consist of two stages: running through the first three partitions is unchanged, while at the fourth partition a change of the way is required. The animal is able to master this task, but thereafter, his entire habit becomes disorganized and errors start to occur at the partitions at which no alteration of pathways is required. Only after a considerable number of additional runs does the animal relearn what he "knew" from the very beginning.

To summarize, we may say that the multiple unit habit represents one single kinesthetic stereotype which is transformed as a whole even if only one of its elements is changed.

These results contradict those obtained by Kogan (1951) who studied the problem of the interpolated learning and relearning. The author used a maze composed of the units with a double choice each, and he found that in reversal learning experiments each unit was learned separately.

Two factors may account for this difference. First, Kogan did not train his animals to a criterion, but instead, he used 8 runs. As seen in Figs. 2, 3, 4, and 5, of the present paper sharp decrease of errors in the unit occurs within 8 runs. Since the author did not follow the further course of reversal training, he could not find errors in the other units. Secondly, in Kogan's experiments the animal after having committed an error, was required to return to the choice point. It is possible that the animal was not able to treat the maze as a whole under such conditions.

SUMMARY

1. The present paper is concerned with the course of reversal learning in relation to the pattern of alterations in the four partition quadruple choice apparatus. The partitions from the starting point to the end of the maze were called A, B, C and D respectively.

2. It was found that if the alterations were introduced in all four partitions, or in partitions A, AB and ABC, the reversal learning required half of the runs necessary for the original learning. On the other hand, when alteration was introduced only in partition D, the reversal learning required 10 percent more runs than the original learning. The experiments with alterations in B, C, BCD, CD took the medium course.

3. An analysis of the considerable prolongation of the reversal learning in which the pathway in partition D was altered has revealed that it occurs in two stages: in the first stage the animal learned to alter his way at partition D, in the second stage, however, the disorganization of the whole habit took place, and the animals committed errors in those partitions in which the doors were not changed at all.

4. The results of these experiments indicate that the animals do not solve this task by learning separately the pathways in each partition, but rather treat it as a single kinesthetic problem.

I wish to express my thanks to Professor Jerzy Konorski for his help in the preparation of this paper.

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CONDITIONING AND DIFFERENTIATION IN THE CHRONIC MIDPONTINE PRETRIGEMINAL CAT

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As shown by Batini, Moruzzi, Palestini, Rossi and Zanchetti in cats (1959), the isolated cerebrum of a midpontine pretrigeminal preparation (MPP), obtained by a pontine transection performed just rostral to the entrance of the trigeminal nerve, is characterized by EEG rhythms and ocular activity suggesting alertness. Affani, Marchiafava and Żernicki (1962a, b, c,) have recently shown, that in the acute MPP habituation of the orientation reflex and conditioning is possible. The experiments, which will be described in this paper, were performed on chronic MPP in which inhibitory CRs were trained in addition to the positive CRs.

MATERIAL AND METHODS

Experiments were carried out on 12 midpontine pretrigeminal cats in which the isolated cerebrum displayed alertness as shown by the vertical movements of the eyes following a finger (Batini et al. 1959, Affani et al. 1962a).

The animals were operated in aseptic conditions under nembutal anesthesia (40 mg/kg). The brain stem transection was performed through cerebellum with a spatula oriented stereotaxically 60° from the horizontal plane. If following the transection a marked oedema of the brain occurred, the middle part of the cerebellum was removed by suction. After the resumption of spontaneous respiration, a chronic monopolar electrode was stereotaxically implanted in the perforical area of the right hypothalamus, at the point which, on stimulation, produced the most remarkable pupillary dilatation (cf. Santibanez-H., Tar-

necki and Żernicki 1960). An indifferent electrode was placed under the skin of the neck and attached to the skull.

The operated animals were housed in cages in an incubated room. The rectal temperature of the cats was maintained at 35° to 39°. Antibiotic therapy was administered in the first three postoperative days, and later, if necessary. The animals were fed by a stomach tube twice daily. However, in the first two postoperative days the food was given by rectum to avoid vomiting. In addition, 15cc. of 5% glucose was given subcutaneously twice daily. Urine was evacuated manually twice daily, and an enema given when necessary. The eyes were washed twice daily with acidum boricum solution. Due to the fact that the lid reflex was disturbed, the eyes were kept closed with bands attached to the lids by Mendelejev's wax and were open only before the experimental session.

The CR experiments were started when animal began to follow a finger with its eyes, which happened on the first or second postoperative day, and were performed twice daily. During the experiment, the cat was firmly fixed in a holder and placed in a small chamber. The chamber was illuminated (from 15 to 35 foot candles in different cats) to produce a moderate myosis of the pupils. The eyes of the cat were kept open wide with bands attached to the lids. During the session the left eye was observed and the right photographed.

As CSi two small visual objects, a rotating blue-red cross ("rotor") and a black horizontal oscillating disk ("oscillator") were used. Both CSi were placed close to each other, directly in front of the animal's eyes at a distance of 8 cm. In five cats, the rotor was used as a positive CS, and the oscillator as a negative (differentiated) CS, and in seven cats, vice versa, the oscillator was reinforced by the US and the rotor differentiated. The US was electrical stimulation of the hypothalamus (cf. Ban and Shinoda 1956, 1960 and 1961, Nakao 1959, and Affani et al. 1962b) which produced pupillary dilatation accompanied in some cats by ocular movements. The pulses were rectangular, rate 50—100/sec, the pulse duration 0.5—1 msec., and duration of the train 2—3 secs. in different cats. The voltage employed was just adequate to produce a sufficient UR. Each experimental session consisted of 10 to 20 trials. The intertrial intervals varied from 1 to 2 min.

After death the brains of the animals were fixed in 10% formaline. Serial frontal sections were made and stained alternately by hematoxylin-eosyn and Heidenhain techniques to ascertain the extent of lesion and the position of the hypothalamus electrode.

RESULTS

Anatomical verification of brains. In ten cats division between the isolated cerebrum and the caudal part of the MPP was complete except for the most basal pyramidal fibers which remained intact in some preparations. In two cats the transection of the brain stem appeared to be incomplete: the pyramidal tracts and some reticular cells lying dorsal and lateral to the pyramidal fibers and in the most lateral parts of the pons remained intact. In all the cats, however, the trigeminal rootlets were destroyed or remained in the caudal part of the preparation.

In the isolated cerebrum, at the level of the inferior collicular commissure, most of the anatomical structures were saved in all preparations; the mesencephalic reticular formation, ventral and medial pontile nuclei, caudal reticular pontile nucleus, raphe nuclei and some cells of the trigeminal mesencephalic nucleus remained intact. The long tracts (cortico-spinal tract, cortico-bulbar tract and medial longitudinal fasciculus) were partially demyelinated. The level of lesion was never so high as to destroy the oculo-motor nuclei.

In the caudal part of the preparation the lesion usually destroyed the tegmentum down to the level of the inferior olivary nuclei. The long tracts were partially demyelinated.

The tip of the implanted electrode was placed in the perifornical area, or just above it.

Survival time. Three cats, which lived from 8 to 9 days were sacrificed after the end of CR training. Other cats died after 3, 5, 6, 6, 8, 8, 8, 27, and 41 days after the transection. In most of these cats, the CR training was not completed. In the two cats which lived for 27 and 41 days, the transection was incomplete.

Caudal part of the preparation. Decerebrate rigidity and phasic reflexes were present from the first postoperative day. The temperature of the preparation followed the changes of the room temperature. When the room temperature was constant, the temperature of the animal was also approximately constant. At a room temperature of about 26°, the rectal temperature could be usually kept at 35°—39° in different cats. Defecation was spontaneous, but usually irregular. In the first days, vomiting occurred in some preparations. Spontaneous urination was absent. Breathing was regular in most cases, and averaged about 20/min.

After about two weeks of survival, the two cats with incomplete lesion could right the head and the anterior part of the body, turn from side to side and swallow food. They could also urinate, but evacuation of the bladder was not complete.

Conditioned-reflex training. At the beginning of the first CR session orientation reactions to the rotor and the oscillator were tested (cf. Affani et al. 1962a). In some cats, these stimuli produced a pupillary dilatation which, when the eyes of the cat were directed upwards or downwards before the stimulus was applied, was accompanied by a vertical eye movement towards the stimulus. In other cats, however, no reaction to the visual stimuli was observed. Since the CR, which was established later, was also a pupillary dilatation, we used as

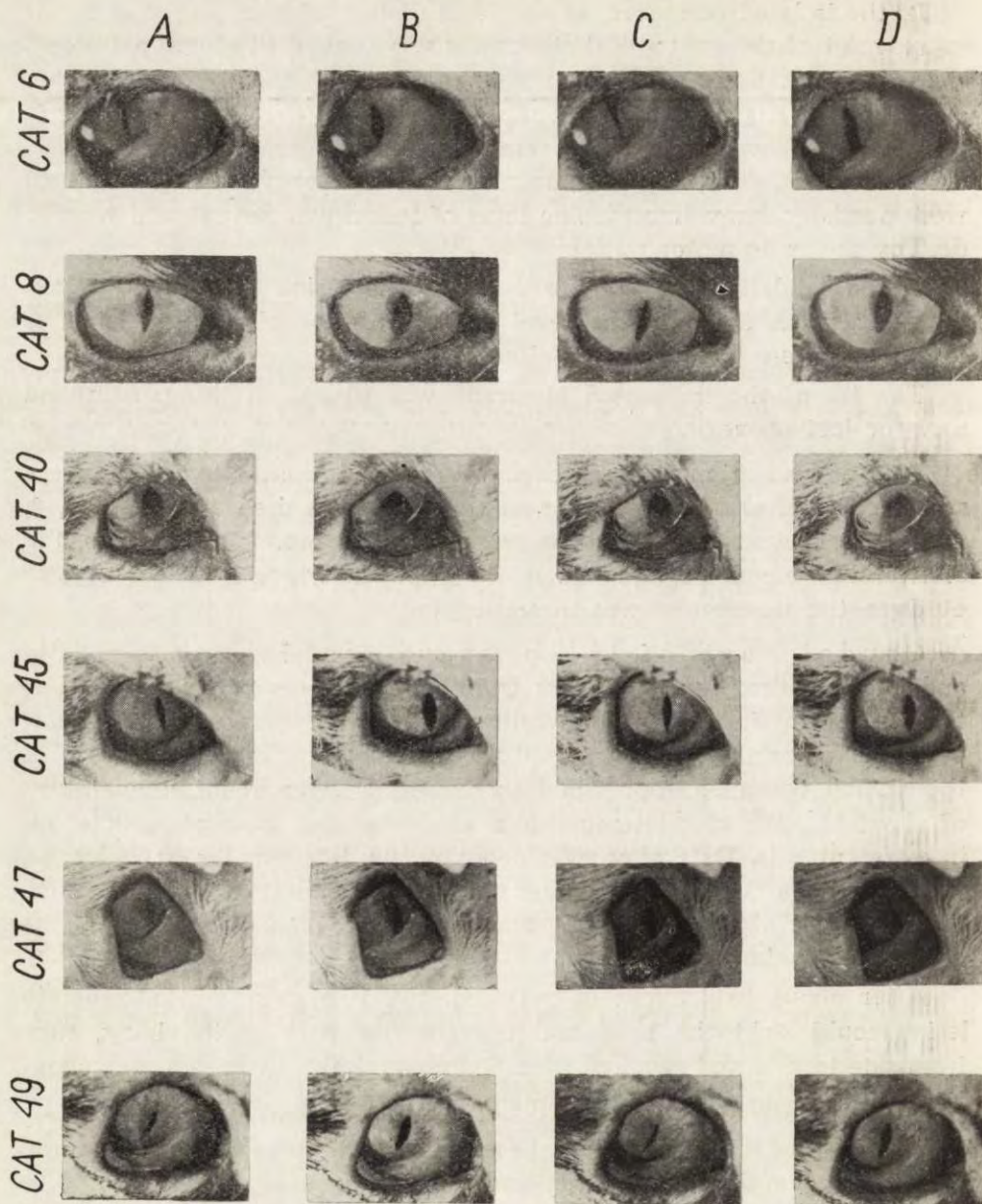


Fig. 1. Conditioning and differentiation

Excerpts of protocols of CR sessions. A, background of pupillary diameter; B and D, pupillary dilatation to the positive CS; C, failure of reaction to the differentiated CS. Pictures in rows B, C and D were taken in three successive trials. The pictures were taken automatically three seconds from onset of the CS. In cats Nos. 6, 40 and 49 the rotor was a positive CS, and the oscillator was differentiated, while in cats Nos. 8, 45 and 47, the oscillator was positive CS and the rotor differentiated. Cat No. 6, there 23th session of testing, cat No. 8 — 15th, cat No. 40 — 12th, cat No. 45 — 6th, cat No. 47 — 3th, cat No. 49 — 10th

a positive CS the stimulus which produced either none or a smaller orientation reflex in relation to the other stimulus.

In the first experimental sessions, only one CS was applied (the rotor in five cats, and the oscillator in seven cats) being always reinforced by hypothalamic stimulation. In the very beginning, the CS was reinforced almost immediately, but later, when pupillary dilatation occurred (or increased, if present previously) the CS-US interval was gradually increased to three seconds. The CS-US overlapping period was always one second. In spite of the fact that in some cats the CR training was too short due to the short time of survival of the animals, and in some other cats the US produced rather weak pupillary dilatation, a definite and constant dilatation to the CS was established in seven cats (Fig. 1), and a weak one, in four cats. Only in one cat was the CR not obtained. The conditioned pupillary dilatation was accompanied by a vertical eye movement towards the stimulus in those cases in which before application of the stimulus the eyes had been directed upwards or downwards. The speed of establishment of the CR varied considerably in different cats. In some animals, a clear pupillary dilatation to the CS appeared during the second experimental session and, occasionally, even in the first session. In other cats, a constant CR could be obtained not earlier than after 5 to 8 sessions.

In seven cats, in which a definite CR to the positive CS had been established, the second visual stimulus was introduced and applied randomly three times during the session among the excitatory trials and without hypothalamic reinforcement. When this stimulus was first given, it produced, as a rule, a considerable pupillary dilatation which could, occasionally, be as strong as, or even stronger than that to the positive CS given in this session. However, this reaction diminished rapidly with successive presentation of the stimulus. After several sessions the reaction to the negative CS was definitely differentiated in six cats (Fig. 1), and partially in one.

In the intertrial intervals the cats reacted vigorously with pupillary dilatation and eye movement to any accidental stimulus, as e.g. to a small movement of the camera. Sometimes, also spontaneous pupillary dilatation and eye movement were observed.

DISCUSSION

The present experiments confirm our previous results obtained in acute preparation (Affani et al. 1962b) that conditioning is possible in the isolated cerebrum of the MPP. We have satisfactory evidence that the pupillary dilatation obtained to the positive CS should be

considered as due to the formation of real conditioned connections, and not to pseudoconditioning related to the sensitization of the orientation reflex on the background of the frequent hypothalamic stimulation (cf. Affani et al. 1962a). First, the pupillary dilatation was gradually increased with the number of CR sessions, while the orientation reflex should be, on the contrary, habituated. Furthermore, the pupillary dilatation to the CS was also present in the first trial of successive sessions, i.e. before the first application of the hypothalamic stimulation. The crucial evidence, however, lies in the fact that the reaction to the negative CS applied without reinforcement but against the same background of hypothalamic stimulation, gradually disappeared completely, or almost completely.

Both visual stimuli used in our experiments were similar: they were represented by small mobile objects located in the same place. Therefore, the strong reaction to the negative CS, when it was first given, may be explained not only as an orientation reaction to the new stimulus, but also as generalization with the positive CS. In consequence, the disappearance or a strong diminution of the reaction to the negative CS with repeated application seems to be due to the differentiation and only partially to the habituation of the orientation reflex. The possibility of the formation of inhibitory conditioned connections in the process of differentiation indicates that in the isolated cerebrum of MPP relatively complicated nervous functions are present.

The speed of formation of CRs (positive as well as inhibitory) varied considerably in different cats. The same phenomenon, however, was observed also in normal animals (Pavlov 1940). It must be added that in our experiments the orientation and conditioned effects were qualitatively the same, and in some preparations, in which positive CR was very quickly obtained, it could be a summation of both orientation and conditioned reactions. Later on, however, when the orientation component of the reaction was habituated and the conditioned connections were strengthened due to the conditioning procedure, the pupillary dilatation should obtain purely conditioned character.

The CR was established in all cats (except for one) who followed a finger with vertical eye movements. This reaction, therefore, can be utilized in practice as a sign that the isolated cerebrum of a MPP is in a satisfactory state for conditioning.

The MPP, owing to its absolute lack of pain and voluntary movements which permit surgical intervention and careful analysis of the ocular responses, has some advantages over an intact animal in CR experiments. Our results show that it is possible to keep a chronic MPP

in good condition for a sufficient time to study its higher nervous functions. However, the survival time of our cats (with complete transection) was shorter than that of the animal in which the brain stem transection has been made more rostrally (cf. Bazett and Penfield 1922, Keller 1932 and 1938, Bazett, Alpers and Erb 1933, Kelly, Beaton and Magoun 1946, Bard and Macht 1958, and Batsel 1959). Our results on cats who survived for a relatively long time after incomplete transection, indicate that it is also possible to utilize such preparations in CR investigations.

SUMMARY

Conditioning and differentiation was studied in the isolated cerebrum of chronic midpontine pretrigeminal cat. As positive and negative (differentiated) CSi two similar visual objects were used. The US was hypothalamic stimulation which produced a pupillary dilatation. In six cats, the definite conditioned pupillary dilatation was established to one of the CS, while the reaction to the second CS was differentiated. The chronic midpontine pretrigeminal cat is a convenient preparation for the study of the higher nervous functions.

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FOOD PREFERENCE AND CONDITIONED REFLEX TYPE II ACTIVITY IN DYNAMIC HYPERPHAGIC RABBITS

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It is generally considered that the medial hypothalamus, particularly its ventral part, exerts an inhibitory influence on food intake since animals subjected to small bilateral lesions in that area become hyperphagic and obese (Brobeck, Tepperman and Long 1943, Anand and Brobeck 1951 a,b, Anand, Dua and Shoenberg 1955).

Teitelbaum (1955) reported an experiment suggesting that obese hyperphagic rats show a preference to certain kinds of food, and that taste characteristics are the major factors determining the food preference.

Miller, Bailey and Stevenson (1950) found that hyperphagic rats following destruction of the ventromedial nucleus of the hypothalamus reduced the frequency of their lever pressing activity despite an increased drive for food. Accordingly, they formulated a dualistic theory of the feeding centre, which recently was extended by Morgane (1961) suggesting that in addition to the feeding system a "hunger motivating system" exists in the hypothalamus. Since, however, the ventromedial rats in Miller's experiment were not tested every day, and between the testing sessions they were fed ad libitum, it might be supposed that they were at a static hyperphagic level. Thus, an investigation has been designed to see the behaviour of dynamic (non-obese) hyperphagic animals maintained on a small constant diet out of the testing cage, and basically fed in the experimental situation in which food was presented in response to the instrumental reflex activity. In addition, the technique of the presentation of two kinds of food was utilized to reflect the food preference of the dynamic hyperphagics.

MATERIAL AND METHODS

Experiments were carried out upon 18 male and female rabbits aging about 2 years and weighing 2 to 3 kgms. in which the type II (instrumental) conditioned reflex (CR) was trained (Konorski and Miller 1933).

The animals were housed individually in small living cages in which they were kept on a standard diet. Their weight was measured once a week before and every day after the ventromedial lesion. Conditioning sessions were conducted in a two food tray testing cage (Fig. 1). All animals were divided into two groups.

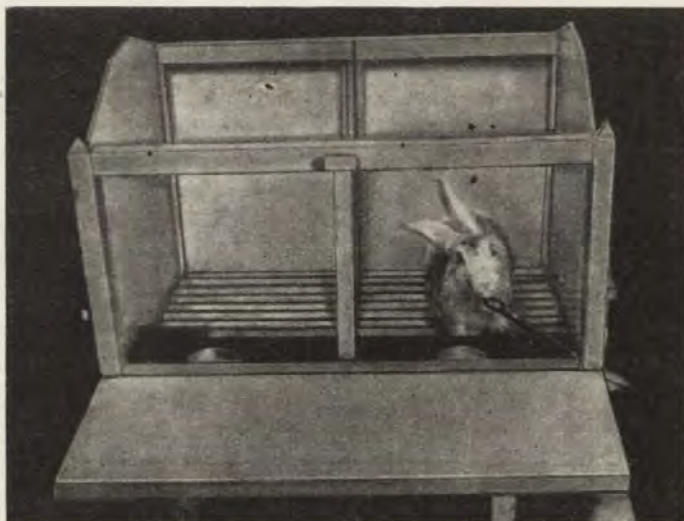


Fig. 1. The picture shows a rabbit in the experimental situation during the performance of the pulling-the-ring response

In front of the cage two cups of two food trays are shown. 5 other food cups in each food tray are hidden under the top lid, and therefore are not seen in the picture. During testing the animal faces one cup. Following conditioned reflex performance the next cup with food is presented by means of an automatic device. In the left part of the cage, the food tray with a small board for the forelimb response is shown. In the right part of the cage, the ring for pulling response

In both groups a forelimb conditioned reflex was trained. It consisted of placement by the animal its right forelimb on a board attached to the food tray which was situated at the left side of the cage. In the initial training, the animal's limb was passively placed on the board, and it was reinforced by the presentation of food. Subsequently, the animal learnt to produce this response actively and obtained food. In Group II rabbits (Nos. 11 to 18) a second type II CR was trained in addition. This was made according to the Malinowski technique (1952): the CR was the animal's pulling a bakelite ring attached to the right side of the cage near the second food tray. Initially, in an attempt to focus the animal's attention on the ring, small pieces of carrot were fastened to, or carrot

juice was put on it. The rabbits sniffed the ring, licked it, and occasionally, they grasped it with their teeth and pulled. This response was immediately reinforced by the presentation of food. From then on, it occurred voluntarily.

In Group I rabbits (Nos. 1 to 10) in which only the forelimb CR was trained, two separate testing series with different food reinforcements were carried out. In one of them the CR was followed by the presentation of carrot, while in the second experiment oats was offered to the animals. Each series persisted for two weeks.

The Group II animals, in which two CRs were trained, were divided into two subgroups. In subgroup A, which comprised the animals Nos. 11, 12 and 13, pulling-the-ring performance was reinforced by carrot, whereas the forelimb response was associated with the presentation of oats. In subgroup B, which constituted of rabbits Nos. 15, 16 and 17, pulling-the-ring performance was, too, reinforced by carrot, but the response of placing the limb on the board was rewarded with cooked purée-type potatoes.

In rabbit No. 14, pulling-the-ring response was reinforced by oats, while the forelimb performance was followed by the presentation of carrot. In rabbit No. 18, pulling-the-ring was reinforced by potatoes, and the limb response was associated with the carrot presentation (Table I).

Table I
Group II animals

Food	Subgroup A		Subgroup B	
	Pulling-the-ring response	Placement of the forelimb	Pulling-the-ring response	Placement of the forelimb
Carrot	Nos. 11, 12, 13	No. 14	Nos. 15, 16, 17	No. 18
Oats	No. 14	Nos. 11, 12, 13		
Potatoes			No. 18	Nos. 15, 16, 17

During testing sessions the animals ate ad libitum, that is, as long as they performed the instrumental CR. Out of the cage, they were maintained on a constant diet which was different from that used in the experimental situation.

Operations. After the animal was anaesthetized with Nembutal (37 to 42 mg/kg) injected intravenously, it was placed in the stereotaxic instrument with the headholder for rabbits described by Sawyer, Everett and Green (1954). Under aseptic conditions the skull was exposed and four holes were drilled in it with a dental trephine. Lesions were made at a depth of 14 to 16 mm. below the surface of the dura at points corresponding to the bregma, 1 mm. posterior to it, and 0.8 mm. lateral to the midline, by passing a direct current of 3 mA for 15 secs.

Testing the animals was resumed a few days after surgery. Three to four months later the animals were sacrificed and their brains fixed in formaline. In an attempt to identify the placement of the lesions brain sections were cut and stained with thionine according to Nissl technique (Fig. 2).

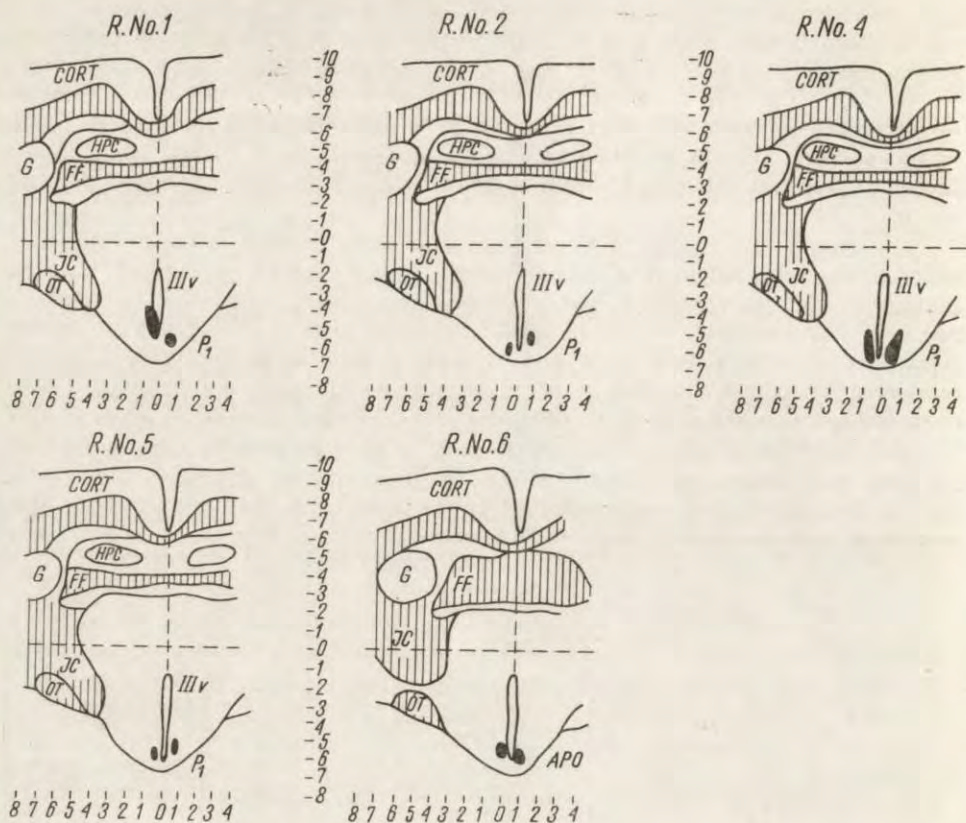


Fig. 2. Examples of reconstructions of ventromedial hypothalamic lesions described in the text

Cort — cerebral cortex, FF — the fimbria of the fornix, G — the mammillothalamic tract, HPC — the hippocampus, IC — the internal capsule, OT — the optic tract, III V — the third ventricle

RESULTS

Food intake. In 16 rabbits out of 18 a striking hyperphagia occurred 2 to 5 days following operation. In addition to overeating the ventromedial animals took food ravenously, and while eating they often insisted on the next presentation of reward. They also were easy to irritate, and some of them displayed aggression or exaggerated reactions to a variety of external cues. Also, it was clearly seen that the dynamic hyperphagic animals showed a marked preference for oats and cooked potatoes. This contrasted with the preoperative behaviour, since prior to hypothalamic destruction the animals preferred to take carrot. Rabbits Nos. 3, 11, 12, 15, 17 and 18, displayed also a slight increase of

carrot intake at the early postoperative stages, but, if they had a free access to carrot and oats, they overate oats, or if carrot and potatoes were available, they preferred to eat potatoes. In the later postoperative period, the carrot intake slightly increased in rabbits Nos. 1, 2, 7, and 17. However, in most instances the intake of carrot in ventromedial rabbits either remained at the preoperative level (rabbits Nos. 5, 8, and 18) or it was reduced (rabbits Nos. 3, 10, 11, 12, 14 and 15). Conversely, the postoperative intake of oats and potatoes progressively increased. Group I rabbits, in which the forelimb response was reinforced either by the presentation of carrot in one experimental series, or by oats in another series, showed an increased drive for both kinds of food, although the preference for oats was much more evident (Figs. 3 and 4). In average, in animals of Group I the carrot intake increased twice

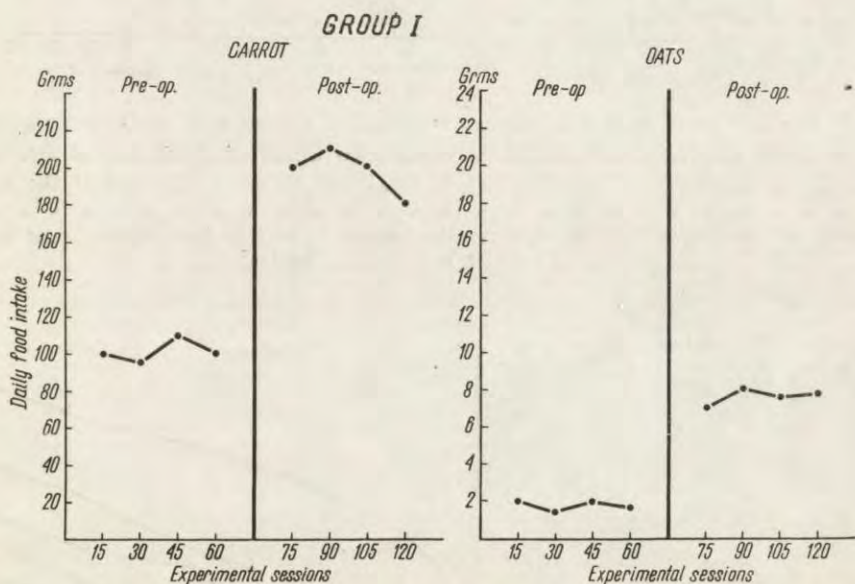


Fig. 3. Average food intake in Group I animals before and after ventromedial hypothalamic lesion in a situation in which either carrot or oats were presented. Each point corresponds to average size of daily food intake obtained from 15 experimental sessions.

postoperatively, whereas the intake of oats amounted up to 400 percent of the preoperative level. Rabbits Nos. 3, 5 and 6 occasionally ate 5 to 7 times as much oats as prior to operation.

In all instances, the dynamic hyperphagic animals gained weight (Fig. 5). Increase of the body weight occurred 5 to 10 days after operation and it progressed within the 3 months of observation.

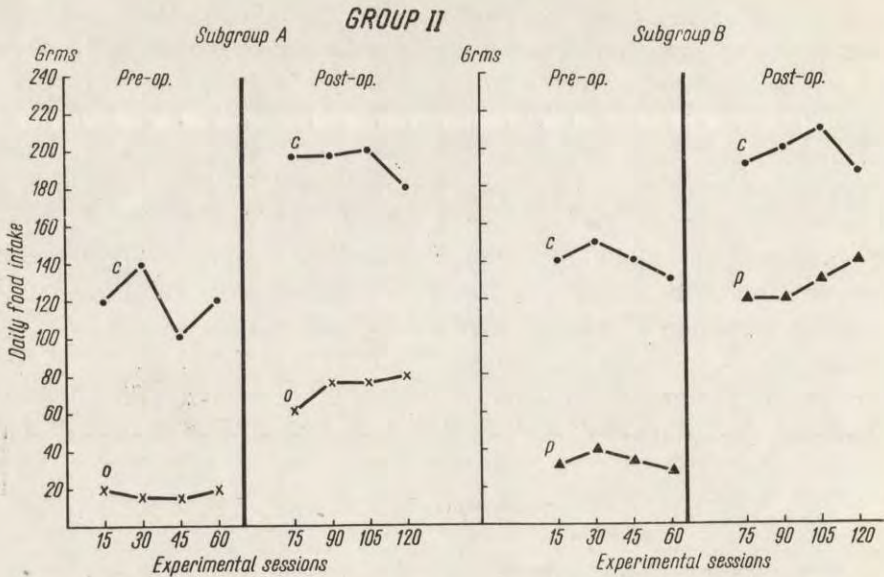


Fig. 4. Average food intake in Group II animals before and after ventromedial hypothalamic lesion in a situation in which two kinds of food (carrot and oats in Subgroup A, or carrot and potatoes in Subgroup B) were offered at the same time

C, carrot; O, oats; P, potatoes. Each point corresponds to average size of daily food intake obtained from 15 experimental sessions

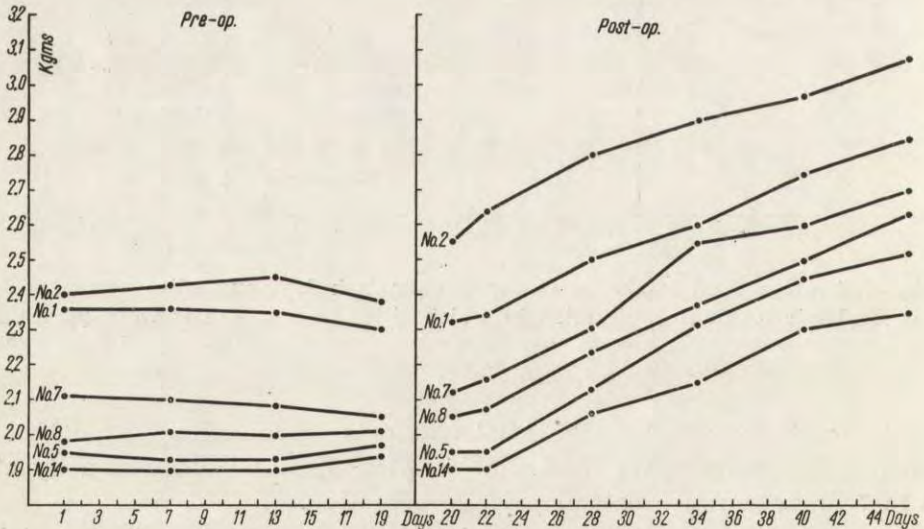


Fig. 5. Increase of the body weight following ventromedial lesion of the hypothalamus in rabbits

Abscissa: days of weight measurements; Ordinate: the body weight in kgms

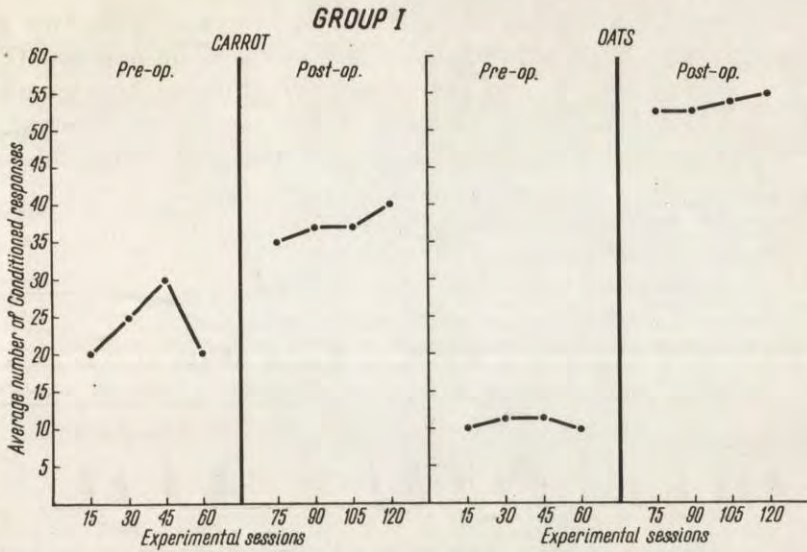


Fig. 6. Increase in conditioned reflex type II activity following ventromedial hypothalamic lesion in Group I animals in a situation in which either carrot or oats were presented

Each point corresponds to average conditioned reflex type II activity obtained from 15 experimental sessions. Note the striking increase in the postoperative instrumental activity in the oats situation

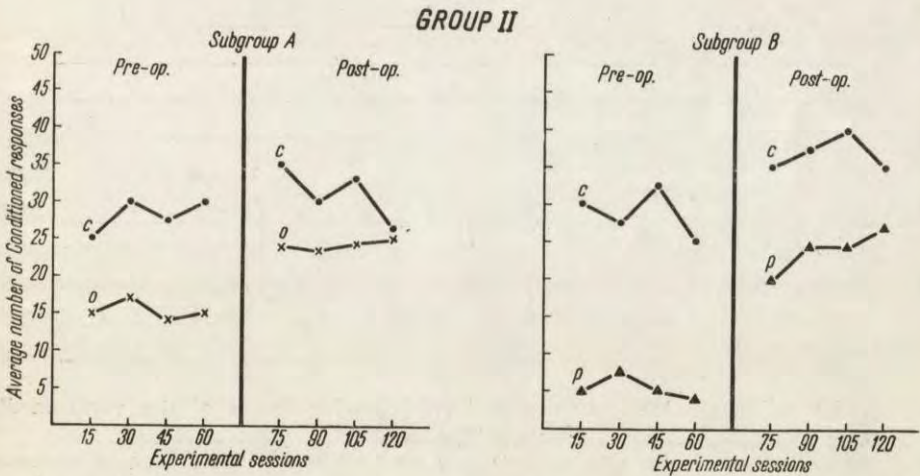


Fig. 7. Increase in conditioned reflex type II activity following ventromedial hypothalamic lesion in Group II animals in a situation in which two kinds of food (carrot and oats in Subgroup A, or carrot and potatoes in Subgroup B) were offered at the same time

Each point corresponds to average conditioned reflex type II activity obtained from 15 experimental sessions. C, carrot situation; O, oats situation; P, potato situation. Note that increase in the postoperative instrumental activity is less conspicuous than that in Group I

Conditioned reflex type II activity. With two exceptions, which will be described later, an increase in number (Figs. 6 and 7) and amplitude of the preoperatively acquired instrumental CR was noticed in dynamic hyperphagics. It was particularly evident in the situation in which the preferred food was presented. Thus, the ventro-

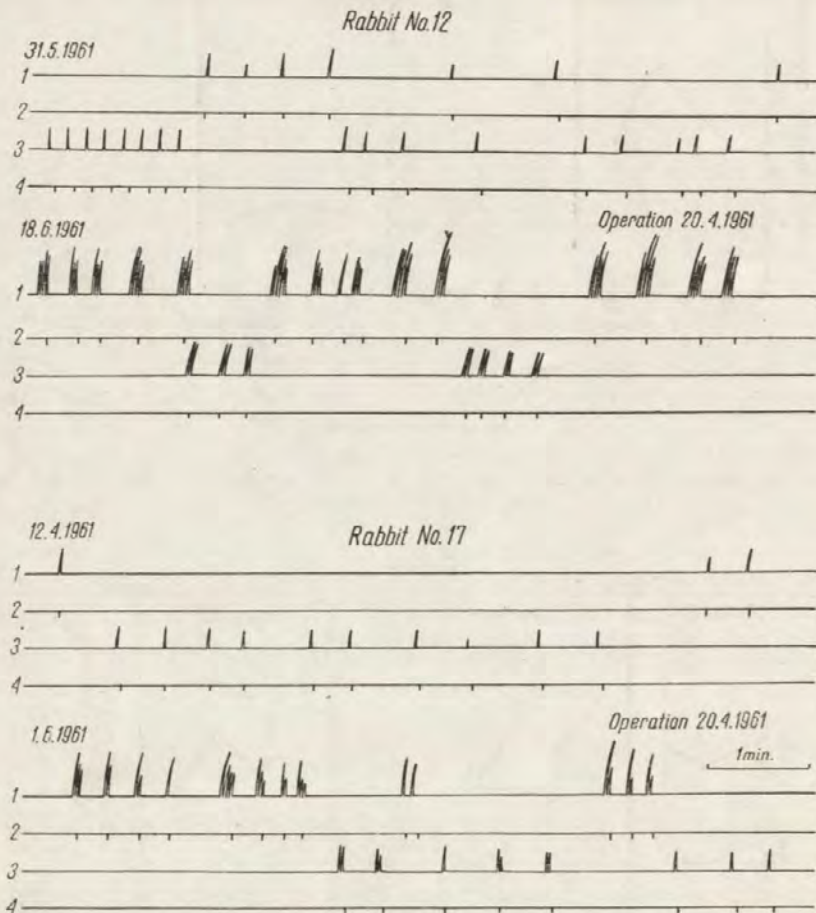


Fig. 8. Alterations in the response pattern following lesion of the ventromedial hypothalamus

A — Typical response pattern in rabbit No. 12 obtained under experimental circumstances described in the text. Tracings represent continuous recordings from experiments before (above), and after (below) operation; B — Rabbit No. 17. The same as in A. The upper tracing (1) is that of the forelimb response; the upward movement indicates flexion and placement of the right forelimb on the food tray. The second line (2) marks the time in which food (oats in rabbit No. 12; potatoes in rabbit No. 17) was presented after the forelimb performance. The third tracing (3) is that of the pulling-the-ring response, and the line which is underneath (4) marks the time of food reinforcement (carrot) for this performance. Note that the postoperative increase in conditioned reflex type II activity is greater in the oats and potato situations than that in the carrot situation

medial animal used to repeat the instrumental reaction, and it did it so rapidly that food reinforcement usually followed a series of responses (Figs. 8a and b.) Occasionally the forelimb response developed into scratching behaviour, or the animal did not take off its limb from the food tray. At times, it also produced the next series of instrumental responses while eating and insisted on the presentation of the subsequent food reward.

Conversely, rabbits Nos. 4 and 13 lost their preoperatively trained CRs. In spite of increased food drive and full preservation of motor performance, they were unable to produce the instrumental response. Instead, they showed a variety of basic feeding patterns in the experimental situation, such as approaching the cup, sniffing, licking etc. In rabbit No. 4 the instrumental response did not recover during the 2-month period of observation. Rabbit No. 13 returned to the preoperative CR performance. The pulling-the-ring habit recovered on the 50th postoperative day, and thereafter the forelimb response re-occurred. From then onward, rabbit No. 13 behaved like the other dynamic hyperphagic animals, i.e., it overate and preferred to take oats. It also produced an increased number of CRs when oats was offered. In the situation, in which carrot was presented, the instrumental responses were slow and less complete.

DISCUSSION

The results of these experiments clearly show that in addition to an increased food intake and preference to certain kinds of food, increase of preoperatively trained instrumental CR occurred in most of the dynamic hyperphagic rabbits. Increase in postoperative instrumental performance was particularly evident in the responses which were associated with the presentation of preferred food. In two animals out of 18, the instrumental response was postoperatively affected with no signs of impairment on motor activity. However, in one of them the conditioned response recovered by the end of the second month after the ventromedial lesion, and once it re-occurred it was as precise as before.

The parallel increase of food intake and instrumental CR reinforced by the presentation of food in ventromedial animals is clear in view of the hypothesis concerning the mechanism of instrumental conditioning (Wyrwicka 1952), suggesting a functional connection between the hypothalamic feeding centre and the centre of instrumental CR. Thus, the effect of the ventromedial lesions in increasing the strength

of the instrumental CR may be interpreted in terms of an abnormal activation of this connection due to the destruction of the hypothalamic satiation centre.

On the other hand, a permanent or transient impairment of the preoperatively established instrumental food CR, which was found in two cases, might be related to an unintentional damage to the medial forebrain bundle. This suggestion bases on the findings described by Miller, Bailey and Stevenson (1950), Miller (1956, 1960), Morgane (1961) and Morrison, Barnett and Mayer (1958) who showed that lesions in this bundle affected the instrumental performance, though food or water intake remained unimpaired or even tended to increase.

It is known that in addition to the increased food intake other changes in emotional behaviour occur after medial hypothalamic lesions. Thus, some of the investigators have reported rage responses (Wheatley 1944, Romaniuk 1962), increased activity and augmentation of the orientation reaction in medial hypothalamic animals. Also in our experiments it has occasionally been noticed that acoustic extra stimuli interfere with the food intake. It is likely that all these changes in behaviour patterns, taken together, exert an influence on the performance of the conditioned reflex type II, and this may explain the decrement of the rate of conditioned reflex type II activity in some of the medial hypothalamic rabbits which has recently been described by Lewińska (1963).

The findings in the present paper demonstrate that dynamic hyperphagic rabbits show a marked preference for oats and cooked potatoes. Since before operation the animals preferred to eat carrot, and there are no indications that dynamic hyperphagics are more sensitive to stimulus aspects of the diet (Teitelbaum 1955), it seems likely that other factors from taste qualities must produce the postoperative alteration of the preference tendency. It may be speculated that dynamic hyperphagic animals prefer to take oats or cooked potatoes due to small content of water in this kind of meal. This suggestion, however, needs additional experimental support.

SUMMARY

1. Experiments were carried out on 18 rabbits. In one animal group, a conditioned reflex type II was trained which consisted of an active placing the right forelimb on the food tray. Two 14-day testing series with either carrot or oats reinforcements in successive order were conducted. In the second animal group, two kinds of conditioned reflexes

type II were trained, viz. the forelimb response and the response of pulling a bakelite ring with the teeth. Each of these responses was followed by a different food reinforcement, namely carrot, oats or purée-type potatoes.

2. Following ventromedial lesions the dynamic hyperphagic rabbits showed preference for oats and potatoes.

3. The performance of the conditioned reflex type II increased after ventromedial lesions.

4. In two ventromedial rabbits the preoperatively trained conditioned reflex type II response was abolished. In one of them, it recovered after 2 months, whereas in the second animal the impairment was permanent.

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THE EFFECTS OF TOTAL AND PARTIAL ABLATIONS
OF THE PREMOTOR CORTEX ON THE INSTRUMENTAL
CONDITIONED REFLEXES IN DOGS

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In one of the previous papers of this series (Stępień, Stępień and Konorski 1960) the effects of lesions of the so-called premotor cortex on instrumental conditioned reflexes in dogs were described. It has been established that bilateral ablation of this area produces no deficit in the execution of skilled movements, although the patterns involved in instrumental conditioned reflexes are much disturbed. In particular, the animals lose the smooth sequence of motor responses displayed to the conditioned stimulus, viz. 1) the orienting reaction toward the stimulus, 2) the food reaction to the bowl and 3) the instrumental movement, and they are not able to integrate them.

The problem of the premotor area is controversial. Most neurologists and some of the neurophysiologists (cf. Fulton, 1949) claim that the premotor cortex possesses its own functional properties which are different from those of the motor area. On the other hand, Woolsey and his group (Woolsey et al. 1950, Travis 1955, Travis and Woolsey 1956, Pinto Hamuy 1956) point out that premotor area consists of two parts: 1) the anterior division of the motor area including representation of the movements of the neck and the body, and 2) the portion which is homologous to the supplementary motor area of Penfield and Welch (1949).

Recent anatomical studies by Adrianov and Mering (1959), and Kreiner (personal communication) have indicated that in front of the motor cortex in dogs there is a distinct area situated on the

lateral as well as on the medial aspects of the hemisphere (Fig. 1), which corresponds to the premotor cortex in primates.

Since during the preparation of our previous paper the borders of this area were not known, it seemed necessary to continue the functional analysis of the premotor area in view of the recent data. This was



Fig. 1. Dorso-lateral and medial views of the frontal lobes of dog's brain

The lateral and medial parts of the premotor cortex are indicated according to Kreiner (1962). L — the medial aspect of the left hemisphere, R — of the right hemisphere

even more desirable because the symptoms of premotor ablations described previously were complex and they needed further elucidation.

In the present investigation both excitatory and inhibitory conditioned reflexes were trained, and an attempt was made to define the functional role of different parts of the premotor area in the dog.

MATERIAL AND METHODS

Experiments were performed on 14 mongrel male dogs, aged from 2 to 4 years, in a soundproof conditioned-reflex chamber. In all animals, the excitatory conditioned reflexes (CRs) were established to auditory stimuli (buzzer, metronom, "tone" and rhythmic whistle). The trained motor reaction consisted of lifting the right foreleg and placing it on the foodtray. This movement, performed in response to the conditioned stimulus (CS), was reinforced by food. After the positive CRs had been firmly established, we proceeded to the elaboration of conditioned inhibition task in all the animals but one (L-4). One of the CSi hitherto applied — rhythmic whistle lasting for 10 seconds — was not reinforced when preceded by the white noise which acted for 5 seconds (conditioned inhibitor, CI). After a number of such trials the inhibitory CR to this compound was established. This being done, we gradually prolonged the interval between both components of the compound up to 5 seconds. The inhibitory training was continued until the animals reached the criterion level of no more than 2 positive instrumental responses in 20 inhibitory trials. Every experimental session consisted of 7 to 9 trials in which 2 trials given in random order were inhibitory, and the others were excitatory.

Surgery was done under aseptic conditions, the cortical tissue was removed by subpial aspiration. Total ablation of the whole premotor cortex including its medial and lateral part was performed in 2 dogs, T-1 and T-2; subtotal ablation, involving the whole lateral and part of medial premotor cortex was performed in 2 other animals, St-1 and St-2. The medial surface was removed in 6 dogs (M-1 to M-6), and the lateral surface in 4 dogs (L-1 to L-4). In all animals except two the ablation was performed bilaterally in one stage, while in dogs M-5 and M-6 the medial part of the premotor cortex was removed first on the left side, and 4 to 5 weeks later on the right side. The extent of lesions verified in postmortem examination of the brains is presented in Fig. 2.

The experiments were resumed one week after surgery. Since in many of our dogs the instrumental CR failed to occur during various periods of time after operation, we feared that the instrumental response might extinguish due to non-reinforcement, or it might be transformed into a classical form of CR while reinforcing every CS. Therefore, only few trials were given in the experimental session, the CSi were sometimes reinforced in spite of the failure of the trained movement, and the experiments were performed not every day but twice a week. When the instrumental CR had recovered, the postoperative training was carried out in exactly the same way as before ablation. The inhibitory conditioned stimuli (ICSi) were postoperatively applied only after the complete restoration of the instrumental response to the positive CSi.

RESULTS

It was found that bilateral lesions in the premotor area produced more or less pronounced disorders in: 1) general motor response to the CSi, 2) instrumental conditioned response and 3) inhibitory processes.

1) The general motor response to CSi was considerably changed in all animals in which the bilateral premotor ablation was performed in one stage (cf. Stępień, Stępień and Konorski 1960). The CSi elicited both exaggerated orienting reaction directed to the source of the sound, and/or alimentary reaction consisting of licking the empty bowl prior to the presentation of food. These disorders were very strong in animals with total premotor ablations, and, in some dogs, after partial lesions, involving either medial or lateral aspects of the premotor area (dogs: T-1, T-2, St-1, M-1, M-3, L-2, L-3). In other animals, they were also present, but less pronounced, i.e. they appeared only at the onset of the CS. Only in dogs M-5 and M-6, in which ablation was performed in two stages, were no changes in general motor response to CSi observed.

With a lapse of time, these abnormalities in motor behaviour decreased and after several weeks they disappeared nearly completely.

2) Instrumental CRs were impaired both after bilateral ablations of the whole premotor area, and after partial lesions. No signs of paresis or ataxia were found in any of the animals.

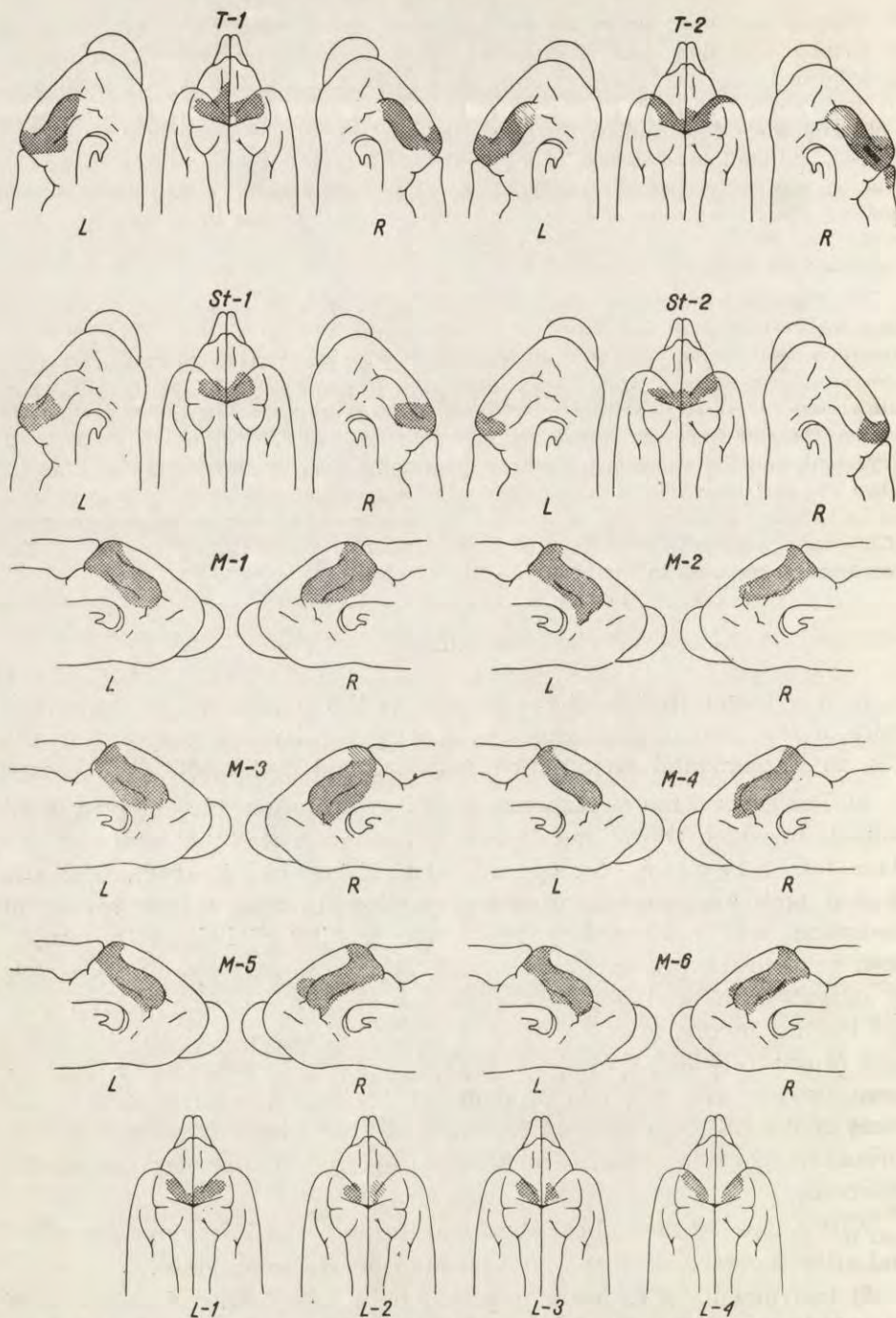


Fig. 2. Location of lesions of the premotor cortex on the lateral and medial aspects of the hemisphere

In 7 dogs, in which either total or partial lesions were performed, the instrumental CRs were abolished for 9 to 30 days (dogs: T-1, St-1, M-1, M-3, L-2, L-3) — Fig. 3. The animals did not perform the learnt movement even when the CS was prolonged to 20—30 secs. Instead, they looked toward the source of the sound or licked the empty bowl. Then, the motor CRs recovered spontaneously, i.e. without any additional training. Occasionally, this also happened after an interval of several days.

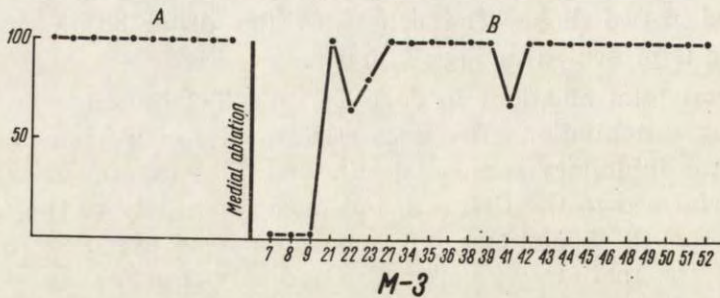
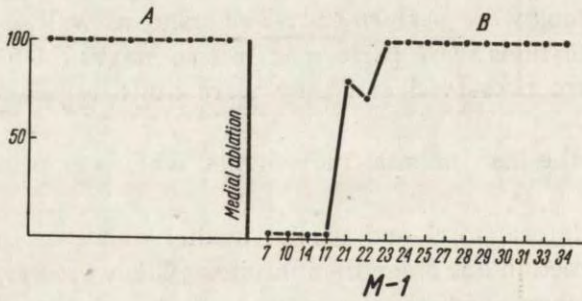
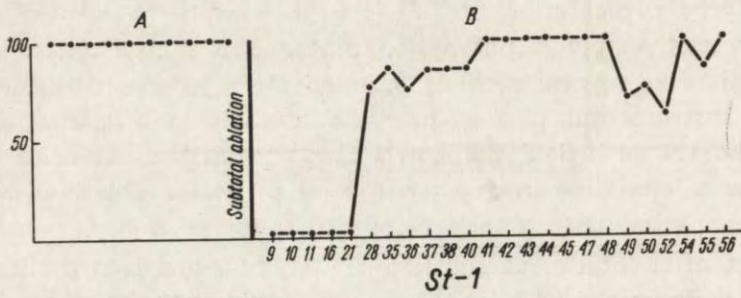
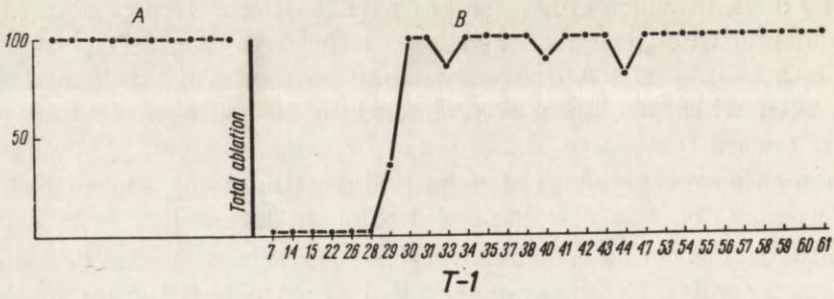
In the initial period after recovery, the instrumental conditioned activity was irregular, i.e. the animals failed to perform the learnt movement to some of the CSi. Later, the conditioned motor responses occurred to every presentation of CSi, but they were performed after longer latencies than before operation. In other words, the instrumental CR appeared after several seconds or even after cessation of the CS. Before the instrumental performance the animals looked toward the source of the CS, or licked the bowl. These prolonged latencies were observed for a long time in dogs with total premotor ablations, while in dogs with partial lesions they recovered quite promptly.

In 3 dogs, after total or partial lesions (T-2, M-2 and L-1) the instrumental CRs were preserved from the very beginning of the postoperative experimentation, but for a period of 4 to 6 weeks they were irregular (Fig. 4) and had prolonged latencies. In 2 other dogs (St-12 and M-4) in which bilateral lesions were performed in one stage, as well as in 2 dogs in which medial ablations were performed in two stages (M-5 and M-6), the motor CRs were preserved and they were quite regular from the very beginning.

In all the dogs the instrumental movements, whenever present, were quite skilful.

3) Following bilateral total and partial medial ablations of the premotor areas performed in one stage the inhibitory CRs were very strongly impaired. On the other hand, they remained nearly normal after removals of the lateral part of this region and also after medial ablations performed in two stages. The course of the disinhibition of ICRs in every dog is presented in Figs. 5 and 6.

Bilateral total ablations in dogs T-1 and T-2 caused a marked and longlasting disinhibition. The dogs performed the instrumental movement to the inhibitory compound stimulus. This movement was, sometimes, performed to the CI itself, but most often only to the CS which followed the CI. Sometimes, the movement was executed in the last second of the inhibitory compound. Gradually, the inhibitory function improved, so that after a period of time the animals were able to solve



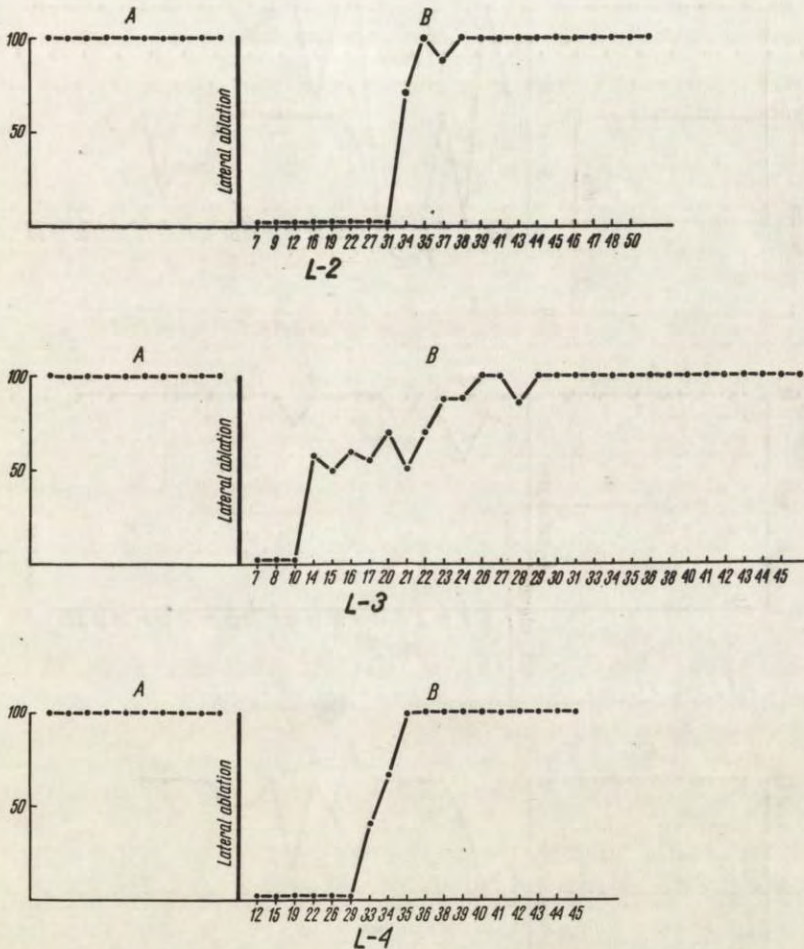


Fig. 3. The absence and recovery of the instrumental CRs after ablations of pre-motor cortex

Abscissa: successive experimental sessions. Ordinate: percentage of instrumental responses to CSi in one experiment. A — last 10 preoperative experiments. B — postoperative period.

The numbers indicate the length of time after operation in days

the inhibitory task. 80 inhibitory trials were necessary in dog T-1, and 50 trials in dog T-2, to reach the preoperative criterion level.

Quite similar disinhibition was observed in one of the two dogs with subtotal lesions of the premotor area (St-1) and in all 4 animals with bilateral ablations of the medial part performed in one stage (dogs: M-1,

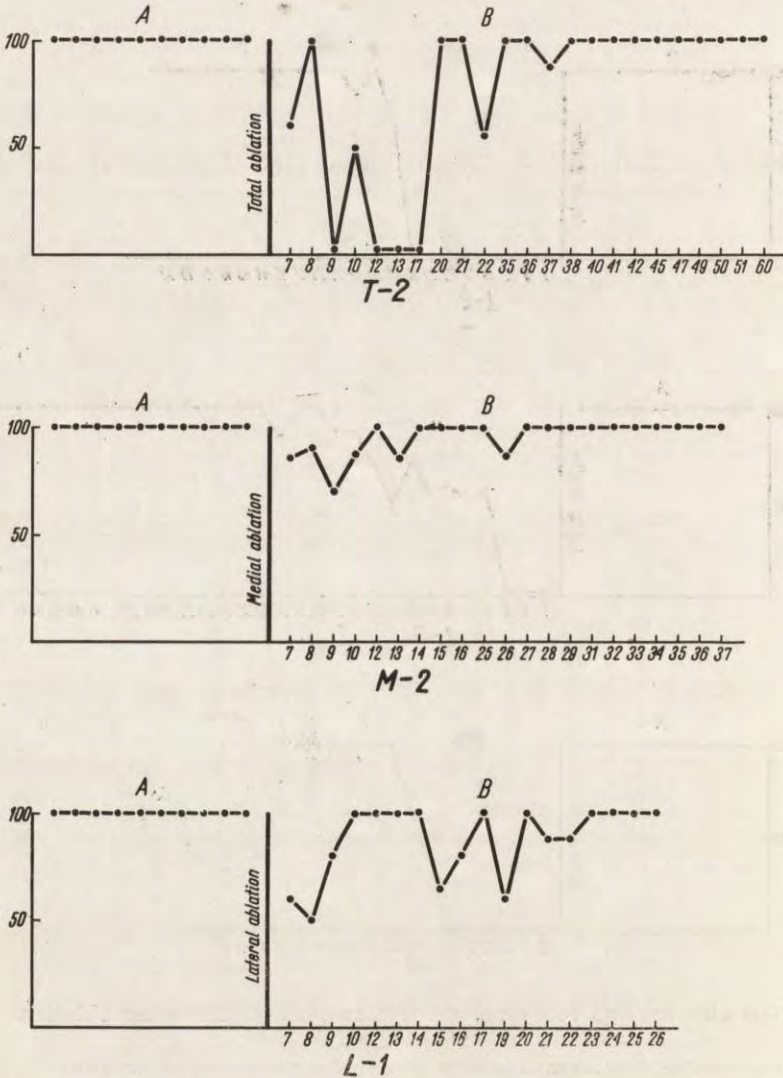


Fig. 4. Irregularity of the instrumental CRs following ablations of the premotor cortex

Explanations as in Fig. 3

M-2, M-3, M-4). In all these dogs but one the inhibitory function improved slowly, reaching the criterion level after 50 to 90 inhibitory trials. Dog M-3 was unable to solve the task even after 140 inhibitory trials,

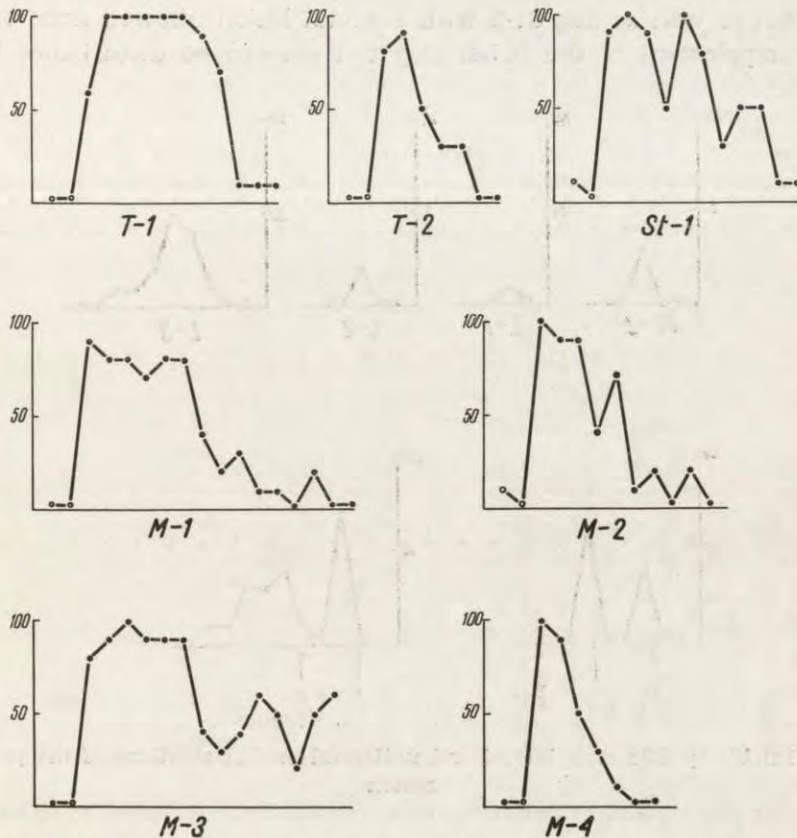


Fig. 5. Inhibitory CRs after total and medial bilateral ablations of the premotor cortex

Ordinate: percentage of errors in 10 inhibitory trials. Two first points — in the last 20 preoperative trials, the next points — in the postoperative period until the criterion level was reached

At the end of the postoperative training the animal became neurotic, and therefore further experimentation was discontinued.

The inhibitory abilities never recovered without training. If only positive trials were given, or if conditioned experiments were temporarily discontinued, the IRCs became even more disinhibited.

Following unilateral medial lesions in dogs M-5 and M-6 very slight disinhibition of ICRs was observed. These animals made 4 and 7 errors respectively only in the first 10 postoperative inhibitory trials. When

the same area of the second hemisphere was removed, the disinhibition of ICRs reappeared: dog M-5 made 6 errors, and dog M-6 made 10 errors.

The dogs in which only the lateral part of the premotor area was removed, as well as dog St-2 with subtotal lesion, showed none or only slight impairment of the ICRs. Dog L-1 showed no disturbance in the

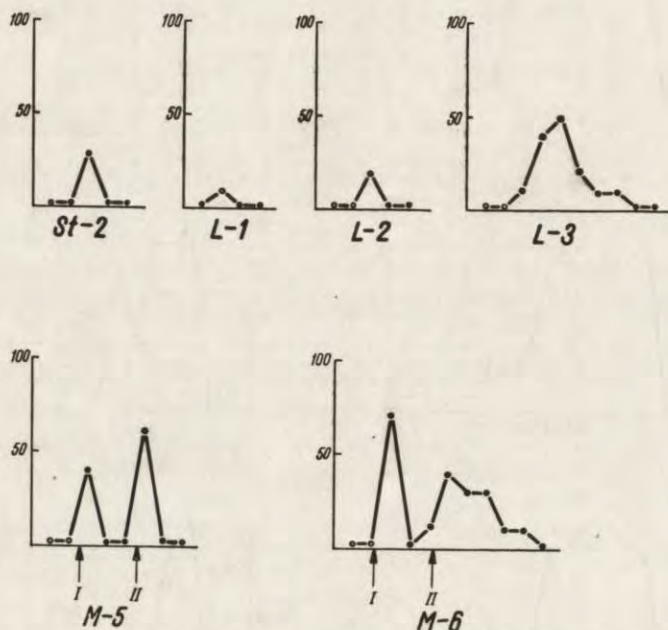


Fig. 6. Inhibitory CRs after lateral and unilateral medial ablations of the premotor cortex

I — left; II — right ablation of the medial area. Other explanations as in Fig. 5

inhibitory function from the very beginning of the postoperative period. Dogs L-2 and St-2 made 2 and 3 errors respectively in the first 10 inhibitory trials, and, henceforward, their inhibitory function was quite normal. Only in dog L-3 a somewhat stronger disinhibition was observed. This animal made 12 errors in 40 inhibitory trials before he reached the criterion level. However, this number of errors is quite small as compared with that made by the animals after medial lesions (see Table I).

The postoperative impairment of inhibitory processes was manifested not only by the performance of the learnt movement in response to the ICSi, but also by performing the intertrial movements. Before operation, the intertrial movements appeared only in the beginning of training. After the lesion, they occurred, in some dogs, much more often than

Table I

Disturbances in conditioned activity after ablations of the premotor cortex

Dog	Extent of lesion	General motor response to CSI	Instrumental CRs	Number of errors in inhibitory trials before criterion level was reached	Maximal number of intertrial movements in one experiment
T-1	total	orienting or strong alimentary	absent for 4 weeks	72	38
T-2	total	strong alimentary	irregular for 4 weeks	29	50
St-1	subtotal	orienting or strong alimentary	absent for 4 weeks	64	6
St-2	subtotal	orienting and alimentary	regular	3	5
M-1	medial	orienting or strong alimentary	absent for 3 weeks	61	18
M-2	medial	orienting or strong alimentary	irregular for 4 weeks	54	20
M-3	medial	orienting or strong alimentary	absent for 9 days reappeared after 12 day interval	criterion level was not reached	5
M-4	medial	orienting and alimentary	regular	27	17
M-5	medial-left	alimentary	regular	4	0
	medial-right	alimentary	regular	6	5
M-6	medial-left	alimentary	regular	7	2
	medial-right	alimentary	regular	10	0
L-1	lateral	orienting and alimentary	irregular for 3 weeks	0	0
L-2	lateral	strong alimentary	absent for 4 weeks	2	4
L-3	lateral	strong alimentary	absent for 10 days	12	0
L-4	lateral	strong alimentary	absent for 4 weeks	not examined	9

before operation. The most frequent movements, amounting up to 50 in one session, occurred in dogs with total premotor ablations. In dogs with medial lesions carried out in one stage (M-1 to M-4) the intertrial CRs were less frequent; they did not exceed 20 in one session. In animals with bilateral subtotal and lateral lesions, as well as in those in which the medial lesions were made in two surgical stages, the intertrial movements appeared very rarely.

All disturbances in conditioned activity observed in our animals after ablations of the premotor area are presented in Table I.

DISCUSSION

The results of these experiments confirm our previous data (Stepień et al., 1960) indicating that instrumental conditioned reaction may be impaired or temporarily abolished after bilateral lesions in the premotor area in spite of the fact that the premotor animals are as skilful as before operation. This motor disfunction may be produced either by damage to the whole premotor area, or it may follow partial ablations of the medial and lateral portions of the premotor region (dogs: M-1, M-3, L-2, L-3, L-4). However, in some dogs the instrumental response is preserved after partial and even after total premotor ablations (M-2, L-1, T-2).

The increased orienting reaction toward the source of the CS, as well as food conditioned response (licking the empty bowl), observed after extensive premotor ablations in our previous experiments, was also seen after both lateral and medial removals of this area. In both cases, each of these two abnormal reactions might interfere with the performance of the instrumental conditioned response. This is why the instrumental CRs were not executed, or appeared irregularly for some time after ablation of this area.

An even more pronounced and clear-cut symptom observed in our premotor animals was a strong impairment of the inhibitory processes, which was manifested in appearance of the instrumental movements in the intertrial intervals as well as in response to the ICSi.

The data presented in this paper seem to show that the medial part of the premotor cortex in dogs plays an important role in the inhibitory function. Ablation of this cortical area, if performed in one surgical procedure, produced a very pronounced and longlasting disinhibition of the ICRs similar to that observed after removal of the whole premotor cortex. It is worthwhile to note that the degree of disinhibition found after medial premotor ablations is similar to that after bilateral prefrontal lobectomies in dogs (Brutkowski et al., 1956).

On the other hand, the inhibitory function remained almost normal following bilateral ablation of the lateral part of the premotor cortex. However, the slight disinhibition of ICRs which may occur after ablation of this area seems to indicate that it may play some supplementary role in the inhibitory functions. This is supported by the fact that the excess of intertrial movements is much greater after ablation of the entire premotor cortex than that following selective lesion of the medial premotor cortex.

Another problem for discussion is the compensation of the disturbances in positive and inhibitory CRs. As far as the positive instrumental CR is concerned, its disappearance in the early postoperative period is connected with exaggerated orienting and alimentary reactions interfering with the execution of the instrumental movement. One may assume that gradual extinction of these reactions occurs both in and out of the experimental situation. This is why the restoration of the positive CRs seems to proceed spontaneously.

On the other hand, the recovery of inhibitory CRs was very clearly connected with the specific postoperative inhibitory training. Intervals in experimentation did not improve, but rather delayed the process of compensation.

The question arises as to the mechanism of this compensation. One possible explanation is that the other cortical areas take over the function of the destroyed premotor cortex. As shown by Brutkowski and Mempel (1961), and Szwejkowska et al. (in preparation) the pregenual, genual and subprereal areas lying in the immediate vicinity of the medial premotor cortex in the dog, are also involved in the inhibitory functions. These cortical areas which were saved in our dogs, may participate in the improving the inhibitory functions after removal of the premotor cortex.

The evidence indicating that the medial premotor cortex and other medial frontal areas are implicated in the inhibitory functions gives ground for the supposition that the entire medial aspect of the frontal lobes is involved in the inhibitory processes at least for alimentary CRs.

SUMMARY

1. The effects of total and partial ablations of the so-called premotor cortex on positive and inhibitory instrumental CRs were investigated in 14 dogs.

2. After bilateral premotor ablations (total, medial or lateral) the general motor reactions and instrumental response were more or less disturbed.

a. The CSi for some time elicited exaggerate orienting and food reactions.

b. The instrumental CRs for some time after operation were either abolished or were preserved but irregular, i.e. they appeared not to every CS and their latencies were prolonged.

3. Inhibitory CRs were strongly impaired following bilateral total or medial premotor removals. On the other hand, they were normal or only slightly disinhibited after whole lateral or unilateral medial lesions.

4. The possible mechanisms of these disturbances are discussed. The absence of instrumental responses seems to be caused by the abnormally strong orienting and alimentary reactions. Instrumental responses recover when these reactions become normal. The ICRs are restored only after postoperative inhibitory training. All animals, but one, reached the criterion level postoperatively.

5. It is concluded that the medial part of the premotor area in the dog plays an essential role in the inhibitory function, while the lateral part of this area may have a supplementary role in the inhibitory processes.

6. The data of the present study taken together with the results of other authors has led us to the conclusion that the medial aspect of frontal lobes in dogs is connected with the inhibitory processes.

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