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Acta Biologiae Experimentalis, Vol. XX

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POLLEN AS A SOURCE OF SOME FOOD CONSTITUENTS OF WAX MOTH (*GALLERIA MELLONELLA* L.) LARVAE

M. RYBICKI

Department of Comparative Animal Physiology, Zoological Institute,
University of Warsaw

(Received 15 October 1959)

The problem of the utilization of various honeycomb components by wax moth larvae has continued to be the object of many-sided research, most of the studies concerning the utilization by larvae of lipids of honeycomb, mainly wax, and, above all, higher fatty acids.

The ability of utilizing any honeycomb component is related to the problem of the source of nitrogen for larvae. This aspect of the problem has received comparatively little attention.

Two research directions, biological and chemical, can be observed in the survey of studies on the nutrition of wax moth larvae. The former direction has been followed by studies based mainly on cultures in which various honeycomb components have been the diet of the wax moth. Dönhoff (1882) fed the larvae with a mixture of wax and pollen. Various diet constituents fed separately yielded a negative result. Metalnikov (1908) fed the larvae with delipidized comb in one of his experiments. The remainder was mostly membranes of bee cells which were consumed by the larvae after they had been moistened. The results of this culture were positive, contrary to those obtained from feeding the larvae on pure wax. Allegret (1956) fed the larvae with crude wax used by beekeepers with mixture of pollen from various plants added to it. The larvae fed on this food grow quickly. The results obtained by Dönhoff and Metalnikov show that the wax* they used is not the principal

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* The name "wax" seems to be wrong and "lipid honeycomb fraction" appears preferable.

constituent of the diet of the larvae. Pollen is an important supplementation of this part of their diet as demonstrated by the results obtained by Dönhoff and Allegret.

Old dark-brown honeycomb is the optimum food of the larvae and its value and chemical composition depend on the number of generations of bee larvae hatched in honeycomb cells (Rybicki 1952). In old comb, fraction insoluble in lipid solvents accounts for nearly 57% of its fresh weight, while membranes of bee cells preponderate in fraction. Microscopic observation of membranes of delipidized bee cells shows that there is a great amount of pollen between the membranes at the bottom of the cell, part of it so closely sticking to the membranes that all attempts at separating pollen grains from the membranes failed. But it was possible to remove loose pollen grains from the bottom of a bee cell (after delipidization) (Rybicki 1952).

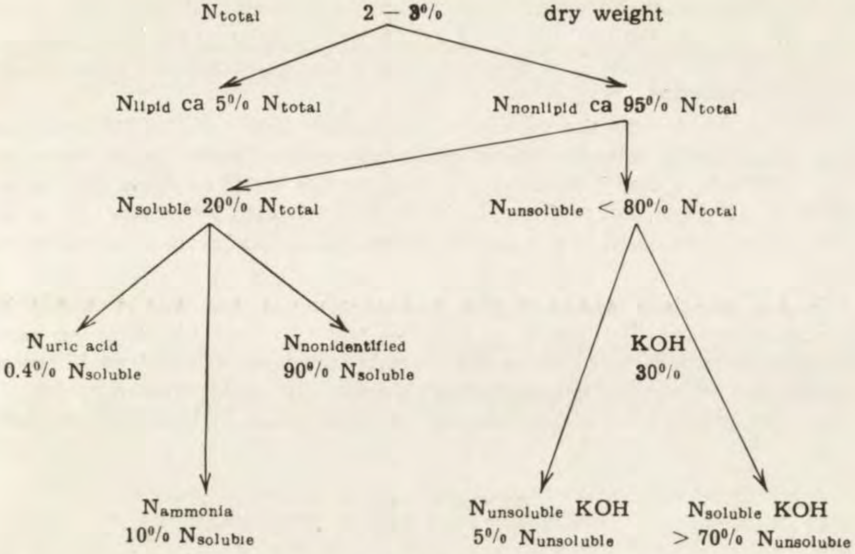
The positive results Metalnikov obtained from his culture after the administration of food consisting of moistened but delipidized membranes of bee cells may suggest that not only the membranes but also pollen was the food of the larvae.

The other direction of research makes it possible to decide to what extent the chemical composition of honeycomb meets the requirements of the larvae, what compounds are assimilable and what changes they undergo. As the value of comb as food depends on its age, its chemical composition in terms of N-content, determined by Kjeldahl's method, varies widely. Old comb from worker cells has the highest N-content (3.17%), while white comb freshly prepared the lowest (0.09%) (Rybicki 1952). More accurate figures are given here by Zielińska (1952, 1955), who has dealt with the fractionization of nitrogen compounds in old honeycomb and whose findings is presented schematically on page 7.

In her discussion, Zielińska leaves the question unanswered of the composition of the nitrogen fraction of honeycomb comprising nitrogen compounds, non-lipid and insoluble, but subject to basic hydrolysis.

Confronted with the results obtained by Zielińska the findings of Dönhoff, Allegret and Metalnikov warrant the supposition that pollen may be one of the most important constituents of the diet of wax moth larvae. To decide to what extent pollen grains of definite plants satisfy the requirements of larvae is the object of the present paper.

HONEYCOMB*



* The above diagram has been approved by Zielińska.

MATERIAL AND METHODS

Galleria mellonella L. larvae were our test material. The test cultivation of larvae was started from a definite number of eggs deposited by three females. Eggs were deposited on pieces of parchment paper checked microscopically to follow their development and transferred together with the paper on to the food a day before the hatching. Microscopical examination determined the actual number of larvae used in the experiments. All cultures were kept in Erlenmeyer flasks of 1.5 litre covered with cotton wool tampons.

The results were obtained from two culture groups. In the first group marked A in this paper, the larvae were fed on a definite amount of freshly prepared white comb and a definite amount of pollen from a definite plant. Six series of experiments were continued in this group and each of the series was named after the plant whose pollen entered into the diet of the larvae. Three parallel larva cultures were kept within each of the series of which cultures I and II had comb and pollen as diet and culture III, a control culture, had comb only. In the second culture group, marked B in this paper, the larvae were fed on a weighed amount of white comb and pollen and a weighed amount of hen egg shell membrane. The series and cultures in this group were marked in the same way and their number was the same as in group A. For the sake of a comparison with the results of culture groups A and B one more group of control cultures was kept in which larvae were fed on old dark-brown honeycomb of worker cells. All cultures were kept at 25—27°C. When the feeding of the larvae was completed, the hatching days of imagines were fixed. The imagines were chloroformed and preserved for further examination.

The pollen fed to the larvae was obtained from wind-pollinated and insect-pollinated plants. To collect pollen from the former twigs were cut from them before they blossomed and put into the water to allow ripe pollen to drop on to paper. Pollen from the latter was collected by means of a small brush. It was then dried in the air and preserved in thin layers in a well-aired place.

To prepare shell membrane, the content of a hen egg was removed, the membrane washed thoroughly in distilled water, separated from the shell and dried in a well-aired place. Dry membranes were crushed as finely as possible in a mortar and passed through a dense silk sack. Nitrogen content in honeycomb, pollen and shell membrane was determined by Kjeldahl's method.

EXPERIMENTS

I. The cultivation of larvae fed on honeycomb and pollen

(Group A)

In these experiments larvae were fed pollen from the following plants: *Populus alba* L., *Betula verrucosa* Ehrh., *Corylus avellana* L., *Helianthus annuus* L., *Cucurbita pepo* L., and *Pinus silvestris* L., its dosis amounting to 10% (cultures I and II) of the amount of honeycomb fed. Only honeycomb was the food of the control larvae in culture III. The results obtained in group A are reflected in the figures contained in Table I.

Remarkable about these figures is the development time (column 6) measured from hatching from the egg till the hatching of the imago. It is the longest in the Pinus series (culture I, 175 days; culture II, 223 days) and the shortest in the Helianthus series (54 days). Even within two parallel cultures (I and II) in the Pinus series the difference in development time amounts to 22%. An even more distinct difference may be observed in the Populus series, where the difference in hatching time between one imago (a) and another (b) in culture II amounts to 78%. The most uniform development time was observed in the larvae of culture II of the Betula series, the difference in time for the second imago (b) amount-

Table I

Results obtained in group A of cultures of *Galleria mellonella* larvae fed on freshly prepared honeycomb and pollen from various plants t° — 25 — 27

Series	Culture	Amount of honeycomb fed g	Amount of pollen fed mg	Number of eggs	Symbol of imago Development time till hatching of imago (days)	Number of imago
1	2	3	4	5	6	7
Populus alba	I	1.92	96	22	—	—
	II	1.97	98	28	(a) 51; (b) 91;	2
	III control	1.85	—	30	—	—
Betula verrucosa	I	3.10	155	24	—	—
	II	3.15	157	22	(a) 92; (b) 96; (c) 99;	3
	III control	2.75	—	32	—	—
Corylus avellana	I	3.67	183	36	—	—
	II	3.15	157	21	—	—
	III control	3.27	—	21	—	—
Helianthus annuus	I	3.85	192	32	—	—
	II	3.60	180	36	(a) 54;	1
	III control	5.02	—	26	—	—
Cucurbita pepo	I	4.75	237	31	—	—
	II	4.30	215	19	—	—
	III control	5.02	—	11	—	—
Pinus silvestris	I	2.06	103	19	(a) 175;	1
	II	2.05	102	25	(a) 223;	1
	III control	2.43	—	33	—	—

ing to 4% and for the third one (c) to 7% of the first (a). The average development time for various larva series is as follows: Helianthus 54 days, Populus 71, Betula 96 and Pinus 199.

The number of eggs used in various cultures (Table I, column 5) varies from 19 (Cucurbita, culture II, and Pinus, culture I) to 36 (Corylus, culture I and Helianthus, culture II) but the number of imagines obtained varies widely and seems to be independent of the number of eggs used.

The greatest number of imagines (3) was obtained from 22 eggs in culture II of the Betula series, while in culture I of the same series not a single imago was obtained from 24 eggs. About the same applies to both cultures of the Pinus series, where one imago was obtained in each, although 19 eggs were used in culture I and 25 in culture II.

No imagines whatsoever were observed in the Corylus and Cucurbita series, although both in the cultures of these series and in culture I of the Populus series, in culture I of the Betula series and in culture I of the Helianthus series small larvae were observed in the first weeks of feeding.

It is interesting to notice that in all cultures of the series in which imagines were obtained neither comb nor pollen was completely consumed. It seems therefore that the reasons for the small number of imagines in various series of the cultures in which the results was positive should be sought for not in the shortage if food but in the deficit of certain constituents unknown now. Despite a painstaking search no dead larvae were found in the cultures of the series in which imagines were obtained. This seems to suggest that the development of the larvae which produced imagines also took place partly by their eating up the rest, hatched from the total of eggs used in each of the cultures.

Painstaking search for dead larvae was successful only in the cultures of the Cucurbita series in which 2 to 3 dry very small larvae were found. No imago was obtained in any of the control cultures either, although traces of feeding were observed on comb in the first weeks.

The number of imagines obtained in various cultures, the whole time that elapsed from hatching to imago, the absence of dead larvae in the cultures in which imagines were obtained and negative results in all control cultures all allow us to suppose that pollen is a source of nitrogen for larvae.

II. The cultivation of larvae fed on white honeycomb, pollen and shell membrane (Group B)

The small number of imagines obtained in the first group of cultures suggested the suspicion that there was no adequate amount of assimilable nitrogen compounds in the composition of the first diet.

This suspicion was warranted by the fact that no dead larvae were found in any of the cultures of group A in which imagines were obtained, which suggested that they had been eaten by the larvae which produced imagines. To enrich the diet with nitrogen compounds we decided to add shell membrane in the proportion of $10^{0}/_{0}$ of comb.

The results obtained in group B are reflected in the figures contained in Table II.

Table II

Results obtained in group B of cultures of *Galleria mellonella* larvae fed on freshly prepared honeycomb, pollen from various plants and shell membrane

Series	Culture	Amount of honeycomb fed g.	Amount of pollen fed mg.	Amount of shell membrane mg.	Number of eggs	Symbol of imago Development time till hatching of imago (days)	Number of imago
1	2	3	4	5	6	7	8
Populus alba	I	2.04	102	102	23	(a)83; (b)86; (c)100;	3
	II	2.03	101	101	42	(a)66; (b)66; (c)66; (d)98; (e)100;	5
	III control	2.06	—	—	25	—	—
Betula verrucosa	I	2.00	100	100	27	—	—
	II	2.02	101	101	26	(a)262; (b)269; (c)391; (d)398;	4
	III control	2.04	—	—	26	—	—
Corylus avellana	I	2.12	106	106	37	(a)99; (b)103;	2
	II	2.18	109	109	17	(a)94;	1
	III control	2.10	—	—	25	—	—
Helianthus annuus	I	2.09	105	105	21	—	—
	II	2.03	101	101	28	—	—
	III control	2.05	—	—	27	—	—
Cucurbita pepo	I	2.06	103	103	25	—	—
	II	2.08	104	104	16	—	—
	III control	2.05	—	—	24	—	—
Pinus silvestris	I	2.08	104	104	22	—	—
	II	2.02	101	101	16	(a)160; (b)162;	2
	III control	2.05	—	—	23	—	—

The results obtained in the cultivation show that the time of development from hatching to imago is the shortest in the Populus series, 90 days in culture I and 79 days in culture II on the average, with an average of 85 days for all imagines of both cultures. The longest development time was observed in culture II of the Betula series and it amounted to an average of 330 days for all imagines in this culture. Two groups of imagines with different development periods may be separated in this culture (II): one period covering two specimens of imago with the average development time of 265 days and the other period, also covering two specimens of imago, with the average development time of 395 days. The difference in development time between these two groups is so great (49%) that this fact cannot be regarded as coincidence. This will be interpreted in the discussion of the results. The average development time in various larva series is as follows: Populus 85 days, Corylus 99, Pinus 161 and Betula 330.

Remarkable about the final result arrived at in group B is the number of imagines of which most were obtained in the Populus series (8 specimens in both cultures) and fewest in the Pinus series (2 specimens in culture II). No imagines whatsoever were observed in the Helianthus and Cucurbita series.

Just as in group A, no close relation between the number of imagines obtained and that of eggs used was observed in group B. The supposition might be justified that the development of larvae, at the beginning in particular, may depend not on the food given but on the consumption of freshly hatched larvae. True, comparison of the numbers of imagines in cultures I (23 eggs) and II (42 eggs) of the Populus series might suggest a certain correlation in this respect, but the results obtained in culture II of the Betula series and culture II of the Pinus series do not bear out this correlation. No dead larvae were found in any of the cultures of group B in which imagines had been obtained, while the food given (white comb, pollen and shell membrane) was not completely consumed in any culture. This may imply that the small number of imagines obtained in various cultures was not due to lack of food but to a certain deficit in some diet constituents we do not know wery well.

Just as in group A, it is remarkable that there were no imagines in the Cucurbita series in group B and there were no imagines in the Helianthus series of this group although traces of feeding were observed in the four cultures of this series during the first weeks of the life of the larvae. A negative result (absence of imagines) was obtained in all control cultures of group B.

Remarkable about group B is the absence of imagines in the Cucurbita and Helianthus series. In the interpretation of the negative results obtained in this group, and in the Cucurbita series in particular, attention was paid to the size of pollen grains, this being shown by the figures contained in Table III.

Table III

Measurements of pollen grains and shell membrane pieces fed to wax moth (*Galleria mellonella*) larvae

Plant	Number	Average size (in μ)
Populus alba	40	27.1μ (24.5 — 32.5μ)
Betula verrucosa	40	26.2μ (24.5 — 28.0μ)
Corylus avellana	40	29.2μ (24.5 — 34.3μ)
*Helianthus annuus**	40	41.3μ (32.5 — 47.2μ)
Cucurbita pepo	40	111.8μ (98.0 — 126.0μ)
Pinus silvestris	40	41.6μ (38.5 — 45μ) x 26.2μ (21.0 — 31.5μ)
Shell membrane	100	23.2μ (7.0 — 227.5μ) x 15.1μ (7.0μ — 192.5μ)

* The size of *Helianthus annuus* pollen grains is given including that of their spines which average 3.5 μ in length.

Comparison of figures for the size of pollen grains from the plants used in the experiments implies that *Cucurbita pepo* pollen grains are larger than any of the others. Their size justifies the supposition that on hatching from their eggs larvae cannot probably feed on them. This interpretation seems correct for the cultures of the Cucurbita series but wrong for those of the Helianthus series, as the size of *Helianthus annuus* pollen grains is the same as in case of *Pinus silvestris* (see Table III), and imagines were obtained in the Helianthus series in the first group only. The analysis of reasons cannot leave out the structure of *Helianthus annuus* pollen grains. In contradistinction from the other plants used in the experiments, *Helianthus annuus* pollen grains have spines (3.5 μ long) which make it difficult for a young larva to swallow them. Observation of faeces showed no chewed pollen grains; they were always whole. The external structure of *Helianthus annuus* pollen would account for the absence of imagines in group B while the presence of one imago in group A could be accounted for by the number of eggs used (36 in group A and 21 and 28 in group B). It could therefore be supposed that the larvae passed the early part of their development in the culture of the first Helianthus series at the expense of other larvae and that when the larvae became larger the size and structure of *Helianthus annuus* pollen were no obstacle in swallowing. It seems however that neither the size of pollen grains nor their external structure was the only factor to prevent larvae from feeding on it. *Corylus avellana* pollen grains are very much the same in size as *Populus alba* ones, while the results obtained in group A were negative (absence of imagines) even when 36 eggs were used (Table I, Corylus series, culture I).

The value of the diet given to the larvae is even more clearly shown by a comparison of the figures contained in Tables I and II. This comparison showed a large increase in the number of imagines in cultures where the larvae were fed on comb, pollen and shell membrane, particularly in the series Populus and Corylus. The increase in the number of imagines in the Betula series was not so large. The addition of shell membrane to the diet of the larvae caused the occurrence of three imagines in the Corylus series in group B (Table II) and the absence of imagines in the same series in group A (Table I).

In order to determine the reasons for the varying number of imagines in the groups the nitrogen content in various constituents of the diet of the larvae was examined. These figures are shown in Table IV.

The figures in this Table which characterize the N-content in the constituents of the diet of the larvae help more accurately to analyze the results obtained. Freshly prepared comb contains least nitrogen (0.09⁰/₀) in the diet of the larvae. The N-content in the pollen of the plants used in the experiments varies widely: it amounts to 1.94⁰/₀ for *Pinus silvestris* and 4.8⁰/₀ for *Cucurbita pepo*. Shell membrane contains most nitrogen (11.66⁰/₀).

The fact that the larvae did not develop in any of the control cultures in both groups (white honeycomb) becomes clear in the light of the figures for the nitrogen content in this constituent of the diet. This part of the diet was very much like the natural diet and was not changed by the action of chemicals. Thus, the whole

Table IV

N-content in constituents of food of wax moth (*Galleria mellonella*) larvae

Material analyzed	N-content as determined by Kjeldahl's method % (average from 10 analyses)	N-content as determined by other authors %	Authors*
Freshly prepared honeycomb	0.09	0.09	M. Rybicki 1952
Populus alba (pollen)	4.58	5.25	K. Lubliner-Mianowska 1956
Betula verrucosa (pollen)	3.20	4.52	K. Lubliner-Mianowska 1956
Corylus avellana (pollen)	3.92	3.68	K. Lubliner-Mianowska 1956
Helianthus annuus (pollen)	3.92	4.08	K. Lubliner-Mianowska 1956
Cucurbita pepo (pollen)	4.86	5.80	C. Sosa Bourdouil 1937, 1938, 1949
Pinus silvestris (pollen)	1.94	2.65	A. von Planta 1885/86
Shell membrane	11.66	—	—

* The N-content determined by other authors was given in a paper by K. Lubliner-Mianowska (1956).

of the lipid fraction of honeycomb consumed by the larvae could not tell on the results of the experiments as all of its physico-chemical properties had been preserved.

A comparison of the number of imagines with the nitrogen content in pollen from various plants is very interesting. The Populus and Cucurbita series in group A are noteworthy in this comparison. The nitrogen content in the pollen given to larvae in these series is very much the same, while imagines were obtained in the Populus series only. It therefore seems that the interpretation of the results obtained in the Cucurbita series presented before in this paper is right.

The comparison of the results obtained in the Corylus and Helianthus series in group A seems to suggest that not only the external structure of pollen grains but also their size and nitrogen content decide the development of the larvae. Pollen grains from *Betula verrucosa* and *Corylus avellana* are very much the same in external structure, the difference in the size of pollen grains amounting only to 3 μ, while as regards the nitrogen content the difference is 0.72% (in favour of *Corylus avellana* pollen). Despite the difference in this respect three imagines were obtained in the Betula series in group A, while there were no imagines in the Corylus series. Even in the Pinus series in group A two imagines were obtained despite a low nitrogen content. In the light of the results obtained hitherto it seems that the normal development of wax moth larvae does not depend on the nitrogen content in the pollen used in the experiments, but on some other additional and indispensible factor contained in the pollen the presence of which stimulates the development of the larvae.

Doubts may be expressed as to whether the nitrogen content in the pollen as determined by the method applied satisfies the requirements of the larvae since not all compounds were assimilable. It was exactly these doubts that made it necessary to enrich the diet used in the first experiment with another source of nitrogen—shell membrane. The choice of shell membrane was prompted by the need for the constituent added to contain as little water as possible and be non-hygroscopic, which might have an adverse effect on dry pollen.

The results obtained in group B disperse some of these doubts only. When added to the food, shell membrane improved the final result obtained very clearly in some of the cultures only. This was particularly clear in the Populus series (cultures I and II) and less so in the Betula series (three specimens in group A, four in group B), while there was no difference in the number of imagines in both cultures of the Pinus series (two specimens in each). The clearest difference was observed in the Corylus series in both groups. The results obtained in the Corylus series in group B confirm the supposition that *Corylus avellana* pollen does not satisfy the requirements of the larvae in terms of the nitrogen compounds it contains and that the addition of shell membrane greatly improves the condition of the culture.

Although the final result obtained in group B (17 imagines) is far better than in group A (8 imagines), the interpretation of these results must allow for the different development time of the larvae in both groups.

In all series of group A except for the Pinus series, development time from the hatching from the egg to the hatching of the imago was shorter than in the series of group B (see Table I, column 6; Table II, column 7). On the average, the development time of the larvae of the Populus series of group B is 14 days or 22% longer than in group A. In the Betula series of group B it is 234 days or 344% longer than in group A, but in the Pinus series of group B it is 38 days or 19% shorter than in group A.

Analysis of the results obtained seems to suggest ultimately that the longer development time of the larvae in group B may be due to a wrong quantitative relation of pollen to shell membrane.

III. The characterization of the imagines obtained in both groups

The biological value of the diet of the larvae and that of pollen in particular is reflected in the size and colour of imagines.

The measurements of imagines are characterized by the figures contained in Table V.

The measurements of the wing span and the thorax were taken when imagines were killed and dried, and so they are somewhat smaller than those of fresh specimens.

Comparison of the size of the imagines whose larvae were kept on the diet (group A) shows that the feature characterizing *Galleria mellonella* imagines (♀♀ being larger than ♂♂) is preserved in the Populus, Betula and Pinus series. The greatest difference (44%) was observed in the Populus series, a smaller one (18%) in the Betula series and the smallest (13%) in the Pinus series. The imagines from group B did not preserve this feature in all series (e. g., the Pinus and Betula series).

It follows from comparison of the figures for the size of ♂♂ and ♀♀ in groups A and B that, on the whole, ♀♀ from group A show far bigger differences in size in various series than ♀♀ from group B.

The big differences in size between ♀♀ and ♂♂ of various series in both groups may perhaps be ascribed to the total of imagines obtained in various cultures, this being suggested by the results obtained in the Populus series of culture II in group B (Table V, Plate 1). This interpretation is called in question by the total of

Table V

Measurements (in mm) of wing span incl. thorax
of *Galleria mellonella* imagines whose larvae were kept on varying diets

Group A				Group B			
Culture	Imago symbol	Measurements mm.	Sex	Culture	Imago symbol	Measurements mm.	Sex
Populus alba							
				I	(a)	21	♂
					(b)	20.5	♂
					(c)	22	♂
II	(a)	23.6	♀	II	(a)	20	♂
	(b)	16.4	♂		(b)	20.6	♀
					(c)	18	♂
					(d)	20	♀
					(e)	17	♂
Betula verrucosa							
II	(a)	19	♀	II	(a)	18.5	♂
	(b)	16	♂		(b)	22.7	♀
	(c)	29	♂		)c)	14	♂
					(d)	16	♂
Corylus avellana							
				I	(a)	22	♀
					(b)	22.4	♂
				II	(a)	21	♂
Helianthus annuus							
II	(a)	24	♂				
Pinus silvestris							
I	(a)	17	♀				
II	(a)	15	♂	II	(a)	19.4	♂
					(b)	18	♀
Control cultures food, old honeycomb							
	(a)	30.9	♀		(b)	30.9	♂

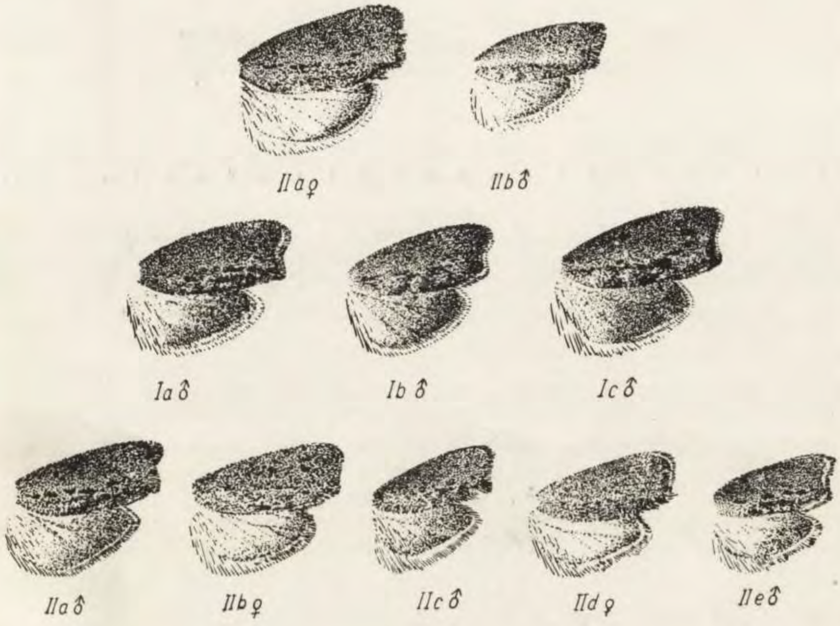


PLATE 1

First row — Imagines of group A, Populus series
♀ of cultures IIa; ♂ of culture IIb;
Second row — Imagines of group B, Populus series
♂ of culture Ia, ♂ of culture Ib; ♂ of culture Ic;
Third row — Imagines of group B, Populus series
♂ of culture IIa; ♀ of culture IIb; ♂ of culture IIc;
♀ of culture IId; ♂ of culture IIe;
All drawings magnified ca 3 times.

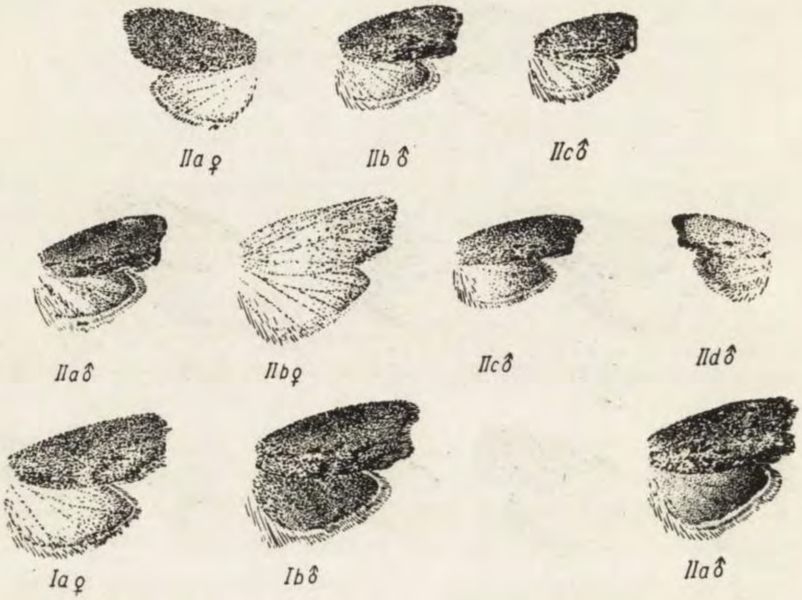


PLATE 2

First row — Imagines of group A, Betula series
♀ of culture IIa; ♂ of culture IIb; ♂ of culture IIc;
Second row — Imagines of group B, Betula series
♂ of culture IIa; ♀ of culture IIb; ♂ of culture IIc; ♂ of culture IId;
Third row — Imagines of group B, Corylus series
♀ of culture Ia; ♂ of culture Ib; ♂ of culture IIa.
All drawings magnified ca 3 times.

imagines in culture II of the Betula series. In culture II of this series of group A three imagines were obtained, the difference in size between ♀ and two ♂♂ amounting to 18%, while in culture II of the same (Betula) series of group B four imagines were obtained, the difference between ♀ and three ♂♂ amounting to 38% (Table V, Plate 2, rows 1 & 2).

Differences in size between ♂♂ and ♀♀ change somewhat if the average measurements of both sexes in groups A and B are compared. The average measurements of five ♂♂ in group A amount to 17.2 mm. (15—24 mm.) and those of twelve ♂♂ in group B amount to 19.2 mm. (14—22.4 mm.); ♂♂ of group B being larger in size by 12%. The average measurements of three ♀♀ of group A amount to 19.9 mm. (17—23.6 mm.), and those of five ♀♀ of group B amount to 20.7 mm. (18—22.7 mm.).

Although comparison of the average measurements of imagines of both sexes seems to suggest that the addition of shell membrane to the diet brings about bigger measurements, but this is not an actual fact. The average measurements efface the very clear differences observed in various series which depend on the plant whose pollen was the food of the larvae. In group A, ♂ of the Helianthus series has the biggest measurements, 24 mm., while in group B ♂ of culture I in the Corylus series 22.4 mm. (see Table V). The biggest measurements (23.6 mm.) are reached in group A by ♀ in culture II of the Populus series and in group B by ♀ in culture II of the Betula series. Thus the addition of shell membrane to the diet seems to increase the number of imagines but pollen and the compounds it contains determine the size of imagines. The imagines obtained in the control culture the larvae of which were fed old honeycomb show the importance of pollen in the diet of the larvae* (see Table V).

The influence of the diet of the larvae is most evident in the colour of imagines. According to Vöhringer (1934) the original colour of imagines kept on normal diet oscillates from yellowish grey through grey-brown to dark-grey. There is one or a few longitudinal dark spots at the lower edge of the upper ring. Drawings in row 3, Plate 3, represent the typical colour of ♂ and ♀ imagines obtained from the control series. Drawing (b) in this plate shows ♂ and drawing (a) shows ♀.

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* The same measurements of ♂ and ♀ of the control culture may be regarded as incidental since ♂♂ are always somewhat smaller than ♀♀.

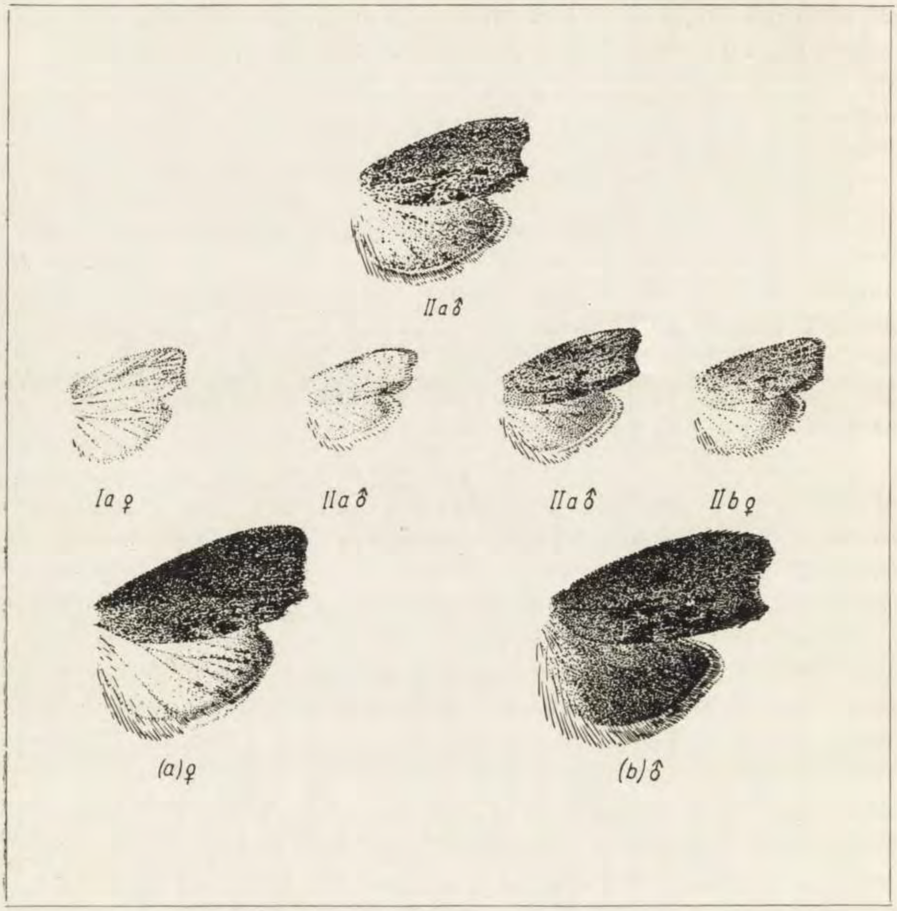

PLATE 3

First row — Imagines of group A, Helianthus series
♂ of culture IIa;
Second row (first section) — Imagines of group A, Pinus series
♀ of culture Ia; ♂ of culture IIa;
Second row (second section) — Imagines of group B, Pinus series
♂ of culture IIa; ♀ of culture IIb;
Third row — Imagines of control series
a — ♀ of control culture; b — ♂ of control culture.
All drawings magnified ca 3 times.

In terms of colour, imagines obtained in our experiments can be divided into two groups: those from group A and those from group B.

In group A ♂ imago of the Helianthus series (Plate 3, row 1, IIa) was of the most typical colour, ♀ of culture II of the Populus series (Plate 1, row 1, IIa) being of a similar colour.

The imagines of the Pinus series of group A were of a completely different colour. The ♀ of this series [Plate 3, row 2, (first section) Ia], was a most white and owing to the absence of scales the wings were transparent and membraneous. The ♂ imago [Plate 3, row 2, (first section), IIa] was of a similar colour.

The imagines of group A of the remaining series were of a colour intermediate between the colour of the imagines of the *Helianthus* and *Pinus* series.

The Betula series differed in colour from the imagines of group B (see Plate 2, row 2, IIa, IIb, IIc, IId). Imagines IIb and IId (♀ and ♂) the colours of which resembled those of ♀ and ♂ of the Pinus series of group A (no scales) were noteworthy in this series.

When the imagines obtained from the two groups were compared a correlation could be observed between colour and size. The larger the imago specimens (except for ♀ of culture II of the Betula series of group B) the darker its colour and more like that of control imagines. The characterization of the size and colour of the imagines obtained seems to suggest that pollen and its components are factors which exert the greatest influence upon those two features. This general observation does not exclude the influence of other constituents of the food making up the diet. However, it seems legitimate to think that what is decisive here is the utilization by the larvae of the constituents inside pollen cells, the access to which is made difficult by the structure of the membranes of pollen grains.

DISCUSSION

The results obtained in group A constitute a sufficient basis for the recognition of pollen as one of the sources, if not the only source of nitrogen for wax moth larvae. The idea of using pollen as one of the constituents of the diet of the larvae was first realized by Dönhoff, Allegret made experiments of the same kind, but the essential difference between their experiments and those made by the present author consists in that mixtures of pollen of various plants and not only of one definite plant were used in the former experiments. Hence the results obtained by the above authors do not show the effect of pollen from definite plants but that of a mixture of pollen from various plants. The results of our experiments seem to suggest that the positive result of those of Metalnikov's cultures in which the delipidized honeycomb fraction (sprinkled with water) was the diet of the larvae cannot be accounted for only by the fact bee cell membranes are the food of moth larvae. The question of the utilization by larvae of various components of old honeycomb is a complicated one, and all attempts at defining the process in quantitative terms by biochemical methods result in a very rough picture, particularly as regards the utilization of nitrogen compounds. The difficulty in solving this problem consists in that, on the one hand, some honeycomb fractions resist the action of chemical factors, and on the other — some components of honeycomb· such as wax (M. Florkin, F. Lozet and Sarlet 1949, Rybicki 1952) and delipidized bee cell membranes get decomposed in vitro by the bacterial flora of the moth (Rybicki 1952). There are therefore reasons to anticipate that the components resistant to the action of chemical factors are decomposed in the intestine of the larvae by its bacterial flora. Consequently, all attempts at feeding the larvae on a changed diet (whether physically or chemically) must have an adverse effect on the bacterial flora, the action of which determines the development of the larvae (Rybicki 1952). For these reasons, as a source of lipid compounds the larvae were given freshly prepared honeycomb, the nitrogen content of which is of no importance for the development of the larvae as shown by the control cultures at various stages of our research, which confirmed the results of earlier experiments (Rybicki 1952).

The results obtained in group A confirm the suggestion that pollen grains may be the only source of nitrogen for larvae. No attention has been paid so far to this constituent of the diet of moth larvae, and the use of pollen as a nitrogen source for larvae in our experiments was suggested by microscopic observations of bee cell membranes (Rybicki 1952) and the results of Dönhoff's and Metalnikov's larva cultures. Although Zielińska (1952, 1955) analysed honeycomb for chitin, but she suspected that this fraction was the source of nitrogen for larvae, as she wrote, "We have up today no answer to the question as to what are the components of this chief fraction of the beecomb, which embraces nitrogen compounds, lipid—free, insoluble, but yielding to an alkaline hydrolysis. This fraction, as it seems, is the most important source of nitrogen to wax moth larvae since in the excreta of feeding larvae the content of insoluble nitrogen does not reach 20% of the total nitrogen of the excreta" (Zielińska 1952, pp. 180—181; 1955, pp. 14—15).

The interpretation of the results obtained in group A to be found in the chapter on experiments should take into account one more factor. There is an essential difference between the pollen fed to the larvae in our experiments and the pollen consumed by larvae in old honeycomb. The pollen fed to larvae in our experiments was collected from plants, dried in the air and fed to experimental larvae. Now the grains of pollen in old honeycomb, particularly those between bee cell membranes, are those which have passed through the alimentary tract of bee larvae and must have undergone changes. This would mean that the moth larvae in group A must have been kept on a better diet than those feeding on old comb. The size of the imago indicates the quality of the food. No imago specimen obtained in our experiments in both groups reached the size of the control imagines [Plate 3, row 3, (a) ♀ and (b) ♂].

Reasons for this fact were sought through and analysis of the scheme of nitrogen fractions of honeycomb as given by Zielińska (1952). Noteworthy in this scheme is the N-soluble fraction which amounts to $<$ 80% of the N-total and changes under the effect of 30% KOH into $>$ 70% N-soluble in relation to N-unsoluble. Considering the findings of the above author and the factor (30% KOH) under the action of which an insoluble fraction changes into a soluble one, it is legitimate to anticipate that the N-fraction soluble under the action of KOH is nitrogen contained in pollen. The results obtained in group A also point to the possibility for moth larvae to utilize nitrogen contained in pollen.

In our first interpretation of the results obtained in group A we suspected that the amount of nitrogen fed to larvae in pollen might be insufficient and that this accounted for too small a number of imagines. This made it necessary to enrich the diet with nitrogen compounds by adding shell membrane to it (the choice of shell membrane is substantiated in the interpretation of the results).

To get a better idea of the correlation between the amount of nitrogen in the diet and the number of imagines theoretical calcula-

Table VI

Amount of nitrogen fed to larvae & number of imagines obtained (based on figures from Tables I, II and IV)

Cultures	Group A		Group B			
	Amount of nitrogen fed in pollen mg.	Number of imagines	Amount of nitrogen fed in pollen mg.	Amount of nitrogen fed in shell membrane mg.	Total mg.	Number of imagines
Populus series						
I	4	—	5	12	17	3
II	4	2	5	12	17	5
Betula series						
I	5	—	3	12	15	—
II	5	3	3	12	15	4
Corylus series						
I	7	—	4	12	16	2
II	6	—	4	12	16	1
Helianthus series						
I	7	—	4	12	16	—
II	7	1	4	12	16	—
Cucurbita series						
I	11	—	5	12	17	—
II	10	—	5	12	17	—
Pinus series						
I	2	1	2	12	14	—
II	2	1	2	12	14	2

tions were made on the basis of the figures in Table IV. Absolute figures for the N-content in the diet and numbers of imagines in the two culture groups (A and B) were quoted in these calculations. These figures are presented in Table VI.

In our experiments the larvae in group A had one nitrogen source and in group B two sources (pollen and shell membrane). As the amount of nitrogen fed to the larvae in shell membrane in all cultures of group B was the same (12 mg.) and the number of imagines varied, the interpretation of this fact must be based on a comparison with the number of imagines obtained in group A. The number of imagines from the Betula and Corylus series in group B would mean that if the food fed to larvae is enriched with nitrogen contained in shell membrane and the nitrogen content in pollen lowered by 2 or 3 mg. in these series as compared with the amount fed to larvae of the same series of group A (see Table VI, column 2 and 4) the number of imagines is higher in these series. The negative result of the Corylus series of group A would mean that nitrogen in *Corylus avellana* pollen is not assimilable at all, so shell membrane would be the source of nitrogen for larvae of the Corylus series in group B. This conclusion is contradicted by the results obtained in the Helianthus and Cucurbita series of group B where no imagines were obtained. This means that the nitrogen of shell membrane may be utilized only in combination with the nitrogen contained in pollen grains accessible to the larvae. This interpretation was borne out by the results obtained in culture II of the Populus series in group B, in which the increase in nitrogen contained in *Populus alba* pollen by 1 mg. resulted in a more than twofold increase in the number of imagines (cf. Tables I and II, the Populus series).

The interpretation of the numbers of imagines in various cultures should take into account the size of imagines (Table V) and the time of their development (Table I, column 6 and Table II, column 7) in various cultures. The figures presented in Table VII illustrate this correlation.

The data presented in Table VII make it possible to state the following new facts:

1. The addition of nitrogen contained in shell membrane to the diet larvae results in a longer development time (from egg to imago), this being particularly well marked in the Betula series of group B.

2. The larvae of group A fed on the pollen of *Populus alba* and *Helianthus annuus* take only a few more days to develop (3 more days in the Populus (a) series and six more days in the Helianthus (a) series) than the control larvae fed on old comb.

3. Imagines of the Populus and Helianthus series of group A do not reach the size of the control imagines, but are largest of all.

The lists and comparisons made so far suggest that the number of imagines, and particularly their development time, size and even

Table VII

Measurements and development time of imagines
(based on figures from Tables I, II & V)

Group A					Group B				
Culture	Imago symbol	Measurements mm.	Sex	Development time (days)	Culture	Imago symbol	Measurements mm.	Sex	Development time (days)
Populus series									
					I	(a)	21	♂	83
						(b)	20.5	♂	86
						(c)	22	♂	100
II	(a)	23.6	♀	51	II	(a)	20	♂	66
	(b)	16.4	♂	91		(b)	20.6	♀	66
						(c)	18	♂	66
						(d)	20	♀	98
						(e)	17	♂	100
Betula series									
II	(a)	19	♀	92	II	(a)	18.5	♂	262
	(b)	16	♂	96		(b)	22.7	♀	269
	(c)	15	♂	99		(c)	14	♂	391
						(d)	16	♂	398
Corylus series									
					I	(a)	22	♀	99
						(b)	22.4	♂	103
					II	(a)	21	♂	94
Helianthus series									
II	(a)	24	♂	54					
Pinus series									
I	(a)	17	♀	175					
II	(a)	15	♂	223	II	(a)	19.4	♂	160
						(b)	18	♀	162
Control cultures food, old honeycomb									
	(a)	30.9	♀	48		(b)	30.9	♂	48

colour cannot be related to the amount of nitrogen in the diet of larvae, a fact to which attention has only been drawn so far. These considerable differences in the development time and size of imagines make it necessary to look for other reasons, undoubtedly connected with nutrition, which influence the speed of development in a decisive way.

The fact of hunger cannot be taken into account here, as all food supplied was not consumed in any of the cultures. The supposition suggests itself that beside nitrogen other biologically very important compounds contained in pollen may be a factor determining the development speed and size of imagines. This is a conclusion from simple calculations. The results of the analysis made by Zielińska (1952) show that every 100 g. of old honeycomb with the N content totalling 3% contains (probably in pollen) 1.68 g. of soluble nitrogen (30% KOH), i. e. each g. of old comb contains 17 mg. of N. Data given in a previous study (Rybicki 1952) indicate that on the average one larva consumes 0.28 g. of old comb which contains 4.76 mg. of N, probably from pollen, in its lifetime. A comparison of the total of nitrogen supplied in various cultures of both groups in our experiments with the amount of nitrogen consumed by larvae in old comb, made it necessary to loock for such other constituents of the diet which would stimulate the development of larvae.

In view of the results of group A, particularly the size and colour of imagines, of the Populus and Helianthus series, in relation to the imagines of the control series, it was decided to make,

1) a survey of the literature of comb analysis not only for lipid and nitrogen content.

2) a survey of the literature of analysis of fresh pollen.

It was already pointed out in the introduction to a previous work (Rybicki 1952) that pigments are also components of old honeycomb. They come first of all from pollen. Jaubert (1927) quotes that 1:3-dihydroxyflavone prevails in honeycomb and pollen of such plants as *Reseda luteola, Calluna vulgaris, Apium petroselineum, Populus nigra.* Tischer (1940) found lutein, lutein ester and 1.28% of carotenoids in honeycomb.

Analysis of pollen confirms the origin of carotenoids. We quote here only one work as a synthetic study based on literature collected as carefully as possible. Lubliner-Mianowska (1955) gives a list of the carotenoid, anthoxanthin, anthocyanin content in pollen of many plants, of which only those were chosen which were used in our experiments. Table VIII presents the results quoted by Lubliner-Mianowska.

A quotation from R. C. Mc Lean and W. R. Ivimey-Cook (1956) should be added to the findings on the presence of pigments in pollen: "Sporopollenin may be identical with the sporonine of Zetsche, which he found in Lycopodium spores and also in the pollen of some Coniferae and in Corylus. In *Pinus silvestris* it makes up nearly 22 per cent of the weight of the grain, but in Corylus only 7.3 per cent. It is of considerable practical interest as the preservation of pollen in peat and in older deposits seems to be chiefly due to the membrane containing this substance" (p. 1363).

Table VIII

Appearance of pigments in pollen*

Plant	Carotenoids	Anthoxanthins	Anthocyanins
Populus alba	traces	+	—
Betula verrucosa	—	+	—
Corylus avellana	—	+	—
Helianthus annuus	+	—	—
Cucurbita pepo	+	traces	—
Pinus silvestris	—	—	—

* After K. Lubliner-Mianowska (1955).

In the light of the results obtained and listed by Lubliner-Mianowska and Tischer and the findings of Mc Lean and Ivimey-Cook, the interpretation of the results obtained in group A must be different. Beside nitrogen contained in pollen the presence of some pigments of vegetable origin is a decisive factor in the development of larvae. Table VIII could explain the size, colour and development time of imagines from the Populus and Helianthus series in comparison with the control imagines. But the results obtained in the Pinus series of group A become impossible to understand. Our knowledge on the presence of pigments in pollen suggests that no pigments examined by Lubliner-Mianowska are present in the *Pinus silvestris* pollen grains, but there is sporopollenin in it. Almost three times as much sporopollenin in *Pinus silvestris* pollen grains in comparison with *Corylus avellana* pollen grains should be even surer to give a negative result in group A as observed in the Corylus series. On comparison of the size, colour and development time it seems possible to evaluate the importance of these compounds from the data on the presence of pigments in pollen.

The presence of carotenoids in food had a decisive influence first of all upon the colour of imagines and their size, and, to a somewhat smaller degree upon their development time. The presence of anthoxanthins and the absence of carotenoids (the Betula series) results in the varying colouring, diminution and longer development time of the imagines. The presence of anthoxanthin and the absence of carotenoids but accompanied by the appearance of sporopollenin completely checks the development of larvae (the Corylus series). On the other hand, the absence of carotenoids and anthoxanthins and the presence of sporopollenin only results in a complete disappearance of pigment, diminution and almost five times longer development time in relation to the control imagines (the Pinus series). The total of imagines obtained in group B was over twice as high as in group A and this was ascribed earlier in this paper to the enriching of the diet with nitrogen compounds. The interpretation of the results obtained in group A explains those obtained in the Populus series of group B. However, a comparison of the colour of imagines from group A (Plate 1, row 1) with imagines from group B (Plate 1, row 2, Ia; Ib, Ic; and row 3, IIa; IIb; IIc; IId; IIe) showed that ♀♀from group B IIb; IId were somewhat lighter and had no dark spots on the front pair of wings in contradistinction from the colour of the female from group A (Plate 1, row 1, IIa). More distinct differences were observed in the Betula series of group B. The exceptionally large ♀ (Plate 2, row 2, IIb) had no colour just as ♀ from the Pinus series of group A [Plate 3, row 2, (first section), Ia]. This fact seems to confirm our belief that carotene in the diet of larvae has a decisive influence upon the colour of imagines, particularly females, which is probably connected with laying eggs. The complete development takes 3—4 times as long as the complete development in group A. As a result of absence of carotenoids in *Corylus avellana* pollen grains and the presence of anthoxanthin and sporopollenin the moth larvae kept under these conditions and fed on the same diet as in group B develop at about the same rate as in the Betula series of group A, the size of imagines of the Corylus series (group B) is generally bigger than that of imagines of the Betula series of group B and the colour of males hardly differs from that of the imagines of the control series. Only ♀ of the *Corylus* series has no dark spots on the front pair of wings (see Plate 2, row 3). The interpretation of the results obtained from the Corylus series in group B apparently contradicts the interpretation made in the case of the Populus and Betula series. The presence of sporopollenin in food in addition to anthoxanthins might seem not only to accelerate the development of the larvae but improve their colouring and bring it closer to that of the control imagines. Such should perhaps be the interpretation if it were not for the results of the *Pinus* series in group B. There are no caretenoids and anthoxanthins but there is sporopollenin in *Pinus silvestris* pollen. Food enriched with nitrogen sometimes shortens development time, and also increases the size of the imagines somewhat, but influences the colour of imagines relatively little, in any case not so decisively as in the Corylus series. ♀ of the Pinus series of group B [Plate 3, row 2 (second section), IIb] has only a few scales on the front pair of wings.

Comparison of the results obtained in groups A and B and their interpretation, which seems justified to a high degree, although there is no direct evidence, allow us to believe that the addition of shell membrane to the food of the larvae is a factor favouring the development of the intestinal flora of moth larvae.

A basis for the acceptance of such correlations is offered by the results of a previous study (Rybicki 1952), according to which aseptic moth larvae fed old aseptic honeycomb develop at 35° C in 97 days on the average, and the amount of honeycomb consumed by them in this time varied from culture to culture and from 1.18 to 0.75 g. (out of 7 g. supplied). From one only out of four parallel cultures one very small imago specimen was obtained the size of which was 57% smaller than that of the imago obtained from a control non-aseptic culture.

Considering the results obtained in the aseptic cultures it is legitimate to suppose that the larvae of group A lacked still other biologically active constituents, of which there was no adequate amount in pollen. The diet of group A did not offer conditions for a proper development of bacterial flora, which developed very intensely on old honeycomb (Rybicki 1952), and consequently it seems that the addition of shell membrane favours the development of the intestinal flora of the moth and only the products of the metabolism of the bacterial flora are probably indispensable for larvae, as the results obtained in group B seem to bear out.

This does not mean however that pollen is an inadequate source of these additional compounds. The results obtained in group A seem to confirm this conclusion, but it seems that proper proportions should be found out of such constituents as: assimilable nitrogen compounds, carotenoids and other biologically active bodies, probably vitamins of grup B.

CONCLUSIONS

1. The pollen of definite plants is an important constituent of the diet of wax moth larvae.

2. Depending on the plant from which it is collected pollen may be the only source of nitrogen compounds for moth larvae.

3. The ability of larvae to utilize pollen grains of a plant depends not only on the content of nitrogen but also on the size and structure of grains.

4. The size, colour and complete development time, from hatching from the egg to the hatching of imago, probably depends on such vegetable pigments as carotenoids and anthoxanthins.

5. The absence of vegetable pigments in the diet of larvae probably causes the colour of moth imagines to disappear, its size to diminish and its development time to extend several times as long as that of the control imagines.

REFERENCES

ALLEGRET P., 1956 — Étude des glandes séricigènes des larves de Lépidoptères. Leur rôle dans la physiologie du développement. Thèses présentées à la Faculté de Sci. de l'Univ. de Paris. Série A. *2925* (3796), 1—345.

DÖNHOFF E., 1882 — Beiträge zur Physiologie. IV. Ein Wachsspaltungsferment im Darm der Larve der Wachsmotte. Arch. f. Physiologie. Physiol. Abt. des Arch. f. Anat. und Physiol. 157—163.

FLORKIN M., LOZET F. et SARLET H., 1949 — Sur la digestion de la cire d'abeilles par la larve de *Galleria mellonella* et sur l'utilisation de la cire par une bactérie isolée du contenu intestinal de cette larve. Arch. Intern. de Physiol. 67, 71—88.

JAUBERT G. F., 1927 — Sur l'origine de la couleur jaune de la cire des abeilles. Compt. Rend. Hebd. de Séances de l'Acad. de Sci. *185* (6), 405—406.

MC LEAN R. C. and IVIMEY-COOK, 1956 — Textbook of theoretical botany. Vol. II. Longmans, Green and Co., London—New York—Toronto.

LUBLINER-MIANOWSKA K., 1955 — The pigments of pollen grains. Acta Soc. Bot. Pol., *24* (3), 609—618.

LUBLINER-MIANOWSKA K., 1956 — Investigations on the chemical composition of the pollen. I Study of the pollen as bee-food. Acta Soc. Bot. Pol. *25* (3), 409—419.

METALNIKOV S., 1908 — Recherches expérimentales sur la chenille de *Galleria mellonella*. Arch. de Zool. Exper. et Génér. IV Serié, *8*, 489—588.

RYBICKI M., 1952 — The participation of the intestinal microflora in the nourishing processes of larvae of *Galleria mellonella* L. Ann. Un. Mariae Curie-Skłodowska, Lublin — Polonia. Sectio C., *8* (2), 15—67.

TISCHER J., 1940 — Über die Herkunft der gelben Farbstoffe des Bienenwachses. Hoppe-Seyler's Zeit. für physiol. Chemie, *267*, 14—22.

VÖHRINGER K., 1934 — Zur Biologie der grossen Wachsmotte (*Galleria mellonella* L.). III. Morphologische und biologische Untersuchungen am Falter der grossen Wachsmotte (*Galleria mellonella* L.). Zool. Jahrb. Abt. Anat. u Ont. der Tiere, *58* (2), 275—302.

ZIELIŃSKA Z. M., 1952 — Studies on the biochemistry of the waxmoth (*Galle*ria mellonella) 8. Nitrogen metabolism of the larvae. Acta Biol. Exper., *16* (13), 171—186.

ZIELIŃSKA Z. M., 1955 — Przemiany azotowe u gąsienic mola woskowego (*Galleria mellonella*). Soc. Sci. Lodziensis. Sectio III, *33*, 1—24.

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THE ABILITY OF WAX MOTH (*GALLERIA MELLONELLA* L.) LARVAE TO UTILIZE POLLEN GRAINS

M. RYBICKI

Department of Comparative Animal Physiology, Zoological Institute,
University of Warsaw

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The pollen of definite plants is an important constituent of the diet of wax moth larvae, and depending on the kind of the plant it may be the only source of nitrogen compounds and a source of vegetable pigments which influence the size, colour and development time of the wax moth (Rybicki 1959). It is a commonly observed fact that wax moth larvae grow best on old honeycomb. On the other hand microscopic observations of delipidized comb show a considerable number of pollen grains between the membranes at the bottom of bee cells. The way the larvae feed (Rybicki 1952) and microscopic observations of delipidized honeycomb membranes suggest that pollen is one of the fundamental components of larvae feeding on old honeycomb. In the previous investigations into the feeding of the wax moth larvae no attention has been paid to the important fact that pollen grains contained in old comb have passed through the alimentary tract of bee larvae. Pollen grains do not decompose in the intestine of bee larvae; nevertheless, in view of their indispensability in the diet of bee larvae, they probably are attacked, at least partly, by some digestive mechanism.

Our research was an attempt at investigating what changes pollen undergoes in various sections of the intestine of wax moth larvae. This may help find out to what degree pollen grains are utilized as food for that organism.

MATERIAL AND METHODS

Galleria mellonella larvae fed on old honeycomb (workers cells) constituted our experimental material.

Mature larvae were etherized, their intestine prepared and preserved in Long's fixative (corrosive sublimate + glacial acetic acid) (Romeis B. 1953). The intestines were kept in the fixative for 12 hours during which the fixative was changed twice. Microtomic sections were stained with Mayer's P. haemalaun and tinted with eosine (Romeis, B. 1953). This method of preservations was applied to the intestines of three mature larvae of about the same size.

We put sections of the intestine on glasses and tried to have roughly the same number of them in each case as this made it possible to compare various segments of the three parallel intestines. Each of the intestine sections was then microscopically examined and the number of pollen grains in a given section carefully counted. While the number of pollen grains in various sections of intestines was determined the grains were split into two groups: those having a preserved protoplast and staining, and those having no protoplast (non-staining), in which case only membrane had been left.

A part of old honeycomb which constitued the food of the larvae was delipidized. When the membranes of the cells were mechanically removed pollen grains were taken out of their bottom and stained with 0.5% erythrosine to find out whether the pollen grains contained in the comb had preserved their protoplasts after having passed through the alimentary tract of bee larvae.

EXPERIMENTS

I. Microscopic examinations of the content of the intestine of larvae fed on old honeycomb

Over 99,000 pollen grains were found in the three larval intestines examined. The number of pollen grains found in various intestines amounted to anything between 29,801 to 39,292. These differences are due first of all to the amount of pollen in various honeycomb cells which is correlated to the number of generations of bee larvae which have developed in the honeycomb.

In spite of considerable differences in the total amount of pollen in the intestines of various larvae it seems legitimate to base the interpretation of results on average figures for the three intestines. Table I illustrates average figures.

Figures for the average number of pollen grains in the three larval intestines examined (Table I, column 5) show that the total number of pollen grains found amounts to 33,162. Of these 25,159

Table I

Average numbers of pollen grains found in the intestine of the wax moth (*Galleria mellonella* L.) larva (average figures for intestines of three larvae)

No. of glass	No. of intestinal sections	Number of pollen grains			Utilization in %% $\frac{3 \times 100}{5}$
		without protoplast	with protoplast	in all (3 + 4)	
1	2	3	4	5	6
1	55	160	128	288	55.6
2	54	932	445	1377	67.7
3	49	6386	607	6993	91.3
4	49	5387	705	6092	88.4
5	49	3822	620	4442	86.0
6	54	3123	842	3965	78.8
7	54	1927	846	2773	69.5
8	47	1146	750	1896	60.4
9	42	733	684	1417	51.7
10	36	548	605	1153	47.5
11	35	393	558	951	41.3
12	33	244	407	651	37.5
13	30	168	336	504	33.3
14	26	110	252	362	30.4
15	29	58	135	193	30.1
16	19	12	38	50	24.0
17	19	10	45	55	18.2
	680	25159	8003	33162	75.6

are grains with no protoplast (Table I, column 3). Consequently, it may be supposed that the protoplast of those cells are digested in the intestine of the wax moth larvae, and this would mean that 75.6$^0/_0$ of pollen grains are utilized in the intestine of the larvae. Both the figures in column 3 and the degree to which pollen grains are utilized (75.6$^0/_0$) give reasons for certain doubt whether these figures really illustrate the process taking place in the intestine of the wax moth larva as the pollen grains it consumend has passed through the alimentary tract of the bee larva. So these figures must be corrected and this will be done later. If we assume that the figures in column 3 reflect the process taking place in the larval intestine a comparison of the figures in the rows of columns

3, 4 and 5 points to the important fact that a pollen grains with a protoplast is utilized by wax moth larvae when the food passes through the intestine. This is also confirmed by the fact that the average number of grains with a protoplast (Table I, column 4) is much lower (but it varies with the segments of the intestine) than that of pollen grains with no protoplast.

Figures in column 5 (Table I) show the total number of pollen grains found throughout the intestine and those in the rows indicate the varying number of grains in the intestine. There may be two interpretations of these figures. If we assume that prior to being killed the larva consumed a piece of honeycomb with the same pollen content per bee cell we must assume that pollen grains are most intensely " digested" in the fore half of the intestine. As the food moves backwards the number of pollen grains in the intestine diminishes, which would mean that they are completely digested. This sharp fall in the number of pollen grains in the hind half of the larval intestine is observed in all of the three histologically examined intestines.

When interpreting these figures (column 5) one should also take into consideration another possibility, i. e., that the large number of pollen grains in the fore part of the intestine may be due to a larger diameter of this part of the intestine. Figures in column 5 show an increase in the total of pollen grains, particularly in the first 207 sections of the intestine (first four glasses) and then a continous decrease in the number of grains. As only whole grains were used in the microscopic examination of the sections of the intestine, the decrease in the number of grains observed in the intestine of the larva would mean that whole grains are digested, i. e., not only the protoplasts but also the membranes. If figures in column 5 (Table I) really illustrated the whole process of the digestion of pollen grains this would be the first case to prove that pollen cell membranes, very resistant to the action of chemical factors may be digested by an animal organism.

Figures in column 2 (Table I) indicate the segment of the intestine in addition to the number of sections and this makes it possible to observe more carefully the changes taking place in each segment. A comparison of figures in columns 2 & 3 of the corresponding rows shows that although there is a considerable number of pollen grains in the fore segments of the intestine (glasses 1 & 2) there are most pollen grains without a protoplast in the next segment (glasses 3 & 4).

This would mean that pollen is intensely utilized already in this segment of the intestine. The terminal segment of the intestine (glases 16 & 17), which is made up of 38 sections on the average, contains some pollen grains without a protoplast and with preserved membranes (column 3, Table I) and even more grains with a protoplast. The pollen grains which preserved membranes are found in faeces.

The number of pollen grains without a protoplast in the intestine varies from 10 to 6,386, while the number of grains with a protoplast (Table I, column 4) varies from 45 to 846. When figures in the various rows of these columns (3 & 4) are compared it is noteworthy that from the tenth glass on there are more pollen grains with than without a protoplast. The last segment (glass 17) contains four and a half times as many pollen grains with a protoplast as without one. This means that not all pollen grains lose their protoplast while passing through the intestine and some of them undergo no changes then. It is difficult to interpret this fact. The following explanation seems most likely: pollen grains in old honeycomb are a mixture of grains from various plants. As the majority of pollen grains in the terminal intestine are grains with preserved protoplast, it is possible to explain this in terms of a difference in the structure of membranes of various pollen grains on the basis of G. Erdtman's (1952) study.

Figures in column 6 (Table I) illustrate the percentage of the utilization of pollen in the respective segments of the larval intestine. The utilization in the first segment of the intestine, which corresponds to 55 histological sections (glass 1) amounts to 56%. It is not likely that the utilization of the content of pollen grains should be so intense in this segment of the intestine. When interpreting this fact one should take into account two alternatives. As the pollen grains contained in honeycomb have passed through the alimentary tract of the bee larva it is legitimate to suppose that part of the pollen grains consumed by the moth larva are already without protoplasts. Nor is the other alternative excluded, i. e., that among the pollen grains without protoplasts found in the first 55 sections of the intestine there are also grains the protoplasts of which have been utilized precisely in that segment before the food content was moved to further segments of the intestine. If we assume that the disappearance of the protoplasts of pollen grains really takes place in this segment of the intestine of the larva it will again be connected with the speed with which the food moves in the intestine and this is not known. It seems most plausible that part of the pollen grains in the segment in question lost their protoplasts while passing through the intestine of bee larvae and part of them were utilized in the intestine of the moth larva.

The material we have had so far warrants the conclusion that only a small part (55) of all pollen grains (33,162) found in the intestine of the larva is not completely digested (Table I, column 5). But in some segments of the intestine of the larva only the protoplasts of some of the grains consumed is utilized (Table I, column 6), while others pass through the intestine without getting decomposed (Table I, columns 3 & 4, glass 17).

II. Distribution of pollen grains in the intestine of the larva

To illustrate more clearly the distribution of pollen in the intestine final absolute figures in columns 2, 3, 4 & 5, Table I are regarded as 100 and various values in each column are expressed in per cents in relation to the total value of each column. These calculations are illustrated by data in Table II.

Figures in columns 3 & 5 make it possible to distinguish very clearly three segments of the intestine with widely varying congestion of pollen grains in the intestinal content.

The three segments of the intestine vary in length, segment I amounting to 16⁰/₀ of the whole length, segment II to 29.5⁰/₀ and segment III to 54.5⁰/₀.

The greatest congestion of pollen grains is observed in the first two segments amounting to 45.5⁰/₀ of the length of the whole intestine and containing 69.83⁰/₀ of all pollen grains found in the intestine. The congestion of pollen grains in this part of the intestine may be due to the processes taking place there and also to its structure (the diameter of the lumen of the intestine).

The division of the intestine was based on absolute figures for pollen grains (Table I, columns 3, 4 & 5), but figures in columns 3 & 4, Table II, call for discussion.

Figures in column 3, Table II, very clearly divide the intestine into three segments (it was agreed to call them fore, mid and hind). 4.34⁰/₀ of all pollen grains without protoplasts were found in segment I, 74.39⁰/₀ in segment II, and 21.27⁰/₀ in segment III. Such

Table II

The proportion of pollen grains in various segments of the intestine of the wax moth (*Galleria mellonella* L.) larva (based on figures shown in Table I)

No. of glass	Length of segments of intestine in relation to its total length %		Number of grains without protoplast in relation to total of grains without protoplast %		Number of grains with protoplast in relation to total of grains with protoplast %		Number of grains in various segments of intestine in relation to total of grains found %
1	2		3		4		5
1	8.1	16.0	0.64	4.34	1.60	7.16	0.87
2	7.9		3.70		5.56		4.15
3	7.2	29.5	25.38	74.39	7.58	34.66	21.09
4	7.2		21.41		8.81		18.37
5	7.2		15.19		7.75		13.39
6	7.9		12.41		10.52		11.96
7	7.9	54.5	7.66	21.27	10.57	58.18	8.36
8	6.9		4.56		9.37		5.72
9	6.2		2.91		8.55		4.27
10	5.3		2.18		7.56		3.48
11	5.2		1.56		6.97		2.87
12	4.9		0.97		5.09		1.96
13	4.4		0.67		4.20		1.52
14	3.8		0.44		3.15		1.09
15	4.3		0.23		1.69		0.58
16	2.8		0.05		0.47		0.15
17	2.8		0.04		0.56		0.17
	100.00		100.00		100.00		100.00

a large amount of pollen grains without protoplasts in the mid part of the intestine is not apparently accidental but seems to be due to digestion in this part of the intestine which constitutes 29.5% of the length of the whole intestine. The disappearance of protoplasts is much slower in the hind segment of the intestine.

The distribution of pollen grains with protoplasts in the intestine is somewhat different. There are 7.16% of these grains in the fore segment (Table II, column 4), 34.66% in segment II, and 58.18% in segment III. Comparison of the congestion of pollen grains with and without protoplasts in various segments of the intestines shows that pollen grains with protoplasts preponderate in the first segment.

As this is the shortest segment (16% of the whole length of the intestine) the congestion of these pollen grains in this segment is not too great in spite of their preponderance. This would mean that food passes rather quickly through this segment to the next one. In the next segment (glasses 3, 4, 5 & 6), which may be regarded as roughly one third of the whole length of the intestine the congestion of pollen grains with protoplasts is five times as high as in the first segment, but the number of pollen grains without protoplasts is more than twice as much in the same segment. In the third segment, which accounts for more that a half of the length of the intestine the congestion of pollen grains with protoplasts is highest in its part adjacent to segment II (10.57%, Table II, column 4). There are always more of these grains than grains without protoplasts in farther segments. The highest congestion of pollen grains with preserved protoplasts is observed at the end of the second and the beginning of the third segments does not fully coincides with our division of the intestine. The division of the intestine into three segments was based on the number of pollen grains without protoplasts (Table II, column 3).

As far as the congestion of pollen grains with protoplasts in the third (hind) segment is concerned, the congestion of pollen grains in the intestinal sections on glasses 16 & 17 is of particular interest. The number of sections is the same on both glasses but the congestion of pollen grains is 0.47% on the former and 0.56% on the latter. The congestion of pollen grains in the last section of the intestine shows that the faeces of larvae fed on old honeycomb should contain far more pollen grains with protoplasts than without.

Our discussion based on figures in Table II for the total congestion of pollen grains (column 5), the congestion of pollen grains without protoplasts (column 3) and of pollen grains with preserved protoplasts (column 4) warrants the conclusion that two processes may take place in the larval intestine. One, consisting in the decomposition of protoplasts of pollen grains and the other, consisting in a marked drop in the total of pollen grains which probably become completely decomposed.

Our results make us doubt whether the numbers of pollen grains found in the intestine reflect the actual manner in which they are utilized by larvae. Pollen grains without protoplasts outnumber those with preserved protoplasts in the fore segment of the intestine consisting of 55 section (Table I, glass 1). Were the former digested

Table III

Analysis of pollen in honeycomb

Type of honeycomb	Number of pollen grains				Total
	without protoplast		with protoplast		
	actual number	%	actual number	%	
delipidized old honeycomb, worker cells	350	26.1	987	73.9	1337
,,	320	27.5	843	72.5	1163
,,	263	22.1	926	77.9	1189
,,	328	25.6	953	74.4	1281
	1261	25.4	3709	74.6	4970

in the intestine of the wax moth larva of consumed by it after they lost protoplasts in the intestine of the bee larva? Table III supplies an answer to this question as it contains the results of microscopic analysis of pollen grains obtained from old honeycomb after delipidization.

About 5,000 pollen grains from four samples of honeycomb were examined and figures in Table III show that 25% of the total of grains examined contain no protoplast and that only membranes are preserved.

Figures in Table III make it possible to correct figures in Table I. We based our corrections of figures in column 3, Table I, on the asumption that honeycomb consumed by larvae contains 25.4% of pollen grains without protoplasts. The corrected figures are presented in Table IV.

The corrected figures in column 3, Table IV, make it possible to maintain the division of the intestine of the larva. Comparison of figures in columns 3 & 4 this time also points to an intense disappearance of protoplasts of pollen grains in the mid segment of the intestine, while pollen grains which have preserved protoplasts stay for a fairly long time in the intestine. They outnumber pollen grains without protoplasts from glass 9 up to the end of the intestine. The utilization of grains (column 6) in the respective segments is

Table IV

Interpolated average numbers of pollen grains found in the intestine of the wax moth (*Galleria mellonella* L.) larva (based on figures shown in Tables I & III)

No. of glass	Length of segments of intestine to total length %	Number of pollen grains			Utilization $\frac{3\times100}{5}$ in%%
		without protoplast number	with protoplast number	in all (3 + 4) number	
1	2	3	4	5	6
1	8.1	119	128	247	48.2
2	7.9	695	445	1140	61.0
3	7.2	4764	607	5371	88.7
4	7.2	4019	705	4724	85.1
5	7.2	2851	620	3471	82.1
6	7.9	2330	842	3172	73.5
7	7.9	1437	846	2283	62.9
8	6.9	855	750	1605	53.3
9	6.2	547	684	1231	44.4
10	5.3	409	605	1014	40.3
11	5.2	293	558	851	34.4
12	4.9	182	407	589	30.9
13	4.4	125	336	461	27.1
14	3.8	82	252	334	24.5
15	4.3	43	135	178	24.2
16	2.8	9	38	47	19.1
17	2.8	8	45	53	15.1
		18768	8003	26771	70.1

lower when compared with figures in Table I and the total utilization (Table IV) is lower only by 5.5% when compared with those figures.

DISCUSSION

Many studies dealing with the utilization by the wax moth larva of various components of honeycomb treat honeycomb as a sum of chemical compounds. Quantitative changes within those compounds after they pass through the alimentary tract of the larva make it possible to draw conclusions about the utilization of various honeycomb components. Only a general idea of the processes taking place in the intestine can be obtained in this way and only one aspect of the problem the quantitative chemical changes, can thus be covered. As chemical compounds form a definite morphological structure, the examination of changes in various honeycomb components considerably supplements the picture obtained with chemical methods.

We already drew attention in our previous research (Rybicki 1952) to the fact that two components of the fraction of honeycomb insoluble in lipid solvents may be distinguished: be cell membranes and pollen. It is very difficult mechanically to separate pollen from membranes of bee cells but it is easier to obtain it from honeycomb in which one generation of bee larvae have developed. It has so far been impossible to establish the importance of pollen in honeycomb for moth larvae.

Initial investigations into pollen of delipidized honeycomb pointed out the great variety of form and size of grains and subsequent attempts at staining such pollen with 0.5% erythrosine have shown that the protoplasts of many such cells are stained.

The above fact and the results of my earlier investigation (Rybicki 1960) called for the application of histological methods which enabled me to examine changes undergone by pollen in various segments of the intestine of the larva.

Figures in Table IV show that:

1) pollen grains without protoplasts outnumber those with protoplasts in the fore segment of the intestine; the respective ratio being 1.42;

2) most pollen grains concentrate in the mid segment, where the ratio between grains without protoplasts and those with protoplasts in 5.03;

3) the number of pollen grains without protoplasts in the hind segment of the intestine diminishes, the same ratio being 0.86.

Interpolated figures in Table IV justify the conclusion that the digestion of the protoplast begins as soon as the larva consumes its first bits of food, which is very clearly reflected in the ratio 1.42. Maximum digestion is observed in the mid intestine. As protoplasts also disappear in the hind segment, this means that protoplasts of various pollen grains are not digested just as quickly. Consequently, the rate of decomposition of protoplasts of pollen grains is not the same throughout the length of the intestine.

Analysis of figures for the total amounts of pollen (Table IV, column 5) makes it possible to distinguish three segments of the intestine, of which the first, fore segment, accounts for 16%, the second or mid segment for 29.5%, and the third or hind segment for 54.5% of the total length of the intestine, while figures for the amounts of pollen in these segments are respectively as follows: 5.2%, 62.5% and 32.3%. (of the total amount of pollen). We based our division of the intestine of the larva on varying congestions of grains in the intestine. This division is somewhat arbitrary and may not correspond to the functional division. Tchang-Yung-Tai (1928, 1929) investigated the division of the intestine of the larva from the functional point of view. According to him (1928) two types of cells, columnar and goblet are characteristic of the mid intestine of the moth larva. The function of absorption is ascribed to the columnar cells. They are situated in all folds of the epidermis at one third or half of the length of the mid intestine. The function of the goblet cells has not been explained. As there are no diagrams it is difficult to find out whether the division of the intestine of the larva as suggested by that author is identical with the one I attempt in this paper.

The following may be observed in the intestine of the larva:

(1) a most intense disappearance of protoplasts of pollen cells in the mid intestine;

(2) only 53 pollen grains of the total of 26,771 (Table IV) found in the intestine are observed in the final 19 sections of the intestine, i. e., over 99% of the grains disappear completely.

Figures in column 3, Table IV show that the disappearance of the protoplasts of pollen cells is not uniform in various grains in the normal conditions prevailing in the intestine of the moth larva. As pollen grains come from various plants the different rates of disappearance of protoplasts seem to be due to the structure of pollen grain membranes.

The disappearance of pollen cell membranes shows all characteristics of lysis. Microscopic observations reveal sharp-edged pieces of membranes. Mechanical disintegration due to tearing or biting would leave the edges uneven or rugged. Such fragments of membranes are observed more frequently in the fore and mid segments of the intestine. Their number distinctly diminishes in the hind segment. On the whole, very few fragments of pollen grain membranes are observed in the hind intestine, especially in its terminal sections and in view of such a large amount of pollen observed in the whole intestine, this also suggests that whole membranes or their fragments become completely decomposed.

CONCLUSIONS

1. Wax moth larvae consume very large numbers of pollen grains contained in honeycomb.
2. A very intense disappearance of protoplasts of pollen grains is observed with the cell membranes left intact.
3. Above 99% of pollen found in the intestine undergoes a complete lysis.

REFERENCES

ERDTMAN G. 1952. — Pollen morphology and plant taxonomy — Angiosperms. Almqvist & Wiksells, Boktryckeriaktiebolag Uppsala.

ROMEIS B. 1953. — Mikroskopiczeskaja tiechnika, Moskwa, Izd. In. Lit.

RYBICKI M. 1952. — The participation of the intestinal microflora in the nourishing processes of larvae of *Galleria mellonella* L. Ann. Un. Mariae Curie-Skłodowska, Lublin — Polonia. Sectio C, *8* (2), 15—66.

RYBICKI M. 1960. — Pollen as a Source of Some Food Constituents of Wax Moth (*Galleria mellonella* L.) Larvae. Acta Biol. Exper. *20*, 22—36.

TCHANG-YUNG-TAI 1928. — Sur l'origine, le développement et la structure des cellules caliciformes dans l'intestin moyen des chenilles de Lepidopteres. Bull. de la Soc. Zool. de France. *53* (1), 56—60.

TCHANG-YUNG-TAI 1929. — Sur la localisation de l'absorption intestinale et le comportement des cellules absorbantes chez les chenilles d'un Lepidoptera (*Galleria mellonella* L.). Compt. Rend. des Séanc. de l'Acad. des Sci. *188* (1), 93—95.

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INFLUENCE OF THE SALINITY AND THE OXYGEN CONTENT IN THE EXTERNAL MEDIUM UPON THE RESPIRATION OF THE SNAIL *THEODOXUS FLUVIATILIS* (L.)*

J. BIELAWSKI

Department of Animal Physiology, Poznań University

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INTRODUCTION

In order to perform their normal functions living cells require a defined medium which may change only to certain limits without harming the organism. In most animals this medium are body fluids.

The hemolymph of stenohaline marine invertebrates is as a rule isotonic to the surrounding water. If the animal passes into water of another salinity its cells must adapt themselves to the changed hemolymph concentration or else the organism must be adapted to maintain an osmotic hemolymph concentration that would be independent of the environment.

A great majority of homoiosmotic invertebrates have a body surface or parts of it permeable to ions and water so that in a unisotonic medium they must actively regulate hemolymph concentration. Publications dealing with this problem have been listed by Schlieper (1930, 1935), Krogh (1939), Prosser (1952).

To maintain a hemolymph concentration that would be independent of the external medium requires a certain amount of energy which must be the greater, the greater the difference in concentration between the external medium and the hemolymph and the greater the permeability of the body surface to ions and water.

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For its vital processes the organism draws energy from respiration. Hence, respiration should be the more intense the greater the osmotic activity performed by the animal (Schlieper 1929).

This hypothesis is supported by a number of facts: in water of different salinity the animals show differences in respiration. Thus e. g. *Carcinus maenas* (Schlieper 1929), *Potamobius fluviatilis* (Schwabe 1933) ond others breathe more intensively in less saline water where the difference in concentration between the hemolymph and the environment is greater; some crabs such as *Ocypode albicans* (Flemister and Flemister 1951) and *Palaemonetes varians* (Lofts 1956 a and b) which regulate excellently hemolymph concentration in a hypertonic as well as hypotonic medium consume the least oxygen in an isotonic medium. A lack of oxygen and substances inhibiting respiration, as e. g. cyanides, reduce the osmoregulating capacities (Beadle 1931, Schwabe 1933, Parry 1953).

However, there exist quite a number of facts which cannot be explained by means of the above mentioned hypothesis: when changes occur in the salinity of their external medium it is not only the animals as a whole that change oxygen consumption but also isolated organs not taking part in osmoregulation (Peters 1936). *Eriocheir sinensis* is a homoiosmotic euryhaline animal living periodically even in fresh water. Its body surface is permeable to ions and water (Conklin and Krogh 1939) but in spite of this it consumes the same amount of oxygen in waters of varying salinity (Schwabe 1933). As examples of animals with well developed osmoregulating abilities and showing no correlation between osmoregulation and oxygen consumption may serve also *Artemia salina* (Gilchrist 1956, Croghan 1958 a and b) and some coelenterates (Palmhert 1933, Lilly 1935).

Nereis diversicolor transferred to more diluted water increases in weight owing to the intake of water. After some time the weight regains its initial value but the difference in osmotic concentration between the external and internal medium is greater than the initial difference. Parallel measurements of respiration showed that the oxygen consumption increases too but returns to the initial value after some time (Schlieper 1929, Beadle 1931).

This fact gave rise to the assumption that a change in the intensity of respiration is not due to a greater demand of energy for osmoregulation but to the hydration of the tissues. Further investigations (Jørgensen and Dales 1957) have shown that in *N. diversicolor* the exchange of ions between the animal and its environment is greater in hypotonic (in relation to the hemolymph) than in isotonic water.

The hypothesis of hydration has been confirmed by investigations on isolated tissues: *Mytilus edulis* is a poikilosmotic animal (Conklin and Krogh 1939) but in spite of this the isolated gills use more oxygen in less saline water (Schlieper 1929, 1955). On the other hand, changes in the respiration of excised gills are accompanied by a change of their hydration (Pieh 1936).

A comparison of the isolated gills of *Carcinus maenas* and *Eriocheir sinensis* yielded interesting results. In the first of these animals a change in the salinity of the water caused a change in the intensity of respiration, in the second — no change was observed. The intensity of respiration and hydration in the isolated gills of *C. maenas* changes when the salinity of the water is changed. In the same conditions the hydration of the gills in *E. sinensis* remains unchanged.

Isolated tissues transferred from sea water isotonic with hemolymph to an equally concentrated NaCl solution show an increase of oxygen consumption and of hydration (Pieh 1936, Hopkins 1949).

The ion content of the medium bears also an influence on the respiration of intact animals. *Viviparus fasciatus* transferred from fresh to diluted artificial sea water having the same concentration as fresh water increases the intensity of respiration to a considerable extent (Obuchowicz 1958, 1959).

In *Mytilus edulis* the adaptation of respiration to a changed salinity of the medium is a long process lasting 4—7 weeks whereas the change of hemolymph concentration takes place within 24 hours (Schlieper 1955).

As may be seen from the above mentioned facts, the correlation between the salinity of the external medium and the respiration of aqueous animals is a complex phenomenon.

Ecological observations quoted by Schlieper (1930, 1935) prove that animals passing from brackish to fresh water settle in places where the current is rapid. The movement of the water in the animal's immediate vicinity facilitates the exchange of gases between the organism and the environment. Thus, animals living in rapidly moving waters as compared with animals in stagnant waters are distinguished by a higher critical oxygen tension, i.e. an oxygen tension above which oxygen consumption depends no longer on its concentration (Fox, Wingfield and Simmonds 1937).

Theodoxus fluviatilis which is the object of investigations presented in this paper lives in rapidly moving waters such as eddies on the shores of lakes and of the Baltic, and on the banks of rivers. According to Boettger (1951) its oxygen requirements are lesser in saline than in fresh water. These observations seem to be indicative of a close relationship between the salinity of the medium and respiration*.

MATERIAL AND METHODS

Snails for the experiments were collected in the Kierskie Lake near Poznań, at first from stones in the shore zone and later, for measurements of hemolymph concentration, from pales driven near the shore into the bottom of the lake. The depth at which the animals were collected was not in excess of 70 cm. The transport to the laboratory took about an hour. Having brought the snails to the laboratory and washed the shells from algae they were placed in 10 l. glass containers with tap water that had been continuously aerated since the day before.

For respiration measurements I applied the polarographic method described in my previous paper (Bielawski 1956). The volume of the respiratory vessel in which the snails were kept during measurements was about 1 ml so that specimens having a length of 1 cm or more could not move freely. The water flowed through the vessel at a rate of 5 ml./hr. and the decrease of oxygen tension amounted at an average to 2.5 mg. O_2/l. (maximum 4.2).

During measurements the whole apparatus was placed in a feebly lighted room. Moreover, the vessel containing the animal was encircled by a rubber ring which eliminated most of the light. The animals were left for 1—2 hours in the vessels to allow their adaptation to the new conditions and then the intensity of respiration was measured. It was found that during measurements there occur some differences in respiration. To reduce errors caused by these differences each measurement was repeated twice at intervals of 20—50 min. and the mean value was calculated. All measurements were made at a temperature of 20°C

In order to make the results of investigations comparable they are calculated in values of the animal's size. This size is usually expressed in live weight, dry weight, body nitrogen or protein nitrogen. In the experiments described below respiration was calculated in body nitrogen. Nitrogen was

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* I received the paper of Lumbye 1958) on the respiration of *T. fluviatilis* in various salinity when part of my experiments had already been made.

determined according to the method of Kieldahl using $CuSO_4$ as catalizer. For determining the nitrogen content in the tissues the shell of the snail should be removed. With a fine scalpel it is relatively easy to remove the operculum. The body is attached to the shell near its mouth and when these attachments are cut through the snail is easily taken out. However, as during these functions the animal is wounded part of the hemolymph becomes lost.

Samples of hemolymph for examining its concentration were taken from the visceral sac after having previously crushed the shell and dissected the mantle on the dorsal side of the snail. Measurements of the freezing point depression (Δ) of the hemolymph and of the external medium were made with an apparatus constructed by Obuchowicz (Obuchowicz 1956, Michejda and Obuchowicz 1956) which is a modification of the Ramsay microcryoscope (1949).

Water of various salinity was obtained by diluting with tap water artificial sea water prepared according to Bogucki (1931) and having the following constituents:

NaCl	28.27 g.	$MgSO_4 \cdot 7\,H_2O$	7.03 g.
KCl	0.76 „	NaBr	0.08 „
$CaCl_2$	1.22 „	$NaHCO_3$	0.21 „
$MgCl_2$ 6 H_2O	5.10 „	water up to	1000.00 ml.

For examining the influence of oxygen concentration on the intensity of respiration water of a defined oxygen concentration is needed which is prepared in the following way:

Air is pumped into the container up to a defined tension. Subsequently tension is increased to a given value by pumping in technical nitrogen or oxygen. The thus prepared mixture of gases with an approximately known percent of oxygen is bubbled for 15 min. through the water used for the experiments. The exact oxygen concentration in the water is defined polarographically.

RESULTS

Influence of the animal's size and of the oxygen content in water upon respiration

The investigations were made in August when the water temperature in the lake amounted to 20°C. After capture the animals were kept in the laboratory at room temperature (20°C) until the next day when their respiration was examined.

Animals of various size were placed in 3—5 respiratory vessels. Snails with a shell length of about 10 mm. and more were kept single, smaller ones either two or three together. The oxygen content of the water flowing into the respiratory vessel was changed three times (in one case twice) either increasing or reducing it gradually. The initial oxygen content amounted to about 9 mg. O_2/l. (in two cases 6.5 and 3.3 mg. O_2/l., respectively). Following the change of oxygen content in the water the snails adapted themselves for an hour to the new conditions and then respiration measurements were made.

The results are shown in Figs. 1 and 2. The relation between the amount of nitrogen inherent in one specimen and its oxygen consumption is represented in Fig. 1. Each point corresponds to the respiration of animals from one vessel with a defined oxygen content. Respiration following a change of the oxygen content is marked by another point. The results shown in Fig. 1 refer only to the respiration of animals supplied with water of a higher oxygen content than 6 mg. O_2/l. As seen in Fig. 2 above that value respiration does not depend on the amount of oxygen contained in the water.

The curve of respiration in *T. fluviatilis* depending on the content of nitrogen is represented in logarithmic scale as a straight line with an inclination equal to 0.89. Further results of investigations on respiration shown in this paper have been corrected by taking account of the size of the specimens, i.e. they were calculated in values of the respiration of animals with a nitrogen content of 1 mg.

Lumbye (1958) examined respiration in *T. fluviatilis* calculating its values in live weight and therefore the absolute values he obteined cannot be compared with those given in this paper.

The occurrence of *T. fluviatilis* in rapidly moving waters implies that the critical oxygen tension should be near the oxygen content of water saturated with air, or even higher. Such exactly are the results obtained by Lumbye (1958). Quite different are the results shown in Fig. 2. The critical oxygen tension is here 5 mg. O_2/l. which constitutes about 50% of the oxygen content of water saturated with air. In water flowing from the respiratory vessel the oxygen concentration for the nearest points above the critical tension amounts to a mean of 4.5 mg. O_2/l. (minimum 3.7). The differences between Lumbye's and my own results may be partly due to the different methods we applied. His investigations were made in water derived from a natural medium. He placed the examined animals in a vessel sufficiently large for them to move but the water in the respiratory vessel was not moved. In my own investigations the snails were kept

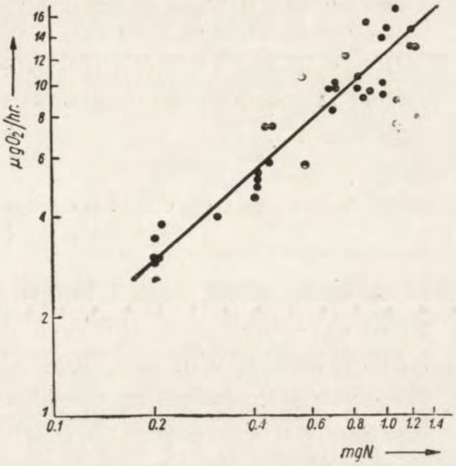

Fig. 1. Relation between the intensity of respiration and the content of nitrogen in the organism. Both coordinates are presented in logarithmic scale

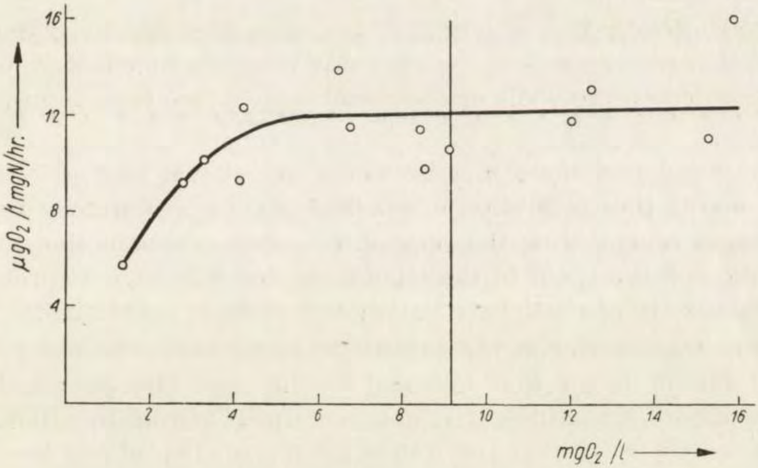

Fig. 2. Influence of the oxygen concentration in water upon the intensity of respiration

The vertical line corresponds to oxygen concentration in air-saturated water. Each point represents the mean measurements of animals taken from 3 — 5 respiratory vessels.

for 24 hours in aerated tap water* and in such water the measurements were made. During measurements the animals were placed in a slow current of water in a vessel so small that they were unable to move. Whether the differences in the results are indeed due to different methods of investigation should be found out by way of experiments.

Influence of the salinity of water upon respiration

Animals with a shell length of 10—11 mm. were collected for these experiments in the second part of October when the water temperature in the lake was 11°C. In the laboratory they were placed in a thermostatic vessel at a temperature of 20°C with fluctuations not exceeding 1°. In this vessel bacteria developed rather soon and therefore in was necessary to wash its walls and to change the water every 2—4 days.

Respiration was measured at defined intervals of time. After five days from the moment of catching the animals part of them was transferred into water of $\Delta = 0.49°C$. Since then at every change of water some animals were dead, more so in saline than in fresh water. However, the majority of the specimens survived over a month. For respiration experiments only those specimens were taken that crawled on the walls of the vessel — two into each respiratory vessel.

The results of these measurements are shown in Fig. 3. Each point marks the respiration of snails from one respiratory vessel. The curve representing the relation of oxygen consumption to the time the animals spent in the laboratory descends at first probably in consequence of partial starvation and thermic adaptation.

After the transfer of the animals to saline water we observe an initial fall of respiration followed by its rise. Though relatively slight, these fluctuations are not contained within the limits of dispersion. After 12 days respiration becomes balanced and identical for snails from fresh and saline water. These data confirm the results of Lumbye (1958) who examined *T. fluviatilis* from natural media of various salinity and found the intensity of their respiration to be identical.

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* Tap water was derived from the river Warta in which *T. fluviatilis* occurs.

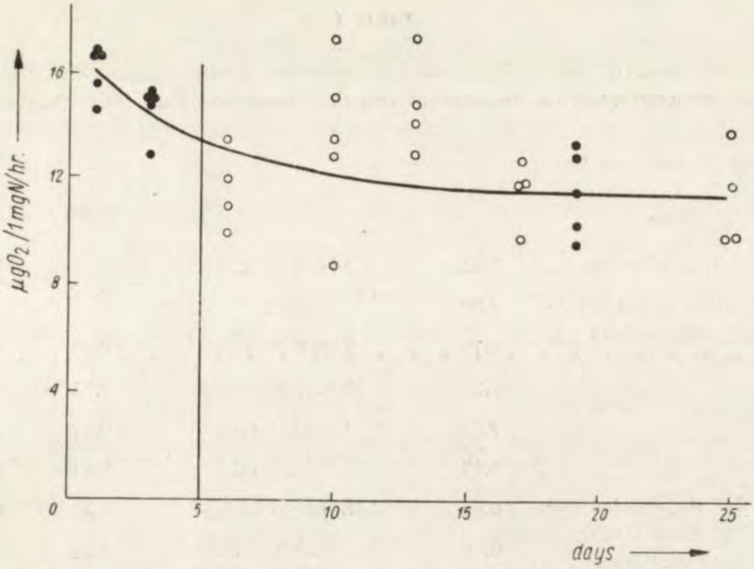

Fig. 3. Influence of the time during which animals are kept in a given water upon the intensity of respiration

The vertical line indicates the time at which the animals were transferred into saline water. ● =respiration in fresh water animals, ○ = respiration in animals kept in saline water. Each point marks the respiration of animals from one respiratory vessel.

To find out whether the difference in osmotic concentration between the external medium and the hemolymph, and thus also the osmotic activity, have not been subjected to changes I investigated the freezing point depression of the hemolymph.

Influence of the water salinity upon the osmotic concentration of the hemolymph

The snails were collected in December. The temperature of the water was at the time not measured but it amounted probably to 0—4°C because shallow and overgrown parts of the lake were frozen. In the laboratory the snails were kept at temperatures from 15 to 17°C. On the day following their arrival the Δ of their hemolymph was measured. After three days the animals were transferred into several containers with water of various salinity and on the next day the Δ of the hemolymph was measured. After further three days the water was changed in order to remove developing bacteria and, finally, after six or seven days in water of a defined salinity the Δ of the hemolymph and of the external medium was examined.

Table I

The influence of the salinity of the external medium upon the osmotic concentration of the hemolymp in *Theodoxus fluviatilis* (L.)

Time of keeping the snails in a given medium	Δ_0	Δ_i	Δ_i mean	$\Delta_i - \Delta_0$
1 day in fresh water	0.02	0.18/0.22/0.26	0.22	0.20
1 day after being transferred into various salinities	0.02	0.22		
	0.10	0.23/0.23/0.23	0.23	0.13
	0.20	0.26/0.27/0.33/0.36	0.31	0.11
	0.34	0.38/0.43/0.45	0.42	0.08
	0.47	0.50/0.56/0.57	0.54	0.07
6 and 7 days after being transferred into various salinities	0.02	0.18/0.21/0.22/0.24	0.21	0.19
	0.10	0.23/0.23/0.25/0.26	0.24	0.14
	0.20	0.29/0.31/0.32/0.33/0.38	0.33	0.13
	0.34	0.37/0.41/0.42/0.45/0.46/ /0.47	0.43	0.09
	0.47	0.50/0.52/0.52/0.52/0.54/ /0.54/0.58	0.53	0.06

Δ_0 — freezing point depression of the external medium.
Δ_i — freezing point depression of the hemolymph.

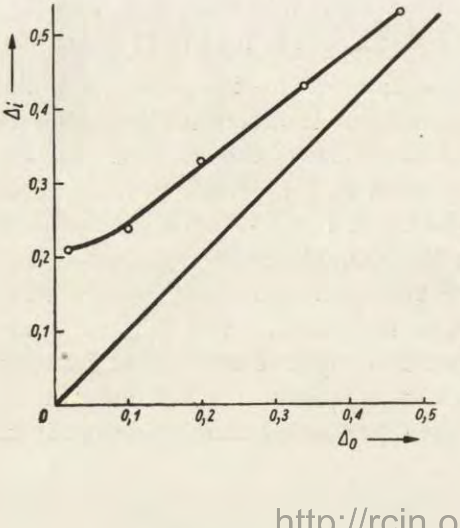

Fig. 4. Influence of the salinity of the external medium = Δ_0 on hemolymph concentration = Δ_i

The diagonal line corresponds to isotonic fluids. Each point marks the mean hemolymph concentration in animals kept for six or seven days in water of a given salinity.

The results are given in the Table and in Fig. 4. At the period of starvation of animals kept in fresh water the Δ of their hemolymph remains constant amounting to a mean of 0.22°C. A change in the concentration of the external medium causes a change in the osmotic concentration of the internal medium while the osmotic concentration of the hemolymph of snails kept for one day and for 6—7 days in the new conditions, remains unchanged.

An increased concentration of the water produces an increase in hemolymph concentration so that *T. fluviatilis* is hypertonic in the examined interval. On the other hand, the difference between hemolymph and water Δ diminishes according to the increase of salinity. If the water Δ equals 0.47°C this difference is three times smaller than in fresh water.

DISCUSSION

Theodoxus fluviatilis is very sensitive to the conditions in which it lives. Thus, for example, specimens kept for a longer period of time in an aquarium consume more oxygen than those examined immediately after having been brought in from their natural environment (Kreczko and Michejda 1956). Investigations on the distribution of aquatic snails in Finland have also shown that *T. fluviatilis* appears in the Baltic whereas it is lacking in fresh water basins situated near the Baltic coast. Segerstråle (1945) attributes this to the very low content of calcium in the fresh waters of Finland.

It may be that the differences between Lumbye's and my own results concerning the influence of the oxygen content in water upon respiration are due to some factors of the external medium that escape our control.

At any rate, the oxygen content of fresh water in which the snails were kept during investigations on the influence of salinity upon respiration was higher than the critical oxygen tension. The fact that specimens from fresh water do not breathe more intensively than specimens from saline water is therefore not due to the too low oxygen content in fresh water.

As shown in the Table and in Fig. 4 the transfer of snails from fresh water to water of Δ = 0.47° C produces a 2.5-fold increase of hemolymph concentration, the hemolymph remaining constantly hypertonic in relation to the medium. The new state of balance between the concentration of the external and internal medium is already established after 24 hours and even much sooner as preliminary measurements have shown. As the examined snails did not take any food they actively took ions from the surrounding water.

A similar reaction to changes in the concentration of the external medium is found in many animals such as *Mesidotea entomon* (Bogucki 1931), *Potamobius fluviatilis* (Schwabe 1933), *Nereis diversicolor* (Schlieper 1929).

On the other hand, the fresh-water snail *Viviparus fasciatus* keeps invariably its hemolymph concentration in water salinities from fresh to isotonic water ($\Delta = 0.21°C$) and in higher concentrations it shows a complete lack of osmoregulation (Obuchowicz 1958, 1959). A similarly stable hemolymph concentration maintains also the fresh-water mussel *Hyridella australis* living in water which is less saline than isotonic water (Hiscock 1953).

Reactions of that type to changes in the salinity of water as in *Viviparus fasciatus* may be regarded as a specialization of stenohaline freshwater animals since it is well known that an increased hemolymph concentration is harmful for such animals (Beadle and Cragg 1940). A second transfer of these animals to water with a higher salinity requires a developed mechanism for maintaining the low hemolymph concentration, as for example in sea-fish or the crustacean *Artemia salina* (Croghan 1958). For stenohaline fresh-water animals the capacity of maintaining a hypertonic internal medium is quite sufficient.

T. fluviatilis may stay in water of various salinity because its tissues, similar as in *Mytilus edulis* or *Nereis diversicolor*, tolerate changes in hemolymph concentration.

In specimens of *T. fluviatilis* living in saline water of $\Delta = 0.47°C$ the difference between the osmotic concentration of the external medium and the hemolymph is three times smaller than in those living in fresh water. Hence, also the osmotic activity is greater in fresh-water animals. However, respiration which supplies energy for osmoregulation is identical in fresh as well as saline water. Also snails from natural localities with various salinity show no differences in the consumption of oxygen (Lumbye 1958). In this respect *T. fluviatilis* resembles such animals as *Eriocheir sinensis* (Schwabe 1933), fresh-water and euryhaline coelenterates

(Palmhert 1933), *Nereis diversicolor* (Schlieper 1929, Beadle 1931) etc.

We may assume that the amount of oxygen corresponding to the consumption of energy for osmoregulation is very small in relation to the total oxygen consumption and is contained within limits of the error of measurement.

These results confirm the view based mainly on investigations of crustaceans that respiratory changes depending on the salinity of the medium are not due to a change in the magnitude of osmotic activity (Schlieper 1935, Krogh 1939, Gross 1957).

SUMMARY

1. *T. fluviatilis* is an euryhaline snail common in rivers and in the littoral zone of lakes and of the Baltic.

2. The critical oxygen tension in freshwater specimes amounts to about 50% of the oxygen content in water saturated with air. The author discusses the probable causes of differences between his own and Lumbye's (1958) results.

3. *T. fluviatilis* which is adapted to saline water with $\Delta = 0.47°C$ consumes the same amount of oxygen as in fresh water.

4. The freezing point depression of hemolymph in freshwater specimens is 0.22°C. A transfer of the snails to water with a higher salinity causes an increase in hemolymph concentration due to the active intake of ions from the water.

5. Changes in the magnitude of osmotic activity produce no perceptible changes in the intensity of respiration.

REFERENCES

BEADLE L. C. 1931 — The effect of salinity changes on the water content and respiration of marine invertebrates. J. Exp. Biol. *8*, 211.

BEADLE L. C. and CRAGG J. B. 1940 — Studies on adaptation to salinity in *Gammarus* sp. I. Regulation of blood and tissues and the problem of adaptation to fresh water. J. Exp. Biol. *17*, 153.

BIELAWSKI J. 1959 — A polarographic method of measuring the intensity of respiration in aquatic animals. Acta Biol. Exper. *19*, 41.

BOETTGER C. R. 1950 — Ein Beitrag zur Frage des Ertragens von Brackwasser durch Molluskenpopulationen. Hydrobiologia *2*, 360.

BOGUCKI M. 1931 — O regulowaniu ciśnienia osmotycznego hemolimfy równonogów morskich (*Mesidotea entomon* L.). Acta Biol. Exper. 7, 61.

CONKLIN R. E. and KROGH A. 1939 — A note on the osmotic behaviou of *Eriocheir* in concentrated and *Mytilus* in dilute sea water. Z. vergl. Physiol. *26,* 239.

CROGHAN P. C. 1958 r. — The osmotic and ionic regulation of *Artemia salina* (L.). J. Exp. Biol. *35,* 219.

CROGHAN P. C. 1958 — Ionic fluxes in *Artemia salina* (L.) J. Exp. Biol. *35,* 425.

FLEMISTER L. J. and FLEMISTER S. C. 1951 — Chloride ion regulation and oxygen consumption in the crab *Ocypode albicans* (Bosq.). Biol. Bull. *101,* 259.

FOX H. M., WINGFIELD C. A. and SIMMONDS B. G. 1937 — The oxygen consumption of ephemerid nymphs from flowing and from still waters in relation to the concentration of oxygen in the water. J. Exp. Biol. *14,* 210.

GILCHRIST B. 1956 — The oxygen consumption of *Artemia salina* (L. in different salinities. Hydrobiologia *8,* 54.

GROSS W. J. 1957 — An analysis of response to osmotic stress in selected decapod Crustacea. Biol. Bull. *112,* 43.

HISCOCK I. D. 1953 — Osmoregulation in Australian freshwater mussels (*Lamellibranchiata*). II. Respiration and its relation to osmoregulation in *Hyridella australis* (Lam.). Austr. J. Mar. Freshw. Res. *4,* 330.

HOPKINS H. S. 1949 — Metabolic Reactions of clams' tissues to change in salinity. I. Ciliary activity, narcotic and cyanide effects, and respiratory quotient. Physiol. Zool. *22,* 295.

JØRGENSEN C. B. and DALES R. P. 1957 — The regulation of volume and osmotic regulation in some nereid polychaetes. Physiol. Comp. Oecol. *4,* 357.

KRECZKO R. and MICHEJDA J. 1956 — Badania nad zapotrzebowaniem tlenowym niektórych ślimaków przodoskrzelnych. Spraw. Pozn. Tow. Przyj. Nauk. II i IV kwartał, 57.

KROGH A. 1939 — Osmotic regulation in aquatic animals. Cambridge, University Press.

LILLY S. J. 1955 — Osmoregulation and ionic regulation in *Hydra.* J. Exp. Biol. *32,* 423.

LOFTS B. 1956 — The effects of salinity changes on the respiratory rate of the prawn *Palaemonetes varians* (Leach). J. Exp. Biol. *33,* 730.

— 1956 — Relationship between blood concentration and environmental salinity in *Palaemonetes varians* (Leach). Nature. *178,* 1003.

LUMBYE J. 1958 — The oxygen consumption of *Theodoxus fluviatilis* (L.) and *Potamopyrgus jenkinsi* (Smith) in brakish and fresh water. Hydrobiologia *10,* 245.

MICHEJDA J. and OBUCHOWICZ L. 1956 — Mikrokryoskopowe metody oznaczania wartości osmotycznej. Zesz. Nauk. U. A. M. Biol. *1,* 147.

OBUCHOWICZ L. 1956 — Nowa modyfikacja aparatu Ramsaya do mikrokryoskopii. Zesz. Nauk. U. A. M. Biol. *1,* 137.

— 1958 — The influence of osmotic pressure of medium on oxygen consumption in the snail *Viviparus fasciatus* (O. F. Müll.) Streptoneura. Bull. Soc. d. Amis d. Sc. et Lettres de Poznań. Ser. B. Livr. *14,* 367.

OBUCHOWICZ L. 1959 — Wpływ stężenia osmotycznego środowiska na zapotrzebowanie tlenowe u *Viviparus fasciatus* (O. F. Müll.) *Prosobranchia*. Zesz. Nauk. U. A. M. *2*, 125.

PALMHERT H. W. 1933 — Beitrag zum Problem der Osmoregulation einiger Hydroidpolypen. Zool. Jahrb. Abt. allg. Zool. Physiol. *53*, 212.

PARRY G. 1953 — Osmotic and ionic regulation in the isopod crustacean *Ligia oceanica*. J. Exp. Biol. *30*, 567.

PETERS H. 1936 — Über den Einfluss des Salzgehaltes im Aussenmedium auf den Bau und die Funktion der Exkretionsorgane dekapoder Crustaceen (nach Untersuchungen an *Potamobius fluviatilis* und *Homarus vulgaris*). Z Morph. Ök. Tiere *30*, 355.

PIEH S. 1936 — Über die Beziehungen zwischen Atmung, Osmoregulation und Hydratation der Gewebe bei euryhalinen Meeresevertebraten. Zool. Jahrb. Abt. allg. Zool. Physiol. *56*, 129.

PROSSER C. L., BISHOP D. W., BROWN F. A., JAHN T. L. and WULFF V. J. 1952 — Comparative animal physiology. W. B. Saunders Co. Philadelphia.

RAMSAY J. A. 1949 — A new method of freezing-point determination for small quantities. J. Exp. Biol. *26*, 57.

SCHLIEPER C. 1929 — Über die Einwirkung niederer Salzkonzentrationen auf marine Organismen. Z. vergl. Physiol. *9*, 478.

— 1930 — Die Osmoregulation wasserlebender Tiere. Biol. Rev. *5*, 309.

— 1935 — Neuere Ergebnisse und Probleme aus dem Gebiet der Osmoregulation wasserlebender Tiere. Biol. Rev. *10*, 334.

— 1955 — Über die physiologischen Wirkungen des Brackwassers. Kieler Meresf. *11*, 22.

SCHWABE E. 1933 — Über die Osmoregulation verschiedener Krebse (Malacostracen). Z. verlg. Physiol. *19*, 183.

SEGERSTRÅLE S. G. 1954 — Über die Verbreitung der Süsswasserschnecke *Theodoxus* (*Neritina*) *fluviatilis* (L.) in Finnland. Societas Scient. Fennica. Commentationes Biol. *9*, 1.

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STUDIES ON HIGHER NERVOUS ACTIVITY IN CHICKENS
I. THE EFFECT OF AGE ON CONDITIONED ALIMENTARY EXCITATORY AND INHIBITORY REFLEXES *

K. ZIELIŃSKI

Department of Biology, Nencki Institute of Experimental Biology, Warsaw

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Most of the research work concerned with the development of higher nervous activity in ontogeny was concerned with the possibility of establishment of permanent conditioned reflexes and differentiation in the first weeks of life. The studies were made chiefly on mammals: rabbits, guinea pigs, dogs, monkeys, or even children. Attempts to establish conditioned motor reflexes in chickens were made by Dietinowa and Krżyszkowski (1925) and Gorszelewa (1936). According to the last author, conditioned alimentary motor reflexes to acoustic stimuli may be established in most 10-day old chickens, but are very unstable and become extinguished when reinforcement fails only a few times. In older chickens, at the age of 47 days, differentiation also was elaborated.

Changes in higher nervous activity in older chickens were investigated by Tuge, Shima and Koga (1956) who studied in chickens 27 to 175 days old the rate at which excitatory and inhibitory conditioned alimentary motor reflexes are established, and the course of transformation of conditioned reflexes. They noted this rate varied considerably between animals as regards differentiation and transformation of reflexes, and found that age had no effect on excitatory conditioned reflex elaboration velocity. The results of this work showed that positive reactions in intertrial

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* This work was performed in Pavlov Institute of Physiology, USSR Academy of Sciences, Pavlovo, Laboratory of Physiology of Breeding Animals.

intervals were extinguished in older chickens more rapidly than in younger ones.

The present paper reports the results of studies on differences between chickens of different age groups in conditioned excitatory and inhibitory alimentary motor reflexes.

MATERIAL AND METHODS

The studies were conducted in two series involving a total of 32 chickens divided in two age groups, one 60 and the other 120-day old. Either group comprised representatives of White Leghorn and Piervomajska breeds, eight of each, pullets and cockerels in equal proportions. All chickens passed through experiments strictly alike, which lasted 2 to 3 months.

All the chickens hatched on the same day and were reared in exactly similar conditions. They all developed normally and differed from control animals neither in weight nor in velocity of maturing, but exceeded weight standards accepted in zootechny.

The experiments were conducted after a method of conditioned alimentary motor reflexes developed by Baru (1953) for adult hens, in a sound-proof camera. A lever inside the camera served to register the animal's conditioned responses, and food in bowls was supplied and withdrawn through a suitable aperture. Optical stimuli consisted of red or green light of equal intensity. The experimental animals were given during experiments a moist mixture of various meals, supplemented with potatoes and meat as reinforcement.

When the chickens became familiar with the experimental situation, a conditioned alimentary motor reflex was elaborated in them: they received food only when they pecked the lever inside the camera. Chickens used to food given in bowls received empty bowls, whereupon the given chick searched the camera for food pecking at the empty bowl, the floor, and so forth. When it hit the lever, food in a bowl was supplied without delay. To make the task easier, bits of food were left on the lever. In these circumstances, the conditioned reflex developed very quickly, sometimes after three to five associations. After this reflex was consolidated, the optical stimuli were introduced. Pecking of the lever was reinforced by supplying food only when it coincided with the optical stimulus: red or green light.

When the trained motor reaction coincided with the excitatory conditioned stimulus, it was immediately reinforced by supplying food. The animal was allowed to eat 30 sec., and the bowl of food was again withdrawn. The normal duration of the conditioned stimulus was 10 sec. If there was no conditioned reaction, it was prolonged to not more than 20 sec. and in absence of reaction switched off without being reinforced with food.

The conditioned stimuli — red and green light — were switched on alternatively, each four times in one experimental session at 1.5 min. intervals.

When the excitatory conditioned responses to both the conditioned stimuli were firmly established, one of these — the green light stimulus — was subjected to chronic extinction beginning with the 41-st trial. It was applied

Table I

Calculation of magnitude of conditioned motor reflexes. Excerpt from the protocol of experiment No. 20,
18 August, 1956, 205 p. m.
Chicken No. 7741, Leghorn, ♀

Time Since 2 05 p. m.	Trial No. (+)	(−)	Conditioned stimulus			Reinforcement	Magnitude of condit. response	
			colour	duration in sec.	latency in sec.		excitatory	inhibitory
0′30″	65		red	11″	11″	+	19	
2′00″		25	green	10″	4″	—		26
3′30″	66		red	10″	4″	+	26	
5′00″		26	green	10″	—	—		0
6′30″	67		red	20″	—	—	0	
8′00″		27	green	10″	—	—		0
9′30″	68		red	10″	3″	+	27	
11′00″		28	green	10″	—	—		0
						Average	18.0	6.5

in each experimental session four times in its usual succession without being reinforced with food.

The main indices under study were:

1) presence or absence of the conditioned alimentary motor reflex;
2) latency period of the conditioned reflex; and
3) the number of positive reactions in intertrial intervals.

The magnitude of the conditioned motor response was defined by a relative figure, depending on presence (or absence) of the reaction and its latency period. The shorter the latency periods and the fewer the failures of the response to the conditioned stimulus, the larger was this figure. Such methods of determining the magnitude of conditioned motor reflexes were used by other authors (Fiodorow, 1951a, 1951b, Ponomarienko, 1958, and others). The magnitude of the conditioned motor reflex was calculated by subtracting the latency period from 30 sec. (i. e., from the maximum duration of the conditioned stimulus in experiments of this type). When there was no reaction, the magnitude of the reflex was zero.

Examples of such calculations are given in Table I.

The results obtained were processed with the aid of statistical methods, analysis of variance in the first place (Romanowski, 1951).

EXPERIMENTAL PART

As is known, the magnitude of excitatory conditioned reflexes changes considerably with increasing number of trial of the positive conditioned stimulus. It was therefore compared for chickens of different age groups separately at each experimental stage. The first stage involved exclusively excitatory stimuli. At the second stage, one of the stimuli — green light — was transformed into an inhibitory stimulus. The third stage covered the period after termination of training of excitatory and inhibitory conditioned reflexes, when their magnitude varied relatively slightly from day to day.

The magnitude of excitatory conditioned reflexes for the specific experimental stages are recorded in Table II, calculated as averages from several days. For the first and second stages, six experiments per animal were taken into account for each, and for the third stage, 20—30 experiments.

Table II

Average magnitudes of the excitatory conditioned motor reflex at different stages of the experiments

Stage of experiments	Series I (2-4 months)	Series II (4-6 months)	P
Both conditioned stimuli positive	16.4	20.7	<0.01
Extinction of response to the other conditioned stimulus begins	18.4	21.7	<0.05
Excitatory and inhibitory conditioned reflexes consolidated	23.3	24.8	<0.01

It follows from the data recorded in Table II that the magnitude of excitatory conditioned reflexes were in later experimental stages greater than in the first one. This shows that with successive experiments positive reactions to the excitatory conditioned stimulus became more regular and their latency periods shorter. The increasing of the magnitude of the excitatory reflex was more pronounced in the younger age group.

The data recorded in Table II indicate conspicuous differences in excitatory conditioned reflex magnitudes between chickens of different age groups. Throughout the three stages of experiments, the value of the index was higher in the older age group, and the difference statistically significant.

The difference in question was most pronounced at the first stage of experiments. This may suggest that they resulted from better adaptations of older chickens to the experimental situation, that is to say, from differences between age groups in the degree of extinction of orientation and defensive reactions to the experimental situation and consolidation of the alimentary motor reflex of pecking at the lever.

However, this surmise is invalid since the preliminary training was continued irrespective of age group till the same criterion was attained, i. e., till the lever-pecking reflex occurred at average alimentary excitability immediately when the food bowl was withdrawn. This criterion was tested during four consecutive experimental sessions. To attain it, the younger chickens proved to require an average of 79.7 min. of training in the camera, whereas the older needed only 63.5 min. (the difference is statistically significant).

Further evidence refuting the surmise referred to is supplied by the statistical significance of the difference in question at all experimental stages.

Lastly, differences in the degree of adaptation to the experimental situation would undoubtedly have a considerable influence on the inhibitory conditioned reflexes.

The inhibitory stimulus applied in the experiments described was originally an excitatory stimulus, which was reinforced with food. The criterion of definitive consolidation of the inhibitory conditioned reflex was correct performance of all excitatory and inhibitory reflexes in the course of two consecutive experimental sessions. When this criterion was attained, positive responses to the inhibitory conditioned stimulus occurred only on changes in the experimental procedure, and in the usual experiments only sporadically.

The results compiled in Table III show that the number of trials needed to attain the criterion of inhibitory reflex consolidation was the same for chickens of either age group. There also is no statistically significant difference in the magnitude of the inhibitory conditioned reflex between the age groups. The magnitude recorded refer to the experimental stage which followed after consolidation of both the excitatory and the inhibitory conditioned reflexes.

As is known, positive reaction in intertrial intervals are elicited by the experimental situation, which is connected by the

Table III

Characteristics of inhibitory conditioned reflexes in chickens of different age groups

Index		Series I (2 — 4 months)	Series II (4 — 6 months)	P
Number of trials of the inhibitory conditioned stimulus till definitive consolidation of the inhibitory reflex		78	77	Not signif.
Magnitude of the inhibitory conditioned reflex		4.3	3.8	Not signif.
Number of positive reactions in intertrial intervals		14.9	15.1	Not signif.
Latency period when inhibitory stimulus prolonged to 90 sec.	First trial	66.7 sec.	61.5 sec.	Not signif.
	Second trial	67.9 sec.	67.9 sec.	Not signif.

chicks with feeding. This significance of the experimental situation is gradually extinguished in the course of experiments, and the conditioned stimulus becomes differentiated from its background. The mechanism of that extinction is analogical to that of differentiation elaboration. It will be seen from the data recorded in Table III that number of positive reactions in intertrial intervals, also failed to reveal any differences between the age groups.

During the experiments in question, positive reactions in intertrial intervals were fairly numerous even after consolidation of the inhibitory conditioned reaction to green light. This was so owing to prior strong consolidation of the alimentary reflex of lever-pecking, which — in agreement with the principle of the primacy of first training (Konorski, Szwejkowska, 1952) — could not subsequently be fully extinguished in intertrial intervals.

Experiments in which the duration of the inhibitory conditioned stimulus was extended from 10 to 90 sec. were carried out twice and separated by several days of the usual procedure. Duration-extension concerned invariably the second stimulus of the given colour (green) in the experimental session involved.

Positive reactions with the prolonged inhibitory conditioned stimulus on were displayed in 14 cases by young and in 16 cases by older chickens. When their average latencies were calculated for either age group separately — assuming that when no reaction was recorded its latency period was equal to the duration of the prolonged conditioned inhibitory stimulus (90 sec.) — comparison showed them to be almost exactly alike, as is shown in Table III.

Tests with prolonged conditioned inhibitory stimuli were followed by irregularities in the conditioned-reflex activity of the chickens: positive response to the excitatory conditioned stimulus was occasionally absent, but was sometimes elicited by the inhibitory stimulus. Such irregularities were recorded in several experimental sessions in almost all the animals, but their intensity depended on the age group. This is shown in Table IV.

Table IV

The effect of age on changes in conditioned-reflex activities induced by extension of the duration of the inhibitory conditioned stimulus

Indices	Series I (2-4 months)	Series II (4-6 months)	P
Number of chickens	16	15	—
Number of negative reactions to the excitatory stimulus	48	5	<0.01
Number of positive reactions to the inhibitory stimulus	92	69	Not signif.

In the younger age group wrong reactions were more numerous. The difference in the number of positive reactions to the inhibitory conditioned stimulus was, however, slight and statistically not significant, whereas that in the number negative reactions to the positive stimulus was very conspicuous and statistically highly significant.

Thus, while there is a distinct difference between age groups in the magnitude of excitatory conditioned motor reflexes, no such differences exist in inhibitory conditioned reflexes.

The question suggests itself, does not the former result from differences in alimentary excitability? The higher magnitudes of the excitatory conditioned reflexes recorded for the older age group might have had their source in higher alimentary excitability.

If this surmise were right, there should be distinct differences in inhibitory conditioned reflexes, since increase of alimentary excitability is conducive to positive reactions to inhibitory conditioned stimuli, while it has little effect on established excitatory conditioned reflexes. In the experiments in question, such effects of increased alimentary excitability would have been very strong, since the inhibitory conditioned stimulus used was originally excitatory in nature. And indeed, relevant changes were noted when the alimentary excitability of the chickens was increased by 24-hours fasting.

The results of experiments made with fasting chicks are compiled in Table V.

Table V

Changes in conditioned-reflex activities of chickens in result of an increase in alimentary excitability

Age group	Feeding	Magnitude of conditioned reflexes		Number of positive reactions in intertrial intervals
		excitatory	inhibitory	
Series I (2-4 months)	normal	24.3	4.0	21.9
	fasting	26.3	15.8	77.4
	P	< 0.05	< 0.01	< 0.01
Series II (4-6 months)	normal	25.2	2.4	9.5
	fasting	26.1	11.1	40.4
	P	not signif.	< 0.01	< 0.01

As will be seen from the data recorded, an increase in alimentary excitability is followed — irrespective of age — by an increase in the magnitude of excitatory and inhibitory conditioned stimuli and in the number of positive reactions in intertrial intervals. The last two changes are very conspicuous and statistically significant in either age group, whereas the first one is so only in the younger age group.

It is worth noting that increase in alimentary excitability had in the younger age group a more pronounced effect on the magnitude of the excitatory conditioned reflex. Similar results were obtained in experiments in which alimentary excitability was diminished.

In these experiments the chickens were satiated with their favourite food, of which they ate an amount equal in calorific value to the daily requirement of chickens of their age. The results are compiled in Table VI.

Table VI

Changes in the conditioned-reflex activities of chickens following a diminution of alimentary excitability

Age group	Feeding	Magnitude of conditioned reflex		Number of positive reactions in intertrial intervals
		excitatory	inhibitory	
Series I (2-4 months)	normal	24.0	2.2	10.8
	satiated	16.0	0.4	5.9
	P	< 0.01	< 0.05	not signif.
Series II (4-6 months)	normal	24.8	1.1	9.5
	satiated	20.3	1.8	7.0
	P	< 0.05	not signif.	not signif.

The results recorded in Table VI indicate considerable diminution of alimentary excitability to have had a relatively slight effect on the magnitude of the inhibitory conditioned reflex and number of positive reactions in intetrial intervals. Changes in the magnitude of the excitatory conditioned reflex, however, were statistically significant in both age groups, in the younger one much more so than in the older. Thus, whether on reduction or increase of alimentary excitability, changes on the positive conditioned reflex were more pronounced in the younger age group.

It is generally known that even when constancy of feeding and rearing conditions is most strictly observed, experimental animals show some vacillations in the magnitude of any, alimentary or defensive, conditioned reflexes, the range of the variability being determined by the animal's individual features. It appeared worthwhile to explore whether age is not a factor affecting variability of excitatory conditioned reflex magnitude. The coefficient of variation of this magnitude was calculated for particular animals from 20—30 usual experimental sessions, after establishment of excitatory and inhibitory conditioned reflexes. The results obtained are recorded in Table VII.

Table VII

The effect of age on the variability of the magnitude of the excitatory conditioned reflex in constant experimental conditions

Magnitude of the coefficient of variation v (%)	Number of cases in age groups	
	younger	older
0.1 — 5.0	—	3
5.1 — 10.0	3	8
10.1 — 15.0	4	2
15.1 — 20.0	5	2
20.1 — 25.0	2	—
25.1 — 30.0	2	—

The results compiled in Table VII are clearly evidence of variability of excitatory conditioned reflex magnitude being higher in younger chickens. Thus — whether under conditions of considerably modified alimentary excitability or under usual conditions with optimum alimentary excitability — the magnitude of the excitatory conditioned reflex was in the younger age group subject to more pronounced changes.

DISCUSSION

The investigations have demonstrated that chickens of different age groups differ in reactions to excitatory conditioned stimuli but not in reactions to inhibitory stimuli.

This result agrees but partly with those obtained by other authors from mammals. Majorow (1929) found excitatory as well as inhibitory conditioned reflexes to be elaborated and consolidated in older puppies more rapidly than in younger ones. Similar results were obtained by Obrazcowa (1952) with rabbits.

It should be observed that in either case the investigations concerned animals less than two months old. The data obtained by Czinka (1953), on the other hand, indicate that distinct differences in the rates of differentiation elaboration and acute extinction of excitatory conditioned stimuli are observed only between puppies not more than three to five months old, whereas between older puppies, the difference — if any — are insignificant. The rate at which excitatory conditioned stimuli elaborated increased with age, the oldest age group being one of 12 months.

In the experiments now discussed, the chickens were used when two months old. It may be surmised that if younger chickens were also investigated, evidence of the effect of age on inhibitory conditioned reflexes could have been obtained. This is all the more likely since hens are birds which develop very rapidly and attain sexual maturity even after roughly 150 days of postembryonal life.

Changes induced by age in excitatory conditioned stimuli have not infrequently been described in literature. Czinka (1953) found that not only did defensive conditioned reflexes develop more rapidly in older puppies, but even their latency periods were in older animals shorter than in younger ones. This author also observes that variability of the latency period of the reflex was greater in younger puppies.

Kolesnikow (1953) claims that variations in the magnitude of conditioned alimentary salivary reflexes and departures from "the law of the force of stimuli" are frequently observed in young puppies.

The results obtained by Czinka (1953) and Kolesnikow (1953) relative to greater constancy of excitatory conditioned reflexes in older animals, agree with the results recorded in this study. Whether at optimum or modified alimentary excitability, the constancy of excitatory conditioned reflexes increased with the age of the chicks, and the latency periods became shorter, which led to an increase in the magnitude of the conditioned motor reflex.

CONCLUSIONS

1. Studies on chickens in two separate age groups showed age to affect the magnitude of conditioned excitatory alimentary motor reflex. Higher magnitudes of excitatory conditioned reflexes indicated

in the older age group fewer failures of positive response to excitatory conditioned stimuli and shorter latencies of such responses.

2. The age of the chickens had no effect on the magnitudes of inhibitory conditioned reflexes. Disinhibition of the negative reaction to the inhibitory conditioned stimulus was of like frequency in either age group. When the duration of the inhibitory conditioned stimulus was extended from 10 to 90 sec., differentiation disinhibition was almost equally frequent in either age group, and the latency period of positive reactions to the inhibitory conditioned stimulus also was more or less the same in either group.

3. Tests with inhibitory conditioned stimuli of extended duration were followed by irregularities in conditioned-reflex activity, which became manifest through differentiation disinhibition and failures of positive conditioned responses to excitatory conditioned stimuli. Disinhibition of differentiation was very much alike in either age group, whereas failures of positive response to excitatory conditioned stimuli were in younger chickens ten times as frequent as in older ones.

4. Changes in either direction in alimentary excitability had in the younger age group a more powerful effect on the magnitude of the excitatory conditioned reflex. Similarly, under conditions of optimum alimentary excitability and constant experimental procedure, higher coefficients of variation of the magnitude of the excitatory conditioned reflex were noted in the younger age group.

5. The differences between age groups in the magnitude of excitatory conditioned reflexes noted at constant experimental procedure and optimum alimentary excitability could not have been elicited by differences in alimentary excitability.

REFERENCES

BARU A. W. 1953 — Metodika issledowanija dwigatielnych piszczewych usłownych refleksow u ptic. Trudy Inst. Fizjoł. im. Pawlowa, *2*, 449.

CZINKA I. I. 1953 — Rozwitije korkowowo wnutrienniego tormożenia w ontogenezie u szczeniat. Trudy Inst. Fizjoł. im. Pawłowa, *2*, 86.

DIETINOWA Ł. W., KRŻYSZKOWSKI K. N. 1925 — K woprosu o diejatielnosti mozgowych połuszarij u ptic. Zap. Leningr. Sielskochoz. Inst. *2*, 310.

FIODOROW WIKT. K. 1951a — K woprosu o trenirowkie podwiżnosti nerwnych processow u myszej pri mnogokratnom peredielywanii bary refleksow. Fizjoł. Żurnał SSSR. *37*(3), 283.

FIODOROW WIKT. K. 1951b — Starczeskije izmienienia podwiżnosti nerwnych processow. Fizjoł. Żurnał SSSR. *37*(4), 446.

GORSZELEWA Ł. S. 1936 — K srawnitielno-fizjołogiczeskoj charaktieristikie wyższej nerwnoj diejatielnosti ptic w swiazi s wlijaniem wozrasta. Arch. Bioł. Nauk. *42*, (1—2), 117.

KOLESNIKOW M. S. 1953 — O rozwitii tipołogiczeskich swojstw wyższej nerwnoj diejatielnosti w ontogenezie. Trudy Inst. Fizjoł. im. Pawłowa. *2*, 173.

KONORSKI J., SZWEJKOWSKA G. 1952. — Chronic extinction and restoration of conditioned reflexes. IV. The dependence of the course of extinction and restoration of conditioned reflexes on the "history" of the conditioned stimulus. (The principle of the primacy of first training). Acta Biol. Exper. *16*, 95.

MAJOROW F. P. 1929 — Usłownyje refleksy u szczeniat rozlicznych wozrostow. Arch. Bioł. Nauk. *29*(3), 341.

OBRAZCOWA G. A. 1952 — Wozniknowienije i rozwitie usłownoreflekternoj diejatielnosti w ontogenezie u krolika. Trudy Inst. Fizjoł. im. Pawłowa. *1*.

PONOMARIENKO W. W. 1958 — Izuczenie swojstw wysszej nervnoj diejatielnosti u kur raznych porod (Autoref. dysertacji). Leningrad.

ROMANOWSKI W. 1951 — Zastosowanie statystyki matematycznej w doświadczalnictwie. Warszawa.

TUGE H., SHIMA I., KOGA K. — 1956 — Studies of the conditioned reflex in the lower vertebrates. III. Motor-food conditioned reflexes in domestic fowls. Sci. Rep. Tohoku Univ., Biol. *22*(2), 115.

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STUDIES ON HIGHER NERVOUS ACTIVITY IN CHICKENS. II. THE EFFECT OF SEX ON CONDITIONED EXCITATORY AND INHIBITORY ALIMENTARY REFLEXES *

K. ZIELIŃSKI

Department of Biology, Nencki Institute of Experimental Biology, Warsaw

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Usually, no major importance is attached to the sex of experimental animals in studies on higher nervous activity. Fiodorow (1953), however, demonstrated that with some strains of mice, transformation of conditioned stimuli is more rapid in males than in females. His experiments involved conditioned alimentary motor reflexes.

This paper concerns the differences noted between chickens of different sexes in studies on the effect of age on conditioned excitatory and inhibitory alimentary reflexes.

MATERIAL AND METHODS

The experiments were run in two series. The first concerned 60-day-old, and the other 120-day-old chickens. In each series there were 4 cockerels and 4 pullets of White Leghorn and Pierwomajska breeds. Il all, 32 chickens were involved, but only 31 completed the whole course of experiments. The chickens of either age group were submitted to exactly similar courses of experiments lasting 2—3 months.

All the chickens were reared in exactly similar conditions, but the sexes were separated.

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* This work was performed in Pavlov Institute of Physiology, USSR Academy of Sciences, Pavlovo, Laboratory of Physiology of Breeding Animals.

The experiments were run in a sound proof camera. Immediately when the animals became accustomed to the experimental situation, they were trained to develop a conditioned motor reflex: a chicken received food only when it pecked a lever. This reflex was consolidated to such a degree that when the food bowl was withdrawn, an animal with normal alimentary excitability carried out almost instantaneously the movement it had learned. Subsequently, optical stimuli were introduced (red and green lights of identical intensity) and the conditioned motor reflex was reinforced with food only when it coincided with the optical stimuli, which were applied alternately at 90-sec. intervals, each four times during one experimental session.

When the two optical stimuli had each been used 40 times with the reinforcement, one of them — the green light — ceased to be reinforced with food and was subjected to chronic extinction.

The magnitude of the conditioned alimentary motor reflex was defined by a relative figure, determined by both the presence (or absence) of reaction and its latent period. The shorter the latency periods and the less frequent the absence of response to the conditioned stimulus, the larger was that figure. The calculation of the magnitude of the conditioned alimentary reflex, and a detailed description of the methods used are given in the preceding paper of this series.

EXPERIMENTAL PART

An analysis was made of three main indices which characterized the conditioned reflex activity of the chicken after establishment of excitatory and inhibitory conditioned reflexes, i. e., the magnitude of the excitatory conditioned reflex, the magnitude of the inhibitory conditioned reflex, and the number of positive reactions in intertrial intervals. The figures given in Tables I — III are averages from 20—30 experiments carried out with each of the experimental animals under strictly constant conditions.

The results compiled in Table I reveal no differences between sexes in the magnitude of the conditioned excitatory reflex. The differences due to age, were described in the preceding paper of this series.

Unlike the age, the sex of the chickens had a significant effect on the last two indices here discussed. The greater magnitude of the inhibitory conditioned reflex in pullets (Table II) shows that disinhibition of the negative response to inhibitory conditioned stimuli was here more frequent than in cockerels.

Coincidence between the results recorded in Tables II and III is due to the mechanism of extinction of response to the experiment

Table I

The magnitude of the conditioned excitatory motor reflex after establishment of excitatory and inhibitory conditioned reflexes

Age group	Breed	Pullets	Cockerels
Series I (2—4 months)	Leghorn	24.4	22.5
	Pierwomajska	23.0	23.6
Series II (4—6 months)	Leghorn	24.1	25.4
	Pierwomajska	24.9	24.2

Analysis of variance

Source of variation	DF	MS	F	P
Age: younger vs. older	1	12.251	10.58	<0.01
Breed: Leghorn vs. Pierwomajska	1	0.080	<1	Not signif.
Sex: pullets vs. cockerels	1	0.361	<1	Not signif.
Interactions				
Age vs. breed vs. sex	1	9.231	7.97	<0.01
All other interactions	3	0.529	<1	Not signif.
Error	23	1.158		

Table II

The magnitude of the conditioned inhibitory motor reflex after establishment of conditioned excitatory and inhibitory reflexes

Age group	Breed	Pullets	Cockerels
Series I (2—4 months)	Leghorn	5.1	2.4
	Pierwomajska	5.0	4.6
Series II (4—6 months)	Leghorn	6.1	2.6
	Pierwomajska	4.7	2.6

Analysis of variance

Source of variation	DF	MS	F	P
Age: younger vs. older	1	0.279	<1	Not signif.
Breed: Leghorn vs. Pierwomajska	1	0.058	<1	Not signif.
Sex: pullets vs. cockerels	1	35.029	12.17	< 0.01
All interactions	4	4.445	1.54	Not signif.
Error	23	2.879		

Table III

The number of positive reactions in intertrial intervals after establishment of conditioned excitatory and inhibitory reflexes

Age group	Breed	Pullets	Cockerels
Series I (2—4 months)	Leghorn	17.0	7.4
	Pierwomajska	21.1	14.1
Series II (4—6 months)	Leghorn	20.7	7.6
	Pierwomajska	17.7	12.3

Analysis of variance

Source of variation	DF	MS	F	P
Age: younger vs. older	1	0.513	<1	Not signif.
Breed: Leghorn vs. Pierwomajska	1	62.609	<1	Not signif.
Sex: pullets vs. cockerels	1	579.941	5.47	<0.05
All interactions	4	30.537	<1	Not signif.
Error	23	105.960		

situation — which response is the cause of positive reactions in intertrial intervals — being analogical to the differentiation mechanism.

After establishment of excitatory and inhibitory conditioned reflexes, a test was carried out which was designed to explore the persistence of negative reaction to inhibitory conditioned stimuli. In these tests, which were carried out twice — i. e. in two different sessions separated by several days of standard procedure — the second inhibitory conditioned stimulus, i. e., the fourth conditioned stimulus applied in a given experimental session, was prolonged from 10 to 90 seconds. In most cases, this extension of the duration of the inhibitory conditioned stimulus caused disinhibition of the conditioned inhibitory reflex, and a positive response was recorded. When the average latency of that positive response is calculated — assuming that in absence of the response its latency is equal to the duration of the inhibitory conditioned stimulus — the values recorded in Table IV are obtained. Since age had no perceptible effect on the results of the test, as was shown by especial analysis, the variability possibly due to age was included in the variability wihin the group, i. e., in the experimental error, when the results recorded in Table IV were analyzed statistically.

Table IV

Latency of the positive response on extension of the duration of the conditioned inhibitory stimulus to 90 sec.

Age group	Breed	Pullets		Cockerels	
		Test 1 sec.	Test 2 sec.	Test 1 sec.	Test 2 sec.
Series I (2—4 months)	Leghorn	63.5	46.8	68.3	82.8
	Pierwomajska	74.3	56.3	62.0	85.8
Series II (4—6 months)	Leghorn	73.0	63.3	64.0	66.7
	Pierwomajska	66.0	49.0	45.3	90.0

Analysis of variance

Source of variation	DF	MS	F	P
Test No: 1st vs. 2nd	1	199.515	<1	Not signif.
Breed: Leghorn vs. Pierwomajska	1	0.015	<1	Not signif.
Sex: pullets vs. cockerels	1	1323.140	1.64	Not signif.
Interactions				
Sex vs. test No	1	5568.892	6.89	<0.05
All other interactions	3	440.557	<1	Not signif.
Error	54	808.249		

It follows from the data recorded in Table IV that with pullets latency of the positive reaction was in the second test shorter than in the first, whereas the reverse holds true for cockerels. This difference is statistically significant, as is demonstrated by the statistical significance of the interaction of sex vs. number of test.

Analogical results were obtained from a comparison of the numbers of disinhibitions: with pullets, there were in the first test six cases of disinhibition of the conditioned inhibitory reaction, and in the second test, twelve, whereas with cockerels the corresponding figures were nine for the first test, and three for the second.

All the results referred to show that persistence of negative reaction to inhibitory conditioned stimuli is in pullets smaller than in cockerels.

In the preceding paper of this series, the evidence was that the magnitude of the response to excitatory conditioned stimuli was in older chickens greater than in younger ones, whereas differences between the age groups in responses to inhibitory conditioned stimuli were virtually nil. It may be supposed that — to use Pavlovian terminology — the balance between the force of the excitation process and that of the inhibition process will be shifted in older pullets in favour of the former. In other words, since the magnitude of the response to excitatory conditioned stimuli increases in chickens with age, inferiority of inhibitory reactions will be in older pullets more pronounced than in younger ones.

Some data obtained from the experiments referred to may corroborate this surmise.

Let us consider the progress of extinction of positive responses in intertrial intervals in the initial stage of the experiments. The results are recorded in Table V.

Table V

The number of positive responses in intertrial intervals when both conditioned stimuli were positive

Age group	Breed	Pullets	Cockerels
Series I (2—4 months)	Leghorn	46.5	40.3
	Pierwomajska	47.7	68.0
Series II (4—6 months)	Leghorn	69.7	49.5
	Pierwomajska	80.9	69.4

Analysis of variance

Source of variation	DF	MS	F	P
Age: younger vs. older	1	2249.525	9.69	< 0.01
Breed: Leghorn vs. Pierwomajska	1	1792.505	7.72	< 0.05
Sex: pullets vs. Cockerels	1	154.443	< 1	Not signif.
Interactions				
Age vs. sex	1	1047.673	4.51	< 0.05
All other interactions	3	260.916	1.12	Not signif.
Error	24	232.253		

It follows from Table V that positive responses in intertrial intervals are more frequent in older chickens at this stage of experiments. This difference between age groups is with cockerels slight, but very pronounced with pullets. It is worth noting that interaction of age vs. sex proved statistically significant. In other words, the effect of age on the number of positive reactions differed between the sexes and was stronger in pullets than in cockerels, the difference being statistically significant.

It needs to be observed that at this stage of the experiments similar differences between the sexes were noted in the magnitude of conditioned excitatory motor reflexes. Relevant data are compiled in Table VI.

Table VI

The magnitude of excitatory conditioned reflexes when both conditioned stimuli were positive

Age group	Breed	Pullets	Cockerels
Series I (2—4 months)	Leghorn	16.7	11.8
	Pierwomajska	13.9	23.3
Series II (4—6 months)	Leghorn	22.0	18.7
	Pierwomajska	22.4	20.0

Analysis of variance

Source of variation	DF	MS	F	P
Age: younger vs. older	1	149.213	12.04	<0.01
Breed: Leghorn vs. Pierwomajska	1	55.388	4.47	<0.05
Sex: pullets vs. cockerels	1	0.813	<1	Not signif.
Interactions				
Age vs. sex	1	57.765	4.66	<0.05
Breed vs. sex	1	113.628	9.17	<0.01
All other interactions	2	2.924	<1	Not signif.
Error	24	12.390		

The data recorded in Table VI show that at this stage of the experiments, the magnitude of the excitatory conditioned reflex increased with age, and more so in pullets than in cockerels, which is strikingly illustrated by the statistical significance of the interaction of age versus sex.

The differences — shown in Tables V and VI — between breeds call for separate discussion and will not be considered in this paper.

Comparison of the results recorded in Tables V and VI confirms the earlier supposition. The increase of the magnitude of excitatory conditioned reflexes with age was more conspicuous in pullets, but was attended by a marked increase of positive responses in intertrial intervals.

A like phenomenon, though less pronounced, became manifest in the course of extinction of the positive significance of one of the conditioned stimuli. Also at this stage of the experiments, age had a significant effect on both the magnitude of excitatory conditioned reflexes and number of positive responses between stimuli. Either of the two indices considered increases with age, more so in pullets than in cockerels, but the difference between the sexes is not statistically significant.

As excitatory and inhibitory conditioned reflexes become established, differences between the sexes in the magnitude of excitatory conditioned reflexes, disappear, whereas differences in the number of positive responses in intertrial intervals continue and attain the values recorded in Table III.

In exploring these differences between the sexes, much useful information is supplied by the control experiments in which the alimentary excitability of the animals was changed. Hence, in Table VII are recorded the magnitudes of the conditioned excitatory reflexes in various groups of chicken in relation to changes in alimentary excitability.

Table VII

The magnitude of the conditioned excitatory reflex in relation to changes in alimentary excitability

Age group	Breed	Pullets	Cockerels
A. Alimentary excitability diminished			
Series I (2—4 months)	Leghorn	17.4	13.7
	Pierwomajska	15.8	17.0
Series II (4—6 months)	Leghorn	22.0	12.7
	Pierwomajska	24.8	20.5
B. Alimentary excitability increased			
Series I (2—4 months)	Leghorn	26.6	26.9
	Pierwomajska	26.3	25.6
Series II (4—6 months)	Leghorn	27.1	26.7
	Pierwomajska	26.3	24.8

The data in Table VII show that decrease of alimentary excitability diminished the magnitude of the excitatory conditioned reflex, in comparison to the data recorded in experiments carried out under the ordinary conditions (Table I). Diminution of that magnitude was least pronounced in the pullets of the older age group.

Increase of alimentary excitability raised the magnitude of the excitatory conditioned reflex in all groups, but with older animals somewhat more in pullets than in cockerels, no such difference between the sexes being noticeable in the younger age group.

It should be observed that in the experiments summed up in Table VII, the influence of sex if any, was in either direction not statistically significant.

DISCUSSION

In the papers devoted to the principle of the primacy of first training, it has been emphasized that when first training was excitatory in character, subsequent establishment of inhibitory reflexes meets with considerable difficulties, and inhibition is not so complete as when the first training was of inhibitory nature (Konorski, Szwejkowska, 1952). The former situation was actually the case in the experiments here discussed. The first training was markedly excitatory in character: the chicken received food immediately on effecting the motor reaction it had learned, even when the bowl with food has only just been withdrawn. In consequence, during the first experimental sessions, the animals were feeding almost throughout their stay in the experimental camera. Introduction of optical stimuli and of intervals between these, combined with reinforcing of the established and consolidated motor reaction only when the latter was displayed against the background of the optical stimulus, faced the animals with the problem of differentiation of conditioned optical stimuli from the background consisting of the experimental conditions in general. Owing to the excitatory character of the first training, this problem was not completely solved, as was shown by the relatively large number of positive reactions between stimuli till the end of the experiments. Similarly, the task of extinguishing the excitatory significance of one of the optical stimuli, and of transforming in into an inhibitory one, was solved incompletely.

The material presented in this paper reveals distinct differences between the sexes in the degree of extinction of the original excitatory conditioned reflex and consolidation of the inhibitory reflex, as well as in the degree of extinction of positive reactions in intertrial intervals. It needs to be emphasized that owing to an increase with age of the magnitude of excitatory conditioned reflexes, inhibition was much more difficult in older pullets. This was especially striking at the first stage of the experiments, and was disclosed by the vast number of positive reactions between stimuli in the older age group. Gradually, however, the difference became less and eventually the degree of extinction of positive reactions in intertrial intervals, and consolidation of the inhibitory conditioned reflex were with pullets the same in either age group.

It should be noted that according to Tuge, Shima and Koga (1956), who worked with similar methods, there was in all pullets during elaboration of differentiation of optical stimuli, distinct generalization, which was in cockerels weak, or altogether absent. The experiments were carried out on 11 chickens, 27 to 175 days old.

How are we to interpret the results obtained when the duration of the inhibitory conditioned stimulus was extended?

When this duration is extended from 10 to 90 sec., the test becomes difficult for the chicken. Submitted to it, even animals which passed it successfully — i.e. without a positive response while that prolonged inhibitory stimulus was on — displayed subsequently irregularities in conditioned reflex activity, manifest in disinhibition of conditioned inhibitory reflexes as well as in absence of positive response to excitatory conditioned stimuli. A second test of this kind was made only after these irregularities had disappeared, nevertheless it had a different conditioned reflex activity background. No conspicuous differences between pullets and cockerels were noted in either the intensity or the character of the irregularities.

Since inhibition is, however, more difficult in pullets than in cockerels, the extended duration of the inhibitory conditioned stimulus must have had a greater effect on conditioned reflex activity in pullets. Therefore, in the repeat experiment, the pullets showed sooner than they had before a positive reaction during the prolonged inhibitory conditioned stimulus. In cockerels, on the other hand, in which inhibition processes develop more readily, the first prolonged inhibitory stimulus may have consolidated the inhibitory conditioned reaction, and the latency period of the positive reaction to the inhibitory stimulus was in the repeat experiment longer.

The question now arises, whether the differences between the sexes in inhibition processes were not determined by differences in alimentary excitability.

It should be borne in mind that the sexes were kept separated, but in conditions exactly alike, and were fed according to the same standard. Thus, possible differences in alimentary excitability could not have arisen from rearing conditions. Relevant studies on chickens of the same flock revealed no differences between the sexes in rates of oxygen consumption in ccm/kg/hour, but showed that because of their weight, the cockerels consumed more oxygen calculated in ccm/min than the pullet (Zieliński, 1960). The higher oxygen and food requirements of cockerels suggest for them a higher alimentary excitability.

However, the question can be answered only by experiments carried out against the background of modified alimentary excitability.

The results recorded in Table VII appear to suggest that in older age groups the pullets have a higher alimentary excitability than the cockerels, whereas in younger chicken this difference does not exist.

Nevertheless it appears that the differences in question between the sexes in inhibition processes cannot be rooted in different alimentary excitabilities. The differences between the sexes in both the magnitude of the inhibitory conditioned reflex and the number of positive reactions in intertrial intervals are alike in character in either age group, whereas in alimentary excitability the sexes differed only in the older age group.

CONCLUSIONS

1. Chickens of different sexes showed conspicuous differences in the progress of inhibitory conditionned reflexes, but none in excitatory reactions. After consolidation of excitatory and inhibitory conditioned reflexes, pullets showed a greater number of positive reactions in intertrial intervals and more frequent disinhibition of the inhibitory conditioned reactions. Extended duration of the inhibitory conditioned stimulus revealed no differences between the sexes on first trial, but when repeated a second time, it showed the

latency period of the positive conditioned response resulting from disinhibition of the inhibitory conditioned stimulus to be shorter in pullets than in cockerels.

2. Since the magnitude of the reflexes to excitatory conditioned stimuli increased with age, inferiority of inhibitory reactions in pullets was more pronounced in the older age group. This was most striking in the initial stages of the experiments, viz., when after a preliminary training of a markedly excitatory character extinction of positive reactions in intertrial intervals, and subsequently of the excitatory character of one of the conditioned stimuli, was begun.

3. Differences between the sexes in the progress of inhibitory conditioned reactions were not the outcome of differences in alimentary excitability, since in the younger age group inhibitory reactions were equally manifest as in the older one, whereas sex differences in alimentary excitability were noted only in the older age groups.

4. It is worth noting that the differences in question between the sexes were equally manifest before sexual maturity (first age group) and after (Leghorns in the second age group).

REFERENCES

FIODOROW WIKT. K. 1953 — K charakteristikie podwiżnosti nerwnych processow u myszej linii A i C_{57}. Biull. Eksper. Biol. i Med. *36* (6, 7).

KONORSKI J., SZWEJKOWSKA G. 1952 — Chronic extinction and restoration of conditioned reflexes. IV. The dependence of the course of extinction and restoration of conditioned reflexes on the "history" of the conditioned stimulus. Acta Biol. Exper. *16*, 95.

ROMANOWSKI W. 1951 — Zastosowanie statystyki matematycznej w doświadczalnictwie. Warszawa.

TUGE H., SHIMA I., KOGO K. 1956 — Studies of the conditioned reflex in the lower vertebrates. III. Motorfood conditioned reflexes in domestic fowls. Sci. Rep. Tohoku Univ., Biol. *22* (2), 115.

ZIELIŃSKI K. 1960 — Intensywność przemiany gazowej kurcząt ras leghorn biały i pierwomajska w zależności od wieku. Roczniki Nauk Rolniczych, 75 B, 3—58.

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THE EXTERNAL CAPSULE IN THE DOG'S BRAIN (MYELOARCHITECTONICS AND TOPOGRAPHY)

L. SYCH

Neuroanatomical Laboratory of the Nencki Institute of Experimental Biology in Warsaw and Department of Comparative Neurology of the Jagiellonian University in Cracow

(Received 25 November 1959)

The aim of this paper was a description of myeloarchitectonics and topography of those systems of nerve fibers which make the external capsule of the dog's brain. We could not find any papers in the literature containing an analysis of the fiber structure of external capsule except some general data relating to the problems of topography (Cajal 1904, Craigie 1925, Gurdjian 1928, and Kreiner 1936).

The microscopic observations were performed on six brains of adult dogs. Sections were made in the three cardinal plains: frontal, sagittal and horizontal. The sections were stained according to Wolters modification of the Weigert method. For examining the borders of some striatal nuclei the frontal sections were used, stained according to a Nissl's method. Four dogs were normal, the two remaining were experimental animals in which some parts of the cerebral cortex (in the region of *fissura cruciata*) were removed. Our observations have shown that there was no difference between these and normal brains.

The external capsule is a structure accompanying the striatal nuclei. It can be seen as a lamina about 0.3 mm thick in the average, built up from the numerous fibers. In spite of small deviation it lies in a parasagittal plain running through the middle of the pyriform lobe. The shape of it is similar to a trapezium (Fig. 1). The longer sides of it form dorsal and ventral borders of external capsule, the

Fig. 1. Projection of the external capsule on the lateral aspect of the brain of dog

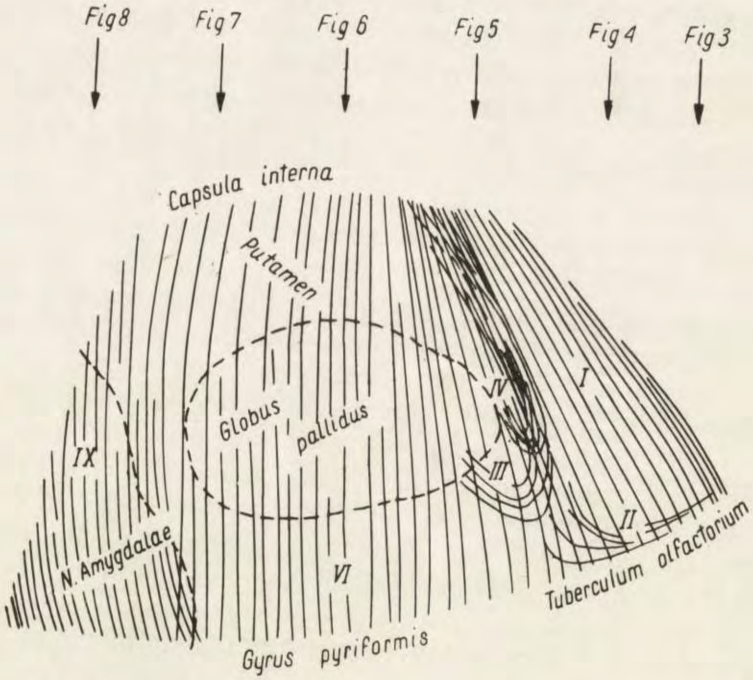

Fig. 2. Diagram of the main fiber systems in capsula externa

shortest one surrounds it orally. The capsule of a middle-size dog is in the caudo-oral dimension about 18 mm long and about 12 mm high.

The dorsal edge of the external capsule merges at the whole length into the internal capsule. The most fibers of internal capsule coming from the top of the hemispheres turn here medio-ventrally while a small amount of fibers runs vertically down and forms the external capsule (Fig. 3—8). The most rostral part of the external capsule bends medially and connects again with the internal capsule.

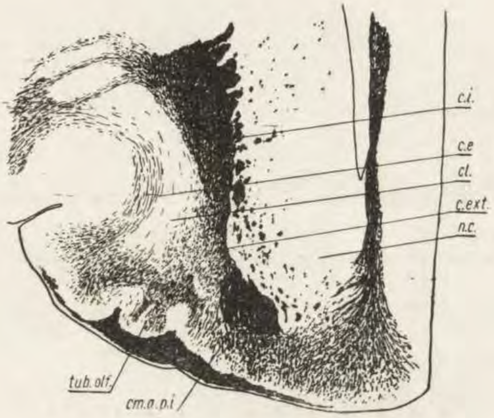

Fig. 3—8. Frontal section through the external capsule of the left hemisphere. Weigert-Wolters, semischematic

Abbreviations: cm.a.p.ib. — commissura anterior pars interbularis; c.a.p.ih. — commissura anterior pars interhemispherica; c.e. — capsula extrema; c.ext. — capsula externa; c.i. — capsula interna; cl. — claustrum; c.p. — cortex pyriformis; gl.pall. — globus pallidus; n.c. — nucleus caudatus; o.n. — openworked net; put. — putamen; s.a. — accompanying system; tub. olf. — tuberculum olfactorium.

The space between both capsules is filled by the lentiform nucleus (Fig. 3—8). This oral border of external capsule extends so far as the head of the caudate nucleus. Caudally the external capsule surrounds the lentiform nucleus and then joins also the internal capsule. The shape of the external capsule is a little convex here, what is caused by the bulk of the lentiform nucleus (Fig. 3, 5). This can be seen in its anterior part where form the medial side it borders the putamen. In the middle of its length the external capsule is

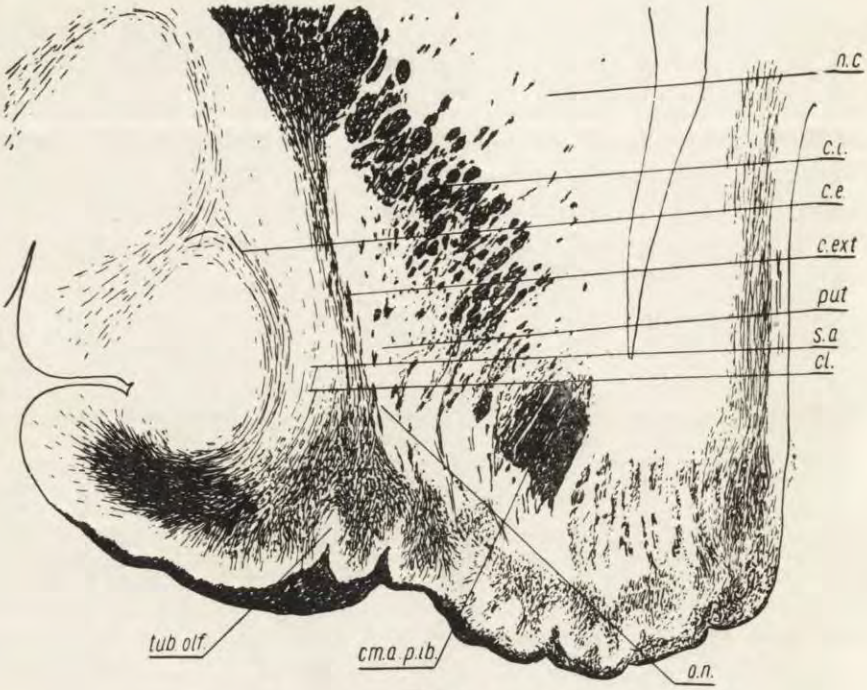
n.c
c.i.
c.e
c.ext
put
s.a
cl.
tub.olf.
cm.a.p.ib.
o.n.

Fig. 4


n.c.
c.i.
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put.
gl.pall.
ca.p.ih.
c.e.
cl.
III
IV

Fig. 5

Fig. 6

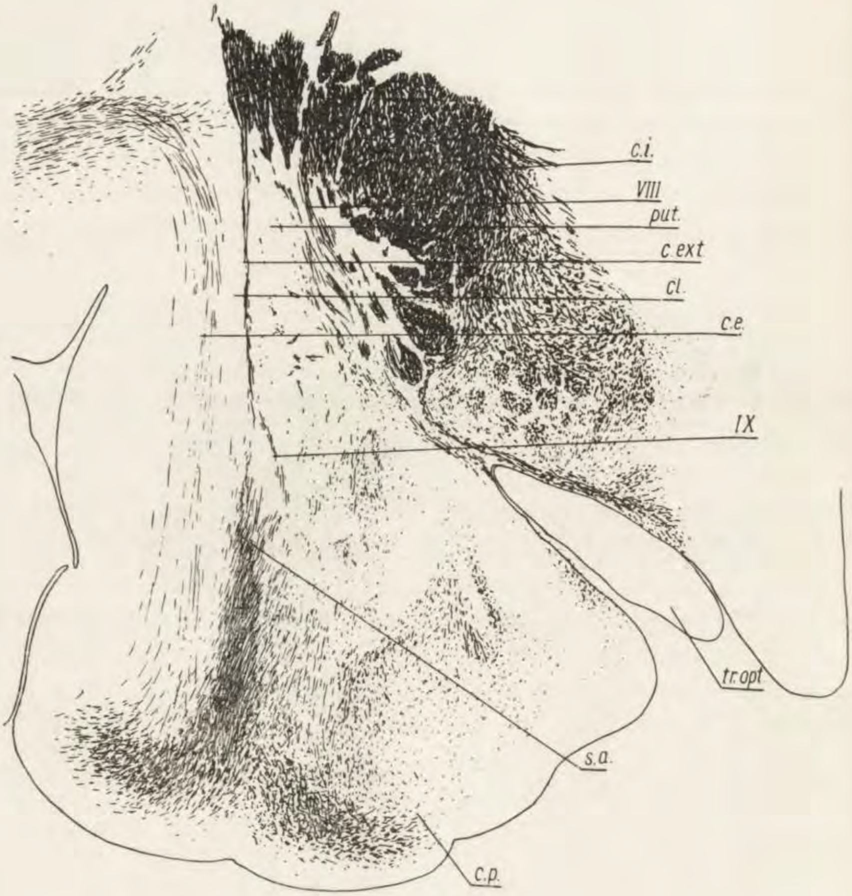

Fig. 7

nearly adjacent to the medial side of the pallidum (Fig. 5, 6). More caudally the pallidum disappears and the posterior part of putamen and nucl. lateralis of amygdala is seen in this place. Laterally, the external capsule along the whole length borders claustrum, the caudal and rostral boundaries of which extend further rostrally and caudally than the external capsule.

Particular attention should be given to the ventral border of capsule as here some fiber systems diverging in different directions can be observed. It is rather a question of agreement to establish in this region the exact boundary of the external capsule. In the

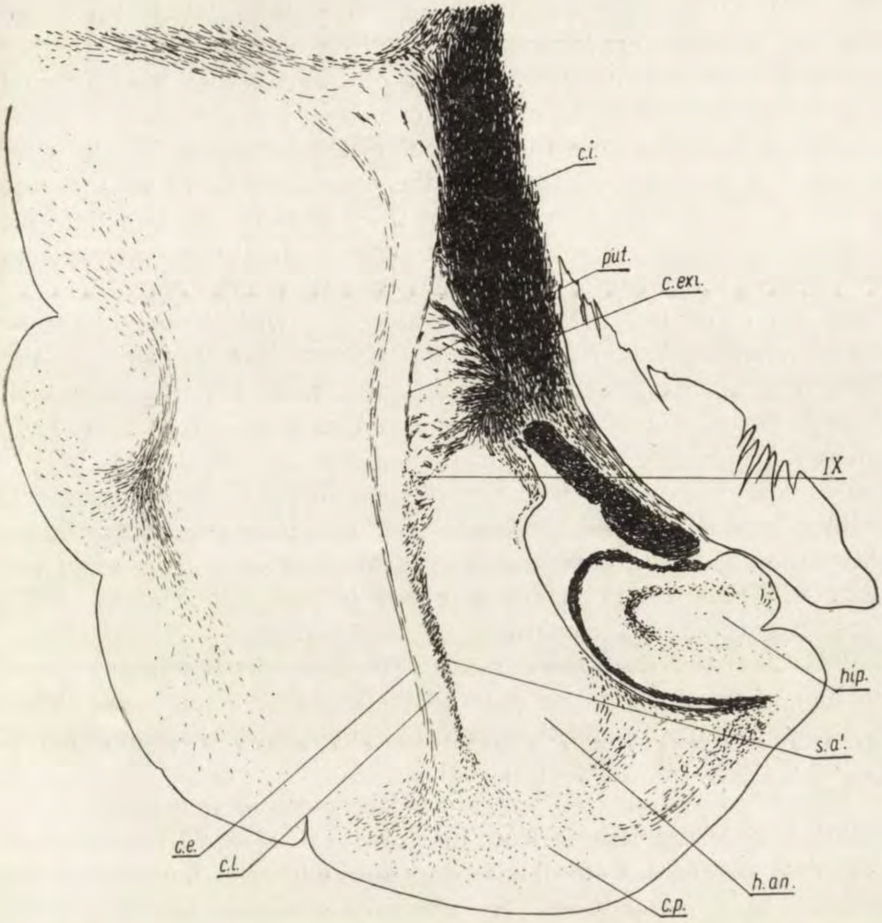

Fig. 8

anterior and middle part of the base of the external capsule it is connected ventrally with olfactory tubercle (Fig. 4), more further caudally it extends along the pyriform lobe (Fig. 7), and the most caudally it touches the antero-dorsal part of amygdala (Fig. 7, 8).

The fibers building the external capsule can be divided in two groups: 1) the main systems building up the capsule and 2) systems only partially accompanying them.

Nearly all systems belonging to the first group have their origin from the region of the olfactory tubercle and the pyriform lobe. Their other ends disappear in the internal capsule, where the dense mass of fibers renders impossible to discover and analyse the further course of the relatively small part of fibers which the internal capsule receives from the external one.

Let us describe now the particular fiber systems. To the first system (I) belong most fibers in the anterior half of the capsule (Fig. 2,I). Those fibers ascend from the region of the olfactory tubercle. Their most rostral part runs postero-dorsally gathering from all sides of the tubercle (Fig. 4). Further they enter into the most lateral part of internal capsule connecting with it the olfactory cortex on its whole length. To the first system belong also the numerous bundles, each containing more than fifty fibers, which come from the medial side of external capsule. After they have emerged from external capsule they pierce the dorsal part of putamen and then enter into the internal capsule. Their course is inclined posteriorly and medially, sometimes they anastomize inside the putamen forming in this way a kind of an openworked net (Fig. 4). These relations are observed in the rostral part of the capsule, posteriorly the openworked net disappears.

The fibers of the second system (II) begin in the latero-dorsal portion of the interbulbar part of the anterior commissure. They run postero-dorsally and then are continuous with the most ventral region of the first system (Fig. 2,II).

The third system (III) appears in the plain of one third of the length of external capsule from its rostral end (Fig. 2,III; 5). It arrives from the contralateral side together with the bundles of the interhemispherical portion of the anterior commissure and runs to the medial side of the first system. Here it turns dorso-laterally and mingles with fibers of the first system (Fig. 5). In this region also a fourth system (IV) was observed. It leaves the latero-ventral part of the internal capsule and turns laterally with a little ventral deviation. Next, it meets the boundle of the third system and together with it runs in the way mentioned above (Fig. 2,IV; 5). In this region the lateral part of the external capsule is made by the first system whose elements run dorso-ventrally, while the medial side consists of fibers originating from the interhemispheric part of anterior commissure and from internal capsule. They run laterally in form of thick boundles.

The interhemispherical part of anterior commissure together with systems III and IV borders the rostral end of the pallidum. The shape of this nucleus in the dog is approximately hemispheric, its flat side being on the medial aspect closely accompanied by the internal capsule, and the convex side pressed into the putamen (Fig. 5). Its lateral aspect practically touches the middle part of the external capsule. From the pallidum two boundles of fibres start laterally toward the external capsule: the first descends from the latero-dorsal part of pallidum and the second from the latero-ventral one. Both boundles join together in their way and make the fifth system (V) not drawn in Fig. 2. This system joins the external capsule just where the capsule approaches the pallidum and then mixes with the fibers of systems described above.

A little more caudally the olfactory tubercle disappears and on its place the pyriform lobe appears. From this lobe the fibers of the sixth system (VI) ascend (Fig. 2,VI). In the ventral part of the pyriform lobe the fibers are dispersed in different directions but mostly in the medial and lateral ones. In the deeper regions of the pyriform lobe they concentrate in the parasagittal plain and leave this lobe as a more compact system making the part of the external capsule.

The fibers of the seventh system (VII) (not visible in the Fig. 2) begin from the lateral surface of pallidum (more exactly from the external medullary lamina). They are concentrated in numerous thin boundles which mostly run latero-dorsally making an openworked net. They pierce the putamen and join the medial surface of the external capsule.

In the vicinity of the place where the pallidum approaches the external capsule there can be observed a small fiber system VIII (Fig. 7,VIII), originating from the dorsal part of pallidum. It runs through the putamen, turns in a dorso-medial direction and enters the latero-ventral part of the head of caudate nucleus. Probably it is one of the most dorsally running fiber boundles which run from the caudate nucleus through the internal capsule to pallidum.

In the caudal region of the external capsule the ninth (IX) system was observed (Fig. 2,IX; 7, 8). It consists of the fibers which in the most ventral region of the capsule are connected with the dorsal part of amygdala (probably with its nucleus dorsalis). More dorsally the fibers incline medio-dorsally with a small rostral deviation and mix with the fibers of the fifth system. The ninth system appears in the form of numerous thin fibers but in a loose contact with each other. The posterior part of external capsule is made just from these fibers.

Besides the systems described above there are systems accompanying the fibers of external capsule only for a short distance. So, in the rostral parts there was observed a system of numerous loose fibers crossing the first and second system. They begin laterally in the orbital gyrus and run medially passing the extremal capsule and claustrum. Then they turn more caudally, cross the fibers of external capsule and at the end enter into the internal capsule. Besides of them in the discussed region there was also observed a small number of fibers accompanying the first system from the lateral aspect. They belong to the extremal capsule, which in the contrast to the external capsule is built from the loosely running fibers. Some of them lose their contact with extremal capsule and retaining the ventro-medial direction run through the claustrum and further medially parallel to the external capsule (Fig. 4, s.a.). They connect the orbital gyrus with the olfactory tubercle.

In the region of the rostral border of amygdala there are additional numerous fibers which seem to be a ventral continuation of the external capsule in its posterior portion. The fibers appear here in the form of a thin capsule reaching dorsally the ventral border of the posterior part of putamen (Fig. 8, s. a.). This capsule consists of three groups of fibers. The first group runs at the beginning (in the neighbourhood of amygdala) laterally and then turns medially on the boundary amygdala-putamen; these fibers are continuous with the system surrounding the caudal part of putamen. The second group connects with the amygdala from its lateral side. The last group originates from the most medial part of extremal capsule some fibers of which bend in the ventral part more medially, and then, after they have passed the ventral region of claustrum, turn dorso-medially, and finally enter into the extremal capsule again. These fibers have no immediate connection with the external capsule although macroscopically they seem to be a continuity of it.

Thus, the external capsule is a structure consisting of several fiber systems, whose topography and course allows us to suppose that it serves previously as a projection system of the olfactory tubercle and pyriform cortex connecting them through the internal capsule with further centres probably including also the cortex.

SUMMARY

An analysis of myeloarchitectonics and topography of external capsule was performed based on six dogs' brains cut in the cardinal directions and stained with the Weigert-Wolters method. The external capsule is a structure surrounding laterally putamen and pallidum and separating them from the laterally situated claustrum. The dorsal border of external capsule joins the internal capsule, the ventral border joins the olfactory tubercle and the pyriform lobe. Caudally, the external capsule is associated with amygdala, rostrally stretches so far as the head of caudate nucleus. Nearly all the systems of external capsule dorsally enter into the internal capsule. Nine systems were distinguished: from the olfactory tubercle, the pyriform lobe, the anterior commissure (from the interbulbar and from the interhemispherical parts), from the internal capsule, pallidum, the external medullary lamine, the caudate nucleus and from the amygdala. Beside those systems there are also accompanying systems which join the external capsule only for a short distance. Such systems are: a) from the orbital lobe through the extremal capsule and claustrum to the internal capsule, b) the single fibers running most medially from the extremal capsule, c) in the most ventral parts the fibers surrounding amygdala.

The fibers which build the external capsule are probably the projection from the olfactory tubercle and pyriform lobe.

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REFERENCES

CAJAL S. RAMÓN 1904 — Textura del sistema nervioso, T. II. Madrid.

CRAIGIE E. HORNE 1925 — Finer Anatomy of the Central Nervous System. Philadelphia.

GURDJIAN E. S. 1928 — The corpus striatum of the rat. (Studies on the brain of the rat. Jour. Comp. Neur. 45(1).

KREINER J. 1936 — Commissura anterior der weissen Ratte. Z. ges. Anat.

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DISTURBANCES OF INHIBITORY PROCESSES DUE TO LESIONS IN THE FRONTAL AREA OF THE BRAIN IN MAN

S. TOCZEK

Department of Neurosurgery, Polish Academy of Sciences, Warsaw

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It is well known, that lesions of the prefrontal area* of the human cerebral cortex result in changes in general behaviour, whereas the so-called purely neurological signs and symptoms remain completely or almost completely absent. So, since the second half of the 19-th century, when Harlow (1868) reported his famous case of a man called Phineas Gage, and for the first time gave a precise description of the frontal syndrome, many a clinicist as well as psychologist have attempted to define the nature and to elucidate the mechanism of appearance of frontal lobe syndrome.

The most frequently described changes in general behaviour are the following: increase in general activity, or on the contrary — apathy, emotional blunting, unjustified light-heartedness, tendency to cheap joking (moria); emotional instability, frequently in form of explosive outbursts of bad temper; increased sexual activity; exaggerated crave for food, etc.

Much psychological testing has been devoted to the investigation of patients with injuries of prefrontal region. All of them showed intellectual deficits, inability of synthesis or abstract thinking, incapacity of planning and others.

Some authors have referred also to impairment of inhibitory function. However, this term has been used rather in a not precisely defined manner. Thus, for instance, Feuchtwanger (1928)

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* According to terminology accepted by many authors, the term prefrontal area denotes the region situated rostrally to the pre-motor area.

speaks of disinhibition of various instincts, whereas Jarvie (1954, 1958) points out the fact, that after prefrontal injuries principally disinhibited become thase inclinations, the inhibition of which had caused much difficulty already before the lesion. Disturbances of the inhibitory process after prefrontal injuries are described by Filippycheva (1954).

Much experimental work has been recently devoted to the effect of prefrontal lobe ablations upon the conditioned reflexes in dogs. It has been pointed out that even small lesions of prefrontal area result in very distinct disturbances of inhibitory processes (Brutkowski et al., 1956; Brutkowski, 1957; Ławicka, 1957).

In the publications mentioned above, the authors have shown, that in dogs after having established alimentary conditioned reflexes, positive and negative (namely differentiation, conditioned inhibition), ablations of the prefrontal areas do not impair excitatory conditioned reflexes, whereas inhibitory conditioned reflexes undergo very distinct disinhibition. This refers not only to salivary conditioned reflexes (type I) but also to instrumental conditioned reflexes (type II).

Taking into account the results obtained from the experiments referred to, the question might arise whether similar impairment of inhibitory processes could be traced also in men suffering from prefrontal injuries of various origin.

The purpose of this paper is just the investigation of this problem.

METHODS OF EXAMINATION

The method applied in the experiments was that of conditioned reflexes as described by Ivanov-Smolensky, in conditions indispensable for a physiological experiment in man.

The motor conditioned reflex consisted in the patient's pressing a rubber bulb or a spring-provider handle connected with an apparatus registering the intensity of reaction and its latent period. The visual conditioned stimuli applied were: multicolour lamps of different intensity of light, placed at different spots on the screen; and the auditory stimuli (sounds from a sound-generator, bells and buzzers). Time-period for each stimulus was 2 sec., and the intertrial intervals varied from 15 to 60 sec. or more. The excitatory (positive) conditioned reflexes were established in association with a given visual or auditory stimulus and the order: "press it". The inhibitory (negative) conditioned reflexes were elaborated by associating a given single stimulus or compound stimuli with the order: "don't press it".

Every correct reaction of the patient, i. e., the pressing of the bulb in case of a positive signal, or not-pressing it in case of an inhibitory one, was reinforced by saying: "Well done".

Had the patient pressed the rubber bulb after an inhibitory stimulus, so he was told: "Don't do that", or: "Too bad.", whereas in case he did not press the bulb after positive stimulus, the instruction was: "Do press it".

The following types of internal inhibition were elaborated:

a) Simple differentiation: in order to establish differentiation, a stimulus, similar to the conditioned stimulus used primarily, was applied together with the order: "Don't press". This stimulus was presented repeatedly among several positive stimuli.

b) Differentiation of compound stimuli: the patient had to differentiate a set of stimuli composed of e. g., three lamps lighted in order, one after the other (ABC), from another (inhibitory) set, composed of the same elements, but applied in a different order (BAC).

c) Conditioned inhibition: after a positive conditioned reflex (to the stimulus P) has been firmly established, a new, previously not applied stimulus was chosen (I); the new stimulus was applied for 2 sec. just before the conditioned stimulus P. This compound stimulus (I + P) was then followed by the order: "Don't press". When the compound I + P has assumed an inhibitory value, and the patient inhibited the motor reaction to the conditioned stimulus P applied after the conditioned inhibitor I, the interval between both components of the compound had been gradually prolonged to 2—60 or more seconds.

d) "Alternation": in these experiments one and the same stimulus was applied throughout each session, and the patient, alternatively, had to press or not to press the rubber bulb, i. e., the same stimulus has been alternatively an excitatory and an inhibitory one. This is a peculiar type of conditioned inhibition in which the memory traces, resulting from excitatory reaction play the role of conditioned inhibitor, conditioning the inhibitory reaction for the next stimulus.

After the examination the patient described the stimuli, he told what task he had to perform and how did he practically solve them. The point was to ascertain whether the patient understood all phases of the experiment, whether his or her errors had not resulted from incomprehension of the orders received. Also, during the experiment itself, the patients were sometimes asked if they understood the significance of the stimuli.

It should be added that the elaboration of inhibitory conditioned reflexes in healthy men is quite easy to be obtained. After one or two stimuli applied together with the order: "Don't press", the patient does not perform motor reaction to that stimulus. The inhibitory stimuli also do not influence the positive reaction in a negative way. The verbal description of the course of the experiment is normal and precise.

RESULTS

The number of patients subjected to the examination was 15; their age was between 14 and 55 years. There were among them 13 cases with unilateral and 2 with bilateral lesions of the frontal lobes due to traumatic injuries (5 patients) and to tumours located in that area (10 patients) — Table I, and Fig. 1.).

In the group comprising those 13 patients with unilateral lesions, there were 10 cases with left-sided and 3 cases with right-sided lesions of the frontal lobes.

The patients have passed their post-operative examination in different time-periods; the shortest was 13 days, the longest — two

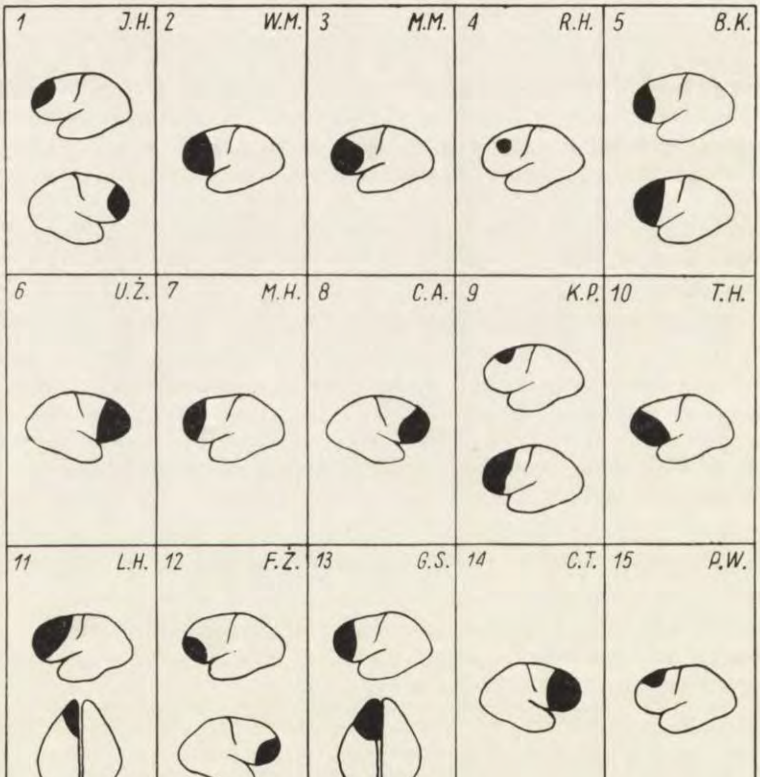


Fig. 1. Demonstrates frontal area lesions of our patients. The cases have been arranged in the same order as in Table I.

years and a half. During the examination the general neurological status of all the patients was satisfactory. There was practically no paresis observed, or if present, it was a very slight one. Only two patients had transistory motor aphasia which diminished subsequently, rendering thus an easy description of the experiment possible.

Almost all the patients were coming willingly for the examination and they regarded it as a training in concentration of their attention, as an examination of their hearing and seeing. Therefore, they have done all their best in order to obtain the best results possible, and they were grieved when they have had any difficulties.

In healthy subjects positive motor conditioned reflexes can be established after 1—3 presentations of the stimuli, accompanied by the order: "press it", and they appear rather regularly in the subsequent experiments. In our patients, however, the process of elaboration of motor conditioned reflex was as follows: in 11 patients the reaction was quite normal, i. e., it appeared after 1—5 associations of the stimulus together with the order: "press it". In the remaining 4 patients the establishment of the reflex required many repetitions of the order. There were also some cases in which the experiment showed a regular course, but after a while the conditioned reaction vanished, but reappeared afterwards. Besides, during the initial phase of the experiments the patients tended to hold the rubber bulb squeezed for many seconds, despite the clear instruction to press and release it immediately. It happened also, however very seldom, that the patients repeated the already learned reaction in intervals between the stimuli.

The elaboration of inhibitory conditioned reflexes in patients with lesions in the frontal area took a regular course only in four of them (Cases No. 12, 13, 14, 15). The remaining patients showed more or less, sometimes even very remarkable, disturbances of the inhibitory process. Thus, they performed the learned movement also after inhibitory stimuli were applied. Inappropriate reactions to inhibitory stimuli were, however, not due to difficulties in understanding of the given experimental test. The examined subjects knew exactly what kind of learned movement had to be performed, and when after the given stimuli the motor reaction should be inhibited. There were also observed such phases of the experiment in which the inhibitory reactions showed a regular course, but after a while the examined patient was unable to inhibit the movement completely. There was indeed "fight going on" between excitation and inhibition in the moment of application of the inhibitory stimulus, for the intensity of inappropriate motor reaction to such a stimulus was usually weaker than the intensity of motor reaction to positive stimulus, and the latent period longer.

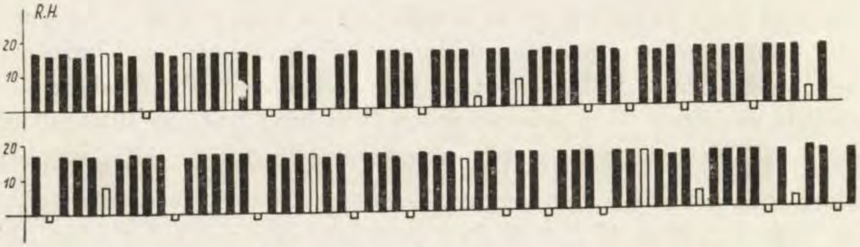


Fig. 2. The process of simple differentiation in Case No. 4.
(Experiment from December, 27th, 1957)

Red light — positive stimulus, green light — negative stimulus. Full columns indicate the reaction to a positive stimulus (reinforced by the words: "Well done"); the empty ones denote the reaction to an inhibitory stimulus (reinforced by the order: "Don't press", or "Too bad"). The intensity of the reaction is represented by the height of the column; an empty square placed under the axis indicates no reaction to an inhibitory stimulus (reinforced by the words: "Well done"). The upper part of the diagram means the first whereas the lower one — the second part of the experiment.

A typical course of differentiation in our patients is demonstrated in Fig. 2. It can be seen therefrom, that even an extremely easy differentiation of visual stimuli can be obtained but slowly. It seems, that after 9 presentations of inhibitory stimuli, the differentiation is already accomplished, nevertheless, there frequently occurs disinhibition, and, as already mentioned, the intensity of the reaction is usually weaker than in case when positive stimuli are being presented. Similar experiments carried out after 6 days have led to analogous results, however, the grade of disinhibition was lower.

The behaviour of some patients during the examination has to be considered as additional proof for the fact that the disturbances just described should be regarded as resulting from disinhibition (but not from difficulties in recognition of the particular stimuli).

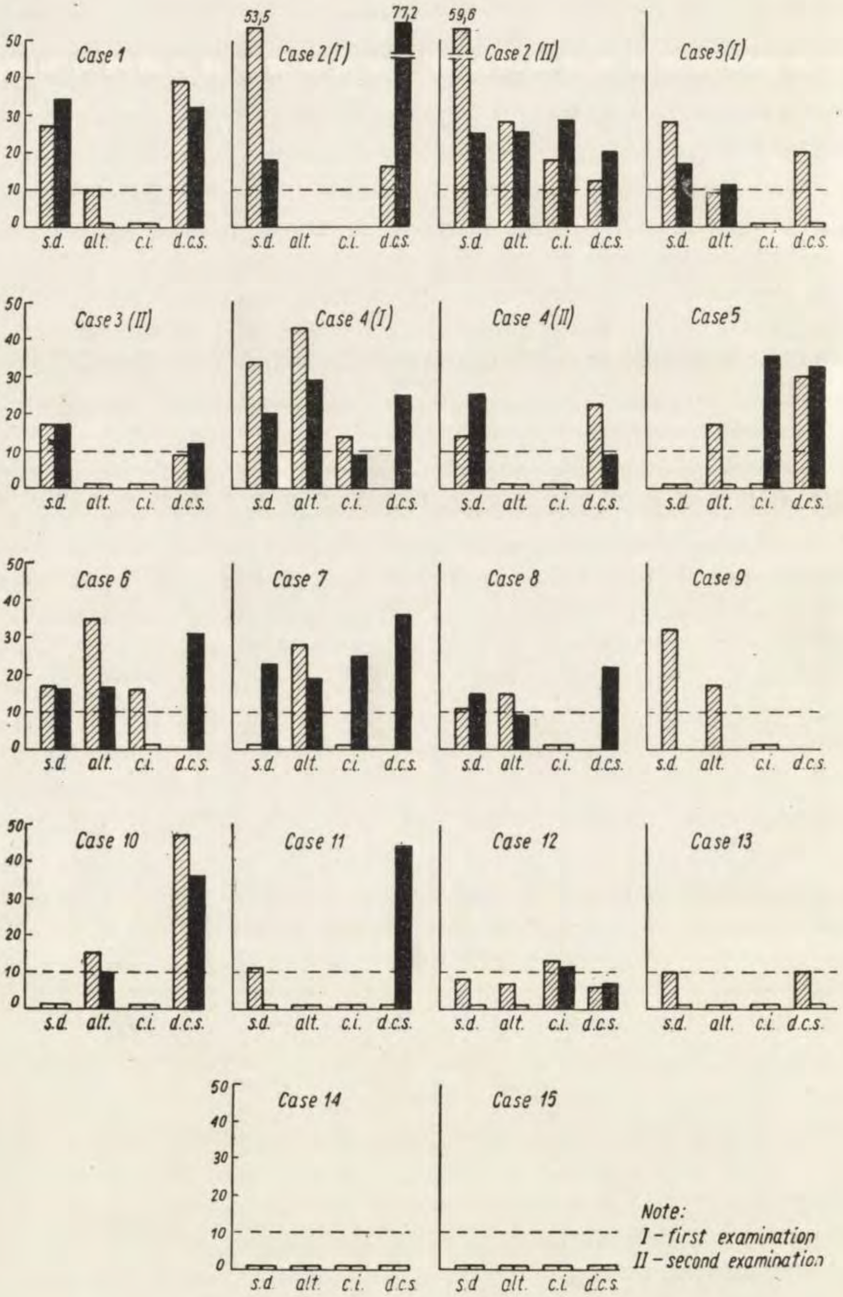
The patient shows frequently that he (she) is not pleased with the result obtained, exclaiming in the moment he has reacted to an inhbitory stimulus: "Oh, dear", "Oh, I've just pressed it a little", "Oh, yes — not to press it at all now..." and so on, or he says directly: "I don't know what has happened, I'am aware of it not to press, but there is something that forces me to do that, and so I'm trying just a litlle..." The patient sometimes tries to conceal his (her) errors for instance, one of them says: "I didn't press it at all", adding after a while: "Oh, yes, this apparatus will but disclose everything..."

The control group was made up of 15 patients with focal but non-frontal lesions of the brain (tumours in the temporal, parietal and occipital region; arterio-venous malformation in the region of posterior cerebral artery; tumours of the posterior fossa, etc.). In this control group it was found, that the reactions to the inhibitory stimuli were normal, or, that the percentage rate of the errors calculated from the total number of stimuli applied in different types of experiments, did not exceed in those patients 10 per cent (Fig. 3B). This percentage of mistakes (errors) can still be included within the range of physiological values.

In the evaluation of the degree of disturbances of internal inhibition in our prefrontal patients, the following factors were taken into consideration: the frequency of the performed motor reaction to the inhibitory stimulus, and whether the errors were repeated in all kinds of internal inhibition, or in some types only (Fig. 3A); the quality of motor reaction to an inhibitory stimulus (latent period and the strength of reaction); the way of reacting of the patients to their errors.

Taking into account all the factors mentioned above, it can be seen that in 5 out of the 11 patients (No. 1, 2, 3, 4, 5), showing signs of inhibition-disturbances, the symptoms of disinhibition were in them more pronounced than in the rest of this group. These five cases had proved to be disinhibited during at least several experiments with various types of inhibition. In general, the patients were at their best in case of conditioned inhibition. As far as other experiments are concerned, it was impossible to establish any rules whatsoever. So, for instance, for the patient No 2 the process of simple differentiation were repeated at some months' intervals and they a two years' period of examination. In this case the experiments with differentiation were repeated at some months' intervals and they always revealed disturbances of the same kind. Nevertheless, the patient always told exactly what colours or sounds she had to distinguish. On the other hand, however, the patient was doing much

A


Case 1
Case 2 (I)
Case 2 (II)
Case 3 (I)
Case 3 (II)
Case 4 (I)
Case 4 (II)
Case 5
Case 6
Case 7
Case 8
Case 9
Case 10
Case 11
Case 12
Case 13
Case 14
Case 15
53,5
77,2
59,6
0
10
20
30
40
50
s.d.
alt.
c.i.
d.c.s.
Note:
I – first examination
II – second examination

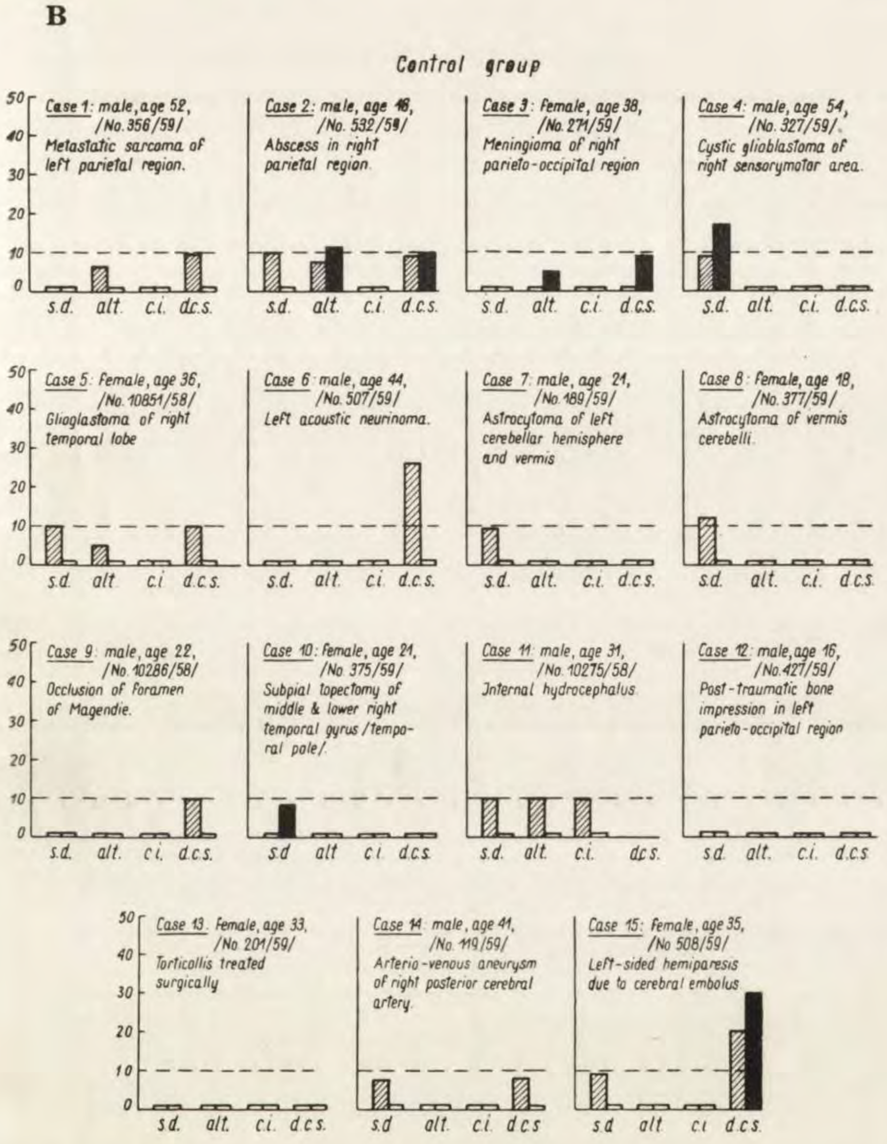


Fig. 3. Percentage of errors from the total number of inhibitory trials in prefrontal lesion (A), and control group (B)

Each column represents the percentage of errors for the particular inhibitory test. Striped columns — visual stimuli. Black columns — auditory stimuli. Interrupted line denotes the 10% limit accepted as physiologically normal value, s.d. — simple differentiation, alt. — alternation, c.i. — conditioned inhibition, d.c.s. — differentiation of compound stimuli.

better with differentiating of compounds, triple stimuli where the reactions could be performed more slowly (Fig. 4a, b).

The differentiation of two auditory stimuli was for patient No. 5 an easy task, even when the sounds were fairly similar, whereas the differentiation of triple compound auditory stimuli was for him very difficult. At the beginning of the experiment he did not react several times to the inhibitory stimuli, what was a proof of his having perfectly distinguished the two compounds. In the succeeding part of the examination the patient, however, performed several times the learned motor reaction as a response to the inhibitory stimulus (Fig. 5a, b).

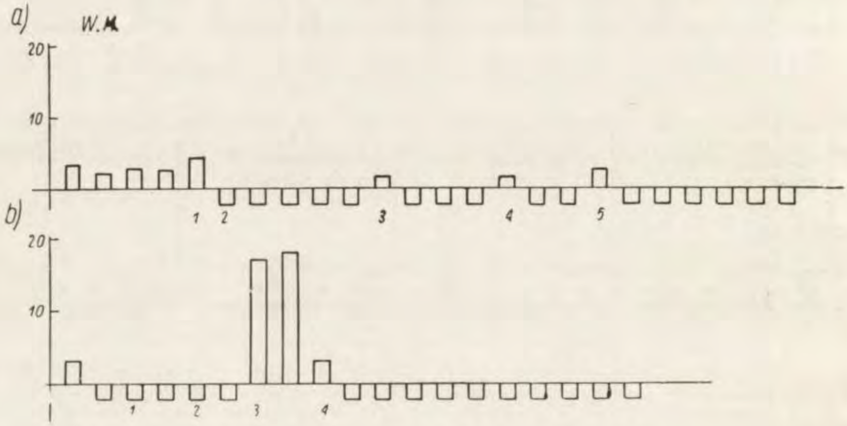


Fig. 4. Differentiation of simple auditory stimuli (a), and of compound stimuli (b) in Case 2

The figure represents reactions to inhibitory stimuli only, whereas those to excitatory ones have been omitted. In the experiment (a) carried out on May, 31st, 1957, the excitatory stimulus was a tone of 6,300 c/s, and the inhibitory one — a tone of 15,000 c/c. In the experiment (b) (June, lst, 1957) the excitatory stimulus was a sequence of tones ABC, whereas the inhibitory one — the tones arranged in different order, namely BAC (tone A — 80 c/s, B — 800 c/s, and C — 8,000 c/s). The numbers inserted under the columns indicate the verbal reactions of the patient: in the experiment (a), 1 stands for: "Oh, dear me"; 2 — "I've succeeded at last."; 3 — "Oh, that is bad, I've pressed"; 4 — "Oh, I just intended to do it", "Oh, I've only pressed a little", "Oh, dear, everything is spoiled"; 5 — "Oh, dear, dear me". In the experiment (b): 1 means: "Well now, I know everything"; 2 — "Ach, I just wanted to press"; 3 — "Oh, I've pressed, I really don't know why?" 4 — "Oh, I've pressed just a little".

The disturbances of inhibition observed in the other group of patients (No. 6, 7, 8, 9, 10, 11) were less distinct and subsided completely after proper training. Nevertheless, these patients too

Table I

Case No.	Initials, sex, age Profesion/ /education Case History No.	Particulars from anamnesis, Localization and nature of the lesion	Neurological status during examination (conditioned reflex method)	Examination was commenced	Patient's attitude towards social life
1	2	3	4	5	6
1	J. H., male, 33 years, Student of Civil Engineering No. 9730/57	In May, 1945 he was wounded by a bullet that passed through both frontal lobes. Operated on September, 10th, 1954 because of generalized epileptic seizures. A cerebral scar of bilateral frontal location, left latero-medial part of the lobe up to the anterior horn of left lateral ventricle, as well as right frontal pole have been removed, and the anterior horn of right lateral ventricle opened. Both medial parts of the frontal lobes up to callosal body were destroyed.	No neurological signs and symptoms were observed before and after operation. However, during 3 weeks time he answers questions reluctantly, is disorientated in time and place, shows impairment of controlled urination and defecation.	After two and a half years following operation	He is explosive, uncontrolled, quarrelsome, impatient, negligent. He is working nowhere, but constantly looking for job, and when employed, he suddenly changes his mind. Financially supported by his mother, he does not care for his only child (though often saying that he likes it). Disinhibited in sexual life. All these features of his character were absent before the injury.
2	W. M., female, 16 years, pupil of Secondary School No. 8136/55	Since 2 years gradually progressing intracranial hypertension and neurological signs and symptoms. **First operation** on May, 16th, 1955, a meningioma of the left olfactory groove, and, at the same time the damaged left frontal pole, mainly its baso-medial parts adhering to the falx have been removed. The resection line passed approximately 4 cm behind the frontal pole.	Post-operative examination revealed impairment of left olfactory nerve. Visual acuity 5/35 bilaterally. In experimental room she distinguishes quite well the colours of lights projected on the screen. Visual field examination shows bilateral concentrical limitation. Leftsided facial weakness of central origin.	After 22 days following first operation	Normal behaviour of the patient in her social life.
		Second operation on November, 14th, 1955. There has been constant rhinorrhea some time after the first operation. Indications to reconstructive operation of the dura mater have been established. During surgical intervention residuing remnants of tumour tissue underneath right frontal pole have been removed.	Neurological status as mentioned above.	After seven and a half months following first operation	
			Left-sided abolishment and right-sided impairment of olfactory sensation. Right-sided blindness. With her left eye she is able to recognize from 2 m. distance the colours of lights on the screen.	After two years following first operation	
3	M. M., male, 13 years, pupil of Elementary School No. 9248/55	The patient prepared an explosive cartridge using a motor sparking-plug. While applying it to electric net, an explosion occurred and the boy was wounded by the plug in his left fronto-orbital region. During the operation on December, 27th, 1955, damaged cerebral tissue of left frontal pole, the plug lying there and its fragments found in the anterior horn of the left lateral ventricle had been removed. This led to a conelike defect the basis of which covered the whole frontal pole, and the top reached up to the anterior horn of the left lateral ventricle.	Post-operative course complicated by purulent meningitis cured with antibiotics. Post-operative central paresis of right facial nerve and somewhat increased reflexes within right extremities.	After 2, 6, 25 months following first operation	Before the injury he was a quiet and diligent boy. At school he made good progress. Now, he is restless, impatient and explosive. (His mother is saying: "he has become terribly mischievous"). According to his school-teacher- "he is incapable and shows little interest in the subjects taught. He retains any new material only with much difficulty and easily forgets it. He is little consientious, often restless. Frequently starts arguments with his playmates".
4	R. H., male, 19 years, farmer, 7 years of Elementary School No. 9978/57	On November, 30th, 1957, he was hit by an axe in his left frontal region. He did not lose consciousness. Operated upon the same day of accident. The fractured bone was removed. The dura mater was found lacerated, and the cerebral tissue contused in prefrontal area, just over the third frontal gyrus. The damaged brain tissue had been sucked out resulting in a cerebral defect of 3×3 cm and approximately 1 cm deep.	There was transitory motor aphasia, but on examination he was able to give freely verbal description of the given test. Central paresis of right facial nerve and slightly increased reflexes in right extremities observed after the operation.	After 27 days following operation After 33 days following operation	Before the injury the patient was quiet and self-controlled. After operation his personality changed; at first indifferent, later on he became restless and showed frequent outbursts of bad temper.
5	B. K., male, 47 years, farmer, 2 years of Elementary School No. 9843/57	Intracranial hypertension increasing rapidly during recent 4 weeks. Operated on May, 10th, 1956. Amputation of left frontal pole infiltrated by the tumour (oligodendroglioma) has been performed. The resection line passed approximately 5 cm behind the frontal pole.	Central paresis of right facial nerve of minor intensity.	After 19 days following operation	Before the disease he was a good father taking much care of his family. He was very interested in social life. After operation he became indifferent, cared neither for his family nor for social matters.
		Second operation on June, 7th, 1957. A large tumour had been removed and further resection of the frontal lobe carried out. The resection line passed approximately 2 cm behind the previous edge of amputation, thus reaching almost the motor area.	Facial weakness as previously. He is talking unwillingly. No speech defects found during examination.	After 13 days following operation	His general status was after the second operation quite satisfactory, but during 2 weeks time he showed impairment of controlled urination and defecation, hiding the dirty bedclothes underneath. He is very indifferent, often does not find his ward.
6	U. Z., male, 25 years, farmer, 6 years of Elementary School No. 10018/57	Since one year he has had steadily increasing headaches and during recent few weeks advancing impairment of visual acuity. On December, 20th, 1957, operated upon because of right frontal pole tumour (astrocytoma); a resection of the frontal pole and the basal parts of frontal lobe was carried out. The resection line passed 2—3 cm to the front of motor area. The tumour most probably infiltrated the callosal body.	During 2 weeks after operation he lost controlled urination and defecation. His general status was, however, satisfactory. Well orientated in time and place. Visual acuity impaired, but he distinguishes form 2m distance colours of lights on a screen. Slight central paresis of left facial nerve. Slight left-sided hemiparesis.	After 31 days following operation	Indifferent, slow-reacting. He is not interested in his family affairs. He often goes astray, unable to find his ward.
7	M. H., male, 19 years, farmer, 7 years of Elementary School No. 10010/57	In 1945 hurt by a pistol bullet in left frontal area. There was suppuration of the wound which healed by itself afterwards. Since 1950 he has had generalized epileptic fits several times every month despite systematic treatment. On February, 7th, 1955, a cerebral scar of left frontal pole was removed. It extended for about 5 cm caudally from frontal pole and reached the anterior horn of left lateral ventricle.	No neurological changes observed.	After 34 days following operation	Slowing; show inclination to perseveration of movements, when asked, he likes to talk, becomes talkative.

Table I (cont.)

Case No.	Initials, sex, age Profesion/ /education Case History No.	Particulars from anamnesis, Localization and nature of the lesion	Neurological status during examination conditioned reflex method)	Examination was commenced	Patient's attitude towards social life
1	2	3	4	5	6
8	C. A., female, 43 years, housewife, Elementary School education, No. 10423/58	Since 5 years she has had periodical headaches, nausea, and vomiting. Since a year increasing somnolence, polyphagia and polydipsia. Since 3 months impairment of visual acuity. Operated on May, 2nd, 1959 a meningioma destructing right pole and basal parts of the frontal lobe has been extirpated. The tumour was growing into cerebral falx.	Visual acuity: V. oc. d. — 7/18 V. oc s. — 7/36 No other changes in neurological status observed.	After 40 days following operation	Euphoric and talkative.
9	K. P., male, 55 years, clerk, Secondary School Education No. 9322/55	Since 7 months he is explosive, irritable, often gets into trouble with his neighbours and at the office. Shortly before hospitalization he showed impairment of controlled urination and defecation. Operated on October, 15th, 1953, and a cystic astrocytoma has been removed. The tumour occupied latero-medial part of the left frontal lobe and went rostrally to prefrontal area. It extended down to the ependyma of anterior horn of left lateral ventricle. During the second operation (September, 25th, 1955) a wide resection of the frontal lobe including the tumour was performed. The resection line approximated the motor area. The IIIrd frontal gyrus and basal parts of the frontal lobe were left intact.	After second operation there was impairment of visual acuity of the left eye, very slight central paresis of right facial nerve, clumsiness of right hand (but no weakening of muscular strength and no increase in tendonal-periosteal reflexes.	After 4 and 7 months following the second operation	After both operations he returned to his previous occupation. His colleagues, knowing him formerly as a very able & consientious employee, much interested in social life, note now that he has become too diligent and pedantic in non-important matters. Formerly very polite and obliging, now often gets angry, using indecent expressions. During conversation he is often laughing without any apparent reason.
10	T. H., male, 31 years, Clerk, Secondary School education No. 10246/58	Intracranial hypertension lasting for 6 weeks. During operation on December, 6th, 1957, a meningioma of a goose-egg size was removed. It based widely on the small wing of left sphenoid bone. The tumour was situated underneath the frontal lobe, destructing its basal parts which had to be resected during tumour extirpation.	Slight right-sided hemiplegia manifested in form of increased tendonal-periosteal reflexes.	After 21 days following operation	No changes in social attitude observed.
11	L. H., female, 38 years, country-woman, Elementary School education No. 10031/57	Since 5 years generalized epileptic seizures occurring repeatedly every few months, also sporadic headaches and vomiting. On January, 8th, 1958, an astrocytoma of left pole and medial part of frontal lobe was removed. The tumour extended up to the anterior horn of left lateral ventricle.	Slight central weakness of right facial nerve. Right-sided hemiparesis slight in degree: weakening of muscular strength in lower right extremity.	After 41 days following operation	No changes in social attitude observed.
12	F. Z., male, 27 years, non-commicioned officer, 3 years of Secondary School (outpatient examination)	On February, 12th, 1957, wounded by a pistol bullet, that passed through both frontal lobes. During the injury he was in state of alcohol intoxication. The operation revealed a destruction of poles and basal parts of both the frontal lobes. Damaged cerebral tissue had been suched out. The ventrical system remained intact. After 3 months since the accident the patient suffered from meningitis.	Besides impairment of smell sensation, there were no focal neurological changes observed.	After 5 months following operation	Slowing, no initiative, melancholic, lacks criticism as to his illnes. Does not remember many facts from his everyday life prior to the injury. During physical examination he confabulates on the causes of his skull deformity.
13	G. S., male, 37 years, graduated engineer No. 8150/55	Headaches, slowing of mental processes, lack of interest in family and professional life lasting for 5 months. Four years ago the patient suffered from a generalized epileptic fit. On May, 24th, 1955, an astrocytoma and a large part of left frontal lobe was removed. The resection line passed approximately 3 cm in front of the motor area; lateral and basal parts of frontal lobe have been preserved. Anterior horn of the left lateral ventricle has been opened.	Motor aphasia and paresis of right upper extremity followed immediately after operation. On examination only central paresis of right facial nerve and more prominent tendonal-periosteal reflexes in the right upper extremity could be noticed.	After 17 days following operation	Before the disease self-controlled, cultural, and interested in many intellectual activities. After the operation frequent outbursts of bad temper. Shows no progress or initiative in his lectures he is giving in old fashion. He has difficulties in making plans and shows weak memory as he cannot repeat what he has read before. Complains of impairment of controlled urination and defecation.
14	C. T., male, 49 years, employee, Secondary School education No. 10248/58	There was a generalized epileptic attack 6 months before the patient was hospitalized. Two months thereafter he complained of headaches and vomiting. On March, 27th, 1958 he was operated upon and the pole and medial parts of right frontal lobe destroyed by tumour (astrocytoma) have been removed. There was a widespread infiltration by the tumour particularly of the anterior part of callosal body.	Slight central paresis of left facial nerve and more prominent reflexes in left extremities resulted from the operation.	After 18 days following operation	Slowing and indifference, not interested in family life.
15	P. W., female, 38 years, nurse, Secondary School education No. 300/59	Suffering form headache and bad memory for 4 months. Since that time a central scotoma of her left eye has been discovered. On May, 6th - 1959 she was operated upon and a meningioma of the left frontal region removed. The tumour was situated somewhat backwards from the frontal pole and rather medialy, touching the cerebral falx.	Visual acuity: V. oc. s. — 7/18 V. oc. d. — 7/36 No other neurological focal signs have been observed.	Examined before the operation and 14 days after surgical treatment	—

encountered difficulties during more complicated examination such as, for instance, differentiation of compound four-fold stimuli (No. 10, 11), or "alternation" (No. 10).

In addition to the experiments, to which reference was made hitherto, also the daily behaviour of the patients has been accurately recorded in order to find out whether they were showing as well

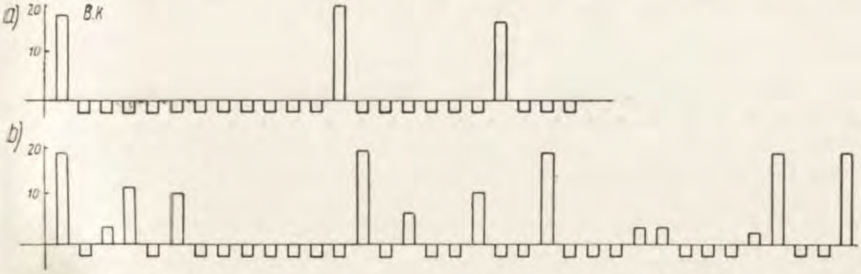


Fig. 5. Differentiation of simple (a) and compound (b) auditory stimuli in Case 5

The figure represents only reactions to inhibitory stimuli, whereas responses to excitatory stimuli have bean omitted. An excitatory stimulus in form of a sound of 6,300 c/s and an inhibitory one — a sound of 15,000 c/s, have been applied in the experiment (a) of June, 20th, 1957; In the experiment (b), carried out on June, 22nd, 1957, the excitatory stimulus was represented by the compound of tones applied in sequence ABC, and the inhibitory one — by the compound of tones arranged in different order, e.g. — BAC. (tone A — 100 c/s, B — 1,000 c/s, C — 10,000 c/s).

changes in their general attitude towards social life, that could be referred to disturbances of inhibition, and, if so, whether their intensity corresponded with that of disinhibition observed during the experiments on conditioned reflexes.

Five patients (No. 1, 3, 4, 9, 13) showed such changes in their behaviour as: increase in general activity (restlessness), tendency to outbursts of bad temper, increased sexual activity, inclination to discuss matters connected with sexual life. All these five patients but one (No. 13) have also demonstrated disturbances of inhibition in experimental conditions. Four other patients (No. 5, 6, 12, 14) revealed symptoms of indifference and slowing of reactions. The remaining patients behaved normally in ward conditions.

For a better illustration of the behaviour of patients included into the "disinhibited" group, a detailed description of case No. 1. will be given: male, J. H. (Case history No. 9730(57), age 33, a student of Civil Engineering. Wounded in 1945, the bullet had passed through both frontal lobes. His mother (a teacher by profession) provided us with the following description of his character: "Before the injury he has always been a very intelligent boy, showing but emotional imbalance. He had no difficulties in learning. It was

11 months after he had been wounded when I saw him again. At first I did not notice any particular changes in his behaviour, but when attending the secondary school, he was restless and prone to making mischief. When admonished — and I have to add that he was very obedient — he became quiet. In a short time after the injury he grew unusually clever, and was able to deal with rather difficult problems of everyday life. He was interested in family affairs, sometimes, even too much. But afterwards I noticed his "attacks" of melancholy; so, for instance, when he once arrived home, he found the door shut and after my return he entered the room with me, became angry, started packing his things and threatened me he would leave the house. It was almost impossible to appease him. Such emotional attacks were now reappearing more frequently, and from time to time I had to leave him alone in order to quieten him.

He liked to talk over matters concerned with girls, and he often returned to this topic, but when admonished, he got embarrassed and ceased to touch this problem".

In 1954 he was operated upon because of generalized epileptic seizures which he has been suffering from for one year and which recurred every six weeks. A cerebral scar was totally removed. It extended over both frontal lobes and reached up to the anterior horns of both lateral ventricles. The post-operative course was uneventful. No epileptic fits were observed during two years and a half after the operation. However, he had to give up his studies because everything became suddenly too difficult for him to be done. "While reading" — he reported — "I always felt the text was too difficult to be understood. Some of the lectures I could not understand at all. When my colleagues explained them to me, I felt that all was so simple, but soon thereafter I forgot all. When dealing with a design of a bridge-pier, I felt I could not solve the problem".

His mother gives the following description of his post-operative state: "he complains of a weak memory, is always negligent and in recent time in particular he is frequently leaving his room in constant disorder. He's continually restless, and feels an inner drive for wandering about. He sometimes pays a visit to his wife and child (he is living in separation), but comes back at once every time. He wants to divorce his wife because of her spelling mistakes he finds in her latters, and as he says — because "she doesn't want to learn anything". He has become brusque and explosive, plying easily into passion. He doesn't work and is constantly looking for new job. He is telling everyone about his plans for the future, but he never tries to realize them".

The patient has been observed in hospital for two years and a half after the operation. He shows increased general activity, easily gets into arguments with other patients or the stuff, he writes love-letters to nurses or tries to court them; he is very talkative, particularly as far as his personal plans are concerned. He is not at all embarrassed while telling that he had married his wife only because of a bet with a friend of his. "I married first — as he says — to win a bottle of pure alcohol".

His general knowledge is quite satisfactory. He is able to solve mathematical problems at a middle school level. During the experiments he is restless, frequently quits his chair saying: "excuse my leaving the room for a moment". This happens particularly so when there are difficulties in solving the experimental test. The differentiation of two red lights of different intensity could not be established in spite of 16 presentations of the inhibitory stimulus accompanied by the order: "don't press the bulb".

In the description of the experiment, however, he rightly says that he has seen two kinds of light, "a red one and the other pink, pale". The same experiment was repeated after a 15 min. rest and it turned out that he could distinguish the stimuli, but in spite of helping himself with the aid of exclamations ('not now", "no", "not", "I see clearly", etc.) he made mistakes, performing already learned movements to some inhibitory stimuli.

Similar tests were carried out once again some days later, and the mistakes occurred again, although the patient was already familiar with the principle of the experiment. The test aiming at differentiation of two auditory stimuli took the same course, although such an experiment has already been undertaken previously. It should be mentioned that the patient has shown some kind of over-sensibility to auditory stimuli, and he reported several times: "I don't like sounds in general", or "this buzzing makes me nervous", or again, "it's a torture, as if you were tearing the guts out of me".

Alternation: after 18 presentations of the stimulus the patient asks: "Why to press one time, and cease to do it the other time?". He received instructions but commits errors once more although he turns his palm upwards after having pressed the bulb, for he prefers not to be "tempted" by the next stimulus. Disinhibition has also been observed while differentiating compound stimuli.

DISCUSSION

It may be concluded from the experiments described here, that patients with lesions in prefrontal area, subjected to examination based on motor conditioned reflexes according to Ivanov-Smolensky's method, very often show more or less pronounced disturbances of internal inhibition. In cases showing more remarkable disturbances, the inhibitory conditioned reflexes are established slowly and remain unstable. In cases with slight inhibitory difficulties, the disinhibition can be seen only while using more complicated tests.

In different cases, various inhibition-tests may present for the patient various difficulties. So for instance, the greatest disturbances in a female patient (No. 2) were to be observed while attempting a single differentiation, undoubtedly owing to the fact, that the examined patient showed great inclination towards pressing the bulb and was unable to inhibit this reaction after the inhibitory stimulus had been presented. In other cases, simple differentiation was, however, preserved, but the greatest difficulties arose during differentiation of compound stimuli or alternation.

It should be added that in the majority of patients all types of differentiation could be attained by proper training. In almost all examined persons the inhibitory conditioned reflexes were much better after many repeated experiments than at the beginning, or even they became quite normal.

The most characteristic feature in our patients was that the irregularity of their reactions was undoubtedly due, first of all, to the inhibitory difficulties, whereas the other factors played a minor role, if any whatsoever.

The following arguments are presented herewith to prove it:

1. The patients very quickly got acquainted with the given task and they were able to explain it perfectly, but they made errors despite of it.

2. The strength of the reaction to the inhibitory stimuli was often minor and the reaction itself occurred after a longer latent period than in case positive (excitatory) stimuli have been applied.

3. The exclamations of the patient reacting in an inappropriate manner were the best proof that the patient's error was due, above all, to the fact that he was unable to inhibit a given reaction; the error was neither caused by the incomprehension of the instruction received nor by not-perceiving of the stimulus.

The above mentioned symptoms show that the patients with frontal lobe lesions perform those tests in quite different mode than do patients with another localization of cerebral lesion.

It has been shown by Stępień and others (1954) and also by Selecki (1959) that in cases with lesions in the parieto-temporo-occipital region of the dominant hemisphere the elaboration of excitatory and inhibitory conditioned reflexes is very difficult. Even simple differentiation can be established only after many presentations of inhibitory conditioned stimulus and the inhibitory conditioned reflex is very unstable. The same was true as far as conditioned inhibition and alternation was concerned. A very striking symptom was that the patient was unable to give the verbal relation as to the experiment itself even in the case when the conditioned reflex has been established. The authors explain the symptom observed by the disturbances of the normal activity of the second signal system.

And, as it is to be concluded from the paper by Koźniewska (1959), the lesions of the temporal area result in difficulties in differentiation of various auditory stimuli, mainly compound stimuli.

In our control group of 15 patients with lesions other than of frontal localization, there could be observed either quite normal reactions to inhibitory stimuli or errors amounting to less than 10 per cent as calculated from the total number of negative (inhibitory) stimuli applied during the separate tests (Fig. 3B). It may be seen from Fig. 3 A, that the percentage rate of errors made by the patients with frontal lesions surpasses the 10 per cent limit, which has been accepted as physiologically normal.

Patients, in whom disturbances of inhibitory reaction have been found in experimental conditions, showed also lack of inhibition in their everyday life.

This correlation has been found in four out of five patients who had shown changes in their attitude towards their social environment.

Speaking of localization of functions in our patients, we maintain, that the results obtained do not permit to deduct any conclusions as to the association of the particular disturbances with any definite localization of the lesion within the frontal lobes, because the material under observation was not homogeneous with respect to the localization and extent of the injured area, characteristic features of the disease, etc.

SUMMARY

The purpose of this paper was to investigate the behaviour of patients with uni- or bilateral lesions of the prefrontal area. The investigation was based on the analysis of conditioned reflexes, on clinical observation and on information supplied by persons knowing the patients personally.

It was found that:

1. The excitatory (positive) conditioned reflexes were rather easy to be established in patients, for only in four subjects out of 15, some difficulties had been encountered.

2. The principal disturbance in the majority of the patients (11) was a more or less impaired inhibition, consisting in a motor reaction as a response to a negative (inhibitory) stimulus. The difficulties

were of various degree and they appeared in the course of the particular experiments. During the training of the patient the difficulties diminished or disappeared completely.

The following data are presented to prove that an inappropriate reaction occurs mainly because of difficulties in inhibition of the motor reaction:

a) quick orientation in a given task and its exact, verbal description;

b) changed reaction to the inhibitory stimuli (weaker reaction and longer latent period);

c) exclamations of discontent uttered immediately after the motor reaction to an inhibitory stimulus.

Such features of behaviour could not be ascertained in experimental conditions in patients with lesions other than of frontal localization, examined with the same method.

3. In some patients, when referred to their attitude towards the social life, there have been observed some features that might be related to impaired ability of inhibition (increase in general activity, outbursts of bad temper, increased sexual activity, etc.). On the other hand, there were also patients showing signs of indifference and slow reactions.

4. The material presented in this paper does not entitle the author to associate the particular disturbances with any definite localization of the lesion within the frontal lobes.

REFERENCES

BRUTKOWSKI S., KONORSKI J., ŁAWICKA W., STĘPIEŃ I., STĘPIEŃ L. 1956 — The effect of the removal of frontal poles of the cerebral cortex on motor cônditioned reflexes. Acta Biol. Exper. 17 (2), 167—188.

BRUTKOWSKI S. 1957 — The effect of prefrontal lobectomies on salivary conditioned reflexes in dogs. Acta Biol. Exper. 17 (2), 327—337.

FEUCHTWANGER E. 1923 — Die Functionen des Stirnhirns. Monogr. Neurol. Psychiatr., 38, 194; cited by Denny-Brown D., The Frontal Lobes and their Functions. Modern Trends in Neurology. 1951, Edited by Feiling A. London, Butterwarth & Co (Publishers) Ltd.

FILIPPYCZEWA H. A. 1951 — Naruszenie wzaimodejstwia dwoch signalnych sistem u bolnych s porażeniem łobnych dolej mozga. K fizjołogiczeskomu obosnowaniju nejrochirurgiczeskich operacjej. Akademija Medicinskich Nauk SSSR, Moskwa, 101.

HARLOW J. M. 1868 — cited by Fulton J. P.: Functional Localization in the Frontal Lobes and Cerebellum. London, Oxford University Press, 1949a.

JARVIE H. F. 1954 — Frontal Lobe Wounds Causing Disinhibition. Journal of Neurology, Neurosurgery and Psychiatry, *17*, 14.

JARVIE H. F. 1958 — The Frontal Lobes and Human Behaviour. Lancet, (London), *2* (7042), 365—358.

KOŹNIEWSKA H. 1959 — W sprawie afazji skroniowej. (in print).

ŁAWICKA W. 1957 — The effect of the prefrontal lobectomy on the vocal conditioned reflexes in dogs. Acta Biol. Exper. *17* (2), 318—325.

SELECKI B. 1959 — Disturbances of Speech and some Mechanism of their Spontaneous Compensation in Focal Injuries of the Parieto-temporo-occipital Region of the Dominant Brain Hemisphere (in print).

STĘPIEŃ L., BRZEZIŃSKI J., MEMPEL E., SREBRZYŃSKA J., STĘPIEŃ I. and ZBROŻYNA A. 1954 — Zaburzenia dynamiki wyższej czynności nerwowej w uszkodzeniach ogniskowych styku płata skroniowego, ciemieniowego i potylicznego dominującej półkuli mózgu. Neurologia, Neurochirurgia i Psychiatria Polska. *4*, 5.

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THE EFFECT OF ELECTRICAL STIMULATION OF THE HYPOTHALAMIC FEEDING CENTRE IN SATIATED GOATS ON ALIMENTARY CONDITIONED REFLEXES, TYPE II

W. WYRWICKA, C. DOBRZECKA and **R. TARNECKI**

Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw

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The discovery of the "feeding centre" in the lateral hypothalamic area (Brügger 1943, Anand and Brobeck 1951, Larsson 1954) was of great importance not only for the physiology of feeding but also for the physiology of the higher nervous activity. It was found that the electrical stimulation of the lateral hypothalamus of cats (Grastyán, Lissák and Kékesi 1956) and rats (Miller 1957) elicited the conditioned instrumental reaction connected with food intake. On the other hand the bilateral lesions of the lateral hypothalamic area caused a disappearance of alimentary instrumental conditioned reflexes (i. e. conditioned reflexes type II) in rabbits (Wyrwicka 1957).

In order to learn more about the properties of the hypothalamic feeding centre and its participation in alimentary conditioned reflexes type II, the present study was undertaken.

METHOD

11 adult goats, 1—2 years old, were used for the experiments. 3 of them (No. No. 4, 9 and 11) were castrated males, the others were females. The experiments were performed in a pen which was a kind of box, 3 × 3 m., with wooden floor and walls, 1.5 m. high, without ceiling. 2.5 m. above floor level a horizontal beam was fixed so that the electric wires used for electrical stimulation could be attached. By one of the walls we placed a food-tray arranged in the form of a little table, 50 cm. high, with 8 bowls for food which could automatically be pushed through.

In the majority of goats a conditioned reflex of putting the left foreleg on the food-tray was at first established. The method of elaboration of the reflex was the same as that used in dogs (Konorski and Miller 1933): a passive movement was followed by the immediate giving of a small portion of food (oats mixed with little pieces of carrot) and this was repeated several times. The active movement appeared on the first day of training, after 10—15 trials. Daily training experiments were then carried out for several weeks.

In one goat (No. 9), besides the movement of putting the foreleg on the food-tray elaborated in usual situation, another movement, kneeling down on the floor was established in another experimental situation which was a little room quite different from the pen.

In most goats the conditioned reflex was established to the experimental situation, i. e. each movement was reinforced by food. In 2 animals, No. 4 and No. 7, the learned movement was reinforced only when it appeared to the sound of a whistle and therefore the animal learned to perform it only in the presence of this stimulus. Another acoustic stimulus, a buzzer, was never reinforced and became inhibitory.

After some weeks of the training the operation of inserting electrodes took place. Under local anaesthesia (Polocain 2%) the goat's skull was perforated by means of a dentist's bore and 3 unipolar electrodes were put into the lateral hypothalamus, after Hess' method (Hess 1949), adapted for goats by Andersson (1951) and described in detail by Larsson (1954). The spot on the skull where perforation was made was 2—3 mm. laterally to the crossing of the coronal and sagittal sutures. Electrodes were inserted about 35 mm. down into the brain. An indifferent electrode was attached to the skin just behind the active ones.

Immediately after the operation the animal was brought to the experimental pen and, usually after several minutes, began to perform the learned movements and eat the food given as a reinforcement. The goats trained to the sporadic conditioned stimulus reacted positively to the whistle and inhibitorily to the buzzer. After some time the animal became satiated, ceased to perform the learned movements and went away from the food-tray.

Then the electrodes were connected with a square-wave generator and stimulation began. A current of 0.5 to 1.5 V, of 50 imp./sec. and of 1 m./sec. duration per impulse was used. The areas corresponding to the uninsulated tip of each electrode were stimulated at several levels until the spot which gave the alimentary reaction, when stimulated, was found.

Afterwards the experiment proper began; this is described in "results".

After the experiment with electrical stimulation the animal was immediately sacrificed and its brain was taken for anatomical control. As the places of stimulation were lightly coagulated at the end of the experiment, they were easily found in the preparation. Some goats were not sacrificed but brought to the home pen to recover, and after several months they were used for a second time in a similar experiment, only then sacrificed. The anatomical examination showed that the spot of stimulation were mainly situated just laterally to the area of mammilo-thalamic tract and columna fornicis desc. (Fig. 1).

RESULTS

I. Establishment of an alimentary conditioned reflex type II during hypothalamic stimulation

This series was performed on 2 goats, No. 8 and No. 10, which were not previously trained. Each animal was at first brought for some minutes every day to the experimental pen where it received food. After several days, when the orientation reflex of the animal to the pen had disappeared, the operation of inserting of electrodes was performed as described above. When, after the operation a spot was found which, when stimulated, caused eating in the satiated animal, the following procedure was applied.

In goat No. 8 stimulation was switched on and when the animal came to the food-tray, its left foreleg was passively put on it by means of a cuff tied round the leg and connected with a cord pulled by the experimenter. This movement was immediately reinforced by food. Several seconds after eating the next passive movement was produced and reinforced by food. This was repeated several times during continuous stimulation. It was found that after 12 passive movements an active instrumental reaction appeared. Then, the 14th and 15th movements were almost active, i. e. with slight help of the experimenter. All the succeeding movements were fully active. When the stimulation was interrupted, the goat stopped performing this movement and went away from the food-tray, but when the current was reapplied, the movement of putting the left foreleg on the food-tray appeared again. This was repeated several times.

After this experiment goat No. 8 was not sacrificed. The electrodes were withdrawn and the skin on the animal's head was sutured. The next day when the goat, unfed, was brought into the experimental pen, it came to the food-tray and performed the movements learned during the electrical stimulation. In the following days, this reaction, which was always reinforced by food, became energetic and regular.

In goat No. 10 a somewhat different procedure was applied. After satiation of the animal stimulation was switched on, then the passive movement of putting the left foreleg on the food-tray was produced, food was given and after several seconds, when the portion of food was eaten, stimulation was discontinued. This was repeated 10 times.

An active movement appeared in the 11th trial. Further repetitions of the stimulation evoked only active movements.

The same goat was then called to the wall of the pen, opposite the food-tray. The stimulation was switched on again, the right hind leg of the animal was passively lifted by the experimenter (by means of a cuff attached to a cord), then food was immediately given in a bowl held by the experimenter and after several seconds the stimulation switched off. This was repeated several times. It was found that an active movement of the hind leg appeared in the 12th trial. Further stimulations evoked only the active movements. The goat was then called to the food-tray again and when the stimulation was applied, putting the left foreleg on the food-tray appeared, i. e. the same movement which was previously learned at this particular place.

Goat No. 10 like goat No. 8 was not sacrificed. We found that in the next days the goat, when brought to the experimental pen, at once performed the movement of putting the left foreleg on the food-tray. The movement of lifting the hind leg was not observed. It must be noted however that the movement of the hind leg was much less trained than that of the foreleg; the goat showed always a tendency to go at once to the food-tray where before it had been fed prior to the experiment with electrical stimulation.

To summarize, it was found that instrumental conditioned reflexes can be established in completely satiated goats during electrical stimulation of the feeding centre.

II. Elicitation of an already established instrumental conditioned reflex type II during electrical stimulation of the hypothalamus

In this experiment, goats were used in which a conditioned reflex type II was previously firmly established. The results here described were obtained on 11 animals.

Electrical stimulation of the feeding centre in satiated goats, in which the conditioned reflex was established to the experimental situation, evoked always the learned reaction. The latency of the reaction varied from 3 to 60 sec., being different in each animal. In goat No. 2 the latency of the reaction was particularly long, 30—100 sec. The latency depended probably on several conditions, the strength of the stimulation, degree of satiation, individual characteristics of the animal and the site of electrodes. The greater the voltage, the shorter the latency and the more energetic the reaction; however the current could not be much increased because then general motor reactions appeared which disturbed the learned movement. As to the state of satiation, it should be noted that during the course of stimulation the animal became more and more satiated; because of this the voltage used at the beginning often proved less effective in a latter part of the experiment and, as a result, the reaction appeared after a longer latency. It was observed also that goats which during the training experiments showed high alimentary excitability, reacted during the electrical stimulation with shorter latency than the others. It is probable that the spot of the stimulation was also of great importance in the time of latency, however we are not yet able to analyse this in detail.

Fig. 1. Frontal section through the hypothalamus of goat No. 9

The arrow indicates the spot of electrical stimulation which evoked the instrumental conditioned reaction and food intake in the completely satiated animal.

If the stimulation was prolonged to several minutes, the animal

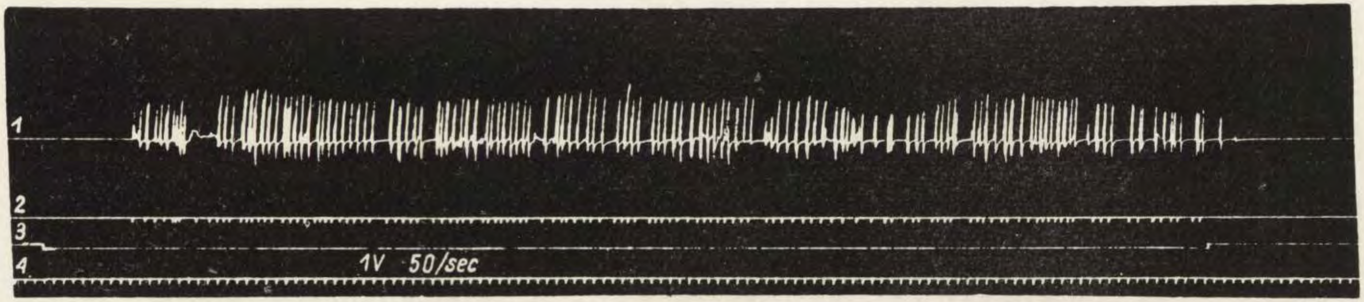

Fig. 2. Elicitation of the instrumental conditioned reaction established previously to the experimental situation, by electrical stimulation of the hypothalamus, in goat No. 5 (kymogram)

1 — conditioned reaction, 2 — food giving, 3 — electrical stimulation, 4 — time every 5 sec. As seen, the conditioned movements appear some seconds after switching on the electrical stimulation (1 V, 50 imp./sec.) and are repeated frequently all the time, so that over 120 movements are performed during 9 min. Every movement is reinforced by a little portion of food which is eaten quickly and voraciously. After stopping the stimulation, the movements cease to appear.

performed the movements all the time; after every movement the given food was eaten, then the next movement followed, etc. (Fig. 2.). The frequency of the movements depended on the same factors as the latency of the reaction. It was observed that after the cessation of a prolonged stimulation sometimes 1 or 2 movements were still performed in spite of the fact that the goat did not take the food presented. The animal then went away from the food-tray.

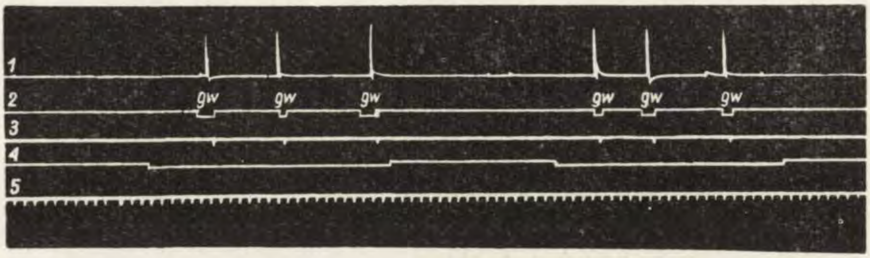

Fig. 3

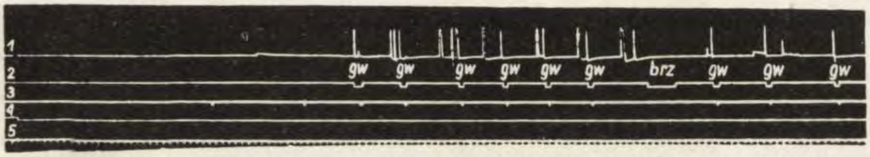

Fig. 4. Elicitation of the instrumental conditioned reaction established previously to a sporadic acoustic stimulus, by electrical stimulation of the hypothalamus on goat No. 4 (Fig. 3) and goat No. 7 (Fig. 4)

1 — conditioned reaction, 2 — conditioned stimulus (Gw — a positive stimulus, the whistle; Brz — an inhibitory stimulus, the buzzer), 3 — food giving, 4 — electrical stimulation, 5 — time every 5 sec. As is seen, the learned reaction appears during the electrical stimulation only when the whistle is applied. There is no reaction to the inhibitory stimulus, the buzzer (Fig. 4).

The behaviour of goats No. 4 and No. 7 in which the conditioned reaction was established to the whistle was somewhat different. When the stimulation was switched on, the animals came to the food-tray but did not however perform the learned movement. Then to the sound of the whistle the goats immediately put the left foreleg on the food-tray, as happened during the usual training experiments (Fig. 3 and 4).

In goat No. 9 in which 2 different movements were established in 2 different situations, stimulation was at first applied in the usual pen, then in the second situation (mentioned in "method"). It was found that in the usual pen the stimulation evoked the putting of the left foreleg on the food-tray, and in the other situation the movement of kneeling down, i. e. in each situation the reaction appeared which had previously been trained in it.

III. Extinction of instrumental conditioned reaction during the electrical stimulation of hypothalamus

When the learned motor reactions evoked by the electrical stimulation were not several times reinforced by food, extinction of these reaction was observed. The experiment with extinction was performed on 6 goats (No. No. 1, 2, 5, 6, 7 and 10).

In goats No. No. 1, 2, 5, 6, and 10, in which the conditioned reflex was established to the experimental situation, extinction of the reflex was conducted in the following way. The stimulation was switched on, and when the few first conditioned movements appeared they were reinforced by food. Then, the movements ceased to be reinforced. At first the reaction grew stronger and the movements appeared more frequently, afterwards they disappeared and the goat went away from the food-tray. However after a while the animal returned and again performed the movements. This was observed in all goats. Going away and returning to the food-tray was repeated usually 1—3 times, then the animal went away and stood far from the food-tray as if it were not interested in food. The kymogram in Fig. 5 illustrates the behaviour of one of the goats in the course of extinction. It should be noted that the course of extinction during the electrical stimulation was very similar to that in normal conditions (cf. Wyrwicka, Dobrzecka and Tarnecki 1959).

When, after the extinction of the conditioned reaction in goat No. 10, the strength of electric current was increased from 1 V to 1.5 V, the learned movements appeared immediately again for some minutes (Fig. 6).

Extinction of the conditioned reflex established to a whistle was carried out in goat No. 7. During a prolonged stimulation the whistle was applied several times for 15 sec. The movements appearing to the whistle were not reinforced by food. It was found that the

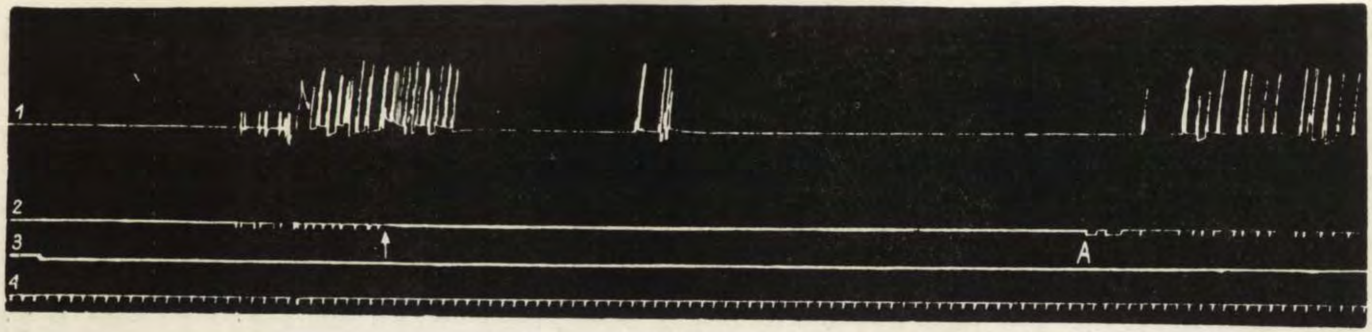

Fig. 5

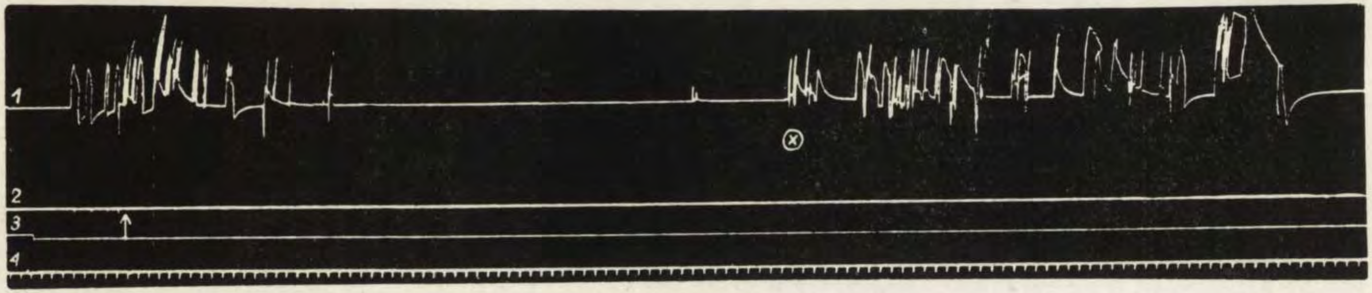

Fig. 6. Extinction of conditioned reaction during the electrical stimulation of the hypothalamic feeding centre in goat No. 5 (Fig. 5) and goat No. 10 (Fig. 6)

1 — conditioned reaction, 2 — food giving, 3 — electrical stimulation, 4 — time every 5 sec. The arrow corresponds ot the moment of withholding of food reinforcement. A, in Fig. 5, indicates the moment in which food is given again. x, in Fig. 6, corresponds to the moment when the stimulation is increased to 1.5 V. As seen in both kymograms, the learned reaction disappears several minutes after the cessation of food giving, in spite of continuous hypothalamic stimulation. The conditioned reaction reappears after the giving of food (Fig. 5) or increasing of the stimulation (Fig. 6).

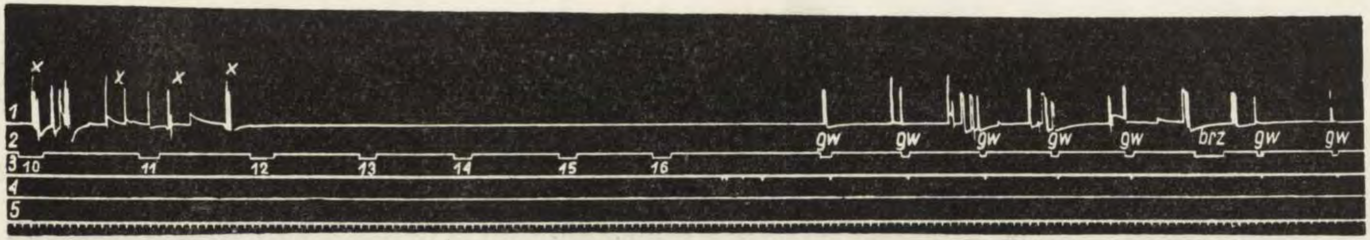

Fig. 7. Extinction and recovery of the instrumental conditioned reaction to the whistle during the electrical stimulation of the hypothalamus in goat No. 7

1 — conditioned reaction, x — untypical movement, 2 — conditioned stimulus (numbers 10 — 16 correspond to successive applications of the whistle without reinforcement, Gw — the whistle, Brz — an inhibitory stimulus, the buzzer), 3 — food giving, 4 — electrical stimulation (continuously lasting and therefore not indicated in this part of kymogram), 5 — time every 5 sec. The conditioned reaction ceases to appear after 11 trials in which food was not given. The instrumental movements however reappear to the whistle as soon as food is given again.

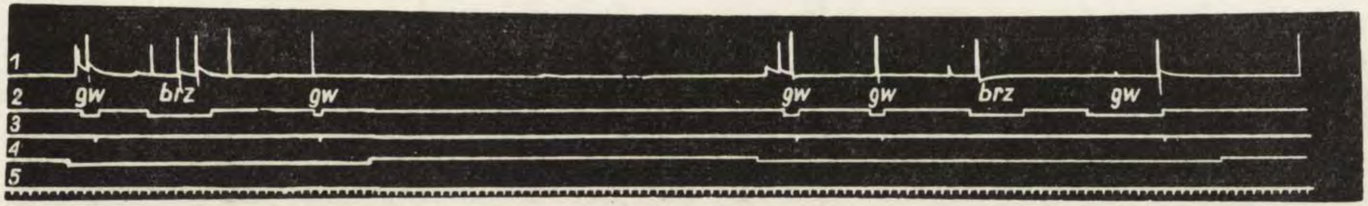

Fig. 8. The effect of increase of electrical stimulation of the hypothalamus on the inhibitory conditioned reflex in goat No. 4

1 — conditioned reaction, 2 — conditioned stimulus (Gw — a positive stimulus, the whistle; Brz — an inhibitory stimulus, the buzzer), 3 — food giving, 4 — electrical stimulation, 5 — time every 5 sec. When stronger electrical stimulation, of 1.5 V, is applied, the learned reaction appears also to an inhibitory stimulus, the buzzer.

learned movement disappeared already after 4 trials. Then, in the 6th and 7th trial the goat returned to the food-tray and again performed the movement. At the same time anxiety was observed in the goat. The last conditioned movement appeared in the 11th trial and then, during the 5 successive applications of the whistle, the animal did not come to the food-tray standing at the opposite wall of the pen. The course of the extinction during the stimulation was quite similar to that in normal conditions in the same goat.

The recovery of the conditioned reflex was accomplished during continued stimulation by giving food. This is indicated in Fig. 7 after the 16th application of the whistle. As is seen, the first application of the whistle after eating, caused the return of the learned movement. Recovery of the extinguished reflex in other goats was also performed by the giving of food during stimulation (Fig. 5).

IV. The reaction to an inhibitory conditioned stimulus applied during the electrical stimulation of the hypothalamus

In 2 goats, No. 4 and No. 7, in which an alimentary conditioned reflex to the whistle was elaborated, the sound of a buzzer was applied, always without reinforcement, for 15 sec. twice a day in both goats. At the beginning, the learned movement appeared always to the buzzer. However, after about 70 applications of the buzzer the conditioned reaction ceased to appear and the goats, to the sound of the buzzer, went away from the food-tray. So the buzzer became an inhibitory stimulus. It was applied over 100 times in each goat.

Then, the same inhibitory stimulus was applied during the electrical stimulation of the feeding centre in both goats. It was found that the reaction of both animals to it was the same as in normal conditions, i. e. the goats went away from the food-tray and stood quietly until the buzzer ceased (Fig. 4). When, however, stronger electric current was used in goat No. 4, the instrumental reaction appeared also during the action of the inhibitory stimulus (Fig. 8).

V. The external inhibition during the electrical stimulation of the hypothalamus

It was found, in all the goats, that the instrumental conditioned reaction elicited by electrical stimulation of the feeding centre was inhibited by such external stimuli as e. g. opening the door, appearance of a new person, the noise of the film apparatus etc. In such cases the animal turned towards the source of the stimulus, i. e. showed the usual orientation reflex and ceased to perform the learned movements as well as refused to eat food.

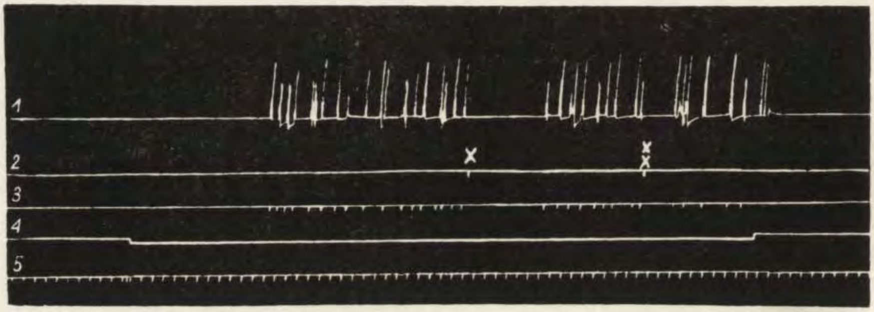

Fig. 9

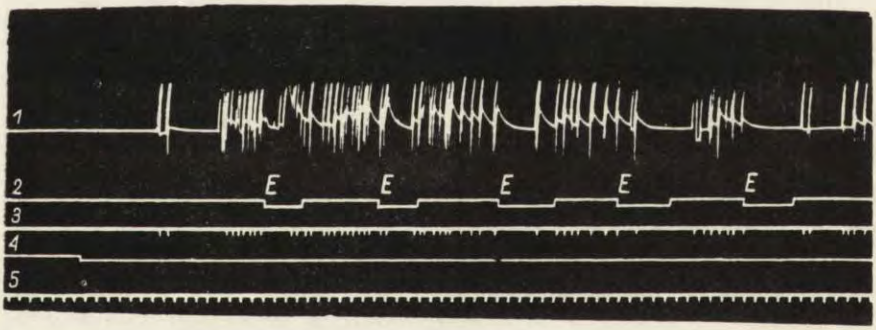

Fig. 10. The effect of external stimulus to the conditioned reactions evoked by electrical stimulation of the hypothalamus in goat No. 5 (Fig. 9) and goat No. 10 (Fig. 10)

1 — conditioned reaction, 2 — external stimulus (X — drawing a curtain aside, $\frac{x}{x}$ — drawing a curtain back, E — a tone), 3 — food giving, 4 — electrical stimulation, 5 — time every 5 sec. As seen in both kymograms, application of external stimulus each time inhibits the conditioned reaction elicited by electrical stimulation of the hypothalamus.

The kymogram in Fig. 9 shows the effect of an extraneous stimulus on the conditioned reaction of goat No. 5. When, during the stimulation, the animal performed conditioned movements and ate food given after each movement, then a window curtain was suddenly drawn aside. The goat reacted by jumping back from the food-tray to the opposite wall of the pen. The animal returned to the food-tray only after 35 sec. in spite of continuous stimulation. When, 50 sec. later, the same curtain was drawn back, the inhibition of the reaction lasted only 15 sec.

In goat No. 10 an external stimulus, a tone, was several times applied during prolonged electrical stimulation of the hypothalamus. Application of the tone each time suppressed the conditioned reaction elicited by the stimulation; however the learned movements reappeared as soon as the tone was discontinued.

DISCUSSION

As was shown in the present paper, the whole course of a normal experiment with alimentary instrumental conditioned reflexes could be reproduced in completely satiated goats by electrical stimulation of the hypothalamic feeding centre. This seems to support our previous supposition that the alimentary instrumental conditioned reaction is dependent on the excitation of a hypothetical alimentary centre (Wyrwicka 1952). It was found, however, that, in spite of unchanged continuous electrical stimulation of the same spot of the hypothalamus, the conditioned reaction could be inhibited by 1) cessation of food giving as reinforcement of learned movements, 2) intertrial intervals in those animals which were trained to react only to the sporadic conditioned stimulus, 3) action of the inhibitory conditioned stimulus, and 4) extraneous stimuli. Moreover if 2 different instrumental reactions were established in the animal in 2 different experimental situations the electrical stimulation of the same spot evoked in each situation only that movement which was previously trained in it. Similarly, during the stimulation of the same point 2 different movements could be learned in 2 different environmental backgrounds.

All this can help us to understand better the role played by the hypothalamic feeding centre in the alimentary conditioned reflex type II. Our results indicate that the feeding centre situated in the lateral hypothalamic area is a centre responsible for the "state of alimentary excitability". In normal conditions, in a hungry animal, this state is produced both by humoral factors affecting directly the feeding centre and by impulses reaching it from internal organs. In a satiated animal which refuses to take food the same state is brought about by electrical stimulation of the hypothalamic feeding centre.

For the sake of simplicity, let us name the state of the feeding centre, produced by electrical stimulation or by internal factors, the "supraliminal level" of alimentary excitability. The "supraliminal level" itself however is not sufficient to evoke the instrumental conditioned reaction. In experiments with electrical stimulation as well as in normal conditions the conditioned stimuli must also be present, which have been connected by previous training with the act of feeding. Owing to the connections established between the centres of conditioned stimuli and the feeding centre, the former excite the feeding centre and through it the centre of the instrumental reaction thus eliciting the learned movement (Wyrwicka 1952).

However the conditioned stimuli are effective only when the "supraliminal level" of excitability is already reached.

When during the electrical stimulation of the hypothalamus or in normal hungry state an inhibitory stimulus is applied which has not been connected with feeding, excitation of the feeding centre does not occur and the instrumental reaction is absent. The state of affairs is somewhat different when the instrumental reaction is extinguished by non-reinforcement; in this case the connections of the centres of conditioned stimuli with the feeding centre become "weaker" and the conditioned stimuli are no longer able to excite the feeding centre to the suitable level.

The "supraliminal level" of alimentary excitability of the feeding centre may however vary. When the state of hunger is very high or electrical stimulation of the hypothalamic feeding centre is increased then the threshold of elicitation of instrumental reaction is lowered; in consequence the animal performs learned movements in intertrial intervals as well as to the inhibitory stimulus (Fig. 8). Similarly, the learned reaction extinguished during the electrical stimulation od 1 V, reappears immediately when the current is increased to 1.5 V (Fig. 6).

As to the inhibition of the instrumental reflex during the electrical stimulation by the external stimuli, the explanation is as follows.

In experiments with the electrical stimulation just as in the usual ones, the extraneous stimuli can produce an antagonistic excitation which suppresses the effect of the conditioned stimuli on the feeding centre. In consequence the learned reaction ceases to appear for some time.

Summarizing, we may conclude that the feeding centre situated in the lateral hypothalamic area is one of the fundamental parts of the pathway of alimentary conditioned reflex type II.

SUMMARY

1. The electrical stimulation of the hypothalamic feeding centre in completely satiated goats a) made possible the establishment of an alimentary conditioned reflex type II in formerly untrained animals, and b) elicited a previously established alimentary conditioned reflex type II.

2. The instrumental conditioned reaction elicited by electrical stimulation appeared only to those stimuli (including experimental situation) to which it had been established before.

3. The instrumental reaction elicited by electrical stimulation became extinguished if food reinforcement was withheld.

4. The inhibitory conditioned stimulus applied during the electrical stimulation elicited an inhibitory reaction, as in previous training.

5. Increasing the stimulating current abolished the inhibition of the instrumental reaction.

6. Extraneous stimuli could inhibit the instrumental reaction elicited by electrical stimulation of the feeding centre.

The authors are greatly indebted to Prof. J. Konorski for his interest in this work and critical reading of the manuscript.

REFERENCES

ANAND B. K. and BROBECK J. R. 1951 — Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med., *24*, 123—140.

ANDERSSON B. 1951 — The effect and localization of electrical stimulation of certain parts of the brain stem in sheep and goats. Acta Physiol. Scand., *23*, 8—23.

BRÜGGER M. 1943 — Fresstrieb als hypothalmisches Symptom. Helv. physiol. pharmacol. acta, *1*, 183—198.

GRASTIÁN E., LISSÁK K. and KÉKÉSI K. 1956 — Facilitation and inhibition of conditioned alimentary and defensive reflexes by stimulation of the hypothalamic and reticular formation. Acta Physiol. Hung. *9*, 133—151.

HESS W. R. 1949 — Das Zwischenhirn. Basel.

KOŇORSKI J. and MILLER S. 1933 — Podstawy fizjologicznej teorii ruchów nabytych (Bases of the physiological theory of acquired movements). Polish text with a French summary. Warsaw.

LARSSON S. 1954 — On the hypothalamic organization of the nervous mechanism regulating food intake. Acta Physiol. Scand. *32*, suppl. 115.

MILLER N. E. 1957 — Experiments on motivation: Studies combining psychological, physiological and pharmacological techniques. Science. *126*, 1271—1278.

WYRWICKA W. 1952 — On the mechanism of the motor conditioned reaction. Acta Biol. Exper. *16*, 131—137.

WYRWICKA W. 1957 — Wstępne badania nad wpływem uszkodzeń podwzgórza na pokarmowe ruchowe odruchy warunkowe u królików (The effect of hypothalamic lesions on alimentary motor conditioned reflexes in rabbits). Polish text. Acta Physiol. Pol., *8*, 575—576.

WYRWICKA W., DOBRZECKA C. and TARNECKI R. 1959 — On the instrumental conditioned reaction evoked by electrical stimulation of the hypothalamus. Science. *130*, 336—337.

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AN EXPERIMENTAL APPROACH TO THE PROBLEM OF MECHANISM OF ALIMENTARY CONDITIONED REFLEX, TYPE II

W. WYRWICKA

Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw

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When a given movement performed by the animal is followed by presentation of food (or any other "rewarding" agent) then it tends to be repeated again and again till the animal gets satiated. On the contrary, if the movement is followed by some noxious stimulus ("punishment"), its performance tends to be suppressed. These well known types of behaviour were first subjected to precise experimental investigation by Thorndike (1911) in the turn of the century. Only many years later, however, Konorski and Miller (Miller et Konorski 1928, Konorski i Miller 1933, Konorski 1948) made an attempt to analyse these responses from the physiological point of view and to elucidate their neural mechanism. They have shown that the behaviour based on "reward" or "punishment" can be expressed in a rigorous conditioned reflex form, i. e. as definite acquired reactions elicited by definite stimuli. They have also shown that the physiological structure of these reflexes is different from, and more complicated than that of classical Pavlovian conditioned reflex. Therefore they denoted this form of conditioned reflex as type II in contradistinction to type I, i. e. Pavlovian conditioned reflex. Similar responses were later called operant behaviour by Skinner (1938) and istrumental responses by Hilgard and Marquis (1940).

In recent years we have been engaged in studying the intercentral structure of conditioned reflexes type II, using the same general experimental procedure as that applied by Konorski and Miller. We were concerned with alimentary conditioned reflexes type II and were not interested so far in the process of their elaboration but in their mechanism after they had already been firmly established. We were also not concerned with the problem of the maintenance of these reflexes and with the signalling nature of the proprioception of the movement performed, which was studied in detail by Konorski and Miller (1933, 1936). And so we exclusively concentrated on the problem of what is going on in nerve centres involved between the moment of application of a conditioned stimulus and the moment of appearance of the instrumental reaction. As our results seem to throw some light on this problem we present here in short all our data collected so far, which were partly published in detail before (Wyrwicka 1952, 1956, 1958).

It is well known that an alimentary conditioned reflex type II reaches its full value only in a hungry animal which shows a sufficiently high level of alimentary excitability. It was found that the instrumental reactions were diminished or completely absent when the experiment took place several hours earlier than usual or when the animal was satiated just before the experiment, i. e. when its alimentary excitability was reduced (Skinner 1938, Skipin 1947, Wyrwicka 1950).

Therefore if we consider the conditioned reflex a process brought about by the acquired connections between certain brain centres the conclusion may be drawn that the pathway of the instrumental reflex goes through a hypothetical alimentary centre. A corresponding scheme may be written as in Fig. 1. In this scheme S is the centre corresponding to a compound: a sporadic conditioned stimulus

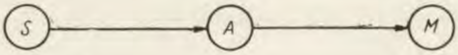

Fig. 1. Scheme I of supposed connections between the centre of conditioned stimulus (S), the alimentary centre (A) and the centre of instrumental reaction (M), active in the course of alimentary conditioned reflex type II.

and its situational background (experimental situation), or an experimental situation alone when sporadic stimulus is not used; A, represent the alimentary centre considerd as a compound of centres responsible for a state of "alimentary excitation"; M, is the centre of instrumental reaction including all motor and sensory centres responsible for accomplishment of this reaction.

The above scheme allows us to understand and even predict the following experimental facts:

1. In dogs, not previously used in experiments, the alimentary instrumental conditioned reflex of putting the foreleg on the food-tray was established to an acoustic stimulus. Then a new stimulus, the smell of ether, was introduced and, in spite of absence of the learned instrumental reaction, food reinforcement was given after 10 sec. After several daily experiments during which the smell of ether reinforced by food was applied twice daily, a salivary reaction at first was obtained and then the learned movement "spontaneously" appeared. In the following days the instrumental reaction to the olfactory stimulus became the same as that to the acoustic stimulus. In the same way the conditioned instrumental reaction to visual and tactile stimuli was established in these and other dogs.

According to our scheme these facts can be explained as follows. Reinforcement of the new stimulus by food causes at first the establishment of the connection S → A (Fig. 1) so that the new stimulus raises the excitation of the alimentary centre A; the latter having been connected by previous training in the same situation with the centre M, sends excitatory impulses to it and the instrumental reaction is evoked.

2. An alimentary conditioned reflex of putting the foreleg on the food-tray was established in dogs (as well as in goats and rabbits) to the experimental situation; every learned movement performed in this situation was reinforced by food. After some days of training the extinction of the reaction was performed, i. e. the learned movement ceased to appear as a result of non-reinforcement by food. When, afterwards, a portion of food was given, the instrumental reaction reappeared immediately.

It may be supposed that the lack of food reinforcement in the course of extinction makes the connection S → A "weaker", so that the conditioned stimulus (the experimental situation in this case) is no longer capable of raising the excitation of the alimentary centre to a suitable level. Giving food after the extinction renews the connection S → A and then the instrumental reaction can again be evoked through the alimentary centre.

3. In a dog which, on account of heat, showed a very low alimentary excitability, the conditioned stimulus was without effect, i. e. the dog did not perform the learned movement to it. When, in the absence of the instrumental reaction, the food reinforcement was given each time to the conditioned stimulus, the learned movement appeared after several trials.

This fact may be explained as follows. Giving food raises the excitation of the alimentary centre and this leads to a rise of excitation of the centre of the learned movement; as a result the instrumental reaction appears.

4. The movement of putting the foreleg on the food-tray to the loud bubbling of water was always reinforced by food while the same reaction to soft bubbling was never reinforced. In consequence the soft bubbling became an inhibitory differential stimulus which did not evoke the learned movement. When, however, the inhibitory stimulus had been reinforced by food several times, it evoked the instrumental conditioned reaction though this reaction was delayed and smaller than to the positive stimulus. After several days of consistent reinforcement the soft bubbling elicited a conditioned reaction of normal value (Wyrwicka 1952). A similar experiment was also performed with other inhibitory stimuli, e. g. with so called "primary inhibitor", i. e. a stimulus different to the others and not reinforced from the very beginning (Konorski and Szwejkowska 1952), and the same result was obtained.

In these cases the connection S → A is inactive on account of non-reinforcement of the stimulus by food (Konorski 1948). When we change the procedure and reinforce the inhibitory stimulus, the connection S → A is renewed (or arises) and therefore this stimulus can excite the alimentary centre and through it the centre of the instrumental reaction.

The existence of the connection S → A → M was also shown with other methods of investigation. As is known a centre of food intake was discovered in the lateral hypothalamic area (Brügger 1943, Anand and Brobeck 1951, Larsson 1954). The properties of this centre suggest that it corresponds to our alimentary centre.

According to our scheme, destruction of the feeding centre resulting in aphagia (Anand and Brobeck, 1951) should abolish all alimentary conditioned reflexes in the animal. Such experiments (Wyrwicka 1957) were performed on 5 rabbits in which 2 instrumental conditioned reflexes were established (i. e. pushing a ring with teeth, reinforced by carrot, and scraping the food-tray with forelegs, reinforced by potatoes). Bilateral coagulation of the lateral hypothalamic area resulted in aphagia lasting 1—2 weeks and in the complete disappearance of both conditioned reflexes for a longer time. In one rabbit the conditioned reflexes did not reappear at all for 3 months, after which time the animal was sacrificed.

Our scheme suggests on the other hand that electrical stimulation of the feeding centre, which causes hyperphagia (Delgado and Anand 1953, Larsson 1954) should excite the centre M and thus evoke the instrumental reaction. Corresponding experiments were performed on unanaesthesized goats (Wyrwicka, Dobrzecka and Tarnecki, 1959). Using Hess' method (Hess 1949) adapted for goats by Andersson (1951), weak stimulation (about 1 V, 50 imp./sec.) of the lateral hypothalamic area, i. e. the feeding centre, in completely satiated goats evoked the previously established conditioned reaction of putting the left foreleg on the food-tray and eating food given as reinforcement. A prolonged stimulation evoked learned movements and eating alternately up to interruption of the stimulation. Similar stimulation of the hypothalamic "drinking centre" (Andersson 1952, 1953) evoked the instrumental reaction which in previous training was reinforced by water (Andersson and Wyrwicka, 1957). Analogous facts were described by Miller (1957). Grastyán, Lissák and Kékési (1956) were the first investigators to obtain alimentary instrumental conditioned reactions by electrical stimulation of some parts of the hypothalamus of the cat. These authors however interpreted the finding as unspecific facilitation of the instrumental conditioned reflex by the electrical stimulation.

The scheme presented in Fig. 1 concerns the case when only one instrumental reaction is established in the animal in a constant experimental situation. It cannot explain and predict the behaviour of an animal trained to perform various movements to various stimuli, e. g. to raise the foreleg to a visual stimulus and the hind leg to an acoustic stimulus; such procedures were applied by Voronin (1948), Norkina (1956), Wyrwicka (1958) and others.

It is also very well known that an animal in free surroundings is able to perform many varied movements connected with finding food in various circumstances and different seasons. If merely S→A→M connection exists, every conditioned stimulus connected with feeding should evoke all these movements. Yet this is not so. Therefore it is reasonable to admit that there is also a direct connection S→M (Fig. 2). If we take into account that, according to the principle of formation of the conditioned connections, the latter arise between all the brain centres excited at the same time, the relation S→M seems to be quite natural because the instrumental movement is performed during the action of the conditioned stimulus.

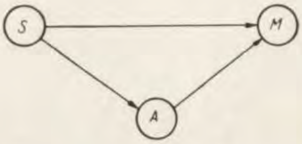

Fig. 2. Scheme II of probable interrelations between brain centres in the course of an alimentary instrumental conditioned reflex

S — centre of conditioned stimulus; A — alimentary centre; M — centre of instrumental reaction

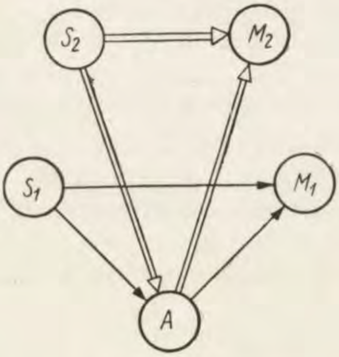

Fig. 3. Schematic representation of probable connections between brain centres in the case of 2 different instrumental conditioned reflexes

S_1, S_2 — centres of conditioned stimuli; A — alimentary centre; M_1, M_2 — centres of learned movements.

Admitting the existence of this connection we can easily explain the appearance of two different movements to two different conditioned stimuli respectively (Fig. 3). Each stimulus elicits only that movement which is directly connected with it by training. The assumption of the direct connection S→M is supported by the following experiments.

1. An alimentary instrumental conditioned reflex of putting the right foreleg on the food-tray to an acoustic stimulus was established in dogs in the usual experimental chamber. When the same stimulus was applied in an empty room quite unlike the chamber, the instrumental reaction did not appear; even when the acoustic stimulus was reinforced several times by pieces of meat which were eaten voraciously by the dog, the learned movement was not elicited and even a simple raising of the right foreleg was not observed. Only when the food-bowl was placed on a low bench somewhat similar to the food-tray, did the instrumental reaction appear (Wyrwicka 1958).

This result indicates that the instrumental reaction cannot be evoked merely by excitation of the alimentary centre (caused by food). Other components of the traininig situation are also needed. This seems to support the view about the direct connection between the centre of conditioned stimuli and the centre of the instrumental reaction.

2. Dogs were trained for about a year to put their right forelegs on the food-tray to various stimuli and the reaction was very firmly established. The animals were completely satiated before entering the experimental chamber and the conditioned stimuli were tested. It was found that the acoustic stimuli which were considered as "strongly motogenic" (Konorski and Wyrwicka 1952) evoked the instrumental reaction in spite of the fact that the animals refused to eat food (Wyrwicka 1950).

This finding shows that the learned movement can appear as a result of the direct connection S→M alone, since lack of alimentary excitation has made the connection S→A→M inactive.

3. In a dog the instrumental conditioned reflex of putting the foreleg on the food-tray was established to 3 stimuli: tactile stimulus applied to the same leg, tactile stimulus applied to the dog's back and sound of metronome. Then the animal was completely satiated and all the stimuli were tested. It was found that the metronome and the tactile stimulus on the back evoked the conditioned reaction in the first trials only and then were without effect, while the tactile stimulus applied to the paw elicited putting this leg on the food-tray much more permanently. However the learned movement to this stimulus was more and more delayed and finally ceased to appear. The animal refused to take food during the whole experiment despite the fact that it was presented after each application of the

conditioned stimulus (Dobrzecka and Wyrwicka). It must be stressed that the appearance of the reaction cannot be considered as a defensive reflex to the tactile stimulus on the leg because the movement elicited was the previously learned complexed movement of putting the leg on the food-tray and not a simple raising leg.

This result indicates that the appearance of the learned movement of the leg to a tactile stimulus acting on the same leg does not depend on the high excitability of the alimentary centre. This fact is easy to understand: the impulses from the tactile stimulus excite the centre of this stimulus which is localized in the area close to the motor centres of the same leg; therefore there is a natural facilitation of the conditioned movement and a preponderance of the direct pathway S→M over the indirect one S→A→M.

To summarize: Impulses generated by the centre of the conditioned stimulus run to the centres connected with it by training, i. e. the alimentary centre as well as the centre of the learned reaction. In turn the alimentary centre sends impulses to all centres connected with it, among them to the centre of the learned movement which already is partly excited. Only then does the excitation of the centre of the learned movement reach a supraliminal level and the motor reaction appears. The impulses running through the indirect pathway S→A→M thus give the general "direction" of the reaction, i. e. alimentary in our case; however the final determinant of which movement is performed depends on the excitation of the direct pathway S→M. As we see, however, the two connections under discussion do not always function equally and simultaneously, and under certain circumstances one of them can predominate.

SUMMARY

The present paper concerns the probable functional connections between some brain centres, necessary to evoke the alimentary instrumental conditioned reflex already established in an animal. The theoretical considerations are illustrated by experimental data.

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REFERENCES

ANAND B. K. and BROBECK J. R. 1951 — Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med. *24*, 123—140.

ANDERSSON B. 1951 — The effect and localization of electrical stimulation of certain parts of the brain stem in sheep and goats. Acta Physiol. Scand. *23*, 8—23.

ANDERSSON B. 1952 — Polydipsia caused by intrahypothalamic injections of hypertonic NaCl solutions. Experientia. *8*, 157.

ANDERSSON B. 1953 — The effect of injections of hypertonic NaCl solutions into different parts of the hypothalamus of goats. Acta Physiol. Scand. *28*, 188—201.

ANDERSSON B. and WYRWICKA W. 1957 — The elicitation of a drinking motor conditioned reaction by electrical stimulation of the hypothalamic "drinking area" in the goat. Acta Physiol. Scand. *41*, 194—198.

BRÜGGER M. 1943 — Fresstrieb als hypothalamisches Symptom. Helv. physiol. pharmacol. acta. *1*, 183—198.

DELGADO J. M. and ANAND B. K. 1953 — Increase of food intake induced by electrical stimulation of the lateral hypothalamus. Amer. J. Physiol. *172*, 162—168.

DOBRZECKA C. and WYRWICKA W. To be published.

GRASTYÁN E., LISSÁK K. and KÉKÉSI K. 1956 — Facilitation and inhibition of conditioned alimentary and defensive reflexes by stimulation of the hypothalamic and reticular formation. Acta Physiol. Hung. *9*, 133—151.

HESS W. R. 1949 — Das Zwischenhirn. Basel.

HILGARD E. R. and MARQUIS D. C. 1940 — Conditioning and learning. New York.

KONORSKI J. 1948 — Conditioned reflexes and neuron organization. Ch. XII. Cambridge University Press.

KONORSKI J. i MILLER S. 1933 — Podstawy fizjologicznej teorii ruchów nabytych. (Bases of the physiological theory of acquired movements). Polish text with a French summary. Warszawa.

KONORSKI J. i MILLER S. 1936 — Uslovnye refleksy dvigatelnogo analizatora. Trudy Fizjol. Labor. Pavlova. *6*(1), 119.

KONORSKI J. and SZWEJKOWSKA G. 1952 — Chronic extinction and restoration of conditioned reflexes. IV. Acta Biol. Exper. *16*, 95—113.

KONORSKI J. i WYRWICKA W. 1952 — Hamowanie następcze w odruchach warunkowych analizatora ruchowego. (Inhibitory after-effect in conditioned reflexes of the motor analyser). Polish text with an English summary. Acta Physiol. Pol. *3*, 63—84.

LARSSON S. 1954 — On the hypothalamic organization of the nervous mechanism regulating food intake. Acta Physiol. Scand. *32*, suppl. 115.

MILLER N. E. 1957 — Experiments on motivation: Studies combining psychological, physiological and pharmacological techniques. Science. *126*, 1271—1278.

MILLER S. et KONORSKI J. 1928 — Sur une forme particulière des reflexes conditionnels. C. R. Soc. Biol. *99*, 1155.

NORKINA L. N. 1956 — Dinamika processa differencirovania dvigatelnih uslovnih refleksov. (Dynamics of the process of differentiation of the motor conditioned reflexes). Żurn. Vys. Nerv. Dieiat. *6*, 710—717.

SKINNER B. F. 1938 — The behaviour of organisms. New York.

SKIPIN G. W. 1947 — O mehanizme obrazovania uslovnih piščevyh refleksov. Moskva.

THORNDIKE E. L. 1911 — The animal intelligence. New York.

VORONIN L. G. 1948 — Analiz i sintez složnyh razdražitelei normalnymi i povreždennymi polušariami golovnogo mozga sobaki. Moskva.

WYRWICKA W. 1950 — The effect of diminished alimentary excitability upon conditioned reflexes of the second type. Acta Biol. Exper. *15*, 205—214.

WYRWICKA W. 1952 — On the mechanism of the motor conditioned reaction. Acta Biol. Exper. *16*, 131—137.

WYRWICKA W. 1956 — On the effect of experimental situation upon the course of motor conditioned reflex. Acta Biol. Exper. *17*, 189—203.

WYRWICKA W. 1957 — Wstępne badania nad wpływem uszkodzeń podwzgórza na pokarmowe ruchowe odruchy warunkowe u królików. (The effect of hypothalamic lesions on alimentary motor conditioned reflexes in rabbits). Acta Physiol. Pol. *8*, 575—576.

WYRWICKA W. 1958 — Studies on the effects of the conditioned stimulus applied against various experimental background. Acta Biol. Exper. *18*, 175—193.

WYRWICKA W., DOBRZECKA C. and TARNECKI R. 1959 — On the instrumental conditioned reaction evoked by electrical stimulation of the hypothalamus. Science. *130*, 336—337.

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TRANSFORMATION OF DIFFERENTIATED INHIBITORY REFLEXES OF TYPE II INTO EXCITATORY REFLEXES

G. SZWEJKOWSKA

Department of Neurophysiology,
Nencki Institute of Experimental Biology, Warsaw

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In our previous paper it was established that if the differentiated conditioned stimuli are transformed into positive conditioned stimuli, the transformation occurs more easily for those stimuli which are more similar to their positive counterpart. These results were based on the experiments with type I salivary conditioned reflexes. According to the experimental evidence obtained in this laboratory (Wyrwicka 1952) the structure of the conditioned reflex type II is more complicated than that of type I, and therefore it was not quite certain whether the same results would be obtained with reflexes of the II type. For this reason the same problem was reinvestigated on conditioned reflexes of the II type.

MATERIAL AND METHOD

The experiments were performed on three dogs in which type II alimentary conditioned reflexes were established. The motor conditioned reaction consisted in lifting the right foreleg and putting it on the foodtray situated before the dog. In one dog the positive conditioned stimulus was a bell (B_1) and the differentiated stimuli were a bell of a different sound (B_2) and a buzzer (B_z). In two other dogs tones of different pitch were used. The tone of 600 Hz (T_1) was positive and two lower tones of 500 Hz (T_2) and of 200 Hz (T_3) were differentiated.

Each experiment consisted of 7 trials; 5 of them were positive and 2 negative. Both negative stimuli were applied in each experiment, usually in the third and sixth place, each day in the reverse sequence. The isolated period of positive conditioned stimuli lasted about 1—2 sec., i. e. immediately after the animal performed the trained movement, the reinforcement was presented. Differentiated stimuli lasted 5 sec. and were not reinforced. The intertrial intervals were 2—3 min.

When the differentiation was firmly established to both differential stimuli, a series of experiments with transformation was started. It consisted in presentation of all the stimuli each day in the same sequence as in the preceding series, but the two differentiated stimuli were now reinforced by food. According to the evidence presented by W. Wyrwicka (1952) such procedure leads normally to the establishment of the instrumental conditioned reflex to new stimuli, as well as to the inhibitory stimuli, without special training. If the animal did perform the trained movement to the differentiated stimulus, even in an abortive form (flexion of the leg without putting it on the foodtray), food was immediately presented. If the animal did not perform the movement, food was given in the third second of the action of the stimulus. As the normal latent period to the positive conditioned stimuli was not longer than one second, such time was quite sufficient for the dog to perform the movement.

RESULTS

Dog No 1. In this dog B_1 was a positive conditioned stimulus whereas B_2 and B_z were differentiated. The series of experiments with differentiation lasted more than 4 months; during that time B_1 was applied 510 times, and B_2 and B_z were applied 100 times each.

The course of differentiation is presented in Fig. 1. We see that differentiation of the buzzer was very prompt. After 4 applications of this stimulus the dog already ceased to perform the trained movement and the differentiation proved to be stable; only once (exp. 25) did the animal perform the trained movement to the buzzer.

The differentiation of B_2 was more difficult. Only after 11 experiments the animal began to inhibit the motor reaction: either the trained movement was performed with a prolonged latent period (1 —1.5 sec. instead of 0.5 — 1.0 sec. to the positive stimulus), or he lifted the leg to various heights without putting it on the foodtray, or he put the leg on the foodtray and then immediately took it off. At the same time the dog became restless and the conditioned reaction to the positive stimulus B_1 was sometimes slower than normally. After about 40 experiments the animal stopped performing the trained movement to B_2 and his behaviour became normal.

In the 101st experiment we began to transform both differentiated stimuli into positive conditioned stimuli. As seen in Fig. 1 the difference between the transformation of the reflex to B_2 and to B_z was very pronounced. As far as stimulus B_2 is concerned the positive reaction to it appeared in the very first experiment after stimulus B_2 was reinforced by food. In following experiments the movement to B_2 was performed in every trial, although in most cases it was defective: either the dog did not put the leg on the foodtray, or he touched it slightly and then put it immediately on the floor. Even in those cases in which a fullsized movement was performed it differed considerably from that performed to B_1: the dog lifted his leg slowly, as if with hesitation, and sometimes put it on the foodtray only after presentation of food. To sum up, the transformation of B_2 into the excitatory stimulus occurred immediately, but till the end of the series (50 experiments) the motor reaction remained incomplete.

The transformation of the second inhibitory stimulus, the buzzer, took a different course. The motor reaction to it appeared first only at the 11th application of the buzzer with reinforcement. Then the movement appeared in some trials but even then it was usually abortive (lifting of the leg without even touching the foodtray). At the end of the series the animal stopped performing the movement at all. The application of a new stimulus with reinforcement led to the formation of the normal motor conditioned reflex after a few trials.

Dog. No 2. In this dog the tone of 600 Hz (T_1) was positive, while the two lower tones, 500 Hz (T_2) and 200 Hz (T_3) were differentiated. As seen in Fig. 1 the differentiation of these two tones was very slow. Till the 22nd experiment the dog performed the movement with both stimuli although the movement to T_3 was sometimes incomplete. Then the dog began to inhibit the motor reaction to T_2 and to T_3, the differentiation to T_3 being slightly easier than to T_2. During the course of differentiation the dog was very excited and when he did not receive food to a negative stimulus, he repeated the movement again and again scratching the foodtray and licking the empty bowl. Only after about 50 trials he became quiet again.

From the 101st experiment both differentiated tones were reinforced by food. As seen in Fig. 1, after one reinforcement

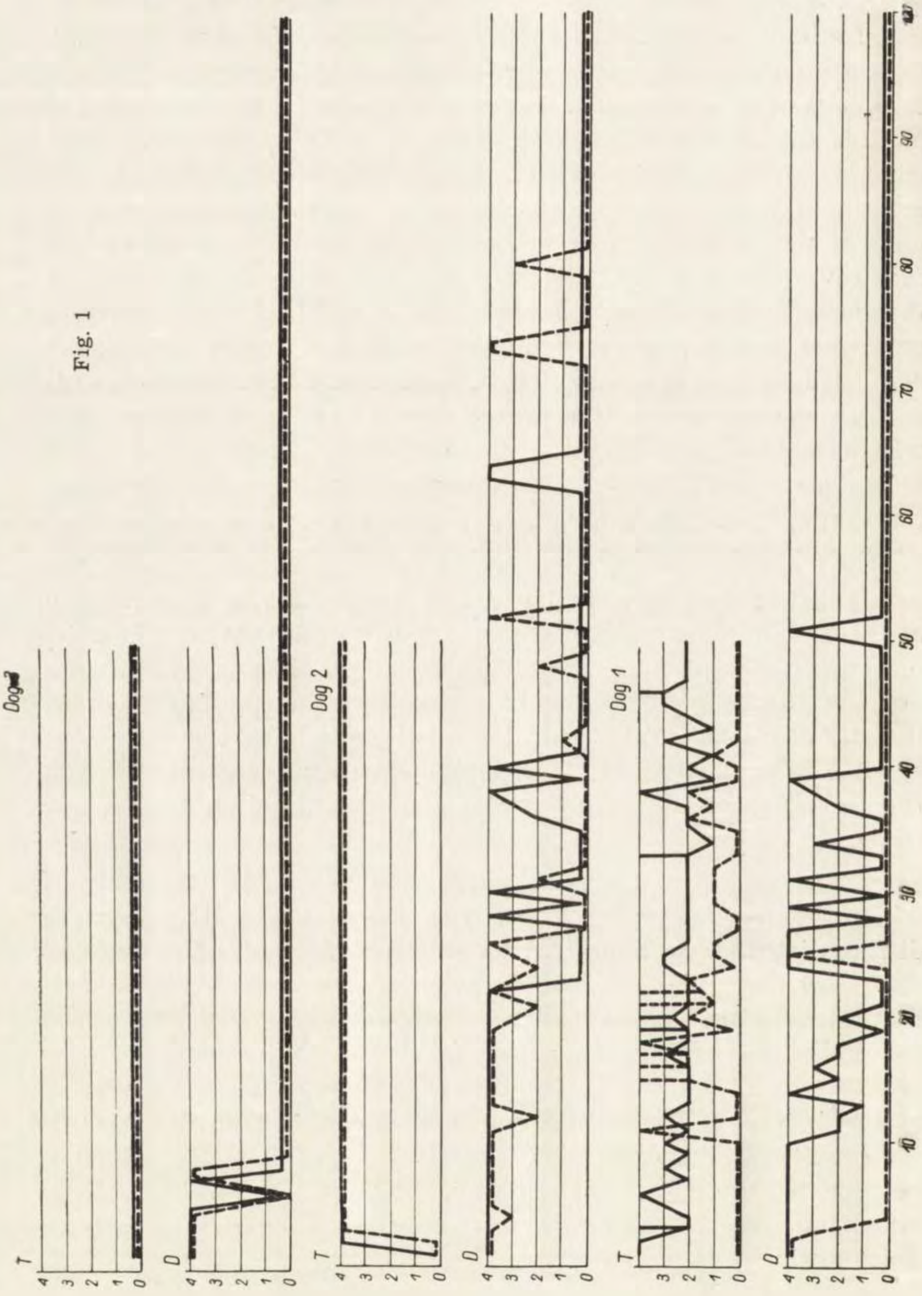

Fig. 1

for T_2 and two reinforcements for T_3, the animal started to perform the movement to both stimuli quite normally, with a very short (0.5 sec.) latent period. And so the transformation took place at once and was complete.

Dog No 3. In this dog, as in dog No 2, T_1 was positive and T_2 and T_3 were negative. As seen in Fig. 1 contrarily to dog No 2, the differentiation of both tones took place very rapidly: after 8 experiments the dog already stopped performing the movement to the both differential stimuli and this reaction was never disinhibited till the end of the series.

From the 101st experiment onwards both inhibitory stimuli were reinforced by food. It was found that the dog did not exhibit any tendency to perform the trained movement to T_2 and T_3, although the introduction of a new stimulus and its reinforcement by food led almost immediately to the formation of the positive motor reflex to it.

DISCUSSION

In our previous papers of this series it was established that the rate of transformation of a given stimulus into the positive conditioned stimulus depends on its "history" and its similarity to other conditioned stimuli. A stimulus quite dissimilar to any of the conditioned stimuli, applied for a long time without reinforcement, is very resistant to the transformation into positive conditioned stimulus: even after a long positive training of such stimulus it continues to elicit a small and irregular conditioned response (Konorski and Szwejkowska 1952). On the other hand when a stimulus, which was first trained as a positive conditioned stimulus and then extinguished is again transformed into the excitatory

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Fig. 1. Course of differentiation of two stimuli similar to the positive conditioned stimulus and their reversal training in the dogs

D — differentiation, T — reversal training. Abscissae: successive experiments. Ordinates: character of movements. O — no movement; 1 — small flexion (abortive movement); 2 — lifting of the leg without putting it on the foodtray; 3 — lifting of the leg and slight touching the foodtray; 4 — full-sized movement. Continuous lines — reflexes to stimuli more similar, broken lines — reflexes to stimuli less similar to the positive conditioned stimuli. In reversal training in dog No. 1 the animal sometimes made a full-sized movement to a stimulus only after presentation of food. These trials are marked by perpendicular lines (continuous or broken) drawn from the given level to level 4.

stimulus, its transformation is very rapid and virtually complete (Konorski and Szwejkowska 1950). These results were explained by the relationship between the excitatory and inhibitory connections established between the "centre" of the stimulus and the alimentary centre. A stimulus not reinforced by food at the beginning of training forms strong inhibitory connections with the alimentary centre. These connections prevent the subsequent formation of the excitatory connections ("primary inhibitory stimulus"). A stimulus primarly reinforced by food forms strong excitatory connections with the alimentary centre, and consequently the formation of inhibitory connections is impeded ("primary excitatory stimulus"). As far as differential stimuli are concerned it was found that again the more similar is a given differentiated stimulus to the positive conditioned stimulus and consequently the more difficult was the process of its differentiation, the easier and more complete is its transformation into the positive stimulus (Szwejkowska 1959).

As we see from the results reported in this paper the same relations hold in type II conditioned reflexes. In dog No 1, one (B_2) of the two differentiated stimuli was much more similar to the positive stimulus than the other (B_z), what is clearly seen from the different rate of differentiation. In consequence the reversal training of B_2 proved to be much easier than that of B_z, although it was also not complete.

In two other dogs in spite of the fact that one differentiated tone (T_2) was more similar to the positive tone (T_1) than the other (T_3), their differentiation was nearly parallel, and consequently their reversal training took also a similar course. But in these experiments it was found that the same differentiation took quite a different course in each dog and consequently the reversal training took also a quite different course. For dog No 2 this differentiation was very difficult and took a long time to be established. In accordance with this the transformation of the inhibitory conditioned reflex into the excitatory reflex was almost immediate. On the other hand the differentiation of the same tones in dog No 3 was very rapid but their transformation into positive stimuli proved to be impossible. The relation between the strength of the given inhibitory reflex, as judged by the easiness of differentiation, and the difficulty of its transformation into the positive conditioned reflex is diagramatically presented in Fig. 2.

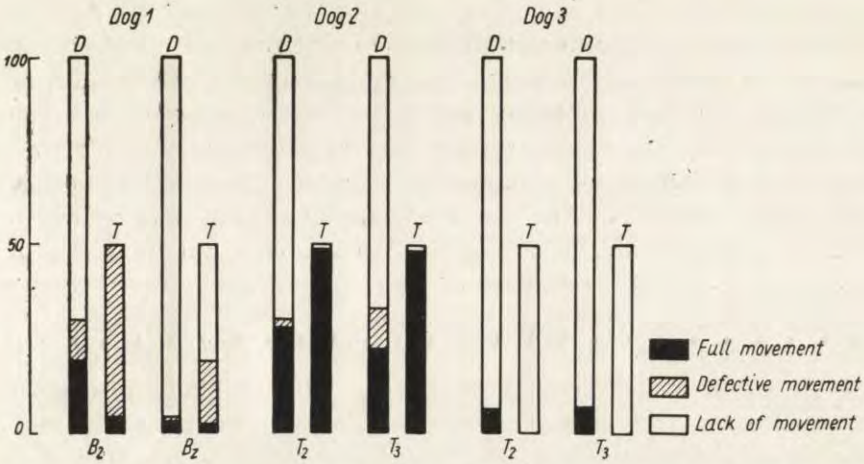

Fig. 2. The various types of reactions to the differentiated stimuli in the course of differentiation (D) and reversal training (T)

Each column shows the number of trials in the given sort of training to a given stimulus (100 in differentiation, 50 in reversal training). Note that the easier the differentiation (which is seen in the high percentage of inhibitory responses), the more difficult the reversal training.

Thus, the general conclusion drawn from these experiments, as well as from those reported in the previous paper, is that the stronger is the resistance to inhibition of the given reflex in a given dog, the less is the resistance to its reversal training, and vice-versa.

Similarly to the data described in the previous paper, in this series of experiments we also observed great irregularities in the course of reversal training and transient neurotic states. These symptoms are due to the conflict between excitatory and inhibitory processes.

The last point to be commented upon concerns the precise mechanism of the inhibitory process involved in differentiation and the cause of the strong resistance of the animal to perform the movement to the differentiated stimulus in spite of its repeated reinforcement. The evidence presented in our previous papers (Konorski and Szwejkowska 1952, Szwejkowska 1959) shows that the primary inhibitory stimulus cannot be completely transformed into the positive conditioned stimulus, since its inhibitory significance is not lost even after a long positive training. In other words such a stimulus is not able, even after many reinforcements, to produce a normal uninhibited alimentary conditioned reaction, as does the primary positive conditioned stimulus. Therefore, we may conclude that in the present experiments, too, the differentiated stimulus, not reinforced by food in many successive trials, acquires a strong inhibitory significance in respect to the alimentary centre and therefore it cannot be completely transformed into the positive alimentary stimulus. And so, the defective performance of the trained movement to this stimulus is nothing else, but the reflection of the inadequate excitation of the alimentary centre, due to contamination by inhibitory process. In the worst case this contamination is so strong that the animal does not perform the movement at all, while in better cases, when inhibition is not so preponderant, the animal performs the movement with much hesitation and uncertainty. Particularly instructive are here those cases in which the animal performs the fullsized movement only after presentation of food i. e. when really this movement is no more "needed". Here the additional strong alimentary conditioned stimulus provided by the sight of food makes the excitation of alimentary centre sufficient to provoke the full-sized movement.

SUMMARY

The transformation of differential inhibitory conditioned reflexes into positive, type II, conditioned reflexes was investigated in three dogs. It was found that the more difficult is the differentiation, the less is its resistance to the reversal training and vice-versa. The resistance to the reversal training is manifested either by performance of defective motor reaction or by a lack of this reaction in spite of repeated reinforcement of the stimulus.

REFERENCES

KONORSKI J. and SZWEJKOWSKA G. 1950 — Chronic extinction and restoration of conditioned reflexes. 1. Extinction against the excitatory background. Acta Biol. Exp. *15*, 155.

KONORSKI J. and SZWEJKOWSKA G. 1952 — Chronic extinction and restoration of conditioned reflexes. IV. The dependance of the course of extinction and restoration of conditioned reflexes on the "history" of the conditioned stimulus (The principle of the primacy of first training). Acta Biol. Exper. *16*, 95.

SZWEJKOWSKA G. 1950 — Chronic extinction and restoration of conditioned reflexes 2. The extinction against an inhibitory background. Acta Biol. Exper. *15*, 171.

SZWEJKOWSKA G. 1959 — The transformation of differentiated inhibitory stimuli into positive conditioned stimuli. Acta Biol. Exper. *19*, 151.

WYRWICKA W. 1952 — Studies on motor conditioned reflexes. 5. On the mechanism of the motor conditioned reaction. Acta Biol. Exper. *16*, 131.

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STUDIES ON THE AVOIDANCE CONDITIONING.
I. RELATIONS BETWEEN CARDIAC (TYPE I) AND MOTOR (TYPE II) EFFECTS IN THE AVOIDANCE REFLEX

S. SOŁTYSIK and **M. KOWALSKA**

Laboratory of Physiology of Nervous System in the Psychoneurological Institute, Pruszków, and Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw

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Although the first experiments in which the avoidance principle was employed (though not recognized) had been performed as early as 1919 by Hamel and also by Carr and Freeman (1919), and later by other authors (Yarbrough 1921, Starcin 1926, Petropavlovsky 1927), it was Konorski and Miller (1928, 1933, 1936), who recognized the instrumental defensive conditioning as such and performed the first systematic study on its physiological mechanism. By appropriately choosing the motor instrumental (type II) conditioned reaction in such a way that it was different from the classical (type I) conditioned response, they could clearly discriminate between these two motor responses and this helped them to analyze the mechanism of defensive type II conditioned reflexes.

The method of elaboration of conditioned avoidance reflexes, as proposed by these authors, was as follows: while conditioned stimulus (CS) alone was regularly reinforced by noxious unconditioned stimulus (US), the compound consisting of CS and the chosen passive or active movement (e. g. passive or active flexion of the leg) was not reinforced.

The physiological mechanism of the avoidance conditioned reflex (CR) is according to Konorski and Miller the following: CS elicits classical defensive CR for it is from the very beginning reinforced (classically) by the US; a complex of proprioceptive and tactile stimuli generated by the passive (or active) movement becomes a conditioned inhibitor (CI) of the classical CR because a compound consisting of the CS and this movement is not reinforced by the US. This is enough for the animal to perform actively this movement in every situation which causes the rise of defensive excitement, be it the application of noxious US or substituting it defensive CS.

Thus, Konorski and Miller came to the conclusion that there are three different components in the avoidance reflex: 1) defensive CR type I (classicaly conditioned) elicited by the CS; 2) defensive inhibitory CR (also classically conditioned) induced by the proprioceptive compound stimulus arisen from the movement, and 3) defensive CR type II (instrumentally conditioned) consisting in the animal performing a given movement in response to the CS, avoiding in this way the noxious US.

Konorski and Miller's hypothesis satisfactorily explains both formation and properties of avoidance reflexes. However, neither the classical defensive CR to the CS, nor its conditioned inhibition called forth by the trained movement, were measured directly in their experiments; classically conditioned components of avoidance reflex were only deduced from experimental conditions.

This work was undertaken to establish, by use of very sensitive index of the classical defensive CR, namely the acceleration of the heart rate, wheather or not: the CS really elicits the classical CR even after the avoidance reflex is firmly established, and secondly, whether the instrumental response really plays the role of a CI of the classical defensive CR.

MATERIAL AND METHOD

The experiments were performed on 9 male dogs (adult mongrels) in a soundproof conditioned reflex chamber. In 4 dogs avoidance reflexes were elaborated while in five other animals cardiac responses to indifferent stimuli were studied. Buzzer, metronome and flashing lamp were used as conditioned stimuli. Electric shock of 1 or 2μF condenser charged from anode battery (120 or 240 V) was applied through electrodes placed between fingers of the right foreleg. Movements of both forelegs, respiration and pulse rate were recorded pneumatically on a kymograph. Pulse waves were received from the carotid artery loop prepared by v. Leersum's method (1911). Conditioned avoidance reflexes were trained according to Konorski and Miller's method (Konorski and Miller 1933), i.e. by the use of passive movement as conditioned inhibitor.

RESULTS

At the start of training a definite "indifferent" stimulus was several times paired with the electrical US. The classical CR was elaborated very quickly and was manifested by a general restlessness and occasionally by flexion of the right, i. e. electrically stimulated, foreleg. Then passive movement, consisting in the lifting of the left foreleg and putting it on the bar, was introduced as conditioned inhibitor (CI): a compound consisting of the CS and the passive movement was not reinforced, whereas the CS alone was always followed by the US. After some time the dogs began to perform this movement actively. Since the animals did not perform the trained movement to every application of the CS, reinforcement by the US was sometimes necessary. However, after a while the motor CR became stable and electric US was no more applied for weeks or months. The intertrial responses gradually decreased in number but their extinction was never absolute.

Latencies of the motor avoidance response varied from 0.5 to 5 sec.; usually they did not exceed 2 sec.

After this first training the avoidance CRs were formed to a number of other stimuli. When stimuli of the same modality as the first CS were used the CR appeared to them at once owing to generalization. When stimuli of other modality were introduced no generalization was, as a rule, seen, but it was enough to reinforce once or twice these new stimuli by the US to obtain the full avoidance reflex. The US also elicited, besides the UR, the conditioned avoidance response. Thus, all facts known from previous studies (Konorski and Miller 1933, 1936, Fonberg 1958) were easily reproduced.

After this preliminary training all dogs were operated on by the v. Leersum method and, from the obtained carotid artery loops, pulse rate was recorded in dozens of experiments. Data from positive trials in which latency of motor response did not exceed 2 sec. are shown for each animal in Fig. 1. In Fig. 2 the pulse curve for all 4 dogs is presented (black circles) and compared with two other pulse curves obtained from 5 control dogs: one of

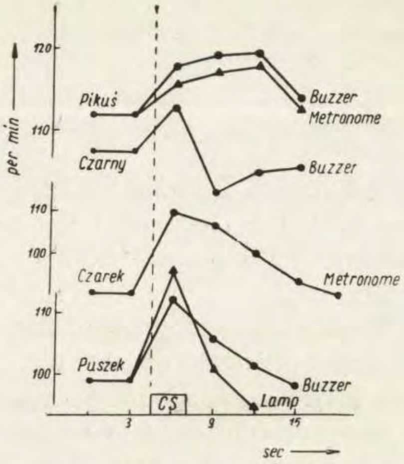

Fig. 1. Cardiac responses accompanying avoidance CRs to CSs in 4 dogs

In all instances latency of instrumental response did not exceed 2 sec. Pulse frequency is shown in 3-sec. intervals.

these curves represents cardiac responses to acoustic stimuli (as loud as the strongest CS in avoidance dogs, and of the same duration), in first 10 trials (white triangles), and the other curve represents cardiac response to the same stimuli after they had been applied 90 times (white circles). It is clearly seen that in all avoidance dogs every CS elicited a steep rise of pulse frequency (from 4.00 per min./3 sec. in Pikuś to 19.87 per. min./3 sec. in Cza-

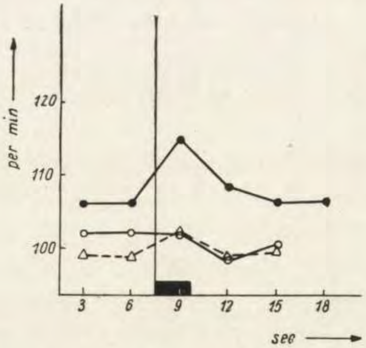

Fig. 2. Cardiac response accompanying avoidance CR (black circles) in four dogs, and cardiac response to indifferent acoustic stimuli (averages from 5 dogs):

white triangles — data from 1st — 10th applications of the stimulus (i. e. before habituation of orienting reflex); white circles — data from 91th — 100th applications of the same stimulus (i. e. after habituation).

rek) which was in all instances highly significant (see Table I). In control dogs indifferent stimuli produced only a small (2.77 per min./3 sec.) and insignificant ($p < 0.30$) rise of pulse rate and this rise disappeared after habituation: Fig. 2 and Table I.

Table I

Cardioacceleratory response to CSs in "avoidance" dogs and cardiac response to indifferent stimulus of the same duration in 5 control dogs: before and after habituation (i. e. before and after 90 applications of stimulus). Pulse rate was measured in 3 sec. intervals. Data concern avoidance reflexes in which latency of motor response did not exceed 2 sec.

Dog	Conditioned stimulus	Pre-stimulus level of heart rate	Pulse rate in 3 sec. period following the onset of CS	Difference and probability level	Mean latency of motor CR
Czarny	Buzzer I	107.78 ± 0.86	113.09 ± 1.31	5.31 $p < 0.001$	0.80 ± 0.01
Czarek	Metronome	90.13 ± 1.26	110.00 ± 2.10	19.87 $p < 0.001$	1.46 ± 0.05
Puszek	Buzzer I	98.85 ± 0.61	112.20 ± 1.68	13.35 $p < 0.001$	1.17 ± 0.03
	Lamp		117.20 ± 1.46	18.35 $p < 0.001$	1.02 ± 0.02
Pikuś	Buzzer I	112.03 ± 0.56	117.92 ± 1.39	5.89 $p < 0.001$	1.06 ± 0.04
	Metronome		116.03 ± 1.45	4.00 $p < 0.020$	1.19 ± 0.04
5 control dogs	Buzzer before habituation	99.95	102.72	2.77 $p < 0.300$	—
	Buzzer after habituation	101.99	101.49	—0.50 $p < 0.800$	—

Table II

Cardiac response following the performance of avoidance reaction

Dog	Conditioned Stimulus	Heart rate during the operation of CS	Heart rate following the performance of CR	Difference	Probability level
Czarny	Buzzer I	113.09	102.44	—10.65	$p<0.001$
Czarek	Metronome	110.00	107.00	— 3.00	$p<0.400$
Puszek	Buzzer I	112.20	105.70	— 6.50	$p<0.001$
	Lamp	117.20	101.10	—16.10	$p<0.001$
Pikuś	Buzzer I	117.92	119.35	+ 1.43	$p<0.900$
	Metronome	116.03	117.43	+ 1.40	$p<0.900$
All dogs	CS	114.79	107.93	— 6.86	$p<0.001$

Another interesting feature of pulse behaviour accompanying the avoidance reflex is that this cardioacceleratory response to CS was changed into a marked deceleration in the next 3 sec. i. e. just after the motor response was displayed and the CS was discontinued. A detailed analysis of pulse changes in each dog reveals that three dogs showed this deceleratory after-effect, while in the fourth one still a slight acceleration was present (Fig. 1 and Table II).

It was important to determine the temporal relations between cardiac and motor responses in the whole avoidance reflex. In

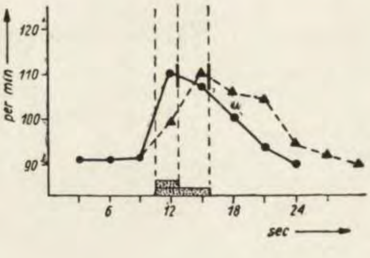

Fig. 3. Dog Puszek: cardiac acceleration to CS precedes the avoidance response Mean values from 5 trials in which latency of instrumental response (to buzzer I) was about 20 sec. Note the dramatic inhibition of cardiac response by avoidance reaction.

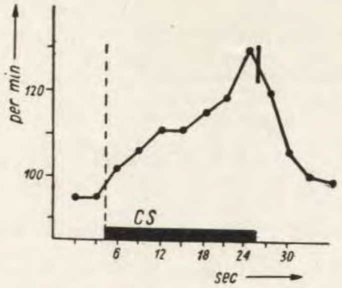

Fig. 4. Dog Czarek: comparison of cardiac responses accompanying avoidance responses (to the same CS: metronome) in two groups of trails:

latencies of motor response in first group were about 2 sec. (black circles) and in second group 5 sec. (black triangles). Slower cardioacceleration in the 5-sec.-group causes the instrumental response to occur at the same absolute level of heart rate as in 2-sec.-latency group.

trials in which the latency of the motor response did not exceed 2 sec. both responses seemed to be simultaneous at least in limits of the accuracy of our method, where pulse frequency was measured not continuously but in 3 sec. intervals. If however, trials with longer latency were selected (Fig. 3 and 4) it became obvious that cardiac acceleration preceded the motor response.

In one dog (Czarek) we had the opportunity to compare the cardiac responses in the period when avoidance reflex was yet fresh with those when it was firmly established (Fig. 5). The main

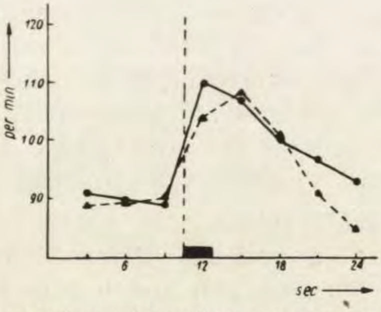

Fig. 5. Dog Czarek: comparison of cardiac responses accompanying the avoidance reflexes in two groups of trials:

in relatively fresh avoidance reflex (black triangles) and in well trained avoidance reflex (black dots). Both groups consist of trials in which the same CS was applied and the latency of motor response did not exceed 2 sec. Firmly established avoidance reactions show greater cardiac acceleration to CS and its immediate inhibition by avoidance response, while in fresh reflexes the avoidance response is still followed by cardioacceleration.

difference is that when the reflex was fresh, the motor response was still followed by cardioacceleration, whereas in well established reflexes cardiodeceleration occurred.

DISCUSSION

The main purpose of these experiments was to study the interrelations between classical and instrumental conditioned responses constituting the whole avoidance reflex. The classically conditioned defensive reflex was measured by the rise in pulse frequency. The motor instrumental response was a simple, phasic motor act, and due to its "all or nothing" nature, the latent period could be used as a convenient parameter of the strength of that response. Cardioacceleratory responses to CSs were shown to be present both in classical defensive conditioning (Sherington 1900, Gant 1942, Froňková and Ehrlich 1959) and in avoidance conditioning (Black 1956). The problem raised in this paper was whether in the well established avoidance reflexes, i. e. in those in which the US is not applied at all, the classical defensive CR is preserved or not. Results obtained in all four dogs are in this respect clear and unequivocal: the classical conditioned reflex, or at least its general visceral component, measured by cardioacceleration, does not extinguish in spite of the fact that the US was not applied for hundreds of trials. On the other hand it is well known that in classical procedure this defensive reflex is extinguishable, at least partially, as shown in Froňkova and Ehrlich's paper. Moreover, it was shown that differential stimuli in avoidance experiments also elicited much less cardioacceleration than positive CSs (Sołtysik 1959, 1960).

This extraordinary resistence to extinction found in avoidance reflexes was explained differently by various authors. Thus, some natural resistance to extinction of defensive CRs is supposed by Solomon and Wynne (1954) and by Mowrer and Lamoreaux (1942). Miller (1951) put forward the hypothesis that the "fear as response" is reinforced (in Hullian sense) by reduction of "fear as drive"; such a reduction takes place in the moment of cessation of CS. The former of these hypothesis, however, fails to explain the rather easy extinction of classical defensive CRs as mentioned above. The latter explanation can hardly be accepted, since a large body of experimental evidence contradicts the assumption that autonomically mediated responses may be conditioned by reduction of drive, as are instrumental CRs.

Konorski's explanation (1948, p. 231) of this preservation of classical defensive CR in avoidance reflexes was that motor avoidance response or, more accurately, proprioceptive and tactile stimuli generated by this response, plays the role of a conditioned inhibitor. As recently was shown in alimentary conditioned reflexes (Chorążyna 1957, Sołtysik 1960a) the conditioned inhibitor exerts a protective influence against extinction of the CR to the CS presented in compound with the conditioned inhibitor.

Thus the fact that the classical CR does not extinguish in avoidance reflexes is easily explained by Konorski's hypothesis.

We think that in this work we have given some evidence supporting the view that the motor avoidance response really plays the role of a CI. It was shown that the motor response is followed by a marked cardiodeceleration (Table II, Fig. 1, 2, 3, 4), or at least (in one dog: Pikuś) by a slowering of cardioacceleratory response to CS. Although this motor response coincides with the cessation of the CS, such marked deceleration, sometimes even below the intertrial level (Czarny and Puszek) cannot be attributed only to release from the CS. In our paper on differentiation of avoidance reflexes (Sołtysik 1960) we have shown that the end of differential stimulus, even in such instances in which the heart rate is not less than that to positive CSs, does not by itself produce such a fall in heart frequency as seen in "positive" trials where the cessation of the CS coincides with the motor response. And in fresh avoidance reflex (i. e. presumably before the elaboration of conditioned inhibition) the termination of CS and occurrence of motor response do not produce such deceleration (Fig. 5. and cf. Black 1956).

On the other hand, we have evidence to show that the motor response itself has no deceleratory influence on pulse rate; this is shown by the fact that in alimentary instrumental reflexes (Sołtysik 1960 in preparation) the same motor response produces cardioacceleration. Obviously the cardiac response to movement-produced stimuli is directly related to their conditioned significance.

There are also some other facts supporting the supposition that the avoidance motor response plays the role of a CI. First, the prevention of this response by curarisation facilitates the extinction of the avoidance reflex (Black 1958). Secondly, very brief application of CS, so that the animal failes to perform the motor CR also leads to extinction (Pakovič 1958). In both cases extinction occurs because the motor response is absent, i. e. because there is no protective action of CI.

Thus, both assumptions of Konorski's hypothesis, namely, 1) that the motor response is a CI of classical defensive CR elicited by the CS, and 2) that this CI protects the classical CR from extinction, seem to be experimentally proved.

Another point of this hypothesis is that the real and direct "provoking" factor of the instrumental response in avoidance reflex is the rise of excitation in the unconditioned "centre" and connected with it the "state of exaltation of the motor cortex" (Konorski 1948, pp. 228—242).

Data obtained in this study confirm this assumption experimentally in the point concerning the excitation of "unconditioned centre". It was shown that the CS really elicits classical defensive CR, which, in its turn, produces the avoidance response. In another work (Sołtysik 1960) it was found that some minute but definite intensity of this classical CR is necessary to obtain the avoidance response. And it seems that the avoidance motor CR is never fully automatized and "disinterestedly" displayed by the dog.

In Fig. 6, a schematic representation of the avoidance-CR-arc is given. Interrupted lines denote intercentral connections of conditioned character, continuous lines represent unconditioned connections. Connection "a" connects centres of CS and US. By this pathway unconditioned centre is activated by impulses elicited by the CS. This is the classical defense CR. Connection "b" is the basis of instrumental avoidance response. Due to this connection any activation of unconditioned defense centre, by USs of noxious character, or by CSs heralding them, or even by direct electrical stimulation (Nakao 1958), produces excitation of the "centre" of a given motor response. Connection "c" is responsible for conditioned inhibition exerted by proprioceptive stimuli generated by avoidance response. For some reason it is more convenient to sketch this connection not directly to the "defensive centre" but to its inhibitory counterpart. Finally, pathway "d" is supposed to exist, by analogy to the same connection found in alimentary conditioned reflexes type II (Wyrwicka 1952). This pathway is supposed to exert a facilitatory influence on the motor centre of instrumental movement, determining which motor act will be elicited by the strong motogenic impulses arriving to the motor centre through pathway "b". Existence of this connection explains why "fear" does not produce the same avoiding response in a completely different situation and also explains the ability to establish two different avoidance responses to different CSs heralding the same danger (Sołtysik unpublished data).

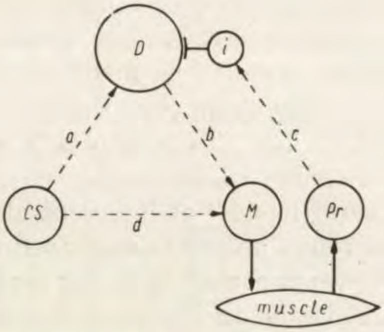

Fig. 6. Scheme of intercentral connections constituting the framework of the avoidance-reflex arc

CS — central representation of conditioned stimulus; M and Pr — executive and proprioceptive centres of instrumental movement; D — defense centre (hypothalamic) and its inhibitory counterpart — i, a — conditioned connection constituting neural basis for classical defensive conditioned reflex; c — conditioned connection constituting classical defensive inhibitory reflex (conditioned inhibition); d and b — connections constituting neural basis for instrumental defensive (avoiding and escaping) conditioned reflex.

As far as the localisation of the "defensive centre" is concerned, recent experiments with brain stimulation in conscious animals provide us with some information. Thus, fear responses were obtained from hypothalamus (Hess 1949, Nakao 1958), from some points within the rhinencephalic centres (medial nucleus of the amygdala, rhinal fissure) and from many points in mesencephalic and diencephalic structures (Delgado et al. 1954, Delgado 1955, Delgado et. al. 1956). These responses may be conditioned classically (Delgado et al. 1954, Nakao 1958) or used as motivating stimuli in instrumental learning (Delgado et al. 1954, Nakao 1958). In animals with previously elaborated avoidance responses (to avoid the electric shock) stimulation of hypothalamic (Nakao 1958) or amygdalar and other diencephalic and mesencephalic (presumably sensory?) structures (Delgado et al. 1956) evoked typical avoidance reflex.

Among these structures, the hypothalamic defense (or "fear") centre seems to play the role of the main, motivating behaviour centre, analogous to the other hypothalamic "primary drives" centres such as "hunger centre" (Wyrwicka, Dobrzecka and Tarnecki 1959) and "thirst centre" (Andersson and Wyrwicka 1957). Other points in the brain, stimulation of which produces fear behaviour, seem to be involved in transmission and elaboration of noxious stimulation, even though some close relationship in organizing the whole "fear pattern", especially for rhinencephalic structures, cannot be excluded.

The proposed "neural model" of intercentral connections involved in the avoidance reflex serves to explain (in the first approximation) some neural events occurring at the moment of application of the CS in well trained animals. The formation of these connections, i. e., the mechanism of elaboration of this reflex, is still not clear and calls for further experimentation.

SUMMARY

1. Heart rate acceleration, as an index of classical defensive conditioned response ("fear") was studied in 4 dogs trained in avoidance reflexes.

2. It was shown that cardioacceleratory response to CS did not become extinguished even in firmly established avoidance reflexes, i. e., in conditions of chronic non-reinforcement of CS by noxious US.

3. Cardiac response was found to precede the instrumental motor reaction showing the classical defensive conditioned reflex as a link, interposed between CS and avoidance instrumental response.

4. Cardiac acceleratory response to CS is immediately inhibited by the instrumental avoidance response, which plays the role of conditioned inhibitor of classical defensive CR.

5. A possible neural mechanism of avoidance reflex is discussed.

REFERENCES

ANDERSSON B. and WYRWICKA W. 1957 — The elicitation of a drinking motor conditioned reaction by electrical stimulation of the hypothalamic "drinking area" in the Goat. Acta Physiol. Scand. *41*(2—3), 194—198.

BLACK A. H. 1956 — The extinction of avoidance responses under curare. Unpublished Ph. D. Thesis, Harvard Univ., Cited in Solomon and Brush 1956.

BLACK A. H. 1958 — The extinction of avoidance responses under curare. J. comp. physiol. Psychol. *51*(5), 519—524.

CARR H. A. and FREEMAN A. S. 1919 — Time relationships in the formation of associations. Psychol. Rev. *26*, 465—473.

CHORĄŻYNA H. 1957 — Some data concerning the mechanism of conditioned inhibition. Bull. Acad. Pol. Sci. *5*(11), 387—392.

DELGADO J. M. R. 1955 — Study of some cerebral structures related to transmission and elaboration of noxious stimulation. J. Neurophysiol. *18*, 261—275.

DELGADO J. M. R., ROBERTS W. W. and MILLER N. 1954 — Learning motivated by electrical stimulation of the brain. Amer. J. Physiol. *179*, 587—593.

DELGADO J. M. R., ROSVOLD H. E. and LOONEY E. 1956 — Evoking conditioned fear by electrical stimulation of subcortical structures in the Monkey brain. J. comp. physiol. Psychol. *49*(4), 373—380.

FONBERG E. 1958 — Transfer of instrumental avoidance reactions in dogs. Bull. Acad. Pol. Sci. *6*(8), 353—356.

FROŇKOVA K. und EHRLICH V. 1959 — Die Aenderungen des Kreislaufs und der Atemfrequenz beim Hunde während des unbedingten und bedingten Abwehrreflexes und dessen Hemmung. Acta Neuroveg. *19*(3—4), 207—220.

GANTT W. H. 1942 — Cardiac conditioned reflexes to painful stimuli. Fed. Proc. *1*, 1.

HAMEL I. A. 1919 — A study and analysis of the conditioned reflex. Psychol. Monogr. *27*(118), 1—65.

HESS W. R. 1949 — Das Zwischenhirn: Syndrome, Lokalisation, Funktionen. Basel: Benno Schwalbe, Pp. 187.

KONORSKI J. 1948 — Conditioned reflexes and neuron organization. Cambridge Univ. Press. Pp. 267.

KONORSKI J. i MILLER S. 1933 — Podstawy fizjologicznej teorii ruchów nabytych. Med. Dośw. Społ. *16*(1—4), 1—168.

KONORSKI J. i MILLER S. 1936 — Uslovnyie refleksy dvigatelnogo analizatora. Trudy Fiziol. Lab. Pavlova *6*(1), 119—278.

LEERSUM G. V. van 1911 — Eine Methode zur Erleichterung der Blutdruckmessung bei Tieren. Pflügers Arch. *142*, 377.

MILLER N. E. 1951 — Learnable drives and rewards. In Stevens' Handbook of Experimental Psychology, New York, 435—472.

MILLER S. et KONORSKI J. 1928 — Sur une forme particulière des réflexes conditionnnels. C. R. Soc. Biol. *99*, 1155—1157.

MOWRER O. H. and LAMOREAUX R. R. 1942 — Avoidance conditioning and signal duration: a study of secondary motivation and reward. Psychol. Monogr. *54*(5), 1—34.

NAKAO H. 1958 — Emotional behaviour produced by hypothalamic stimulation. Amer. J. Physiol. *194*(2), 411—418.

PAKOVIĈ B. I. 1958 — pers. comm.

PETROPAVLOVSKY B. P. 1927 — Metodika uslovnogo myŝeĉnogo tonusa i opyty ugaŝenia. Metiko-biol. Zhurn. *2*, 43—57.

SHERRINGTON C. S. 1900 — Experiments on the value of vascular and visceral factors for the genesis of emotion. Proc. Roy. Soc. *66*, 390—403.

SOLOMON R. L. and BRUSH E. S. 1956 — Experimentally derived conceptions of anxiety and aversion. Nebraska Symposium on Motivation. *6*, 212—305.

SOLOMON R. L. and WYNNE L. C. 1954 — Traumatic avoidance learning: the principles of anxiety conservation and partial irreversibility. Phychol. Rev. *61*(6), 353—358.

SOŁTYSIK S. 1959 — On the physiological mechanism of the avoidance conditioned reflexes. XXI Int. Congr. Physiol. Sci., Buenos Aires, Abstr. of Comm., 258—259.

SOŁTYSIK S. 1960 — Studies on the avoidance conditioning. 2. Differentiation and extinction of avoidance reflexes. Acta Biol. Exper.

SOŁTYSIK S. 1960a — Studies on the avoidance conditioning. 3. Alimentary conditioned reflex model of the avoidance reflex. Acta Biol. Exper.

STARICIN S. J. 1926 — Metodika vospitania socetatelno-dvigatelnych refleksov u sobak pri razdrazenii w podusecnuin cast' lapki. Sbornik posviasc. V. M. Bechterevu k 40-letiu professorskoi deiatelnosti. L.

WYRWICKA W . 1952 — On the mechanism of the motor conditioned reaction. Acta Biol. Exper. *16*(10), 131—137.

WYRWICKA W., DOBRZECKA C. and TARNECKI R. 1959 — On the instrumental conditioned reaction evoked by electrical stimulation of the hypothalamus. Sci. *130*(3371), 336—337.

YARBROUGH J. U. 1921 — The influence of the time intervals upon the rate of learning in the white rat. Psychol. Monogr. *30*(135), 1—52.

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STUDIES ON THE AVOIDANCE CONDITIONING.
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S. SOŁTYSIK

Laboratory of Physiology of Nervous System in the Psychoneurological Institute, Pruszków, and Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw

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A peculiar property of avoidance conditioned reflexes (CRs) is that these reflexes once established do not require reinforcement by the noxious unconditioned stimulus (US) for their maintenance. As shown in our previous paper (Sołtysik 1959, Sołtysik and Kowalska 1960) even after a long avoidance training when reinforcement by the US has not been applied for a long time, the CS, nevertheless, elicits a normal classical defensive response (as measured by the increase of the pulse rate), which after attaining a definite intensity produces the instrumental response. Since this response (or rather its proprioceptive feedback) plays a role of conditioned inhibitor (CI) the defensive classical CR decreases immediately after the performance of this movement. The sequence: CS — classical CR — instrumental CR — conditioned inhibition of classical CR, develops in few seconds, so that all the avoidance reflex represents a peculiar self-stabilizing, or self-protecting from extinction, system of positive and inhibitory CRs. (cf. Chorążyna 1957, Sołtysik 1960).

The important consequence of this state of affairs is that a firmly established avoidance CR cannot be subjected either to extinction or to differentiation since these procedures are based on non-reinforcement of the hitherto reinforced CS or a stimulus similar to it. Therefore Konorski and Miller (1936) postulated that the only way of differentiation of avoidance reflex is to establish it before the training of instrumental response was begun, i. e. during the preliminary classical aversive conditioning.

However, Bregadze (1953) and independently Fonberg (1953) found a procedure for obtaining an extinction and differentiation of avoidance reflexes. It consists in that the action of CS is prolonged for several seconds after the instrumental response was displayed.

It was interesting to elucidate by which mechanism the process of differentiation and extinction in avoidance CRs occurs. This seemed to be possible by using a concurrent recording of cardiac responses as indicator of classical defensive CR, which method was applied in the preceding paper.

MATERIAL AND METHOD

The experiments were performed on 4 male dogs (adult mongrels) in a soundproof conditioned reflex chamber. In all the dogs first avoidance reflexes were elaborated and firmly established to acoustic and visual CSs according to Konorski and Miller's method (1928, 1933) i. e. by the use of passive movement as conditioned inhibitor (cf. previous work: Sołtysik and Kowalska 1960). Than differentiation were trained using the procedure of prolonged presentation of the stimuli to be differentiated.

Movements of both forelegs, respiration and pulse rate were recorded kymographically. Pulse waves were received from the carotid artery loop prepared by the v. Leersum method (v. Leersum 1911). In one dog pulse was not recorded since the animal destroyed its arterial loop. Instrumental response consisted in lifting the left foreleg and pressing the bar. A noxious stimulus avoided by this movement was electric shock from a condenser applied through electrodes placed between fingers of the right, "not instrumental" foreleg.

RESULTS

Elaboration of differentiation proceeded differently in different dogs.

In Czarek, after elaboration of avoidance CR to metronome, fan was introduced and applied for 10 sec. twice or three times in daily session. The operation of the metronome did not usually exceed 2—3 sec., since this was the latent period of instrumental reaction.

During first 7 trials the dog performed the instrumental CR to fan and then it became extinguished.

In Puszek buzzer II was differentiated from buzzer I, i. e. buzzer I was discontinued immediately after the performance of the trained movement, while buzzer II lasted always 10 sec. For a long time it was impossible to extinguish the CR to this differential stimulus (DS). The dog used to perform the avoidance movement as long as DS was acting and the CR did not diminish during 250 trials. Then another stimulus, a fan was introduced as DS. In this case, despite a very vigorous CR, elicited by this stimulus at first applications, differentiation was obtained in 6 trials and afterwards, almost at the same time, the CR to buzzer II was also extinguished.

In Pikuś buzzer I and metronome were used as "positive" CSs, while buzzer II and fan were differentiated. Extinction of motor CR to these stimuli was very difficult and even after several hundreds of trials it did not reach 100% criterion. The general behaviour of this animal also differed from that of other dogs. During the operation of either DS Pikuś was extremely excited: he howled and struggled and in spite of the fact that in the later stage of training the motor avoidance CR usually was absent he seemed to be just as frightened as during the action of the positive CSs. On the contrary, an indifferent stimulus — flashing lamp — did not elicit either restlessness or avoidance CR.

In Rex buzzer II was differentiated from buzzer I. Differentiation was established in 85 trials.

Cardiac responses to the positive CSs and to DSs, as well as heart rate level in intertrial intervals are presented in Table I, and in Fig. 1—4. As seen in table and figures the course of the cardiac responses accompanying the CSs and DSs differed in Czarek and Puszek from those in Pikuś.

In Czarek and Puszek (Fig. 1 and 2) the increase of pulse rate to DSs was much smaller than that to CSs. The absolute level of heart rate in response to DSs did not attained the level of heart rate to CS, in spite of the fact, that the CSs lasted less than 2 sec. and the DSs lasted 10 sec.

In Pikuś the cardiac responses to DSs were, on the contrary, greater than those to CSs. The difference is seen (Fig. 3) even in first 3 sec. but becomes significant during further operation of DSs. It can also be seen in this Fig. 3 that the indifferent stimulus

Table I

Heart rate during the action of differential stimuli compared with the cardiac response to positive conditioned stimuli and with the heart rate in the intertrial intervals

DOG	Differential stimulus	Heart rate during action of CS (shown above) and heart rate in intertrial intervals (below)	Heart rate during 10-sec. action of DS, taken in three 3-sec. intervals, and differences between it and cardiac response to CS (shown above) and heart rate in intertrial intervals (shown below)		
Czarek	Fan	110.00	— 10.40 $p<0.010$	— 8.00 $p<0.050$	— 9.40 $p<0.010$
			99.60	102.00	100.60
		90.13	+ 9.47 $p<0.001$	+ 11.87 $p<0.001$	+ 10.47 $p<0.001$
Puszek	Buzzer II	112.20	— 6.80 $p<0.020$	— 3.00 $p<0.300$	— 3.80 $p<0.400$
			105.40	109.20	108.40
		98.85	+ 6.55 $p<0.050$	+ 10.35 $p<0.010$	+ 9.85 $p<0.010$
	Fan	112.20	— 13.40 $p<0.001$	— 10.20 $p<0.010$	— 3.80 $p<0.400$
			98.80	102.00	108.40
		98.85	— 0.05 $p>0.900$	+ 3.15 $p<0.400$	+ 9.55 $p<0.010$
Pikuś	Buzzer II	117.92	+ 3.85 $p<0.100$	+ 6.31 $p<0.010$	+ 8.15 $p<0.010$
			121.77	124.23	126.07
		112.03	+ 9.74 $p<0.001$	+ 12.20 $p<0.001$	+ 14.04 $p<0.001$
	Fan	116.03	+ 4.11 $p<0.100$	+ 5.56 $p<0.050$	+ 5.78 $p<0.020$
			120.14	121.59	121.81
		112.03	+ 8.11 $p<0.001$	+ 9.56 $p<0.001$	+ 9.78 $p<0.001$

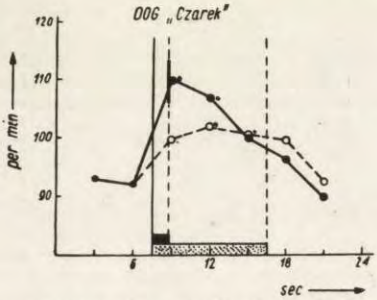

Fig. 1. Heart rate accompanying the application of CS (metronome) — black dots, and DS (fan) — white circles. Small dots denote the significant difference between heart rate level at given point and pre-stimulus level of pulse frequency. Black rectangle above the time scale shows duration of CS, the spotted rectangle — duration of DS.

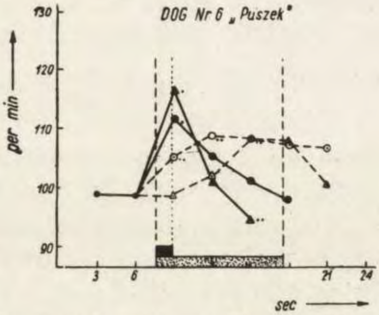

Fig. 2. Heart rate accompanying the application of CSs (buzzer I — black circles; flashing lamp — black triangles) and DSs (buzzer II — white circles; fan — white triangles)

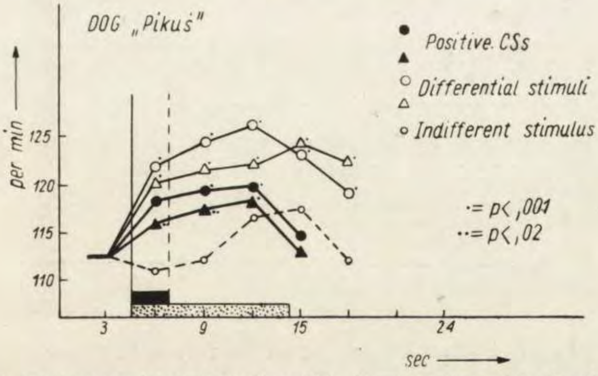

Fig. 3. Heart rate accompanying the CSs (buzzer I — black circles; metronome — black triangles) and DSs (buzzer II — white circles; fan — white triangles). Small white circles show the cardiac response to an indifferent stimulus — lamp.

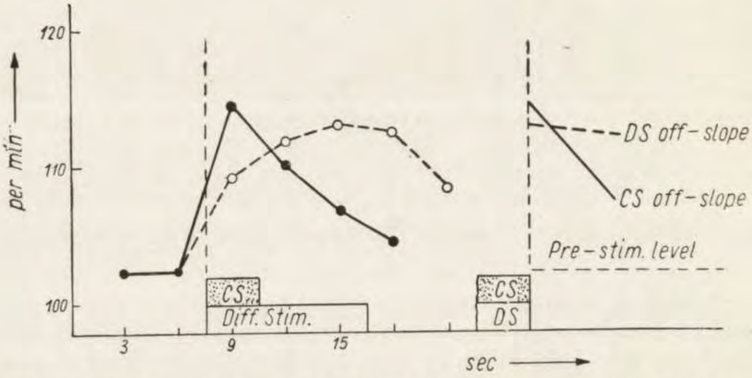

Fig. 4. Heart rate accompanying the CSs (black dots) and DSs (white circles) in all dogs

On the right, the cardiodeceleratory response in first 3 sec. after the termination of CS (simultaneous with instrumental response) — continuous line, and DS — interrupted line.

(a flashing lamp) did not produce any significant change in pulse rate.

In Fig. 4 data from all 3 dogs are summarized. It is seen in this fig. that not only the on-slope of cardiac curve (i. e. the acceleration of pulse following the stimulus onset) is much greater to CSs than to DSs, but also off-sloppe to CSs is significantly greater (difference being 6.29 per min./3 sec., $p < 0.001$;).

Since the elaboration of differentiation in Czarek was relatively rapid and the carotid artery loop was already prepared, it was

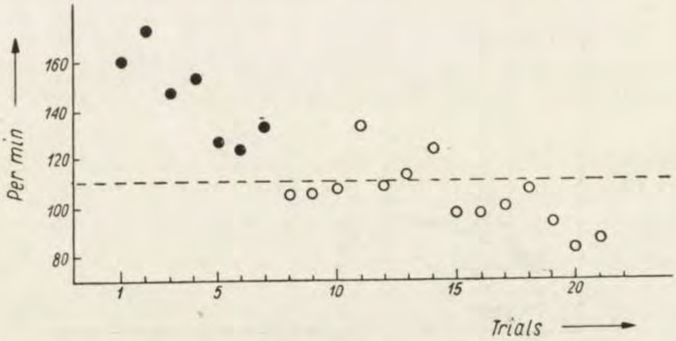

Fig. 5. Extinction of avoidance reflex in Czarek

Each circle represents the maximal heart rate (measured in 3 sec. intervals) observed during the 10-sec. action of differential stimulus (fan). Black circles represents heart rate in trials in which fan elicited the instrumental response; white circles represent those trials in which the motor response was inhibited. The interrupted line shows the level of pulse frequency (110 per min.) at which the motor CR occurred to positive CS.

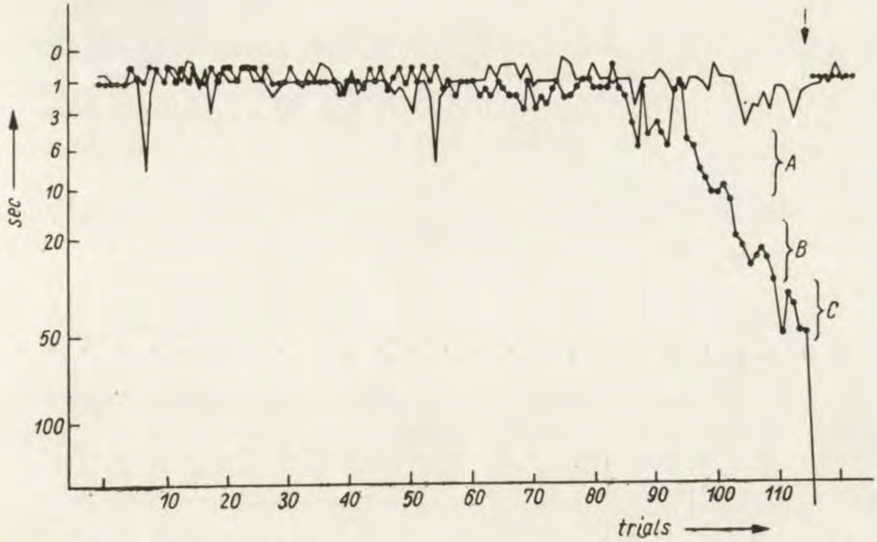

Fig. 6. Extinction of avoidance reflex to positive CS (buzzer I) in Puszek

Dotted line represents latencies of motor CR to buzzer I in successive trials. Plain line shows latencies of CR to another positive CS (flashing lamp). Note the selective extincion of CR to buzzer I and immediatete recovery of this CR following the single reinforcement by electric shock (arrow). Cardiac responses to buzzer I in trials denoted as group A, B, and C, are shown in Fig. 7.

possible to trace pulse behaviour in the course of extinction of instrumental motor response. As seen in Fig. 5 the cardiac response to DS gradually decreased and after 7 trials dropped beneath the level of 110 per. min. i. e. beneath that level which was attained in response to positive CS just before the execution of the trained movement. This means that the instrumental response to DS disappeared exactly at the moment when decreasing cardiac response fell beneath the pulse level corresponding to the cardiac response to CS.

Finally we would like to describe here the process of disappearance of avoidance reflex to CS (buzzer I) in Puszek. This happened unexpectedly and was undoubtedly due to the elaboration of differential inhibition to buzzer II.

This "extinction" of CR to buzzer I took quite a regular course. In spite of the fact that buzzer I was always discontinued when the animal performed the instrumental response, latency of this response became longer and longer and finally even amounted to 3 minutes (Fig. 6).

The behaviour of the heart rate in the course of this extinction is represented in Fig. 7, in the form of 3 pulse rate curves corresponding to three successive periods of "extinction". It is seen that in the two first periods (curve A and B) when the latency of instrumental reaction did not exceed 22 sec. the cardioacceleratory responses to CS were by no means diminished and lasted right up to the moment of execution of the trained movement. Their onset was as steep as in normal responses to CS. Since the performance of motor CR was delayed the eventual pulse rate at the moment of its execution was even higher than the pulse rate in normal avoidance responses when the latency of motor response did not exceed 2 sec.

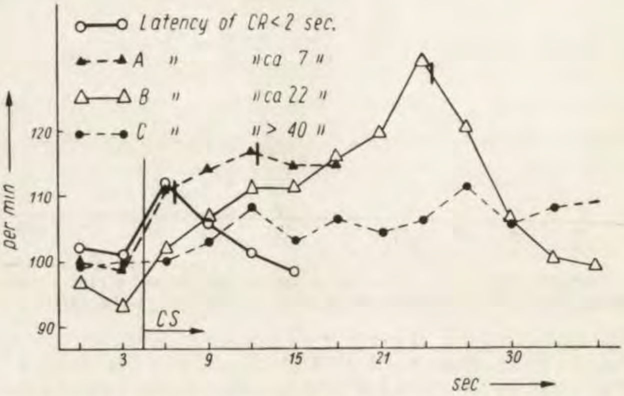

Fig. 7. Pulse behaviour accompanying the avoidance CRs to buzzer I in Puszek

Trials with increasing latency of motor CR are selected in four groups, thus showing the changes in cardiac CRs during the course of extinction of avoidance reflex as shown in Fig. 6.

However in the third period of "extinction" (curve C) the buzzer no longer produced cardiac acceleration during first 20—30 sec. and the instrumental reaction appeared much later.

In other words, in the two first periods, inhibition is seen exclucively in the motor component of the whole avoidance reflex but not in the cardiac component. Thus the relations between cardiac and motor components of the response to buzzer resemble the relations found in response to DSs in Pikuś.

On the other hand, the relations found in the third period of extinction, resemble those obtained in response to DSs in this dog and also in Czarek.

It is worth mentioning that when the avoidance CR to buzzer I became totally extinguished it was sufficient to administer a single shock reinforcement to buzzer I, to reestablish the reflex for an indefinite period.

DISCUSSION

The aim of the present paper was to elucidate the mechanism of differentiation of the avoidance CRs, obtained by the use of method described in Bregadze's paper (1953). This method consists in applying the DS for a definite number of seconds and discontinuing it without reinforcement, independently of whether and when the animal performs the avoidance movement.

As seen from our results, after differentiation by the aid of this method was achieved, the cardiac response to DS was twofold: in two dogs the pulse rate accompanying the DS was significantly lower than that accompanying the CSs; in the third dog, on the contrary, the pulse rate accompanying the DSs was higher than that to CSs. These two results we shall discuss separately.

The results obtained in the first 2 dogs may be easily explained. The prolongation of the stimulus beyond the moment of the performance of the avoiding reaction means that this reaction is not followed by the release of the animal from fear (i. e. classical defensive CR) as happens in normal avoidance procedure. The continuation of fear-producing stimulus may be considered as secondary reinforcement substituting the noxious US. Such a negative reinforcement following the performance of a given movement leads to its suppression. In consequence the DS becomes gradually less motogenic.

As was shown in the preceding paper the performance of the avoidance movement followed by the cessation of CS has a protective role maintaining the positive warning significance of this stimulus (Sołtysik and Kowalska 1960). Therefore if this movement is no longer performed, or performed with considerable delay, and the CS is not reinforced by the noxious US — the conditions arise for the extinction of the defensive classical reflex to this stimulus. As seen from our data this extinction, at least partially, does in fact occur and is manifested by the decrease of heart rate in comparison with positive CSs.

And so the process of differentiation (or extinction) of the avoidance CR (by the Bregadze's procedure) theoretically consists of two stages: one is the inhibition of the avoidance movement itself by negative secondary reinforcement provided by the continuation of negative CS; the second stage is simple extinction of the classical defensive CR which is neither reinforced nor protected by the avoidance movement. It is clear that these two stages may in some cases occur nearly parallelly. This was seen in experiments with Czarek (Fig. 5) where the process of extinction of cardiac response already began in the first presentations of the DS.

More difficult to understand are the results obtained in the third dog in which the avoidance movement to the DS was also eliminated in the course of differentiation, but the cardiac acceleration to DS remained and was even greater than to positive stimulus. The increase of the pulse rate in the first stage of differentiation is evident in the light of our previous consideration. But the problem arises now why the classical CR did not become extinguished in spite of the lack of the protective factor (CI) provided by the movement. Since the animal in response to the DS was very restless and performed a more or less stable sequence of defensive movements (such as howling and struggling) it may be that some of these movements, being accompanied by the cessation of stimulus became true avoidance reactions and replaced the previous response elaborated by us. This would easily explain why the cardiac response was totally preserved. Similar result was obtained deliberately by Page (1955) who showed that preventing of the performance of one response leads to spontaneous elaboration of the another one.

On the basis of these considerations it is also easy to explain the rather incidental series of experiments in which the original avoidance CS, being very similar to the differentiated one, gradually lost its motogenic property: the animal performed the avoidance movement to this stimulus with increasing latency and then stopped performing it altogether. As seen in Fig. 6 and 7, in the first stages of such "extinction" the cardiac response was still very prominent, although the movement was much delayed. This was because the motogenic value of CS decreased, owing to the fact that this movement was inhibited in response to the similar stimulus in the preceding and contemporary training (cf. analogical "generalization of differentiation" in classical conditioning: Siratsky 1924, Fursikov 1921).

On the other hand, the prolonged operation of this stimulus, neither accompanied by the movement itself nor by the US, led to the extinction of classical defensive CR to this stimulus, which was manifested by the decrease of cardiac response. This extinction in its turn made the CS increasingly inefficient in respect to the elicitation of the motor avoidance response.

However a single reinforcement of the stimulus by the noxious US restored immediately its conditioned defensive significance and as the result of this restoration, the avoidance conditioned reaction returned (Fig. 6).

Another result of the present experiment that should be commented upon is the marked difference between cardiodeceleration following the cessation of positive CSs and DSs (Fig. 1 and 2). This result can be easily explained by the inhibitory role of the instrumental avoidance response in respect to the classical defensive CR.

SUMMARY

1. The aim of this work was to elucidate the mechanism of differentiation and extinction of avoidance conditioned reflexes by the prolongation of the conditioned stimulus beyond the execution of the instrumental movement.

2. It was shown that extinction of the instrumental response occurs by the two mechanisms: 1° — the inhibition of the movement itself due to its negative secondary reinforcement by the continuation of fear-producing stimulus, 2° — extinction of the classical defensive conditioned reflex to this stimulus, due to the removal of the protective (from extinction) role of the instrumental response playing the role of conditioned inhibitor.

3. Thus, while avoidance reflex is considered as circulus-vitiosus-like self-stabilizing system of positive and inhibitory conditioned reflexes, the impairment of either its excitatory or inhibitory link causes its progressive destroying and finally a complete abolition.

REFERENCES

BLACK A. H. 1958 — The extinction of avoidance responses under curare. J. comp. physiol. Psychol. *51*(5), 519—524.

BREGADZE A. N. 1953 — K voprosu vyrabotki oboronitelnogo uslovnogo refleksa u sobak. Trudy Inst. Fiziol. I. S. Beritashvili, *9*, 43—59.

CHORĄŻYNA H. 1957 — Some data concerning the mechanism of conditioned inhibition. Bull. Acad. Pol. Sci. *5*(11), 387—392.

FONBERG E. 1953 — Unpublished data.

FURSIKOV D. S. 1922 — Staticeskaya irradiacya tormozhenia. Russk. Fiziol. Zhurn. *5*, 333.

KONORSKI J. 1948 — Conditioned reflexes and neuron organization. Cambridge Univ. Press.

KONORSKI J. and MILLER S. 1933 — Podstawy fizjologicznej teorii ruchów nabytych. Med. Dośw. i Społ. *16*(1—4), 1—168.

KONORSKI J. and MILLER S. 1936 — Uslovnyje refleksy dvigatelnogo analizatora. Trudy Fiziol. Lab. Pavlova, *6*(1), 119—278.

LEERSUM G. V. v. 1911 — Eine Methode zur Erleichterung der Blutdruckmessung bei Tieren. Pflügers Arch. *142*, 377.

MILLER S. and KONORSKI J. 1928 — Sur une forme particulière des réflexes conditionnels. C. R. Soc. Biol. *99*, 1155.

PAGE H. A. 1955 — The facilitation of experimental extinction by response prevention as a function of the acquisition of a new response. J. comp. physiol. Psychol. *48*, 14—16.

SIRATSKY V. V. 1924 — O mozaikie vozbudimych i tormoznych punktov v kore bolŝich poluŝarii. Russk. Fiziol. Zhurn. 7, 316.

SOŁTYSIK S. 1959 — On the physiological mechanism of the avoidance conditioned reflexes. XXI Int. Congr. Physiol. Sci. Buenos Aires 1959, Abstr. of Comm. 258—259.

SOŁTYSIK S. 1960 — Studies on the avoidance conditioning. 3. Alimentary conditioned reflex model of the avoidance reflex. Acta Biol. Exper. *20*.

SOŁTYSIK S. and KOWALSKA M. 1960 — Studies on the avoidance conditioning. 1. Relations between cardiac (type I) and motor (type II) effects in the avoidance reflex. Acta Biol. Exper. *20*.

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STUDIES ON THE AVOIDANCE CONDITIONING.
III. ALIMENTARY CONDITIONED REFLEX MODEL OF THE AVOIDANCE REFLEX

S. SOŁTYSIK

Laboratory of Physiology of Nervous System in the Psychoneurological Institute, Pruszków, and Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw

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A peculiar property of the so called avoidance conditioned reflexes (CRs) is that these reflexes, once established, need no reinforcement by the unconditioned stimulus (US) for their preservation. In fact, the gist of avoidance behaviour is that the conditioned stimulus (CS) is never reinforced when the conditioned instrumental response is displayed. This is why the mechanism of these reflexes raised much controversy (cf. Solomon and Brush, 1956) and is still considered as poorly understood.

Konorski and Miller (1933, 1936) and Konorski (1948) attempted to explain the mechanism of avoidance reflexes on the basis of two the following assumptions: first, they postulated that the proprioceptive stimulus generated by the performance of the avoidance movement is a conditioned inhibitor (CI) of the classical defensive CR elicited by the CS; secondly, Konorski (1948) supposed that when CI is applied in compound with CS without reinforcement, this does not lead to the extinction of CR to CS. In consequence, CS followed by the learnt avoidance movement (CI) preserves its excitatory properties and hence does not loose its capacity to elicit this movement.

The second of these assumptions according to which repeated application of CI-CS compound does not lead to the extinction of the positive CR to CS applied alone, has recently been proved by Chorążyna (1957) on alimentary CRs. She found that, after the compound CI-CS was differentiated from CS applied separately, even repeated application of the compound CI-CS over a long time without any application of its positive counterpart CS, does not lead to the extinction of CS. Thus, Konorski's prediction that CS applied together with CI without reinforcement is, so to speak, "protected" by the latter stimulus from being extinguished, was verified. On the other hand Sołtysik and Kowalska (1960) presented experimental evidence showing that the conditioned avoidance response does in fact play the role of CI in respect to the defensive CR.

The difference between the experimental setting in Chorążyna's study and that in normal avoidance conditioning was that while in her experiments the sequence of the inhibitory compound was CI-CS, in avoidance reactions it is just the reverse. In fact, the animal performs the trained movement only after the application of the stimulus. Therefore it was planned to imitate as closely as possible the general structure of avoidance responses by using alimentary reflexes and exteroceptive stimuli both as CS and CI. In such a way an exact alimentary CR "model" of avoidance CR could be produced. Since in our previous experiments concerning avoidance CRs, the autonomic responses (changes in heart rate) were examined, we decided to record them also in the present experiments.

The aims of the present experiment were in the first place to test the inhibitory effect of CI when it is applied not before but during the operation of CS, and secondly to see whether CI applied in this way protects the CS from extinction.

METHOD

The experiments were performed on one dog in a soundproof conditioned reflex chamber. Both classical and instrumental conditioned effects were observed. The isolated period of CS was 10 sec. Salivation from the shortened Stensen's duct fistula (Sołtysik and Zbrożyna 1957) was recorded by the Kozak method (Kozak 1950). Instrumental conditioned reaction consisted in lifting the foreleg and pressing a bar on the foodtray. Intertrial intervals were 3—5 min. and usually 6—8 trials were given in each experimental session. Movements of both forelegs, respiration and pulse rate were recorded kymographically using the Marey tambours. Pulse waves were received from carotid artery loop prepared by v. Leersum's method (v. Leersum 1911).

RESULTS

Experiments began with the elaboration of salivary and motor CR to the visuo-acoustic stimulus supplied by a rotating Fan. After the reflex was firmly established the latency of both salivary and motor reaction was very stable and amounted to 4—6 seconds. Then, we introduced a new stimulus, Buzzer, as CI. Buzzer was applied simultaneously with Fan for 10 sec. and was not reinforced by food, while Fan alone continued to be reinforced. Usually the inhibitory compound was applied once or twice daily among positive trials. After about 2 months of daily training the compound Buzzer-Fan did not elicit either salivary or motor reaction even when CI preceded the CS by 5—10 sec.

When this preliminary training was accomplished, a new series of experiments began, in which only the inhibitory compound was applied 10 times in each experiment. The sequence of both stimuli in the compound was reversed: first Fan was applied for 3 sec. and then Buzzer was added to it for another 10 sec. The series consisted of 12 experiments, so that 120 inhibitory trials were altogether given.

In spite of the fact that this new combination was never used in this dog, no additional training for the elaboration of the inhibitory reflex to it was needed. In the previous training the latency of salivary and motor CRs was usually longer than 3 sec. so the application of the Buzzer took place as a rule before these responses started and fully prevented their appearance. In only four trials (the 20th, 25th, 28th and 34th ones) did the dog start the alimentary motor response in the first 3 sec. of isolated action of the Fan, but as soon as the Buzzer was added the CR became suppressed. In the remaining 116 trials neither salivary nor instrumental responses were seen during the presentation of this CS-CI compound.

At the end of the 12th experiment, when the target number of 120 inhibitory trials was completed, Fan alone was applied four times in succession and reinforced by food. As seen in Fig. 1, Fan applied alone elicited a quite considerable salivary and motor CR even in the first trial.

Data concerning the cardiac response to application of CS-CI compound for each experimental session in this series are presented in Fig. 2. It is seen that during the first 3 seconds of operation of

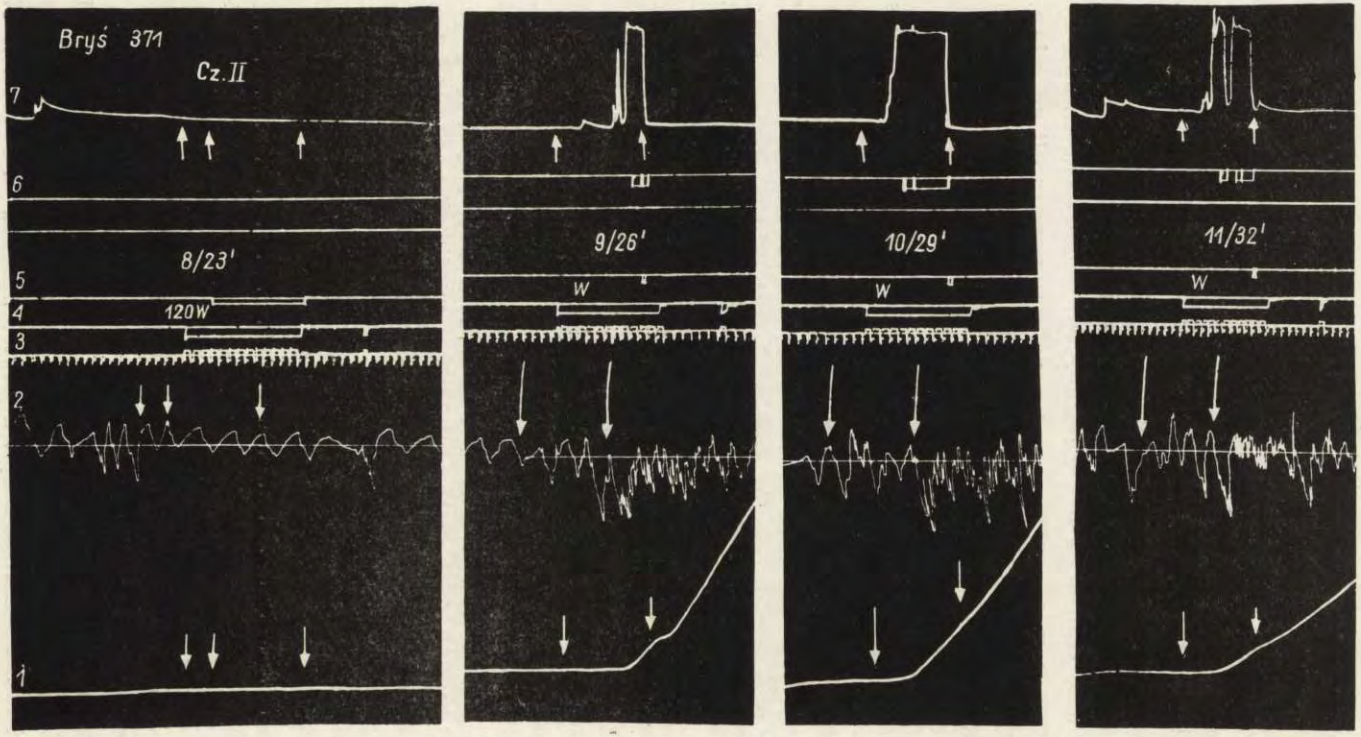

Fig. 1. Preservation of alimentary CR to CS applied repeatedly without reinforcement together with conditioned inhibitor

From left to right: the last, 120th — presentation of CS-CI compound and three following positive CS-US trials. 1 — salivation, 2 — respiration, 3 — time in sec., 4 — conditioned stimulus (Fan), 5 — conditioned inhibitor (Buzzer), or presentation of food (in positive trials), 6 — lever, pressed by the dog (instrumental CR), 7 — movements of the left foreleg. Arrows show beginning and termination of stimuli.

Fan, there is a marked rise of pulse rate which immediately drops during the following 10 sec. when Buzzer is added. It may be also seen from Fig. 2 that the rise of pulse rate during the 3 sec. of isolated action of CS is preserved during the whole series in spite of the fact that the stimulus was never reinforced by food.

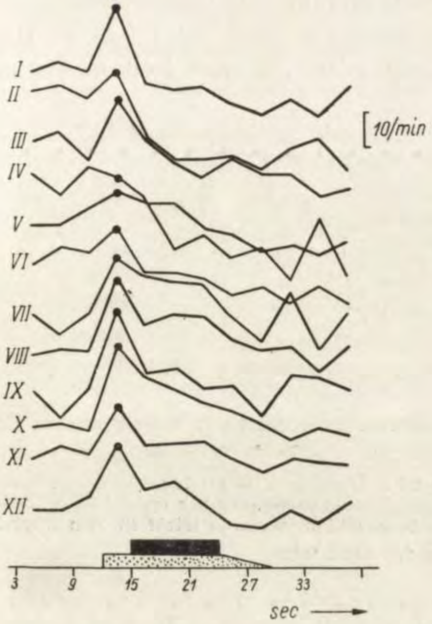

Fig. 2. Heart rate accompanying the CS—CI compound in 12 experiments

Duration and temporal relationships between CS and CI are seen above the time scale: spotted strip represents CS (Fan — its inertia caused prolonged termination), black strip represents CI (Buzzer). On the right above, scale of changes of pulse rate is given. On the left: numbers of experiments. Pulse rate is shown in 3 sec. intervals. Black dots represent pulse rate during 3 sec. of CS acting alone.

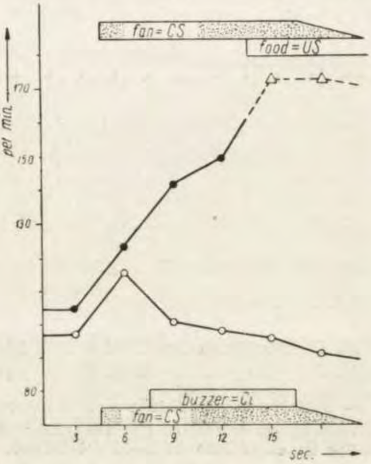

Fig. 3. Heart rate accompanying positive CS—US trials in 12th experiment—black circles, and heart rate accompanying CS—CI compound (mean values of 116 trials) in 12 experiments — white circles

In Fig. 3 the average pulse behaviour from this series is compared with that at the four positive trials in the 12th experiment. It is clearly seen that the effect of the addition of the CI to CS really consists in suppressing the rise of the heart rate elicited by the CS alone. Similarly to the results obtained in the avoidance reflexes (Sołtysik 1959, Sołtysik and Kowalska 1960) this suppression is immediate, i. e. the change from acceleratory into the deceleratory response occurs just after the application the CI.

It was interesting to examine whether or not there were some changes in the pulse rate during intervals between trials in the course of the above series of experiments. As seen in Fig. 4 and Table I the pulse rate in intertrial intervals gradually diminished and was lowest in last experiments of the series. On the other hand the pulse rate in the first 3 sec. following the onset of the CS (acting alone) remains the same. In consequence the acceleration of the pulse rate to the CS rather increases in the course of the series. It is further seen in Fig. 4 that as soon as positive trials were reintroduced the pulse rate in intertrial intervals raised steeply even above the initial level.

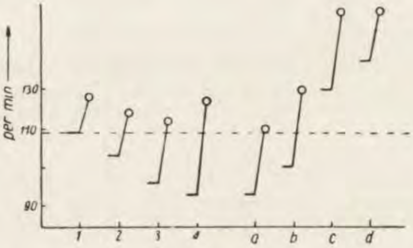

Fig. 4. Intertrial level of heart rate and cardioacceleration in first 3 sec. of CS is shown for:

1 — positive trials before the 12 experiments with compound (40 trials); 2 — CS-CI compound in experiments 1—4 (40 trials), 3 — the same in experiments 5—8 (40 trials), 4 — the same in experiments 9—12 (40 trials), and a, b, c, and d, positive trials in 12th experiment (one drawing for each trial).

The last fact we would like to draw attention to is the evolution of the level of heart rate just after the application of CS-CI compound. It is represented in Table I. As seen from it the post-compound level of the pulse rate is in first experiments of the series much lower than the prestimulus (or pre-compound) level. In the experiments following it gradually increased and, as the pre-compound level decreased during the course of experiments, these two levels eventually became nearly equal.

Table I

Changes in heart rate just before and just after the presentation of CS-CI compound in the course of "inhibitory" experiments

	Exp. 1-4	Exp. 5-8	Exp. 9-12
Pre-compound heart rate level	103.66	95.98	92.98
Post-compound heart rate level	84.11	89.41	90.33
Difference	19.55	6.57	2.65
Probability level	$p<0.001$	$p<0.010$	$p<0.100$

DISCUSSION

The data presented in this paper unequivocally show that 1°. the CI added to the CS (in the course of its operation) does in fact suppress its conditioned effects both autonomic and somatic, and 2°. that the CS covered in this way by the CI in many successive trials does not loose its positive conditioned significance. These two results should be commented upon.

1°. The conditioned effects to the CS we observed in this study were as follows: i) conditioned salivary reaction (type I), ii) conditioned motor reaction (type II), iii) conditioned cardiac response (type I). The first two had generally a rather long latent period amounting to 4—6 sec. Therefore, the CI started to act usually before the beginning of these reactions, and its effect consisted in the total precluding of their manifestation. But in those trials in which these reactions had started before the onset of CI they were immediately suppressed by it. On the other hand the cardiac conditioned response proved to be much more sensitive and less "inertial" (Gantt and Hoffmann 1940) than the two preceding ones and its latency was quite negligible. Therefore this response revealed the excitatory significance of the positive CS and showed that this significance did not change at all in the course of experiments.

One might ask whether the rise of the pulse rate to the CS is indeed the manifestation of the positive alimentary CR. This question should be raised because even indifferent acoustic stimuli very often produce cardiac acceleration which is sometimes resistant to extinction (cf. Sołtysik et. al. 1960). This objection is, however, excluded by the following facts: i) in Gantt and Hoffmann's (1940) and in Froňková, Ehrlich and Ŝlegr's (1957) experiments it has been shown that inhibitory (differential) CSs produce much less cardioacceleration than that produced by positive CSs; ii) We found (Sołtysik and Kowalska 1960b) that satiation producing the disappearance of both salivary and motor reaction to CS also caused disappearance of cardiac acceleration to this stimulus; iii) Finally, the very fact that the addition of CI to CS (i. e. loud Buzzer to Fan) elicits an immediate fall of the pulse rate shows that in turn its rise to the CS has a specific CR character.

2°. The full preservation of the excitatory significance of the CS despite its repeated non-reinforcement in the CS-CI compound is revealed by two facts: i) by unchanging cardiac acceleration in the first 3 sec. of the action of CS in all 12 experiments, and ii) by the full salivary and motor reaction to the CS applied after 120 inhibitory trials.

The last point to be commented upon concerns the behaviour of the pulse rate in the intertials intervals. As seen in Fig. 4 and Table I, the level of the pulse rate just preceding the application of the CS-CI compound gradually decreased in the course of this series, the level of the post-compound pulse rate on the contrary increased.

The relatively high level of the pulse rate in intertrial intervals (in periods preceding the application of the CS-CI compound) is due to the fact that, as generally known, the whole experimental situation becomes alimentarily conditioned, since in normal experiments the excitatory trials largely exceed the inhibitory ones. The cardiac effect of this "latent" CR to the situation is confirmed by the observation (cf. Sołtysik and Kowalska 1960b) that it is seen only in hungry animals and when the dogs are satiated the level of the pulse rate is lowered. Now, in the course of the inhibitory series, where the situation is not reinforced by food day after day, this environmental CR is gradually extinguished and this is manifested by the lowering of the intertrial pulse rate. So we see that protective role of the CI extends only to the CS itself, but not to the environmental stimuli.

As far as the post-compound heart rate is concerned, its low level may be attributed to the strong inhibitory effect of this compound. Why this effect seems to be attenuated in the course of inhibitory series is not clear.

SUMMARY

1. When the conditioned inhibition compound is applied in such a way that the conditioned inhibitor is added to the already acting positive conditioned stimulus, it produces a dramatic inhibition of all excitatory effects of this stimulus (salivary — type I, cardiac — type I, and motor — type II).

2. Conditioned inhibitor applied in such a way exerts a powerful protective influence against the extinction of the positive conditioned reflex to conditioned stimulus applied alone, even when inhibitory trials are repeatedly applied in a number of experiments in which no excitatory trials are given.

3. The environmental conditioned reflex, measured by the intertrial heart rate level, is gradually extinguished in the course of purely inhibitory experiments.

4. The results of these experiments throw some light on the physiological mechanism of avoidance conditioned reflexes.

REFERENCES

CHORĄŻYNA H. 1957 — Some data concerning the mechanism of conditioned inhibition. Bull. Acad. Pol. Sci. *5*(11), 387—392.

FROŇKOVÁ K., EHRLICH V. and ŠLÉGR L. 1957 — Die Kreislaufänderung beim Hunde während des bedingten und unbedingten Nahrungsreflexes und seiner Hemmung. Pflügers Arch. *263*, 704—712.

GANTT W. H. and HOFFMANN W. C. 1940 — Conditioned cardio-respiratory changes accompanying conditioned food reflexes. Am. J. Physiol. (Proc.) *129*(2), 360—361.

KONORSKI J. 1948 — Conditioned reflexes and neuron organization. Cambridge Univ. Press.

KONORSKI J. and Miller S. 1933 — Podstawy fizjologicznej teorii ruchów nabytych. Med. Dośw. i Społ. *16*(1—4), 1—168.

KONORSKI J. and MILLER S. 1936 — Uslovnyie refleksy dvigatielnogo analizatora. Trudy Fiziol. Lab. Pavlova. *6*(1), 119—278.

KOZAK W. 1950 — Method of graphic registration of salivary secretion. Acta Biol. Exper. *15*(14), 185—192.

LEERSUM G. V. v. 1911 — Eine Methode zur Erleichterung der Blutdruckmessung bei Tieren. Pflügers Arch. *142*, 377.

SOLOMON R. L. and BRUSH E. S. 1956 — Experimentally derived conceptions of anxiety and aversion. Nebraska Symposium on Motivation. 212—305.

SOŁTYSIK S. 1959 — On the physiological mechanism of the avoidance conditioned reflexes. XXI Int. Congr. Physiol. Sci. Buenos Aires, Abstr. of Comm. 258—259.

SOŁTYSIK S. and KOWALSKA M. 1960a — Studies on the avoidance conditioning. 1. Relations between cardiac (type I) and motor (type II) effects in the avoidance reflex. Acta Biol. Exper. *20*, 98.

SOŁTYSIK S. and KOWALSKA M. 1960b — in preparation.

SOŁTYSIK S., KOWALSKA M. and RADOM S. 1960 — in preparation.

SOŁTYSIK S. and ZBROŻYNA A. 1957 — The chronic fistula of shortened Stensen's duct in dogs. Acta Biol. Exper. *17*(2), 339—344.

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THE EFFECTS OF UNILATERAL ABLATIONS OF SENSORI-MOTOR CORTEX ON TYPE II CONDITIONED REFLEXES IN CATS.

I. NATURAL CONDITIONED REFLEXES

E. JANKOWSKA and **T. GÓRSKA**

Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw

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From the very beginning of investigations on the higher nervous activity, the Pavlovian school distinguished between "natural" conditioned reflexes, i. e. those which are established in the course of the normal life of the animal, and "artifical" conditioned reflexes, i. e. those which are elaborated by the experimenter under laboratory conditions (Pavlov, 1952). To give an example, salivary reaction to sight or smell of food represents a natural conditioned reflex, whereas salivation to a buzzer reinforced by food is an example of an artificial reflex. The instrumental (type II) conditioned reflexes may be divided in the same way. Such reactions as the drawing in of food by the foreleg from outside a cage or from a hole may be considered as natural instrumental reactions. They are formed in the course of an animal's life by trial and error in all situations where food is not directy available. As far as artifical instrumental reflexes are concerned, they may be formed under laboratory conditions by appropriate reinforcement of any movement of the animal (Thorndike, 1911; Konorski and Miller, 1933). Although the division between natural and artificial conditioned reflexes is by no means sharp, it is however very useful because these two categories of reflexes present some different properties probably connected with different ways in which they are formed.

In natural instrumental reflexes the effect is not necessarily obtained by a determined movement. In its life, the animal learns to perform various movements in order to reach a given goal. These movements may be mutually replacable. Artificial instrumental reflexes used in the laboratory are, on the contrary, most often very determined since the experimenter trains a definite movement and reinforces only this very movement by food. By reinforcing movements of only one extremity we can transform the complex natural instrumental response into a reaction limited to a single definite movement like in usual artificial instrumental reflexes.

The present paper is concerned with the effects of unilateral sensori-motor lesions on the natural alimentary instrumental reflexes, consisting in movements of either forelimb or of only one of them.

MATERIAL AND METHODS

Experimental animals

Experiments were performed on 26 cats, males and females, from one to a few years old. In some of the cats, other surgical interventions (deafferentation of the hindlegs) had been previously performed. These were in connection with some other experiments.

Tests

Two instrumental reflexes were used: (i) the reflex of stretching out the forelimb between the bars of a cage, to reach food which was placed outside, and (ii) the reflex of putting the forelimb into a trough with a narrow groove, in which the food was placed, and drawing it out of the groove. The first of these tests will be refered to as cage test, the second — as trough test. The experimental situation is shown in Fig. 4.

The distance between neighbouring bars of the experimental cage was 3.5 cm. The food was placed several centimetres in front of the bars. The groove in the trough was 10 cm. long, 3 cm. wide, and 3 cm. deep. The food was usually laid in the middle of the groove. In the trough there was a special device which enabled the experimenter to reinforce the movements of only one of the limbs, and thus if the animal performed movements with the other extremity, they were unsuccessful.

In order to make the cat react to the sight and/or the smell of food, and not to the movement of the experimenter's hand, the food was, as a rule, placed before the cat while it was still eating the previous portion. Bits of boiled meat were used as reinforcement.

The experimental procedure

In the majority of cats the experimental procedure was as follows. When the cat became accustomed to the experimental situation, the percentage of movements of drawing in the food with the left and the right forelimb was determined in about 20—50 trials. In some animals the movements of only one forelimb were reinforced. In these animals the preoperative training was carried out up to the stage where no more than 5% of movements of the non-reinforced forelimb appeared in the last hundred trials preceding the operation.

The operation was performed usually contralaterally to the limb most often used in the preoperative period. After a lapse of 5 days to several weeks after operation, the experiments were undertaken to test the state of reflexes. At first the cat was permitted to draw food with either limb ("free-choice behaviour"), but if the limb contralateral to the lesion was not used, we tried to force the cat to execute movements with this limb ("training"). The movements performed with the limb ipsilateral to the lesion will be called "ipsilateral movements", those performed by the contralateral limb — "contralateral movements".

The surgical procedure

The operations were made under aseptic conditions in somnifen (0.4 ml./kg.), or nembutal (35—45 mg./kg.) anaesthesia. Cerebral cortex was removed by suction. In some cats, pia mater and vessels over the part of the cortex to be removed were coagulated electrically. In others, subpial suction was used and care was taken not to damage the vessels. The lesion was covered with dura mater (not sutured). Then the muscles and skin were sutured in layers.

Lesions

The cerebral cortex removed involved forelimb somatic afferent area I (Pinto Hamuy, Bromiley and Woolsey, 1956), forelimb motor area I (Travis and Woolsey, 1956) and some other adjacent regions, depending on the variant of operation. In the first variant these additional regions included the head somatic afferent area I and head motor area I; in the second — hindlimb somatic afferent area I, hindlimb motor area I and at least a part of supplementary motor area for the hindpart of the body (Woolsey, Settlage et al., 1950); in the third variant all regions enumerated here were removed.

Fig. 1 shows boundaries of the three variants of lesions. The widest (3rd variant) involved: *g. cruciatus ant.* and *post.*, *g. sigmoideus ant.* and *post.*, *g. coronalis*, anterior parts of *g. compositus ant.*, *g. lateralis*, *g. fornicatus* and *g. presplenialis*, nad posterior part of *g. proreus*. When head areas were spared, the lateral boundary of lesions reached the bottom of *s. coronalis*. When the hindlimb areas were spared the median boudary of lesion was a line running parallel to the midline and cutting through the middle of *s. cruciatus*. The cortex was carefully removed from the surface of the hemisphere, as well as from the depth of the sulci. Special care was taken

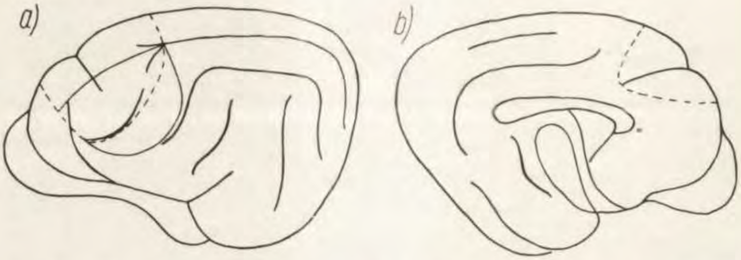

Fig. 1. Diagram showing the extent of lesions on the lateral (a) and medial (b) surface of the brain

Continuous line shows the boundaries of lesions when the forelimb and head areas were removed (variant I). Interrupted line shows the boundaries of lesions when the forelimb and hindlimb areas were removed (variant II). The extent of the widest lesions (variant III), including the forelimb, hindlimb and head areas is given by the sum of both previous variants.

not to leave the cortex hidden in the *s. cruciatus, s. coronalis* and posterior 1/3 of *s. presilvius*, including areas for hindlimb, forelimb and head (Chusid, Guttierez and Robinson, 1949, Delgado and Livingston, 1956).

RESULTS

Postoperative state of reflexes in the free-choice behaviour

In the first postoperative experiments, 17 cats in the trough test and 17 in the cage test were allowed a free-choice of limbs for drawing in the food. This procedure was used both for cats in which the movements of both forelimbs were reinforced before operation and for those in which only the movements of the limb contralateral to the future lesion were reinforced. All the cats drew food only with the limb ipsilateral to the lesion independently of which limb they used before operation. However, besides movements of the ipsilateral limb, the only successful ones, some movements of the contralateral limb were also performed. They were the abortive reactions, which never resulted in reaching the food. These movements were present in only 6 out of 17 cats in the trough test and in 3 out of 17 in the cage test (see Table I, Fig. 2 and 3).

In Table I results obtained in different groups of cats are compared. In the group III, in which only the movements of the

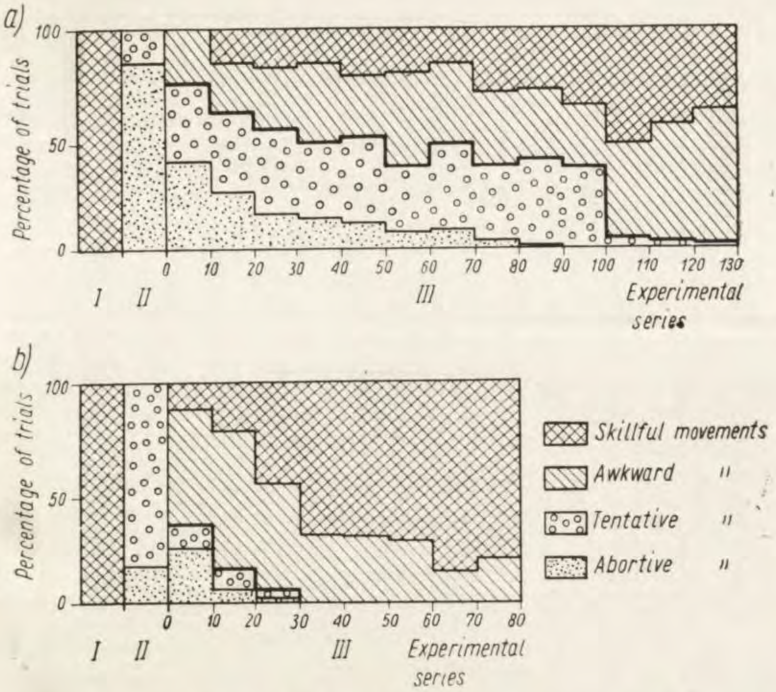

Fig. 2. Percentages of trails in which ipsi- and contralateral limb movements were performed in the:

I — preoperative period, II — first five trials of the free-choice behaviour after operation, III — postoperative training with nonreinforcement of the ipsilateral limb movements (in ten successive trials). a — trough test, b — cage test.

limb contralateral to the lesion were reinforced before operation, movements of this same limb appeared in all 4 cats and were performed in about half of the trials. In groups I and II, in which free-choice reactions were allowed before operation, the movements of the affected leg were present in only some cats and the percentage of trials in which they appeared was much smaller. There was a marked difference in the trough test between the groups II and III although they had the same percentage of contralateral movements before operation. On the other hand, the difference between the groups I and II with different percentages of contralateral limb movements before operation, but without any restrictions in the use one of the limbs, was small, especially in the cage test.

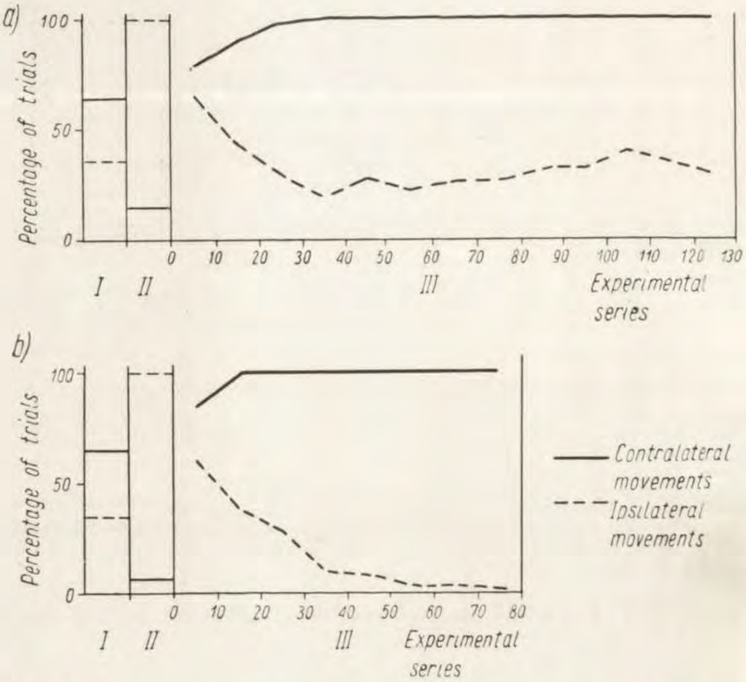

Fig. 3. Percentages of different kinds of the contralateral limb movements in the same experimental series as in Fig. 2. The heavy line separates the unsuccessful movements from the successful ones. Other denotations as in Fig. 2

No dependance between the behaviour of the animals in these experiments and the extent of lesions, or the duration of recovery (ranging from 1 to 16 weeks) was found.

The data presented above refer to the first 5 trials after operation. These were thought to be the most characteristic, because the continuation of experiments in free-choice conditions resulted in fixation of those forms of behaviour which were successful at the beginning. Thus if the cat was drawing food with the ipsilateral limb, it continued to do so and never tried to use the contralateral limb. If some movements of the contralateral limb were present at the beginning, their evolution varied according to the experimental procedure: if the cat was given food immediately after performance of such movement (although it did not draw it) and before any movement of the ipsilateral limb appeared, then these movements remained for some time. If, however, the experimenter waited for

Table I

Comparison of number of movements of the contralateral limb in the first 5 trials of the free-choice behaviour after operation in cats with different percentages of movements of this limb in the preoperative period. Groups I and II — cats in which movements of both forelimbs were reinforced before operation; Group III — cats in which only the movements of the limb contralateral to the future lesion were reinforced in the preoperative period

Tests	Groups of cats	Number of cats in a group	% of trials with contralateral movements before operation.	Number of cats in which contralateral movements were present after operation	% of trials with contralateral movements after operation
Trough test	I with 0—80% of contralateral movements.	9	37	1	2
	II with 80—100% of contralateral movements.	4	90	1	15
	III with 80—100% of contralateral movements.	4	91	4	45
Cage test	I with 0—80% of contralateral movements.	11	51	7	2
	II with 80—100% of contralateral movements.	6	91	6	1

successful movements, performed only with the ipsilateral limb, then the movements of the contralateral limb, non-reinforced, disappeared. It should be noted that whenever movements of the contralateral limb appeared, they were abortive and always unsuccessful.

Changes in the state of reflexes resulting from the non-reinforcement of the ipsilateral movements

In the experiments just described it was found, that after unilateral removal of the sensori-motor cortex, movements of the contralateral limb were present in some cats only, and they were performed only in an abortive form. So, another series of experiments was undertaken to see: (i) whether the cats which performed no contralateral movements could be forced to execute them, and if so, (ii) whether it would be possible for these cats, as well as for those in which some movements of the contralateral limb appeared spontaneously, to perform full movements with this limb.

In order to provoke movements of the contralateral limb, the movements of the opposite limb were extinguished. As we know, when one instrumental reflex is extinguished, other reflexes which were normally reinforced in the same or in a similar situation (Wyrwicka, 1955; Ajrapetjan, 1958) emerge. Therefore, if our animals were able to perform movements with the contralateral limb, extinction of movements of the ipsilateral limb should create favourable conditions for their appearance.

To extinguish movements of the ipsilateral limb we fixed on it a bag, big enough to prevent the animal from putting this limb between the bars of the cage or in the groove of the trough. In the preoperative period no special disturbances were observed when such a bag was put on. Cats ceased to use this leg in 2—3 trials even if there was a great preference for its movements before. The effects of this procedure on movements of ipsi- and contralateral limbs in the postoperative period are shown in Fig. 2. The data concern 14 cats in the trough test and 10 in the cage test. As movements of both the contralateral and ipsilateral limb could appear in a single trial, their sum may be higher than 100%. We see from this figure that the movements of the ipsilateral limb rapidly and seriously decrease when non-reinforced. Already in the first 10 trials their number dropped to 60% and then to 2—8% in the cage test, and to 20—40% in the trough test. Decrease of movements of the ipsilateral limb is followed by the appearance of movements of the affected limb, occurring for the first time between the 1st and 7th trials. In the first 10 trials they were present in about 80% of cases, and then very quickly reached 100%.

As it was already stated, the movements of the contralateral limb in the free-choice behaviour after operation, if present, were always unsuccessful after operation. As a consequence of non-reinforcement of the ipsilateral movements, successful movements of the contralateral limb began to appear in all cats, and they became able to get food by themselves. Fig. 3 shows percentages of different kinds of contralateral movements performed in every 10 successive trials of the training. The movements were classified into: (i) unsuccessful movements, which could be divided into abortive and tentative, and (ii) successful movements which were either awkward or skillful (see Fig. 4). In the cage test the abortive movements consisted in lifting the limb or scratching the floor of the cage; tentative movements — in scratching the bars or making an effort to stretch the limb out of the cage; awkward movements consisted in stretching the limb out of the cage through the bars but not straight towards food and/or not with reaching food immediately; skillful ones — in stretching the limb out of the cage in front of the meat and drawing it in immediately. In the trough test the movements were considered abortive when the animal scratched the table near the trough; tentative — when it scratched the top of the trough; awkward movements consisted in putting the leg into the groove but without precision, with missing the meat and throwing it out only after several attempts; skillful movements— in putting the limb into the groove and throwing it out with one or two movements.

In calculating the percentages of particular kinds of movements, the best movements from each trial were used. For example, if the cat performed at first the movements classified as abortive, and then drew in the meat with an awkward movement, only this last served for computation. Therefore, besides the movements represented in Fig. 3 there was also a certain number of worse movements. The sum of awkward and skillful movements shows the number of successful movements by which the cat was able to draw out the meat by itself. The sum of abortive movements and tentative movements shows the number of unsuccessful ones. It has to be added, however, that these latter were also reinforced. As a rule, the experimenter waited some time (from several seconds to

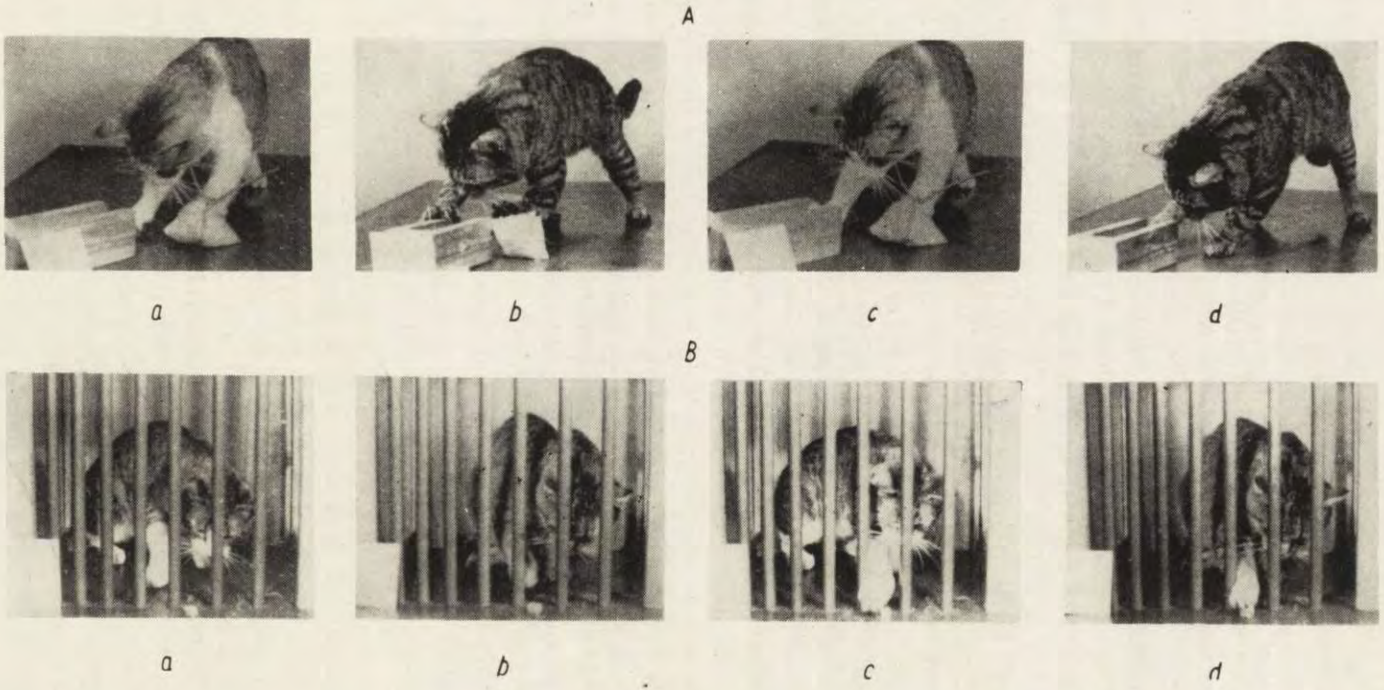

Fig. 4. Examples of different kinds of the contralateral (right) limb movements;

a — abortive movements, b — tentative movements, c — awkward movements, d — skillful movements. A — trough test, B — cage test. Pictures with the bag on the left forelimb were taken during the training period, the other ones before or after the training.

about half a minute), for animal's more successful movements and gave the food immediately only when the cat seemed to give up the attempts.

A gradual decrease in the percentage of unsuccessful movements, and increase of the successful ones, can be observed in both test. The first successful movements appeared in the cage test between the 1st and 13th trial, and in the trough test between the 1st and 49th trial. In the cage test they increased from more than 60% in the first 10 trials to 100% in the next 10 trials, and in the trough test — from 23% to 97%. In the latter test there were some cats which never reached the 100% level during this training. The increase in the total number of successful movements was accompanied by an increase in the ratio of skillful movements to awkward ones. In the cage test the number of skillful movements at the end of the training was 4 times as high as that of awkward movements; in the trough test they were then almost equal. As far as the unsuccessful movements are concerned, the abortive movements diminished faster than the tentative ones, but in the trough test this process was slower.

If we analyse the changes in number of ipsi- and contralateral movements, as well as the changes in number of different kind of contralateral movements, we can find no statistically significant differences between those cats which performed some movements with the contralateral limb in the free-choice behaviour, and the cats which did not. Also no differences were evident between the cats that had the cerebral cortex removed in different variants of lesions.

State of reflexes in the free-choice behaviour after training with non-reinforcement of the ipsilateral movements

When the experiments described above were over, in 5 cats all restrictions on the use of the ipsilateral limb movements were removed. No bag was applied and the cat was allowed to draw in food with either limb. The resulting changes are shown in Fig. 5 and Fig. 6.

If we compare the first 10 trials of this free-choice behaviour with the end of the preceding series, an increase in the number of ipsilateral and a decrease in the contralateral movements are

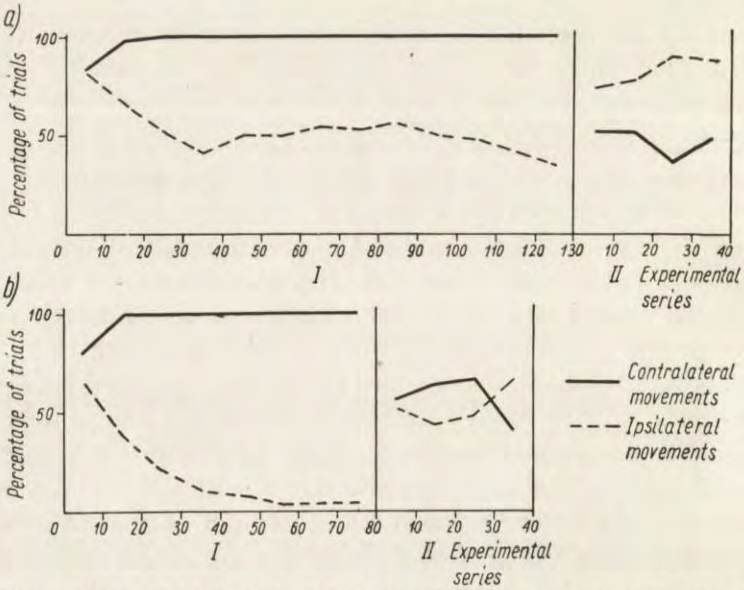

Fig. 5. Percentages of trials with the ipsi- and contralateral limb movements in 5 cats in which the free-choice behaviour (II) was studied after training with non-reinforcement of the ipsilateral limb movements (I)

Denotations as in Fig. 2.

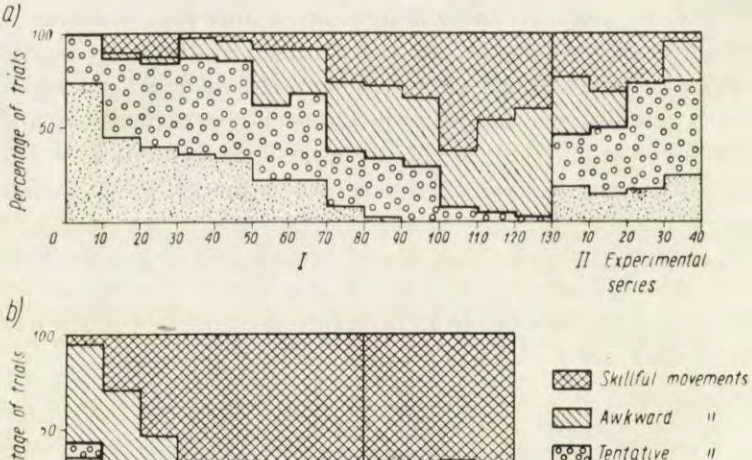

Fig. 6. Percentages of different kinds of contralateral limb movements in the same cats as in Fig. 5

I — training with non-reinforcement of the ipsilateral limb movements, II — free-choice behaviour after the training. Denotations as in Fig. 3.

seen. The numbers lie between those of the free-choice behaviour immediately after operation, and those observed at the beginning of training. At the same time serious deterioration in the quality of the contralateral movements took place. In the trough test, the abortive movements, absent in 40 trials or more, reappeared and the number of tentative movements greatly increased. In the cage test tentative movements absent in the last 50 trials or more, also reappeared. It is worth noticing, that the same cat could perform very different kinds of movements e. g. tentative and skillful, abortive and awkward, and even abortive and skillful in successive trials. Such diversity was not seen in the earlier period of experiments.

The contralateral movements in animals with some difficulties in the use of the ipsilateral limb

In 2 cats, the sensori-motor cortex was removed some weeks after an extensive deafferentation of the hindlimbs. As a result of this deafferentation, animals were unable to stand. When sitting they had both their hindlimbs stretched out to one side. In order to keep an upright position they were obliged to use one foreleg for support. When the limb contralateral to the cortical lesion was used for this purpose, food was drawn only with the ipsilateral limb, both in the trough test and in the cage test. But when the cats supported themselves with the ipsilateral limb, then two forms of behaviour were present: either they performed the movements with the contralateral limb, or used the ipsilateral one. In the latter case the ipsilateral limb did not support the body and a loss of balance followed. In this situation, from the very beginning of experiments, the movements of the contralateral limb were performed in 5—10 trials out of 10. They were always successful, though in one cat skillful, and in another awkward and preceded by tentative movements. In about half of the trials some attempts to use the ipsilateral limb were observed before the movements of the contralateral limb. These consisted in changing the position of the body so as to free the ipsilateral limb and were accompanied by slight flexions of this limb. As these data were obtained on 2 cats only, no detailed comparison with experiments described above is possible. So the Fig. 7 serves only as an indication of the trends. In comparison with cats which were allowed to use both limbs after operation, the animals described here, show a much greater number of contralateral movements and less of ipsilateral ones. The most important point, however, is that their contralateral movements were successful in all trials in which they were present, whereas none was successful in other groups. When we compare cats with difficulties in using the ipsilateral limb with cats in which the training with non-reinforcement of the ipsilateral movements has already started, they show a similar number of movements both of ipsi- and contralateral limb, but the quality of the contralateral movements in the cats described here was better.

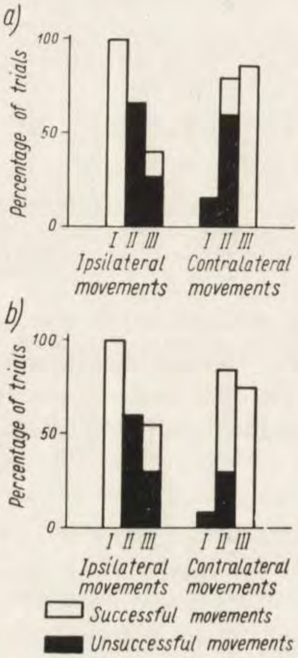

Fig. 7. Percentages of trials with the ipsilateral and contralateral limb movements in the trough test (a) and in the cage test (b)

I — first 5 trials of the free-choice behaviour after operation, II — first 10 trials of training with non-reinforcement of the ipsilateral limb movements, III — first 10 trials of experiments in cats with difficulties in using the ipsilateral limb.

DISCUSSION

Experiments presented in this paper have shown that unilateral ablations of sensori-motor cortex (within the limits described above), do not abolish the natural instrumental reflexes of drawing food with the contralateral forelimb. In spite of the fact that movements of this limb did not appear in the free-choice behaviour, or at best, appeared only in an abortive form, the animals were able to perform them regularly and even skillfully in favourable conditions. The aim of this paper was to elucidate what were the conditions enabling the animal to perform movements with the affected extremity, and which factors determined the degree of skillfullness of their performance.

When the normal animal is allowed to perform the movements of drawing food from outside the cage or from the trough with both forelimbs we have two instrumental conditioned reactions elicited by the same stimuli (sight and smell of food) to deal with. Whether the animal performs the movement with the right or left foreleg depends on, as we assume, which of the two motor executive centres receives more impulses from the higher centres at the given moment.

According to the results obtained by I. Stępień and L. Stępień (1957) on dogs, and our own unpublished data on cats, the instrumental reactions of the given limb depend not only on the contralateral sensori-motor area, but also, partly, on the ipsilateral area. This is shown by the fact that when the ipsilateral sensori-motor area is removed after ablation of the contralateral one, the instrumental reflex, present after the first operation, becomes either abolished or seriously deteriorated. So, after the unilateral sensori-motor ablations, the executive centres of the ipsilateral limb should receive only slightly diminished number of impulses from the cortex, while the centres of the contralateral limb receive much less impulses, because of the removal of their main sensori-motor area. In the free-choice behaviour after operation, our animals used, as a rule, only their ipsilateral leg for drawing food. Taking into account that the contralateral movements could be also performed in some conditions, this fact may be explained as due to a change of balance between the strength of the two instrumental reflexes. Therefore, the disappearance of the reflex of the limb contralateral to the cortical lesion, should be considered in these cases not as a loss of this reflex but rather as its ousting by reactions of the ipsilateral leg.

It may be concluded from these considerations that if, for some reason or other, the preference for performance of ipsilateral movements is abolished, the animal may use its contralateral leg. This was indeed found to occur under following circumstances: (i) when the movements of the ipsilateral limb were not reinforced before operation, (ii) when the tendency to perform movements of the ipsilateral limb greatly decreased as a result of postoperative training in which these movements were not reinforced, (iii) in cats that had some difficulties in the use of the ipsilateral limb. In the first two cases the instrumental conditioned reaction of the ipsilateral limb became extinguished. In the third case, we had to do with the inhibition of the spinal motor centres of the movements of the ipsilateral limb, because it was engaged in a strong postural reflex, antagonistic to the instrumental response.

As far as the skillfulness of movements of the contralateral limb is concerned, serious deterioration was observed in the first experiments after operation and then again when, after the experiments with non-reinforcement of the ipsilateral limb movements, a free-choice of limb was allowed. In the first case destruction of the chief cortical centres and inability of the remaining cortex to take over their functions at once, could be a cause of this deterioration. In the second case, however, no further surgical interventions preceded this deterioration. So its cause ought to be sought rather in the changes of the experimental conditions.

At the end of the training a tendency to draw food with the ipsilateral limb, although greatly reduced, remained, and the animal, time and again, returned to this movement. When the movements of the ipsilateral limb were again reinforced by food, their inhibition was removed, and, in consequence, the tendency to use this limb increased. This led again to the relative preponderance of the movements of the ipsilateral leg, which seems to be in this case the main cause of the deterioration of movements of the affected limb. Thus the fact that in many trials instead of full movements of this limb only the abortive movements appeared or attempts stopped before food was attained, could be explained as due to the cutting short of these movements by the competitive reaction of the opposite limb.

The same mechanism may be also involved in the deterioration of the contralateral movements in the initial experiments after the operation. The suppressing effect of the movements of the ipsilateral limb might be then even stronger.

As seen from these considerations the defective performance of instrumental movements with the limb contralateral to the cortical lesion as well as their absence, may be attributed to the interfering effects of the movements performed with the ipsilateral limb. This hypothesis will find additional support in our further experiments concerning the artificial instrumental reflexes.

SUMMARY

In experiments on cats, the effects of unilateral ablations of the sensori-motor cortex on natural instrumental reflexes, which consisted in drawing food with one of the forelimbs, were studied. It was found that, after operation, the animals drew food only with the leg ipsilateral to the lesion. Movements of the contralateral leg could appear, however, in favourable conditions, i. e. when movements of the ipsilateral limb were extinguished in the pre- or post-operative training, or when this limb was engaged in an antagonistic reflex. The quality of the movements of the contralateral limb was much better when the ipsilateral limb could not be used. The results obtained were discussed from the point of view of balance between the reflexes of both limbs. A hypothesis was put forward by which the disappearance of movements of the contralateral limb or the impairment of their skillfullness could be explained, at least partly, by the interfering influence of the preponderant reflex of the ipsilateral limb.

REFERENCES

AJRAPETJAN A.A. 1958 — K woprosu o meżcentralnych otnoszenijach w korkowom otdele dwigatelnogo analizatora u sobak. Awtoreferat dissertacii. Leningrad.

CHUSID J. G., GUTIERREZ-MAHONEY C. G. and ROBINSON F. 1949 — The "motor" cortex of the dog. Fed. Proc. *8*, 25.

DELGADO J. M. R. and LIVINGSTON R. B. 1955/1956 — Motor representation in the frontal sulci of the cat. Yale J. Biol. Med. *28*, 246.

KONORSKI J. and MILLER S. 1933 — Podstawy fizjologicznej teorii ruchów nabytych. Ruchowe odruchy warunkowe. Warszawa.

PAWŁOW I. P. 1952 — Dwadzieścia lat badań wyższej czynności nerwowej (zachowania się) zwierząt. Warszawa.

PINTO HAMUY T., BROMILEY R. B. and WOOLSEY C. N. 1956 — Somatic afferent areas I and II of dog's cerebral cortex. J. Neurophysiol. *19*, 485.

STĘPIEŃ I. and STĘPIEŃ L. 1957 — Wpływ jednostronnych uszkodzeń okolicy czuciowo-ruchowej mózgu na ruchowe odruchy warunkowe u psów. Acta Physiol. Pol. *8*, 532.

THORNDIKE E. L. 1911 — Animal intelligence. New York.

TRAVIS A. M. and WOOLSEY C. N. — 1956 — Motor performance of monkeys after bilateral partial and total cerebral decortication. Amer. J. Physiol. Med. *35*, 273.

WOOLSEY C. N., SETTLAGE P. H., MEYER D. R., SENCER W., PINTO HAMUY T. and TRAVIS A. M. 1950 — Pattern of localization in precentral and "supplementary" motor areas and their relation to the concept of a premotor area. Res. Publ. Ass. Nerv. Ment. Dis. *30*, *238*.

WYRWICKA W. 1955 — Badania nad odruchami warunkowymi analizatora ruchowego. Łódź.

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THE EFFECTS OF BILATERAL LESIONS IN THE MOTOR CORTEX ON TYPE II CONDITIONED REFLEXES IN DOGS

I. STĘPIEŃ, L. STĘPIEŃ and **J. KONORSKI**

Nencki Institute of Experimental Biology, Department of Neurophysiology, Warsaw

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The present series of papers deals with the influence of various lesions in sensorimotor cortex upon motor, type II, conditioned reflexes. In the previous paper (Stępień and Stępień 1959) it was established that after bilateral ablations of sensory areas I and II the motor conditioned reflexes are abolished for a number of weeks and then they are gradually restored. The general behaviour of animals in the conditioned reflex chamber is, however, unimpaired: to conditioned stimuli the dog displays a pertinent alimentary reaction, and when the trained motor reflex is restored his state hardly differs from that before operation.

The present paper is concerned with the effects of bilateral ablations of the so called motor cortex on instrumental conditioned reflexes. According to the abundant evidence obtained by the method of electrical stimulation of the cerebral cortex since Fritch and Hitzig (1870), it is generally accepted that in dog this area forms a transversal strip of the cortex situated approximately between the cruciate sulcus and the central sulcus (and their lateral prolongations). The lateral boundary of this area is formed by the rostral prolongation of the suprasylvian sulcus. Thus, the motor area in dog comprises the following gyri: *postcruciatus*, the anterior part of *sigmoideus posterior* and anterior part of *coronalis* (Fig. 1A). The electrophysiological data are in good agreement with morphological description of the cortex of the dog. In fact, according to recent data (Morin 1951, Adria-

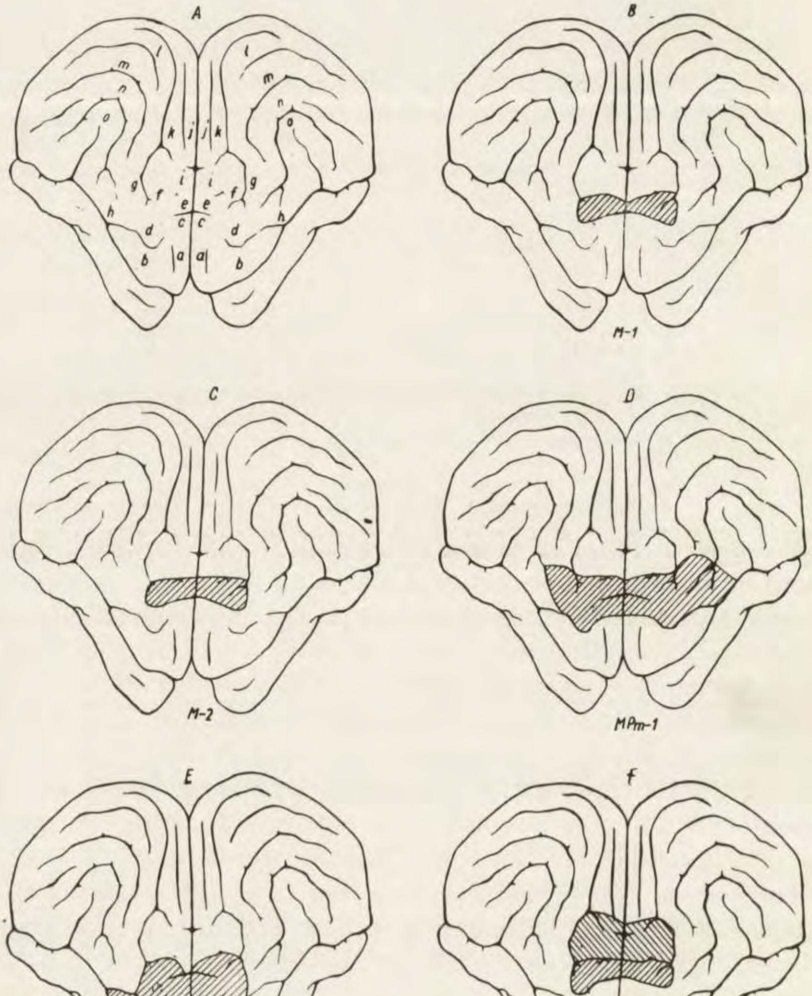

Fig. 1. The cerebral cortex of the dog flattened on the plain, and sites of lesions in experimental dogs. Note that we have subdivided the precruciate and postcruciate regions into two parts: the medial parts of these regions are called gyrus precruciatus and postcruciatus, the lateral parts gyrus sigmoideus anterior and posterior

A — normal cerebral cortex. Denotations: a — *gyrus proreus*, b — *g. orbitalis*, c — *g. precruciatus*, d — *g. sigmoideus anterior*, e — *g. postcruciatus*, f — *sigmoideus posterior*, g — *g. coronalis*, h — *g. compositus anterior*, i — *g. postcentralis*. B — F, lesions of the cerebral cortex in various dogs as indicated in text.

nov and Mering 1959) this area comprises the greatest percentage of giant pyramidal cells. The number of these cells falls down dramatically both rostrally and caudally to the motor area.

Studies concerning the motor area carried out either by electrophysiological methods, or by methods of ablations in chronic animals, show unequivocally that it is chiefly, or perhaps even exclusively, concerned with the control of movements of extremities and mouth. On the other hand, it is now believed that the so called premotor area controls the axial musculature and movements of the head (Woolsey et. al. 1950, Woolsey and Settlage 1950). Whatever the real functional significance of the premotor area, the symptoms produced by its ablation and those obtained after ablation of the motor area sensu stricto are quite different. This difference has been observed by a number of authors on monkeys and, as will be seen from the present series of papers, it also exists in dogs. For this reason it was decided to analyse the effects of lesions in these two areas separately. However, as the boundaries of these areas and their mutual overlapping are not precisely known, in some dogs ablations encroached into adjacent areas. Knowing the symptoms of removals of these other areas the results of the lesions of motor area itself could be understood.

MATERIAL AND METHODS

The lesions in motor areas were performed on five mongrel male dogs, two to four years old, weighing from 14 to 20 kg. In two of them M-1 and M-2, the lesions were limited to motor areas, in two other dogs, MPm-1 and MPm-2, they included both motor and premotor areas, and in one dog (MS-1) motor and sensory areas were removed. The method of experimentation was exactly the same as that applied in our previous paper (Stępień and Stępień 1959). We used acoustic conditioned stimuli (buzzers, bells, whistles etc.), and the motor conditioned reaction of the dog consisted in lifting of the right foreleg and putting it on the footray.

Surgical procedure was also described in detail in the previous paper. Postoperational experiments were started usually about one week after operation.

In those cases in which conditioned reflexes proved to be abolished or strongly impaired after operation the experiments were performed only once a week, or so, and consisted only of a few trials, some of which were reinforced by food and some were not (for details of this procedure see Stępień and Stępień 1959). Our aim was to observe the "spontaneous" restoration of motor conditioned reflexes, and therefore re-training was carefully avoided.

RESULTS

Dog M-1. Ablation of the motor area only (Fig. 1B)

In this dog the lesion comprised *gyrus postcruciatus* and anterior part of *gyrus sigmoideus posterior*. The anterior part of *gyrus coronalis* was spared in order not to impair the movements engaged in eating.

Several days after operation the dog manifested a very strong impairment in his motor performances, and besides that his general behaviour was rather chaotic, which symptom is characteristic for premotor ablations (see the next paper). This chaotic behaviour lasted only for a few days and was undoubtedly due to the transient oedema of the adjacent regions of the cortex. On the other hand the symptoms of motor disability were longlasting and although they somewhat improved in the course of time, they were clearly seen till the end of observation (i. e. during 7 months). These disorders consisted chiefly in the sliding of all four legs apart with the body falling limply to the floor. This occurred chiefly either when the dog ran on a smooth floor, or during the act of eating. He was not able to stand for a long time, as his hind legs bent slowly and he was compelled to sit down. Sometimes he crossed his forelegs or put the dorsal aspect of the foot to the ground. These last symptoms were only temporary and they practically disappeared after some weeks.

Nearly immediately after operation another prominent symptom appeared, namely hyperkinesis of extremities, seen chiefly in the forelegs. It was best seen when the animal was lifted in the air or when he stood on the stand in the conditioned reflex chamber. This hyperkinesis consisted in constant alternate lifting of both forelegs ("pedalling movements"). When the animal was lifted these movements were so strong that it was practically impossible to test his placing reaction. However, except a few days after operation, the dog did not exhibit any general disorder of his motor behaviour: in spite of hyperkinesis of the legs, no locomotor hyperactivity was observed, he kept his head in normal position (not bent down as is the case in premotor animals) and reacted adequately to all external stimuli.

When brought to the experimental chamber (nine days after operation) the dog performed the trained movement from the very beginning, although it was very awkward and atactic. Most often the movement appeared immediately after the application of the conditioned stimulus, but sometimes its latency was prolonged which was due to the "technical" difficulties of its performance. To the conditioned stimuli the dog displayed a more distinct orienting reaction than was seen before operation, a symptom which is also strongly developed after premotor lesions.

The awkwardness of trained motor conditioned reactions gradually disappeared, but some traces of it remained till the end of observation. The hyperkinesis of the forelegs was seen during the intervals between trials: every few seconds the animal lifted either his left or his right leg to about 10—20 cm. above the floor of the stand. These movements had nothing to do with the trained motor conditioned response and they were not accompanied by any alimentary reaction such as displayed to the conditioned stimuli.

Another defect observed in the conditioned reflex chamber in the first period after operation was that the dog, after putting his foreleg on the foodtray to a conditioned stimulus, was not able to take it off. Therefore after having eaten his portion of food, he stood for a long time with his leg on the foodtray, and only when he turned away did the leg passively fall to the floor.

But the most prominent symptom which appeared immediately after operation and remained during the whole period of observation was that the dog confused his forelegs and put on the foodtray either his right foreleg, trained before operation, or his left foreleg, or both. It should be noted that before operation the animal never performed the trained movement with his left foreleg. Now, after performing this movement, the dog displayed a normal alimentary reaction to the foodtray, as if not noticing that he used the "wrong leg".

It was soon observed that whether the animal lifted to the conditioned stimulus his right of left leg depended chiefly on the position of his body in relation to the foodtray. If, before the application of the stimulus, he was turned with his left side towards the foodtray, then to the sound of the conditioned stimulus he made a turn to the left and in this case his right leg was lifted and put on the foodtray. Vice-versa, if he stood with his right side directed to the foodtray, then in response to the stimulus he turned right and lifted his left foreleg (Fig. 2). We made some attempts to compel the animal to lift only the right foreleg by not reinforcing the movements of the left foreleg. However this measure was not successful and the movements of the left foreleg appeared as often as the dog stood with his right side turned to the foodtray before application of the stimulus.

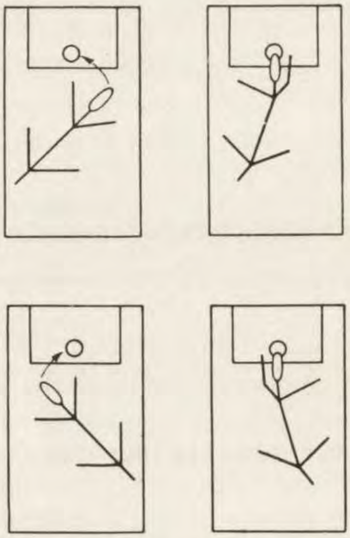

Fig. 2. Schematic representation of the mechanism of confusing legs in the performance of trained movement in motor dogs

Explanation in text.

Dog M-2. Ablation of the motor area (Fig. 1C)

The boundaries of the lesion were similar to those in dog M-1.

The general behaviour of the dog after operation, as well as his motor conditioned reactions, were very similar to those seen in dog M-1, except some details. Immediately after operation he did not display the premotor symptoms seen in dog M-1, but on the other hand his motor conditioned reflexes were almost absent in the first experiments: the dog displayed very clear and distinct alimentary reaction to the conditioned stimuli, but his forelegs remained on the floor. Only in the 7th experiment (one month after operation) conditioned motor reactions became regular. The movements were at first awkward and then they became nearly normal.

The hyperkinesis in this dog consisted in frequent lifting of either right or left foreleg, and holding them in the air for several seconds. These movements were quite different from the instrumental conditioned reactions. Owing to this hyperkinesis it was not possible to test the presence of the placing reaction.

The symptom of confusing the legs in the performance of motor conditioned reactions was as clearly seen in this dog as in the first one and it also persisted during the whole period of observation. He was also not able at the beginning to take off his leg actively from the foodtray.

After two months a premotor ablation was performed, the results of which will be described in the next paper.

Dog MPm-1. Ablation of the motor and premotor area (Fig. 1D)

The lesion in this dog included: gyri *postcruciatus* and *precruciatus* (bilaterally), anterior parts of *sigmoideus posterior*, and the whole of *sigmoideus anterior* (bilaterally), anterior parts of *gyrus coronalis* (bilaterally), and *compositus anterior* on the left side.

This dog displayed, besides the symptoms seen in the two previous dogs, also additional symptoms, due to premotor lesion which will be described in the next paper. To the first group of symptoms belonged awkwardness of movements, sliding of the legs apart, their abnormal positions and hyperkinesis. To the second group belonged chaotical and inadequate behaviour, locomotor hyperactivity with the head bent down and a strong tendency to stereotyped movements. Because of the abnormal general behaviour, the conditioned reflexes were for a long time extremely chaotical: although the animal was able to perform the trained movement from the very beginning, his conditioned reactions were most irregular; often the dog performed the movement in the intervals between trials, but did not perform it to the conditioned stimulus itself (conditioned "disreflexia"). All this confusion in conditioned responses gradually decreased, but the motor hyperactivity and stereotypy of movements remained till the end of observation (the dog was sacrificed 7 months after operation).

From the very beginning of the post-operational period, as soon as motor conditioned reflexes reappeared, the dog started to perform the trained movement either with his right or his left foreleg or with both of them. This confusion was observed both in responses to the conditioned stimuli and in the intervals. It persisted during the whole time of observation.

Dog MPm-2. Ablation of motor, premotor and partially prefrontal areas (Fig. 1E)

The lesion in this dog included bilaterally: the lateral part of *gyrus postcruciatus, gyrus precruciatus,* the anterior part of *sigmoideus posterior, sigmoideus anterior,* the posterior parts of the *proreus* and medial parts of *orbitalis*; on the right the lateral part of *gyrus orbitalis* was also removed.

As in this dog the lesion of the motor area was not extensive, the motor disability was rather slight. There was no awkwardness of movements, but the dog manifested hyperkinesis of the forelegs. The motor conditioned reactions were changed in such a way that, instead of putting the right foreleg on the foodtray, the animal always jumped onto it with both forelegs. Besides he manifested hyperactivity and a tendency to stereotyped movements.

Dog SM-1. Ablation of the sensory and motor cortex (Fig. 1F)

The dog was subjected first to the limited bilateral sensory ablation in which *gyrus postcentralis,* the posterior part of *gyrus sigmoideus posterior* and posterior part of *gyrus coronalis* were removed, and then, five weeks later, to the bilateral motor ablation in which *gyrus postcruciatus* and the anterior part of *gyrus sigmoideus posterior* were destroyed. After the first operation the dog was atactic, and his motor conditioned reflexes were abolished for 2—3 weeks. Thereafter they became normal. After the second operation the conditioned reflexes were again abolished for 3—4 weeks and then, when they were restored, the animal started to perform the trained movement either with his right or left foreleg, which was never seen after the first operation. This state persisted during the period of observation which lasted three months. The dog also had difficulties with taking his leg off the foodtray and did it passively when turning aside.

In general the deficit of motor performances seems in this dog to be stronger than in other dogs. This was due to the more extensive lesion in the sensori-motor cortex.

Table I

The chief symptoms observed in dogs after operation

Dogs	M-1	M-2	MPm-1	MPm-2	SM-1
Extent of lesion	motor	motor	motor premotor	motor premotor prefrontal	sensory motor
Postoperational period in months	7	2	7	1.5	3
Disorders in movements of legs					
awkwardness	strong* slight	strong slight	strong slight	absent	strong
sliding apart	strong slight	strong absent	strong medium	slight absent	strong
abnormal positions	slight absent	strong absent	strong absent	absent	strong
hyperkinesis	strong medium	medium	strong	strong	present
hyperactivity stereotypy	absent	absent	strong	strong	absent
Conditioned activity					
conditioned responses to CS	regular	irregular regular	irregular	irregular	absent regular
confusion of forelegs	present	present	present	present	present
intertrial movements	present absent	present absent	frequent	present	absent

* The first characteristic denotes the early stage after operation, the second characteristic denotes later stages.

The chief symptoms of our dogs after operation are summarised in Table I.

DISCUSSION

The general impairment of motor activity seen in our dogs after bilateral ablations of the motor cortex (except in dog MPm-2 in which the lesion was small) is so well known that there is hardly any necessity to discuss it in detail. The only point worth mentioning is the difference between the motor defects seen in the "motor dogs", and in the "sensory dogs" described in our previous paper (Stępień and Stępień 1959). Whereas the chief defect of the latter dogs results from the impairment of the sense of position of the legs and consists in such symptoms as putting the dorsal aspect of the foot to the floor, crossing the forelegs etc., these symptoms are not so clearly seen in our "motor dogs". Instead their chief deficit consists in sliding of extremities and falling down when the dog is eating or running on a smooth floor. These symptoms may be considered as depending rather on the impairment of the motor performance itself than on lack of the sensory input.

The next symptom characteristic for "motor dogs" is hyperkinesis of forelegs. It consists in incessant lifting of the legs to various height, mostly in the form of "pedalling". This symptom was never seen in our "sensory dogs". The origin of it is not quite clear; it depends probably on removal together with the motor area itself, also of the suppressor area 4s, which may just control and inhibit this sort of movements.

It is very important to draw attention to the difference between the symptom of hyperkinesis, characteristic of dogs with motor lesions, and that of hyperactivity and stereotyped movements, observed after premotor lesions. Whereas the first symptom consists in incessant small movements of the legs, unrelated to the general behaviour of the animal, the second symptom concerns the animal's motor acts and consists in their repeated performance. As we shall see in the next paper locomotor hyperactivity is only one of the instances of this sort of disorder.

As far as motor conditioned reflexes are concerned their performance after motor lesions was more or less defective and was parallel with the general impairment of movements resulting from the operation. It seems that after pure motor lesions the animals are in general able to perform the trained movement from the very beginning (cf. dog M-1) in contrast to the state observed after sensory lesions when the movement is abolished for a certain time*.

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* The disappearance of the motor conditioned reflexes in dog SM-1 was probably due to the fact that in this animal not only the motor area but also the sensory area was removed.

However the movement of taking the leg off the foodtray was generally much impaired and returned only after some time.

The chief symptom found after lesions of the motor cortex consisted in confusion of legs, in the performance of the trained movement. This symptom observed in all our dogs seems to be characteristic of these lesions, since it was not seen either after pure sensory lesions or premotor ones. How remarkable and extra-ordinary this symptom is, may be clearly understood if we take into account that not only in normal dogs such an exchange of movements does not occur, but it even does not happen in dogs with contralateral cortical lesions. In fact, following left sensori-motor ablations or even full left hemispherectomy, the animals, trained to perform the instrumental movement with their right foreleg, continue to do so, although this movement may be very defective, and do not display any tendency to substitute the movement of right by that of left foreleg (Stępień and Stępień, in preparation).

The problem arises how this symptom of confusing the legs may be explained. If we assume that performance of the trained movement by a given foreleg, and not by the symmetric one, is due to some sort of differentiation of two flows of afferent impulses generated by performance of movements with each leg, then we may say that this differentiation is lost after bilateral motor ablation. There is an anatomical evidence to support the view that the so called motor area represents a receptive field concerned with the analysis of movements, as contrasted to the sensory area which is concerned with the analysis of positions of limbs. As the representation of proprioception of movements is both contralateral and ipsilateral, after contralateral ablation of the motor area, the ipsilateral area is sufficient for differentiation of movements of both extremities. On the other hand bilateral removal of this area makes this differentiation impossible. The symptom of confusing legs seems to be permanent, as even after a long period of time after operation the tendency to perform the trained movement with the "wrong" foreleg remains. Although we were not specially concerned with the possibility of differentiation of both movements, we have evidence that this differentiation would not be easily obtained.

It was noticed that whether the animal performed the trained movement with his right or left foreleg depended on the turn the animal made just before its execution. If the turn in the direction of the foodtray was to the right, then the animal's body was supported by the right legs while the left foreleg was "free". Then it is with this leg that the animal performs the trained movement in such an occasion. It is well known that, according to the Sherrington's principles of postural reflexes, turning right produces the extension of the right foreleg and flexion of the left one. Therefore it may be concluded that this postural reflex of flexion is in our experimental condition allied to the instrumental reflex of putting the leg on the foodtray and consequently determines with which leg this movement is executed. The very interesting point is that this alliance, if existing in a normal animal, is totally suppressed by the training of the movement of a particular leg (e.g. the right one) and can be revealed only after the bilateral lesion of the motor cortex, when the proprioceptive feedback from the performance of the movement is abolished.

SUMMARY

The effects of bilateral ablations of the so called motor area of the cerebral cortex in dogs on their motor activity, and in particular on type II conditioned reflexes (consisting in lifting the foreleg and putting it on the foodtray), were investigated.

The following symptoms were observed:

1. The impairment of movement of extremities consisting chiefly in sliding of the legs apart and, in a lesser degree, in atactic symptoms.
2. Hyperkinesis manifested particularly in forelegs ("pedalling movements").
3. Confusion of the forelegs in the performance of motor conditioned responses.

The above symptoms are compared to those observed after either sensory lesions or premotor lesions and their origin is discussed.

REFERENCES

ADRIANOV O. C. i MERING T. A. 1959 — O morfofizjologiczeskich osobiennostiach kory bolszowo mozga sobaki. Żurn. Wys. Nierw. Diejat. A. N. SSSR. 9, 471.

FRITSCH G. und HITZIG E. 1870 — Über die elektrische Erregbarkeit des Grosshirns. Arch. Anat. Physiol. Wiss. Med. 37, 300.

MORIN G., POURSINES Y. et MAFFRE S. 1951 — Sur l'origine de la voie pyramidale documents obtenus par la méthode de dégénéréscences descendantes chez le chien, *43*.

STĘPIEŃ I. and STĘPIEŃ L. 1959 — The effect of sensory cortex ablation on instrumental (type II) conditioned reflexes in dogs. Acta Biol. Exper. *19*.

STĘPIEŃ I. and STĘPIEŃ L. — The effects of contralateral ablations of the cerebral cortex on type II conditioned reflex in dogs, in preparation.

WOOLSEY C. W. and SETTLAGE P. H. 1950 — Pattern of localisation in the precentral motor cortex of *Macaca mulatta*. Fed Proc. *9*, 140.

WOOLSEY C. W., SETTLAGE P. H., MEYER D. R., SENCER W., PINTO HAMUY T. and TRAVIS M. 1950 — Patterns of localisation in precentral and "supplementary" motor areas and their relation to the concept of a premotor area. Proc. Ass. Res. Nerv. Ment. Dis. *30*, 238.

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THE EFFECTS OF BILATERAL LESIONS IN THE PREMOTOR CORTEX ON TYPE II CONDITIONED REFLEXES IN DOGS

I. STĘPIEŃ, L. STĘPIEŃ and **J. KONORSKI**

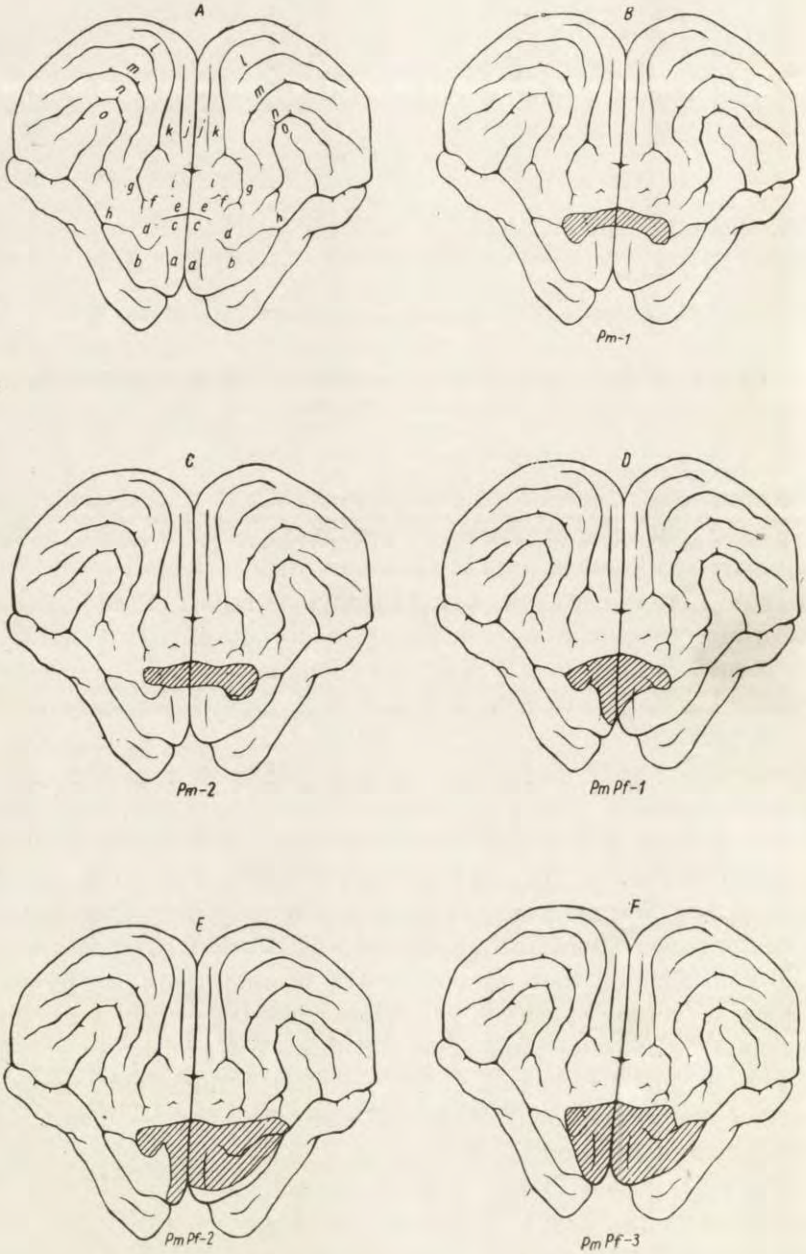
Nencki Institute of Experimental Biology, Department of Neurophysiology, Warsaw

(Received 5 November 1959)

In most descriptions of the cerebral cortex of monkeys and apes it is stated that in front of the so called motor area sensu stricto (or area 4) another area is situated which differs from the first one both anatomically and physiologically and is called premotor area or area 6. Its chief anatomical characteristic is that it is thinner than the motor area, that it possesses a smaller amount of pyramidal cells and has no giant pyramidal cells in layer V. Electrical stimulation of this area in anesthetized animals does not evoke discrete movements of extremities, but rather coordinated movements of the whole body.

The opinions concerning functional role of the premotor area are controversial. On the one hand it is considered as an integrative region controlling complex motor acts elicited by intermediary of the motor area. Chief protagonist of this view was Jacobsen (1936) who claimed that removal of the premotor area in monkeys produces a "motor apraxia". The same view is held by many neurologists. On the other hand other authors (Woolsey et al. 1950, Woolsey and Settlage 1950) consider this field simply as a part of motor area controlling movements of the head and axial musculature of the body.

Whatever view will prove to be correct, since ablations of the premotor area produce in monkeys symptoms very different from those produced by lesions of the motor cortex, it seemed necessary to study in dogs the symptoms of ablations of these two areas separately.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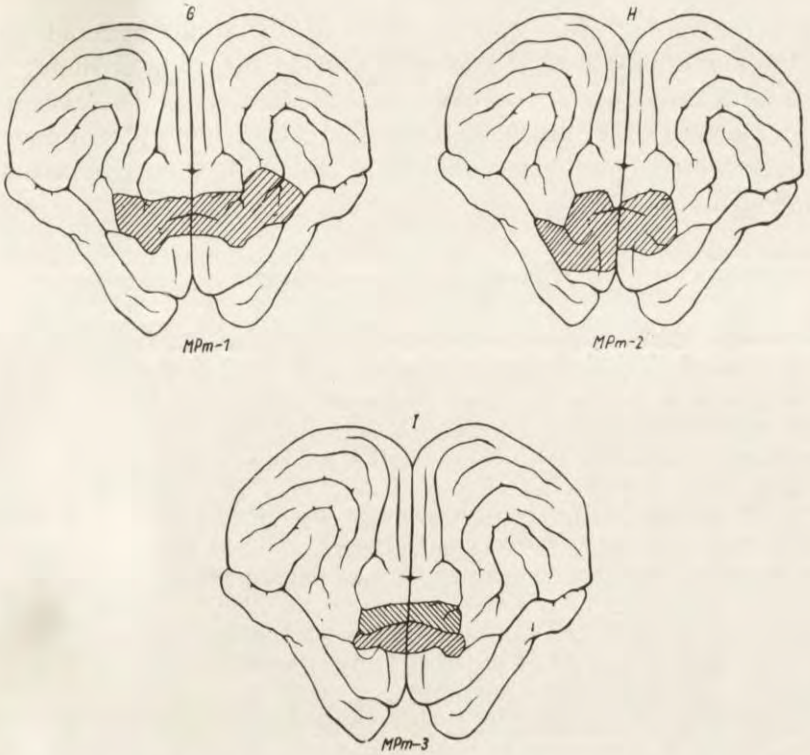

Fig. 1. The cerebral cortex of the dog flattened on the plain, and sites of lesions in experimental dogs

A — normal cerebral cortex. Denotations: a — *gyrus proreus*, b — *g. orbitalis*, c — *g. precruciatus*, d — *g. sigmoideus anterior*, e — *g. postcruciatus*, f — *g. sigmoideus posterior*, g — *g. coronalis*, h — *g. compositus anterior*, i — *g. postcentralis*. B — I, lesions of the cerebral cortex in various dogs as indicated in text.

Anatomical limits of the premotor area in dog are so far not well defined (for literature see Adrianov and Mering 1959), and therefore they must be chosen rather arbitrarily. We consider the premotor area in dog as a region limited caudally by the cruciate sulcus and its prolongation, rostrally by the presylvian sulcus, and laterally by the prolongation of the coronal sulcus. Thus, according to our definition, this area consists of precruciate gyrus and sigmoid anterior gyrus (Fig. 1A). As those boundaries are not certain, we performed in some dogs more extensive lesions including either parts of prefrontal areas (proreal gyrus and partially orbital gyrus), or motor areas (postcruciate and posterior sigmoid gyri). As the symptoms observed after the ablations of both motor and prefrontal areas were already investigated in this laboratory (Brutkowski et al. 1956, Stępień et al. 1960) it was possible to assess which symptoms should be attributed to the region situated between them.

MATERIAL AND METHODS

Experiments were performed on 8 dogs, weighing 12—20 kg., aged 2—4 years. In two dogs Pm-1 and Pm-2 only premotor areas (boundaries as described above) were removed, in three other dogs PmPf-1, PmPf-2 and PmPf-3, parts of the prefrontal areas were also ablated, whereas in three remaining dogs, MPm-1, MPm-2 and Mpm-3 the lesions included motor areas (in MPm-2 also part of the prefrontal area).

In all the dogs instrumental alimentary conditioned reflexes to various acoustic stimuli were established before operation. The trained motor reaction consisted in lifting the right foreleg and putting it on the foodtray. This movement performed in response to conditioned stimuli was reinforced by presentation of food. Other details of the method and surgical procedure were described in a previous paper (Stępień and Stępień 1959). After operation the experiments started as soon as the animals were sufficiently recovered. When after operation the conditioned motor reflexes were found to be present, then the experimental sessions took their normal course. When they were absent the conditioned stimulus was sometimes reinforced and sometimes not, and after a few trials the experiment was discontinued. The next experiment was performed only after a week or so. This procedure was adopted to avoid any re-training of the animal after operation (for details see Stępień and Stępień 1959).

RESULTS

I. Ablations of the premotor area only

In two dogs Pm-1 and Pm-2 only small lesions including precruciate and anterior sigmoid gyri were performed.

Dog Pm-1 (Fig. 1B). From the first days after operation the dog was able to walk quite well without ataxia or sliding. Placing reaction was positive. Brought into a room he walked around during long periods of time. This walking had a stereotype character, and there was no tendency to explore the environment, as is typical for normal dogs. When called he displayed only a slight orienting reaction, but did not stop walking. Sometimes unexpectedly he stopped in the middle of the room, and after a while he set out to walk again. While walking his head was bent down to the level of the back and the dog exhibited a tendency to pass beneath and not above the obstacles. These symptoms disappeared gradually within two weeks.

On the 5th day after operation, when brought into the conditioned-reflex chamber, he behaved more or less quietly, but performed some oscillating movements in front of the foodtray; such movements were never observed before. To conditioned stimuli the dog exhibited a strong orienting reaction towards the source of the sound, not seen before operation; then he turned towards the foodtray and performed alternately the movements towards the stimulus and towards the bowl several times without any tendency to lift the foreleg and put it on the foodtray. Once, however, during the first experiment he performed this movement in the interval between conditioned stimuli; it was performed quite skillfully and without any "technical" difficulties. During following experiments a similar picture was observed: to the conditioned stimuli the dog exhibited alternately orienting and alimentary reaction but the trained movement did not appear. Then in the 9th experiment (38th day after operation) on the third trial he executed the trained movement without any perceivable difficulty and with a very short latent period. In following experiments the trained movement appeared in every trial, although the orienting reaction towards the stimulus preceded it nearly always. Sometimes the movement appeared also during intervals between conditioned stimuli. This state remained almost without change throughout the period of observation. On 65th day after operation the dog was sacrificed.

Dog Pm-2 (Fig. 1C). Like dog Pm-1 this dog was also unimpaired in his motor performances from the first days after operation but he displayed a strong hyperactivity even more pronounced than that of the previous dog. He persistently walked about the room and, when on leash, made oscillating movements of the body. When walking, his head was bent and he tended to slip underneath tables and chairs. These symptoms gradually disappeared within a few weeks. Placing reaction was normal.

In the first experiment, in response to the conditioned stimulus, the animal displayed a very strong alimentary reaction but the trained movement did not appear. However, already in the next experiment (9th day after operation) the animal started to perform the trained movement, although in some trials its latency was prolonged, due to the strong direct alimentary reaction which obviously inhibited the instrumental response. At the same time the animal started to perform the trained movement in intervals between trials and did so every few seconds. Both putting the leg on the foodtray and taking it off were quite skillful. Sometimes the intertrial movements were only abortive. They were most often not accompanied by alimentary reaction which was always observed with the movements executed to the conditioned stimuli. In the course of the experiment the intertrial movements decreased in number but the general restlessness of the dog and oscillating movements of the head persisted up to the end.

Such state continued day after day during the whole period of observation (3 months), although the intertrial movements became less frequent and appeared chiefly in the beginning of each experiment.

II. Ablations of the premotor and prefrontal areas

Dog PmPf-1 (Fig. 1D) In this dog in addition to the removal of precruciate and anterior sigmoid gyri, the proreal gyrus was also ablated. The motor efficiency of this dog was, as in previous animals, perfect from the first days after operation. He displayed a strong locomotor hyperactivity with tendency to repeat again and again the same itinerary round the room. His head was bent below the line of the back and he tended to pass underneath the obstacles. Placing reaction was normal.

When brought to the experimental chamber (5th day after operation) he incessantly walked around the stand and frequently climbed the foodtray with his forelegs or even with all four legs. To the conditioned stimuli his first reactions were quite different than those before operation. He stretched out his head towards the source of the stimulus (situated behind the foodtray), climbed on the foodtray with his forelegs and paid no attention to the food presented in the bowl. If he noticed it accidentally, he started immediately to eat it.

In the third experiment (8th day after operation) besides the orienting reaction towards the source of the stimulus, the proper alimentary reaction towards the bowl appeared also and after several seconds the right foreleg was put on the foodtray. In the next stage this movement appeared immediately after the application of the stimulus. The movement was performed either with the right foreleg or (rarely) with both.

In the intervals between trials the dog walked round the foodtray, licked the empty bowl, climbed repeatedly the foodtray and performed many times the trained movement. This abnormal behaviour remained unchanged throughout the observation period which lasted two months.

Dog PmPf-2 (Fig. 1E). The ablation in this dog included precruciate, anterior sigmoid and proreal gyri bilaterally; on the left side parts of orbital gyrus and of coronal gyrus as well as anterior compositive gyrus were ablated. The chief symptom which appeared immediately after operation was a very strong locomotor hyperactivity: on the fourth day after operation the dog walked incessantly around the room for 9 hours, till complete exhaustion. He also displayed a strong tendency to walk again and again along the same itinerary. His head was bent beneath the line of his back. He often slipped underneath the obstacles and entered the corners of the room staying there for a long time. He displayed no exploratory reactions towards the environment and reacted very poorly to extraneous stimuli. On the other hand his motor efficiency was perfect and placing reaction was normal. This state of affairs gradually improved, but the locomotor hyperactivity was permanent and lasted throughout the period of observation (one year).

In the first experiments (first week after operation) in the conditioned-reflex chamber the dog behaved as if the experimental situation were quite new to him. To conditioned stimuli he displayed only an orientation reaction and only accidentally found food presented to him. Throughout the experiment he incessantly walked round the foodtray (as far as the length of the leash allowed) and often climbed the foodtray from various sides.

Gradually the conditioned stimuli began to elicit alimentary reaction in addition to the orienting reaction. The alimentary reaction consisted in bending the head very low to the empty bowl and licking it, which was never seen before operation. The trained movement appeared for the first time in the 8th experiment (21 days after operation), but only after the cessation of the stimulus. It was quite skillful and did not differ from that before operation. In the following experiments the trained movement appeared more and more often but nearly always with a long latent period: the stimulus evoked first the orientation reaction, then the direct reaction to the bowl and only after several seconds, when the food was not presented, did the animal put his leg on the foodtray. Only after several months the conditioned reactions improved to such a degree, that most of them were displayed immediately as before operation.

As soon as the trained movements began to appear to the conditioned stimulus, the animal started to perform them very often in the intertrial intervals. In consequence his whole behaviour in the conditioned-reflex chamber appeared to be most chaotical and unpredictable. The latent periods of conditioned reactions were variable, and it happened sometimes that to the conditioned stimulus the movement did not appear at all. On the contrary, during the intervals between trials the dog was in permanent motion, he performed the trained movement again and again, very often climbed the foodtray with his forelegs or with all four legs, and so on. The majority of intertrial movements had not an alimentary character. Such state of affairs changed but little up to the end of observation. Although conditioned reactions became more regular, the whole behaviour of the animal during intervals seemed to be quite "stupid".

Dog PmPf-3 (Fig. 1F). The lesion in this dog was nearly the same as in the previous one, and all his symptoms were so similar that they will be only very briefly described. The dog displayed locomotor hyperactivity, connected with stereotype repeating of the same itinerary, with a tendency to enter into corners of the room and to pass underneath the obstacles. His motor efficiency and placing reaction were perfect. In the first experiments the motor conditioned reflex was absent, but the dog was very restless and often climbed the foodtray. To the conditioned stimuli he displayed first orienting and then alimentary reaction. The trained movement appeared about three weeks after operation, its latency was variable and he performed it very often in intervals. Such state lasted during the whole period of observation (8 months).

III. Ablations of the premotor and motor area

Dog MPm-1 (Fig. 1G). The lesion in this dog included bilaterally postcruciate and precruciate gyri, anterior parts of posterior sigmoid, the whole of anterior sigmoid and anterior part of coronal gyrus; on the left side anterior composite gyrus was removed.

The symptoms connected with the removal of the motor cortex were described in the previous paper (Stępień et al. 1960). They included disorders of motor acts, sliding apart of legs, hyperkinesis of the forelegs and confusion of legs in execution of the trained movement. In addition to these symptoms the dog displayed a locomotor hyperactivity with his head bent and entering the corners of the room. This state disappeared within two weeks.

During the first days after operation the animal's behaviour in the experimental chamber was so chaotical, restless and awkward that conducting experiments was impossible. Then, gradually his behaviour improved: the dog began to perform the trained movement to conditioned stimuli, but this movement appeared only occasionally and with variable latency. To the conditioned stimuli the dog displayed both the orienting reaction and the direct reaction to the bowl. These reactions interfered with the performance of the trained movement. The direct reaction to the bowl was soon converted into persistent gnawing of the border of the bowl, manifested even when the dog was satiated. During such gnawing the animal did not react at all to conditioned stimuli. In those periods in which gnawing of the bowl was not present, the dog performed the trained movement in the intervals again and again, he climbed the foodtray etc. After several months the motor reactions to the conditioned stimuli became more regular and the dog was able to perform them immediately after the application of the stimulus. However in intervals the "stupid" behaviour with gnawing the bowl and other forms of hyperactivity remained till the end of observation i. e. 7.5 months.

Dog PMm-2 (Fig. 1H). The lesion in this dog included bilaterally: the lateral part of postcruciate gyrus, precruciate gyrus, the anterior part of posterior sigmoid, anterior sigmoid, the posterior parts of proreal and medial parts of orbital gyrus; on the right side the lateral part of orbital gyrus was also removed.

The impairment of motor acts was in this dog only slight because of the limited extent of the lesion in the motor cortex (cf. Stępień et al. 1960). On the other hand all the premotor symptoms were manifest.

Dog MPm-3 (Fig. 1I). This dog was first subjected to the ablation of the motor cortex and was described in the previous paper as dog M-2. (Stępień et al. 1960). Two months later the second operation was performed in which precruciate gyrus and anterior sigmoid were bilaterally removed. The second operation produced striking changes in the animal's behaviour. At first the trained movement appeared to the conditioned stimuli very irregularly and unpredictably. It was noticed that to the stimulus situated in front of the animal the motor reaction was prompt, while to the stimulus situated in the corner of the chamber the response did not appear. This was caused by the fact that in the first case the orienting reaction towards the stimulus was allied with, while in the second case it was antagonistic to the trained motor reaction. As soon as the motor reaction to the conditioned stimulus became more or less regular, the animal began to perform it in intervals. As the dog was not able to take his leg off the foodtray without help (the result of the first operation), he stood for long periods of time with his leg on the foodtray, but if the leg was taken off he immediately lifted it again. The motor reactions both to the conditioned stimuli and in intervals were performed either with right or with left foreleg, or with both. Such behaviour was due to the lesion in the motor area.

Till the end of observation, lasting two months, this state did not change: to the conditioned stimuli the trained movement was executed with variable latent periods, since orienting reaction or the direct alimentary reaction appeared before it. On the other hand, in intervals the dog raised his leg immediately after it was taken off the foodtray.

The chief symptoms observed in all our dogs are presented in Table I.

DISCUSSION

The experimental data presented in this paper, together with the results described previously (Brutkowski et al. 1956, Stępień et al. 1960, Stępień and Stępień 1959) allow us to draw some conclusions as to which symptoms produced by

Table I

The chief symptoms observed in dogs after operation

Dogs	Pm-1	Pm-2	PmPf-1	PmPf-2	PmPf-3	MPm-1	MPm-2	MPm-3
Extent of lesion	premotor	premotor	premotor* prefrontal	premotor prefrontal	premotor prefrontal	motor premotor	motor premotor	motor premotor
Postoperational period in months	2	3	2	12	8	7	1.5	2
Disorders of movements								
awkwardness	absent	absent	absent	absent	absent	strong slight	absent	present
head bent	present absent	present absent	present absent	present absent	present absent	present absent	present absent	present absent
sliding apart of legs	absent	absent	absent	absent	absent	strong medium	slight absent	absent
abnormal position of legs	absent	absent	absent	absent	absent	strong absent	absent	absent
hyperkinesis	absent	absent	absent	absent	absent	strong	strong	present
hyperactivity and stereotypy	present absent	strong	strong	strong	strong	strong	strong	present
Conditioned activity								
conditioned responses to CS	absent regular	absent irregular regular	absent irregular regular	absent irregular	absent irregular	irregular	irregular	irregular
confusion of forelegs	absent	absent	absent	absent	absent	present	present	present
intertrial movements	rare	frequent	frequent	frequent	frequent	frequent	frequent	present

* The first characteristic denotes the early stage after operation, the second characteristic (or the third one) denotes later stages.

ablations of the so called premotor area may be considered as specific for this site of lesions.

The most spectacular difference between the lesions in the premotor area and those in sensori-motor area (in the stricter sense) is that whereas the latter lesions always produce prominent, and more or less persistent, defects in movements of the legs, the premotor animals, even immediately after operation, are as skillful as before. Neither abnormal positions of the legs, nor awkwardness of movements, nor sliding of legs apart are ever seen. Placing reaction, which is totally absent after sensory lesions and dubious after motor lesions (owing to the perpetual "pedalling" movements), is prompt and unmistakable in the premotor animals.

On the other hand, if we draw attention to the behavioural aspects of motor activity we see that the premotor dogs are much more changed in comparison with their preoperational state than the sensory or motor dogs. The sensory ablations make the animals awkward and atactic but their general pattern of behaviour is unchanged. After motor ablations the animals are behaviouristically less normal, as confusion of legs and putting the left foreleg instead of the right one on the foodtray may be considered as a disorder of the whole motor act. But the strongest disorders of behaviour are seen after premotor lesions. These disorders may be divided into the following groups:

1) Perseveration or stereotypy of movements. All our premotor animals exhibited a symptom which is generally called hyperactivity, although it would be better to call it: "stereotypy or perseveration of motor acts". This symptom consists in that the animal when starting some sort of activity repeats it again and again for a long time. Its simplest form is the so called "locomotor hyperactivity" consisting in persistent walking around a room, most often along the same itinerary. The periods of walking are interspersed with periods of complete rest.

But it must be stressed that locomotor stereotypy is not the only sort of perseverative behaviour observed in the premotor dogs. In the experimental chamber they performed repeatedly hundreds of times the following movements: putting the right foreleg on the foodtray (the trained movement); jumping with both forelegs, or with all four legs on the foodtray and then back on the floor of the stand; oscillating movements of the head or the body; incessant gnawing of the border of the bowl.

It should be noted that, when the dog performed continuously the trained movements in the intervals between trials, these movements did not have a clear alimentary significance. It was noticed that the animal performed them in quite a different manner than he did to the conditioned stimulus itself, namely he did not display any explicit alimentary reaction, always present in response to the conditioned stimuli. (Unfortunately salivation was not examined in those dogs).

It was observed that not all of our dogs presented the symptom of stereotype movements in the same degree. In dog Pm-1 this symptom was seen only for a short time after operation. On the other hand in dog Pm-2 with nearly the same lesion it was very well marked. As, generally speaking, in all our premotor-prefrontal dogs the tendency to hyperactivity and perseveration of motor acts was very strong and persistent, it might be concluded that ablations of these two areas jointly are responsible for this symptom. But, taking into account that dogs Pm-2 and MPm-1 did manifest strong perseverative tendency in spite of the fact that their prefrontal areas were left intact, and, on the other hand, "pure" prefrontal animals do not display this symptom at all, we are rather inclined to believe that there is some intermediate zone, including some parts of our premotor area and of prefrontal area, ablations of which produce this sort of disorder. After all one should remember that the boundary between our premotor and prefrontal area is taken rather arbitrarily, so we do not know how much the region possessing the physiological role of the premotor area involves the prefrontal area. Perhaps a careful investigation of the anatomical subdivisions of the frontal region combined with the detailed analysis of lesions produced in our dogs could answer this question.

To summarize this point it should be once more pointed out that the increased tendency to perseveration is a general symptom and it concerns all possible kinds of motor acts and not only the walking. In the dogs in which we observe locomotor "hyperactivity" in an empty room, we may notice oscillating movements of the head in the stand and/or gnawing of the bowl and/or putting the leg on the foodtray, etc. In this connection it is worthwhile to mention that in Ławicka's experiments (1957) in which vocal conditioned reflexes were established in a dog, the animal displayed a quite clear vocal hyperactivity (or rather perseveration) after premotor-prefrontal ablation: in the experimental situation in which vocal conditioned reflexes to the sporadic stimuli were trained (but not elsewhere), the dog barked almost incessantly throughout the experimental session and this symptom appeared to be permanent; it was quite analogous to the symptom of incessant putting the right foreleg on the foodtray established in this dog in another experimental situation and repeatedly executed in that situation.

2) The next symptom of premotor dogs is the tendency to walk with head bent beneath the level of the back, to pass underneath the obstacles instead of jumping over them, as do normal animals, to slip under furniture and to enter the corners of the room. Again this symptom seemed to be more prominent in premotor-prefrontal animals than in "pure" premotor animals.

3) The third symptom observed after premotor ablations consists in alteration of reactions to the conditioned stimuli. Before operation the animals put the leg on the foodtray as quickly as possible. After operation they display at first a very strong orientation reaction toward the source of the stimulus (as if it were quite new to them), then after a number of reinforcements of this stimulus by food, they manifest a not less intensive but abnormal alimentary reaction toward the bowl consisting in licking it, gnawing its borders, etc. These reactions strongly interfere with the execution of the trained movement, which at this stage either does not appear at all, or it appears with a very long latency, or appears in intertrial intervals but not to the stimulus. In consequence, the conditioned-reflex activity is highly chaotic and unpredictable in striking contrast with the quite normal character of the performance of the movements themselves. We have called such a state "conditioned disreflexia". Owing to it even on superficial observation the behaviour of these animals makes the impression of being silly and absurd.

It remains to compare the symptoms ensuing from premotor ablations with those following prefrontal ablations, or with those obtained after combined lesions of these two areas. When the lesion includes only the frontal poles rostrally to presylvian sulcus, the condition of animals is quite different from that described in this paper. Most often these animals are quite quiet even immediately after operation and in the majority of cases they do not display any signs of hyperactivity or stereotypy, either locomotor or other.

Therefore, their general behaviour appears to be quite normal and it hardly differs from that before operation. Their positive conditioned reactions (classical or instrumental) are also normal or somewhat increased. On the other hand they display a marked disinhibition of inhibitory conditioned reflexes manifested in all sorts of conditioning: instrumental alimentary (Brutkowski et al. 1956, Ławicka 1957), classical alimentary (Brutkowski 1957), instrumental drinking (Żernicki 1960), classical defensive (Auleytner and Brutkowski 1960). As a rule, after a sufficiently long postoperative retraining inhibitory reflexes can be restored.

Since the prefrontal animals in the first period after operation do execute the instrumental conditioned reaction also in intervals between trials as the effect of disinhibition, it is worthwhile to compare this symptom to the analogous one seen after premotor, or premotor-prefrontal lesions. The prefrontal animals perform the trained movement in intervals only occasionally in a similar way as they did it in the first stage of the pre-operational training. These intertrial movements are accompanied with an alimentary reaction and after several days they disappear. On the other hand, as described in this paper, in premotor dogs these intertrial reactions, as well as other manifestations of motor stereotypy are very frequent and abundant. They remain either permanently, or for a very long time, and do not present a clear alimentary character.

In spite of the fact that the symptoms of premotor lesions are very clear-cut and distinct, their explanation encounters great difficulties and needs perhaps much more experimental work. The fact is that in contrast to the impairment of the discrete movements of the legs, characteristic for pure sensori-motor ablations, the premotor lesions produce the impairment of the general behaviour and of whole motor acts. This could be explained either by accepting the older hypothesis of destruction of some "higher" centres controlling the behaviour, or else by Woolsey's conception of the destruction of the head-body cortical representation. The symptoms of hyperactivity and motor stereotypy seem to be analogous to the symptoms of hyperkinesis observed after motor ablations (cf. Stępień et al. 1960). May be that in the vicinity of both motor area and premotor area two adjacent suppressing areas are situated whose functional role is to refrain and check the motor performances either on the level of simple movements (area 4s?) or on the level of the whole motor acts.

The symptom of locomotor hyperactivity after lesions in the frontal region was often described in other animals such as rats (Richter and Hawkes 1939), cats (Langworthy and Richter 1939) and monkeys (Richter and Hines 1938, Kennard et al. 1941, Ruch and Shenkin 1943 and others). The precise localisation of lesions producing this symptom is not clear, however we have some evidence that they do involve our premotor area. For, according to our results (Ławicka and Konorski in preparation) cats with strictly prefrontal lesions (rostral to presylvian gyrus) do not exhibit any hyperactivity. All this experimental material seems to indicate that there exists in various species of animals an area situated somewhere in the frontal lobes rostrally to the motor cortex, whose function consists in inhibiting superfluous motor acts, or rather in cutting short those activities which are no more necessary. Hence the ablations of this area lead to motor perseveration and stereotype movements. The probable physiological mechanism of this impairment was discussed in detail by Konorski elsewhere (Konorski 1957).

SUMMARY

The present paper deals with the effects of ablations of the premotor cortex on the general behaviour and motor conditioned reflexes in dogs. The chief symptoms observed after these ablations were as follows:

1. The tendency to repeat again and again the same motor activity such as: walking in a room along the same itinerary, performance of the trained movement in the experimental situation, gnawing of the bowl, etc.

2. The tendency to slip beneath the obstacles and to stick in the corners of the room.

3. The disorders of the conditioned-reflex activity consisting in the loosening of the bond between the conditioned stimulus and the conditioned motor reaction. To the conditioned stimulus the animals display an exaggerated orienting reaction towards the source of the stimulus and an exaggerated and abnormal direct alimentary reaction. These reactions interfere with the performance

of the trained movement. On the other hand this movement is repeatedly performed in intertrial intervals.

These symptoms seem to be more pronounced if in addition to the premotor area parts of the prefrontal area are removed but they are not observed after removals of prefrontal area alone. On the other hand no impairments of discrete movements of extremities are seen after prefrontal ablations.

REFERENCES

ADRIANOV O. C. i MERING T. A. 1959 — O morfofizjologiczeskich osobiennostiach kory bolszowo mozga sobaki. Żurn. Wys. Nierw. Diejat. A. N. SSSR. *9*, 471.

AULEYTNER B. and BRUTKOWSKI S. 1960 — Effects of bilateral prefrontal lobectomy on the classical (type I) defensive conditioned reflexes and some other responses related to defensive behaviour in dogs. Acta Biol. Exper. *20*, 151.

BRUTKOWSKI S. 1957 — The effects of prefrontal lobectomies on salivary conditioned reflexes in dogs. Acta Biol. Exper. *17*, 327.

BRUTKOWSKI S., KONORSKI J., ŁAWICKA W., STĘPIEŃ I. and STĘPIEŃ L. 1956 — The effect of the removal of frontal poles of the cerebral cortex on motor conditioned reflexes. Acta Biol. Exper. *17*, 167.

JACOBSEN C. F. 1936 — Studies of cerebral functions in primates. Comp. Psychol. Monogr. *13*.

KONORSKI J. 1957 — O giperaktivnosti żiwotnych posle udalenija łobnych dolej bolszych połuszarii. Problemy Fiz. Centr. Nierw. Sistemy, A. N. SSSR. Inst. Fizjol. im. Pawłowa, 285.

KENNARD M. A., SPENCER S. and FOUNTAIN G. 1941 — Hyperactivity in monkeys following lesions of the frontal lobes, J. Neurophysiol. *4*, 512.

LANGWORTHY O. R. and RICHTER C. P. 1939 — Increases in spontaneous activity produced by frontal-lobe lesions in cats. A. J. Physiol. *126*, 158.

ŁAWICKA W. 1957 — The effect of the prefrontal lobectomy on the vocal conditioned reflexes in dogs. Acta Biol. Exper. *17*, 317.

ŁAWICKA W. and KONORSKI J. The effects of prefrontal lesions on delayed responses in cats, in preparation.

RICHTER C. P. and HAWKES C. D. 1939 — Increased spontaneous activity and food-intake produced in rats by removal of the frontal poles of the brain. J. Neurol. Psychiat. *2*, 231.

RICHTER C. P. and HINES M. 1938 — Increased spontaneous activity produced in monkeys by brain lesions. Brain, *61*, 1.

RUCH T. C. and SHENKIN H. A. 1943 — The relation of area 13 of orbital surface of the frontal lobe to hyperactivity and hyperphagia in monkeys. J. Neurophysiol. *6*, 349.

STĘPIEŃ I. and STĘPIEŃ L. 1959 — The effect of sensory cortex ablations on instrumental (type II) conditioned reflexes in dogs. Acta Biol. Exper. *19*, 257.

STĘPIEŃ I., STĘPIEŃ L. and KONORSKI J. 1960 — The effects of bilateral lesions in the motor cortex on type II conditioned reflexes in dogs. Acta Biol. Exper. *20*, 135.

WOOLSEY C. N. and SETTLAGE P. H. 1950 — Pattern of localisation in the precentral motor cortex of *Macaca mulatta*. Fed. Proc. *9*, 40.

WOOLSEY C. W., SETTLAGE P. H., MEYER D. R., SENCER W., PINTO HAMUY T. and TRAVIS M. 1950 — Patterns of localisation in precentral and "supplementary" motor area and their relation to the concept of a premotor area. Proc. Ass. Res. Nerv. Ment. Dis. *30*, 238.

ŻERNICKI B. 1960 — The effect of prefrontal lobectomy on the water instrumental conditioned reflexes in dogs. In preparation.

Acta Biologiae Experimentalis, Vol. XX, 1960

EFFECTS OF BILATERAL PREFRONTAL LOBECTOMY ON THE CLASSICAL (TYPE I) DEFENSIVE CONDITIONED REFLEXES AND SOME OTHER RESPONSES RELATED TO DEFENSIVE BEHAVIOUR IN DOGS

B. AULEYTNER and **S. BRUTKOWSKI**

Department of Neurophysiology
Nencki Institute of Experimental Biology, Warsaw

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In a series of papers recently published by several authors of this laboratory (Brutkowski et al., 1956, Ławicka 1957, Brutkowski, 1957, 1959a, 1959b) it was established that bilateral prefrontal lobectomy results in a partial disinhibition of various alimentary inhibitory CRs. Żernicki (1960), using water instead of food as the UCS, lately confirmed these findings of a temporary impairment of conditioned inhibition in prefrontal dogs.

The purpose of the present research was to investigate the influence of prefrontal lobectomy on defensive CRs. These were reinforced either by electric shock applied to the leg or by the introduction of a solution of acid into the dog's mouth.

EXPERIMENTAL PROCEDURE

Experiments were carried out on 4 experimentally naive dogs: D-12, D-13, D-14 and D-22. The animals were about 2 years old except for D-14 which was about 6. In D-13 and D-14 both electro-defensive and acid-defensive CRs were trained. D-12 was trained only in CRs reinforced by acid (A-experiments), and D-22 received training in CRs reinforced only by electric shock (E-experiments). Both kinds of defensive CRs were established in the same sound-proof conditioned-reflex chamber. During testing each dog stood on a stand in a harness. Training involved excitatory as well as inhibitory defensive CRs.

The excitatory (positive) CRs reinforced by acid (A-CRs) were trained in the following way: A 1000-cps Tone was used as the first positive CS.

At the beginning of training the Tone was reinforced by the immediate introduction of 6 cc. of 0.33 per cent lactic acid into the dog's mouth. The acid was injected in 3 equal portions in succession during 5 seconds. It was supplied from outside the chamber by a system of tubes leading to a small steel tube fixed to the dog's right cheek witch Mendeleev's wax. The end of this small tube was curved and directed into the animal's mouth. The acid was injected by means of a calibrated veterinary syringe. The salivary effect following both the CS and the introduction of acid was observed. Salivation was recorded from the right parotid gland fistula prepared according to Sołtysik and Zbrożyna (1957). Kymographic registration of the salivary responses followed Kozak's method (1950).

After a number of trials with immediate reinforcement the reinforcement was increasingly delayed until the isolated period of the CS was 20 sec. In the meantime other positive acoustic CSs were also introduced.

The animals rejected the lactic acid used in the experiments and even refused to take meat or other food sprayed with the acid.

The excitatory electro-defensive CRs (E-CRs) were reinforced by a single condenser discharge of 2 μF and 90 V applied to the right hindleg in D-14 or to the right foreleg in D-13 and D-22. The first positive CS used was the vibration of a telephone membrane. After several trials the positive E-CR was established such that at the sound of the CS the animal lifted its leg with a delay of a few seconds and held it in this position until the noxious reinforcement was given. The application of electric shock usually produced an enhanced flexion often accompanied by a whine. Sometimes the CR itself was also accompanied by whining, barking and general restlessness of the animal. The movement of the leg was recorded on the kymograph. Both the latent periods and the amplitudes of motor reactions were measured.

In each experiment the animal's respiratory movements were also recorded by means of a simple pneumatic apparatus. Finally, in D-12 and D-13 tongue-movements were counted since in the A-experiments these dogs intensively licked their lips during the 20-second isolated period of the CS.

When positive (excitatory) CRs had been firmly established, inhibitory (negative) CSs were introduced which were never reinforced by the UCS (acid or electric shock). There were two inhibitory tests: 1) differentiation of Tones (in A-experiment) or Vibrations of the telephone membrane (in E-experiments) and 2) conditioned inhibition. The conditioned-inhibition test consisted of a combination of two stimuli, a conditioned inhibitor and a positive CS, separated from each other by an interval of 5 sec. This combination was never reinforced while the CS alone was always reinforced. The conditioned inhibitor was a Rattle in the A-experiments and a Bell in the E-experiments. Each inhibitory CS was applied for 20 sec. in A-experiments and for 10 sec. in E-experiments. The correct inhibitory E-CR consisted in the animal performing no movement to the inhibitory stimulus. Very poor or delayed movements to the inhibitory CSs were considered as partial errors. Complete errors were movements performed in the same manner as to the positive CSs. In the A-experiments salivation to the inhibitory CSs gradually diminished with training so that a difference between excitatory and inhibitory CRs was apparent.

Daily sessions usually consisted of 6 excitatory trials and 2 inhibitory trials, although in some experiments 3 or even 4 inhibitory trials were used. The intertrial intervals in A-experiments were 4 min., in E-experiments they were about 1 min. There were 3—6 experimental sessions a week.

The preoperative training of A-CRs lasted a year and a half, that of E-CRs about 3 months. The excitatory CR was usually established in several sessions. However, the training of inhibitory CRs was always much longer, and, as a rule, it was longer in A-experiments than in E-experiments.

The number of inhibitory trials given to each dog before the operation is presented in Table I.

Table I

	Differentiation		Conditioned inhibition	
	A-experiment	E-experiment	A-experiment	E-experiment
D-12	279	—	347	—
D-13	215	95	209	75
D-14	224	87	216	132
D-22	—	53	—	52

When the animals had reached a criterion of about 90 per cent correct inhibitory CRs out of 20 inhibitory trials in E-experiments they were regarded as ready for operation. The preoperative criterion in A-experiments was not so exact because of the difficulty, generally encountered when using the salivary procedure, of maintaining salivary responses at a constant level for a long period. However, as a rule, the operation was performed when there had been no extreme oscillations in the excitatory and inhibitory CRs, and when the ratio between them (hereafter called I/E ratio) was relatively constant in at least 25—30 sessions. The salivary effects were measured in mm, and the I/E ratio was computed by determining the mean responses to the differentiated stimulus or to the positive CS in the conditioned-inhibition compound, and the mean responses to the excitatory CSs during 5 successive experimental sessions. It is seen from Fig. 1, 2, and 3 that for all the animals the conditioned-inhibition compound was much more difficult than differentiation. The salivary inhibitory CR to the differentiated stimulus as well as the I/E ratio for differentiation were always smaller than the corresponding response and ratio for the conditioned-inhibition compound.

The operations were carried out with aseptic precautions under Nembutal anesthesia. The grey and white matter rostral to the presylvian sulcus was removed by suction. The lesions, which included the proreal, subproreal and antero-orbital portions of the frontal lobes were bilaterally in one stage. The olfactory tracts were left intact. The animals recovered uneventfully except for D-14 which for about 4 days postoperatively showed a reduction in locomotor activity and a marked decrease in appetite (presumably due to an oedema which developed in the nasal parts of its head). A recovery period of a week was allowed before testing was started. However in D-14 some disorders in salivation were observed during the first postoperative experiments (both its CRs and UCRs were irregular), suggesting that its recovery from the effects of the oedema was still incomplete. In view of this the performance of D-14 on the first postoperative experiments was discounted.

The results presented in this paper were obtained during the postoperative period of about 2 months in D-12, D-14 and D-22, and 4 months in D-13.

RESULTS

In agreement with our previous findings (Brutkowski et al., 1956) no gross changes in the animal's general behaviour were observed postoperatively. The dogs did not show any signs of hyperactivity, perseverative movements nor any other symptoms commonly observed after more extensive frontal lesions.

Post-operative changes in A-experiments

The comparison of A-CRs before and after operation for each dog is presented in Fig. 1, 2, and 3. It is seen that out of the three dogs tested, D-12 and D-13 showed an initial increase in the excitatory A-CRs. However, these reflexes very soon returned to the preoperative level or even dropped below it. The third dog D-14 showed no significant change in positive A-CRs.

The analysis of the performance on the inhibitory trials shows that both types of inhibitory A-CRs were disinhibited, with postoperative impairment in differentiation being less marked than that in conditioned inhibition. Whereas the salivary response to the positive CS in the inhibitory combination (conditioned inhibition compound) reached or even exceeded the CR to the positive CS alone, the salivary response to the differentiated stimulus was not increased as much. It is interesting to note that even in the initial postoperative period correct inhibitory CRs were sometimes observed. In some instances the performance on inhibitory trials was good during initial postoperative testing and only on the later inhibitory trials was an impairment observed. Moreover, except for conditioned inhibition in D-12 and D-14, the postoperative disinhibition of the inhibitory A-CRs was never complete. The salivary

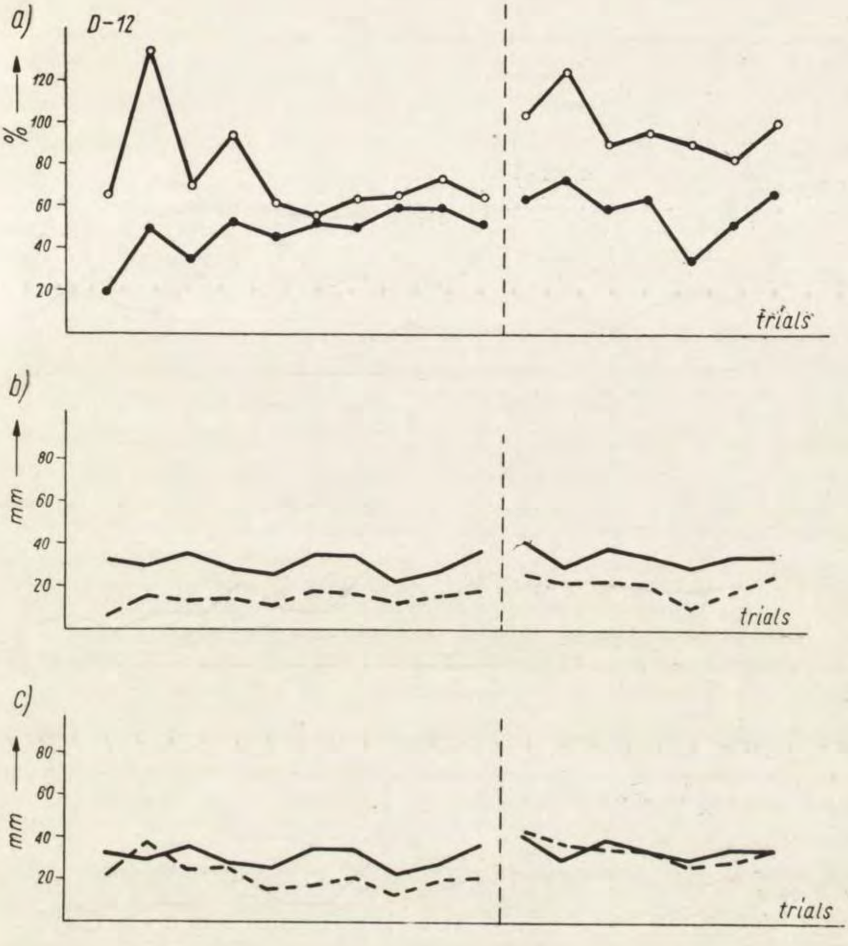


Fig. 1. Disorders of inhibition in acid conditioned reflexes in dog D-12 after prefrontal ablation

a — Inhibitory reactions in percentage of excitatory reactions. Each point denotes the average level of inhibitory reaction in 5 successive experiments. Open circles — conditioned inhibition, full circles — differentiation. The day of operation is represented by perpendicular line. The data of 50 experiments before operation and 35 after operation are represented. b — Absolute amounts of salivation to excitatory and inhibitory stimulus (differentiation). Continuous line — reactions to excitatory stimulus; broken line — reactions to inhibitory stimulus. Other explanations as in a. c — Absolute amounts of salivation to excitatory and inhibitory stimulus (conditioned inhibition). Explanations as in b.

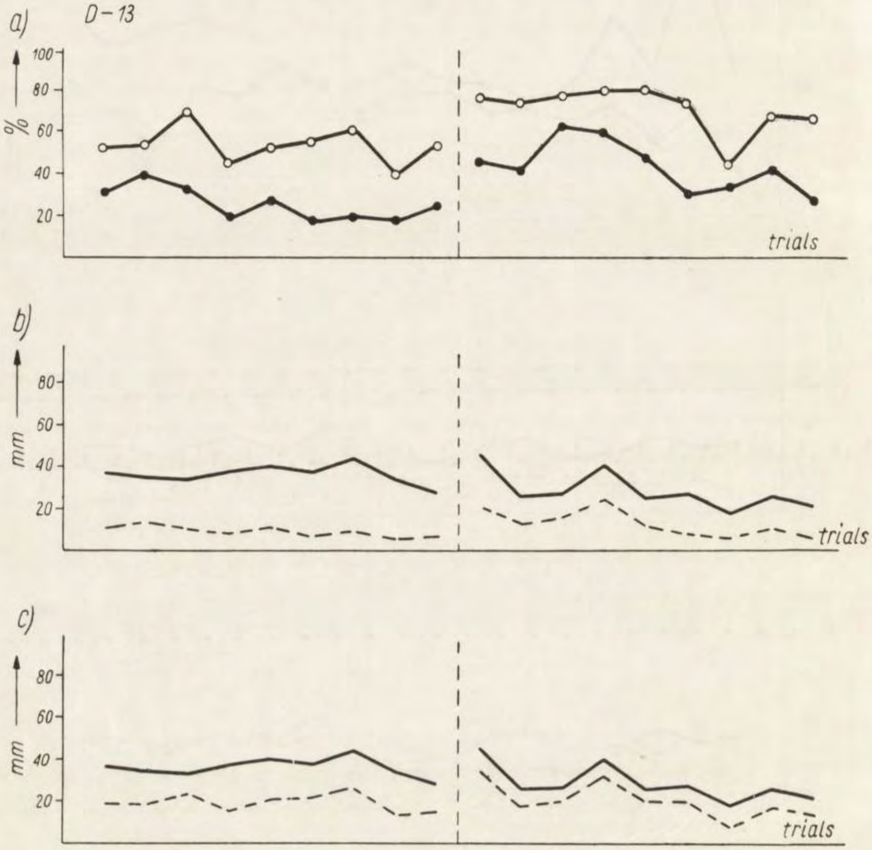


Fig. 2. Disorders of inhibition in acid conditioned reflexes in dog D-13 after prefrontal ablation

Explanations as in Fig. 1.

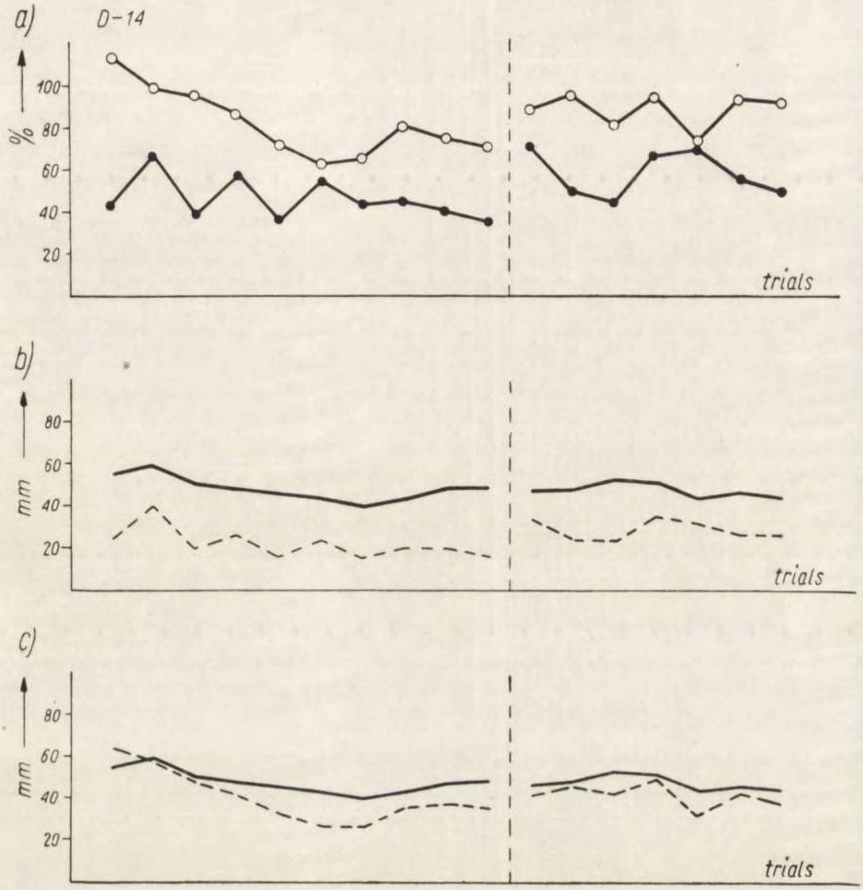
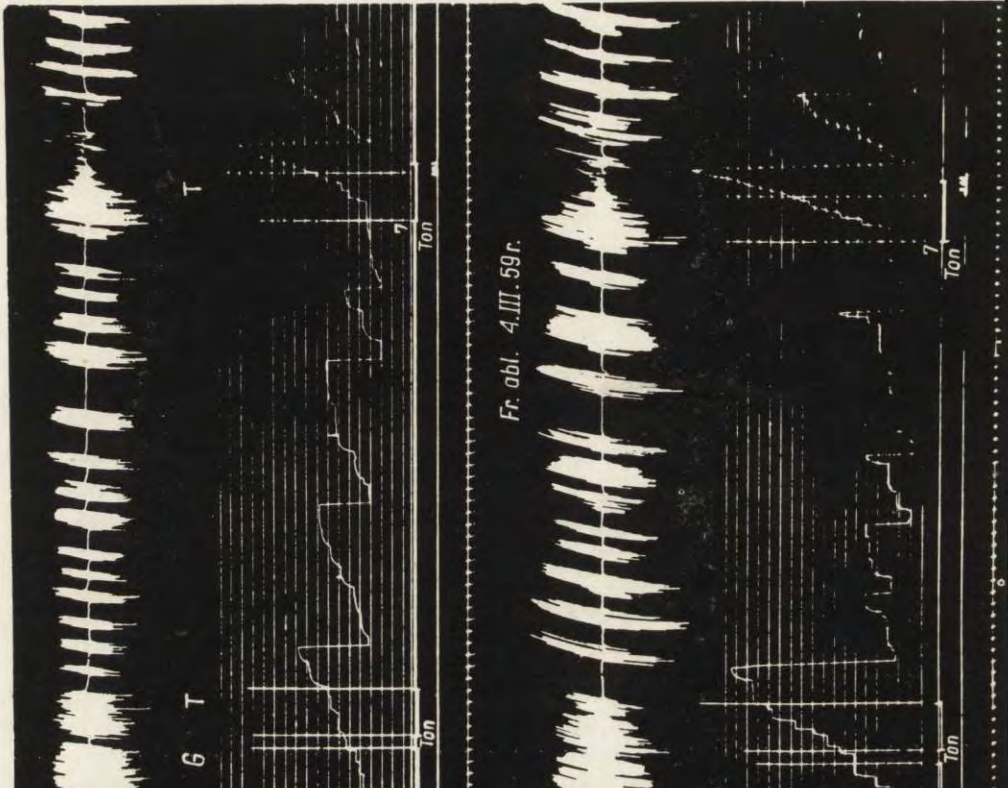


Fig. 3. Disorders of inhibition in acid conditioned reflexes in dog D-14 after prefrontal ablation

Explanations as in Fig. 1.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responses in inhibitory trials were in most cases smaller than the positive CRs though they were increased in comparison with the preoperative level.

The results presented in Fig. 1, 2 and 3 show that the inhibitory A-CRs, affected by prefrontal lobectomy, can be at least partially retrained. It is difficult, however, to determine the exact time of complete recovery of the inhibitory ability, since no abrupt sustained improvement was observed. Thus, poor performance on an inhibitory trial was sometimes followed by a sudden improvement but this was often followed in turn, by a new disinhibition. Such a "wavy" course of recovery of the inhibitory A-CRs was particularly apparent in the differentiation test.

In addition to the postoperative impairment in A-CRs, disorders in other reactions of the animals were also observed. First, changes in respiration occurred, which consisted in an increase in respiratory rhythm or amplitude (Fig. 4). Next, an increase in chewing and licking to the inhibitory CSs was observed. The increase was marked and on occasion reached the level of licking to the excitatory CSs which seemed to be maximal (Table II).

Table II

Average number of licking movements

	Before operation (10 Sessions)			After operation (10 Sessions)		
	exc. CS	diff. CS	cond. inh.	exc. CS	diff. CS	cond. inh
D-12	7.0	1.7	2.1	7.2	3.3	3.5
D-13	4.9	1.4	3.0	4.8	2.0	4.1

Furthermore, following the lobectomy an increase in the amount and rate of salivation during intertrial intervals was observed. It was particularly apparent in the initial period after reinforcement, i.e. chiefly as a prolongation of the response to the UCS (Fig. 5).

Fig. 4. Typical experiments with A-CRs before (above) and after (below) operation

From top to bottom: respiration, salivary responses. conditioned stimuli, reinforcement, time (5 sec.). T — tone — positive conditioned stimulus. G — rattle — conditioned inhibitor. Note the following changes occurring after operation: increased respiration, increased salivary after-effect after positive trials, full disinhibition of inhibitory compoud.

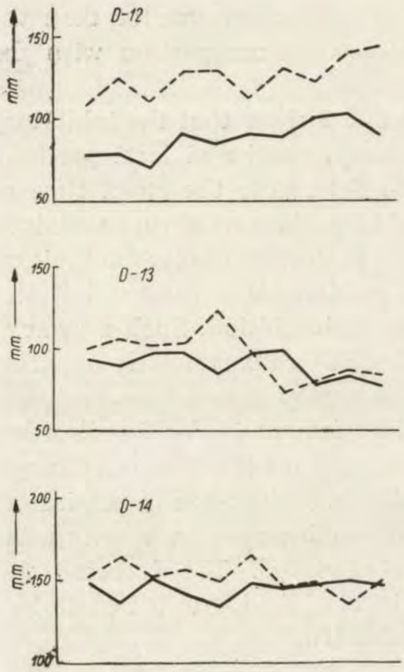

Fig. 5. Salivation during 40 sec. after introduction of acid into the mouth in dogs D-12, D-13 and D-14

Each point denotes average amount of salivation in 6 positive trials in the course of one experimental session. Continuous line — before operation, broken line — after operation.

Finally, the animals were often irritable under the experimental conditions and tried to tear off the tube supplying the acid. These last changes were transient and 2—3 weeks after the lobectomy they gradually disappeared.

Post-operative changes in E-experiments

In the initial postoperative period marked changes in the excitatory E-CRs were observed. The positive E-CRs became more intensive than before surgery: the latent period was often shortened, the amplitude increased and the responses persisted sometimes even after the reinforcement (Fig. 6). The E-CRs appeared, furthermore, not only to CSs but also between trials. This was particularly apparent in D-13 and D-22 both of which performed a large number of intertrial E-CRs in succession throughout the whole postoperative period of observation (4 and 2 months, respectively). In D-14 however, only a few intertrial responses were observed, and these occurred during the first few postoperative sessions. D-13 began to respond with both forelegs alternately

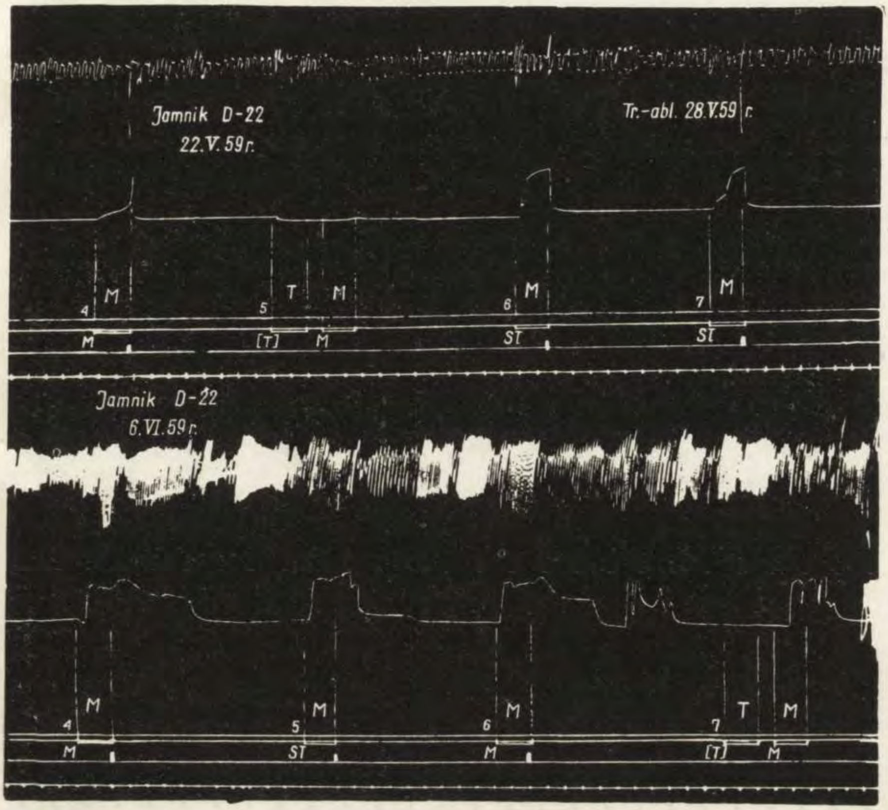

Fig. 6. Typical experiments with E-CRs before (above) and after (below) operation

From top to bottom: respiration, motor response, conditioned stimuli, reinforcement, time (5 sec.). Note increased respiration, increased positive CRs and URs and disinhibition of inhibitory reflex to CS following conditioned inhibitor after operation.

although the electric shock was always applied to the right leg only. After a number of weeks the response of the left foreleg disappeared.

Performance on the inhibitory E-CRs is illustrated in Fig. 7. A comparison for each animal between its pre- and postoperative performance on the differentiation test indicates no differences for D-14 and D-22 but a marked disinhibition for D-13. On the other hand the inhibitory E-CR to the positive CS in the inhibitory combination (conditioned inhibition) was impaired at least initially in all the animals. In some instances a positive response occurred even to the conditioned inhibitor. Except for D-13 the impairment of conditioned inhibition persisted only for several

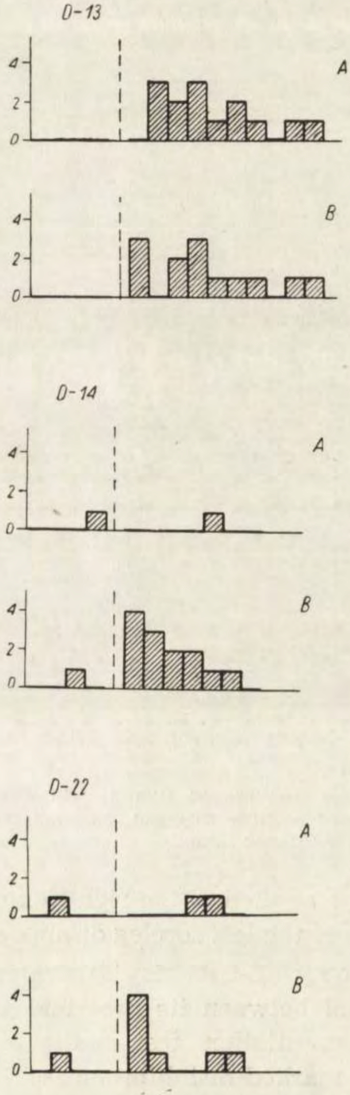

Fig. 7. Disorders of inhibitory reflexes after prefrontal ablations in E-CRs

Each column represents the number of "errors" to inhibitory stimulus in 5 consecutive inhibitory trials, 20 inhibitory trials before operation are shown: A — differentiation, B — conditioned inhibition. Note strong and lasting impairment of conditioned inhibition and slight impairment of differentiation after operation.

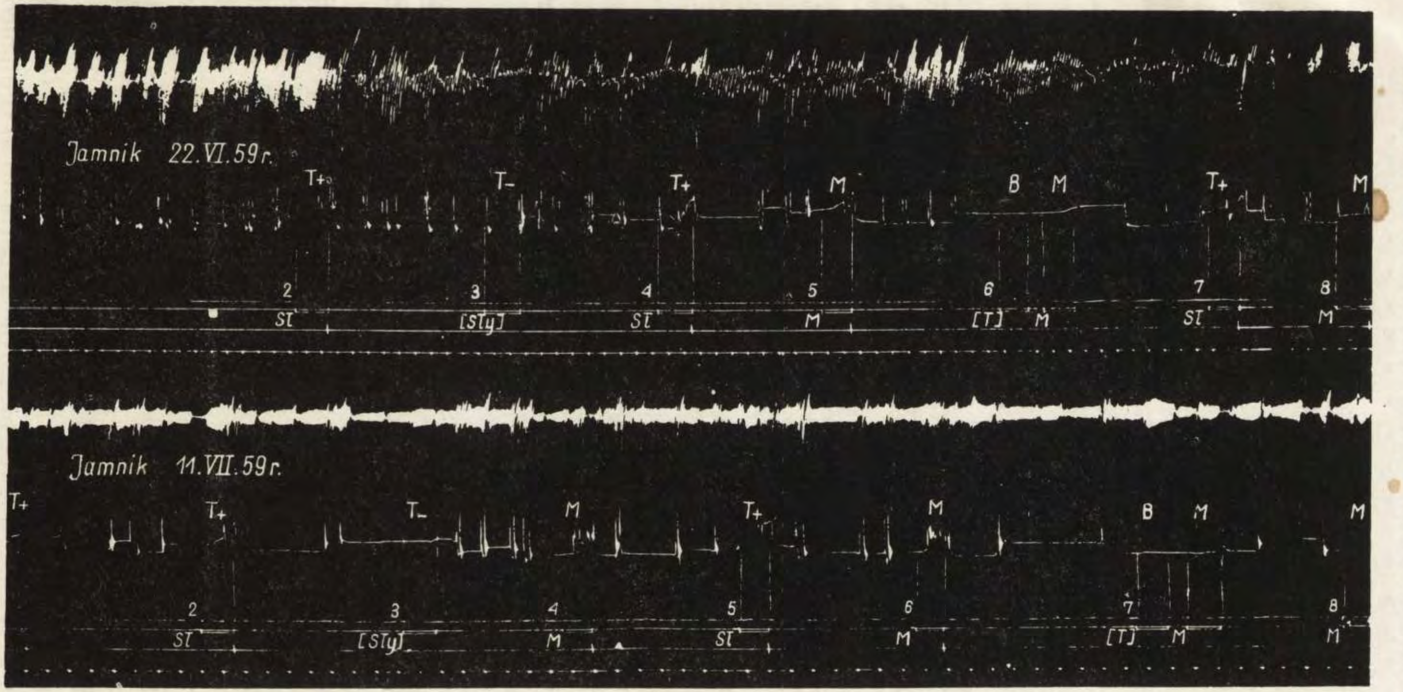

Fig. 8. Examples of experiments with E-CRs after operation in which the inhibitory reaction to inhibitory compound is preserved but the animal displays conditioned reaction in intervals

Explanation as in Fig. 6.

trials. As in the A-experiments, sometimes no impairment in inhibitory E-CRs was observed during the first postoperative testing and only subsequently, when the animals become excited, they did exhibit poor performance on some inhibitory trials. However, on occasion, and in spite of a marked increase in the number of intertrial E-CRs, an enhancement in breathing, and maximal excitement, no impairment was observed in inhibitory trials; the inhibitory CSs even seemed to make the animals quiet (Fig. 8).

Examination of other reactions accompanying E-CRs indicates that the animals showed marked changes in various responses related to the defensive behaviour. Sometimes, there were striking changes in respiration. For example, following operation in D-22 a very slow respiratory rhythm was replaced by a fast intensive one (Fig. 6). A change in respiration was also apparent in the other dogs but it was less pronounced because of their relatively fast intensive breathing before the operation. Some of the postoperative changes suggest that the animals were afraid of the experimental situation as never before. They growled, grimaced, barked and tried to escape or to struggle with the experimental equipment on the stand, biting the harness and lines. During the first few postoperative days testing with D-22 was possible only if an assistant was present with him in the chamber; if not, the dog demolished the equipment on the stand. Such behaviour was completely absent before the operation. Although these changes gradually disappeared, they recurred from time to time even several weeks after operation.

DISCUSSION

As seen from the foregoing data bilateral prefrontal lobectomy in 4 dogs produced changes in various responses related to defensive behaviour. Objective observations carried out on defensive CRs reinforced by electric shock or by the introduction of acid into the animal's mouth showed in most instances an increase in excitatory CRs and a disinhibition of inhibitory CRs. The increase in positive defensive CRs consisted of an enhancement of E-CRs to CSs and the appearance of intertrial E-CRs in E-experiments, and in an augmentation of the salivary effects in A-experiments. These changes in CRs were accompanied by an increase in respiration and by changes in other somatic as well as autonomic reactions (e. g. intensive licking in A-experiments, biting the experimental equipment, tearing off the tubes attached to its cheeks, grimacing, growling etc.) suggesting that the anxiety state in prefrontal dogs was enhanced. Moreover, the fact that immediately after lobectomy the dogs were often unchanged, and only after a number of reinforcements became excited and disinhibited, seems to indicate that they were more sensitive to the noxious stimuli (i. e. UCSs) postoperatively.

Of the two inhibitory tests used in these experiments, conditioned inhibition was impaired in all instances while differentiation showed either relatively slight or no disinhibition. Also, the impairment in the inhibitory CRs was more marked in A-experiments than in E-experiments. Thus, in A-experiments differentiation was disinhibited in all dogs, while in E-experiments this test was impaired only in one dog out of three.

Most of these changes tended to disappear in time, suggesting, that, in addition to the frontal lobes, other structures moderate defensive behaviour and contribute to the inhibitory ability in defensive conditioning.

In none of the operated dogs were all the changes which were listed above observed. However, in each dog many of these changes appeared so that the postoperative increase in defensive behaviour was apparent.

The postoperative impairment in defensive CRs closely resembles changes in alimentary CRs described previously. First of all, this relates the disinhibitory effects found in prefrontal dogs under the two (alimentary and defensive) conditions. There were, however, dogs in which no disinhibition of defensive inhibitory CRs was observed, or a disinhibition which appeared only in the later postoperative period. Moreover, in some sessions during which there appeared to be an enhanced level of defensive activity (when the animal performed a large number of intertrial CRs and its rate of breathing was greatly increased) performance on inhibitory trials was found to be quite adequate. Thus, in some instances, a postoperative change was observed only in positive responses (e. g. UCRs, conditioned salivation etc.). It is difficult to explain a postoperative increase in positive responses in terms of the hypothesis of disinhibition, proposed earlier (Brutkowski, Konorski, Ławicka, Stępień, I., and Stępień, L., 1956). It might be reasonable to suppose, however, that prefrontal disinhibition is a secondary effect which appears as the result of an enhanced level of positive defensive activity.

It is of interest to note certain dissimilarities between the results obtained in the A-experiments and E-experiments. From the data presented it seems to follow that A-CRs were more affected by the lobectomy than E-CRs. In our opinion, however, this difference may be explained by disparities in the experimental procedures used in the two situations. The method for recording the salivary effects which detects even slight alterations of the animal's responses is more precise by far than the procedure used in motor reflexes where it can be determined only whether the movement was performed or not. Hence, certain small motor reactions (e. g., responses to inhibitory CSs) are simply not recorded and thus not taken into account. A second factor seems to relate to differences in the preoperative training in the two experimental situations. The data of Table I show that the training of inhibitory A-CRs required about 3 times as long as that of E-CRs. This disparity probably results from the fact that the salivary response is much more difficult to suppress than is the motor response.

It further appears that the postoperative impairment in conditioned inhibition is more marked than that in differentiation. In A-experiments this difference seems to be due to the fact that, preoperatively, differentiation was not as difficult for the animals as conditioned inhibition. As seen from Fig. 1, 2, and 3 the curve for salivary responses to the differentiated stimulus always lies below that for the CS preceded by the conditioned inhibitor (in conditioned-inhibition compound). Following the operation the distance between these curves was maintained but on a higher level. This suggests that the postoperative impairment is a function of the extent of inhibition involved in the test. Moreover, the conditioned-inhibition compound, such as has been used in all our studies, is made more difficult by the interval of several seconds introduced between conditioned inhibitor and CS.

The relatively slight postoperative impairment in the differentiation of E-CRs may have another cause. It is a well-known fact that the classical motor defensive CRs reinforced by electrical stimulation (E-CRs) often tend to disappear so that only about 90 per cent of the positive CSs are effective. There is some evidence indicating that such disappearance of positive E-CRs results from the relatively weak noxious reinforcement used in the majority of E-experiments (to avoid too harmful stimulation). However, the inefficacy of a number of positive defensive CSs suggests that at least in some dogs the defensive excitability is not very high. There are some experimental findings which seem to confirm this point of view indicating that in many situations alimentary behaviour may predominate over defensive behaviour. Yerofeeva (1912) was the first to establish that the defensive UCR evoked by electric shock may be suppressed if the noxious stimulus is followed by the presentation of food. Furthermore, it was found by this author that instead of a pain reaction to strong electrical stimulation an alimentary CR appeared consisting of both salivation and a motor reaction. In many other studies it was clearly shown that in contrast to the E-CRs only rarely does the CR to a positive alimentary stimulus fail to appear. Consequently, the lack of response to some defensive inhibitory CSs may thus be considered not as an active inhibitory CR, but rather as the absence of an excitatory CR (while the lack of a response to the alimentary inhibitory CS may certainly be regarded as an active inhibitory reflex).

Returning now to the results obtained in our prefrontal dogs, the absence of impairment in differentiation in two of them (D-14 and D-22) might be explained by their relatively low level of defensive excitability since in these dogs only about 90 per cent of the positive defensive CSs were effective. The same explanation may perphaps be applied to Ławicka's results obtained in a dog in which both alimentary CRs and E-CRs were trained in two different situations. After prefrontal ablation neither an impairment in differentiation nor in conditioned inhibition in E-experiments was observed while under the alimentary conditions the same inhibitory tests were markedly impaired (unpublished results). The electrical stimulation used in those experiments was even weaker than that of ours. In consequence, even positive E-CRs in this dog were often absent or delayed. Therefore, it may be supposed that the absence of responses to a number of inhibitory CSs in this dog was due to a low level of defensive excitability resulting from an insufficiently strong noxious reinforcement, and not to a well-established inhibition. It is understandable, then, that prefrontal lobectomy did not affect the E-CRs of this dog.

Our results confirm Afanasev's (1913) findings obtained on prefrontal dogs in which E-CRs were trained preoperatively. The author found a temporary impairment in inhibitory E-CRs to acoustic, visual, tactile and kinestetic CSs in 6 dogs. In addition to these changes the animals became irritable and restless. 3—4 weeks after the lobectomy inhibitory responses recovered. Allen (1949), using similar experimental technique, found impairment in olfactory differentiation only.

Recently, a number of papers has been published (Lichtenstein, 1950, Streb and Smith, 1955, Pribram and Weiskrantz, 1957, Waterhouse, 1957) indicating that damage to the frontal lobes results not in an increase in defensive activity, as our findings seem to suggest but, on the contrary, in a reduction of defensive behaviour. However, the present results cannot be compared with those of many other authors because of differences in subjects, experimental procedures, operative techniques, and frontal lesions. It is likely that one or another of these differences is responsible for the contradictory conclusions. For example, the avoidance technique used in most of the studies as an indicator of "emotional" behaviour differs from our classical defensive procedure in certain essential aspects. The most important difference is the absence of a "real" noxious reinforcement under the avoidance conditions. Our findings tend to indicate that prefrontal dogs are more sensitive to defensive UCSs. It was shown that a postoperative increase in excitatory CRs and a disinhibition of inhibitory CRs, often appear only after a number of noxious reinforcements. Moreover, in order to see the effects of prefrontal lobectomy clearly, the reinforcement must be rather strong in order to maintain defensive responses on a high level. If the preoperative level of defensive behaviour is low, the changes resulting from lobectomy may be too slight to be observed. This, we believe, is the case when avoidance technique is used. The decrease in the performance of avoidance responses may be considered as an impairment in instrumental reaction rather than a decrease in emotional reactivity. We have recently found (Brutkowski, 1959b) that under alimentary conditions in a number of prefrontal dogs the instrumental reflex is lost initially, but recovers rapidly after several reinforcements. On the other hand, the classical salivary CR and various primary alimentary responses are present af first postoperative testing. This evidence suggests that the loss of the instrumental reflex does not necessarily reflect a decrease in emotionality.

In some studies the experimental procedure was complicated by the fact that in addition to defensive CSs, alimentary CSs were used (Waterhouse, 1957) or food was presented to the animals under the defensive conditions (Lichtenstein, 1950). Due to the predominance of alimentary over defensive behaviour in centain situations, as discussed above, and the possible interference between defensive and alimentary responses when these are stuied in the same experimental session, the conclusions to be drawn from the experiments cannot be clear. Loss of "feeding inhibition" in Lichtenstein's lotomized dogs may result from the postoperative increase in alimentary activity observed in prefrontal animals (cf. Brutkowski, 1959) and so need not favour the hypothesis of fear reduction. Finally, the very interesting findings of Streb and Smith (1955) indicating that in lobotomized rats there was an overresponding to the CS instead of the crouching and immobility preoperatively acquired, seem to support our suggestion of an increase in defensive reactivity in prefrontal animals.

SUMMARY

1. The aim of this paper was to study the effect of prefrontal lobectomy on the defensive classical conditioned reflexes reinforced either by introduction of acid into the animal's mouth (A-experiments), or by stimulating the leg by electrical shock (E-experiments).

2. It was found that in both groups of experiments the following changes in the animal's behaviour were manifested: a) the unconditioned reflexes to the noxious stimuli (A or E) were in most cases increased; b) the positive conditioned reflexes to conditioned stimuli were also either increased or remained on the same level as before operation; c) the inhibitory conditioned reflexes were disinhibited, the disinhibition being more pronounced in conditioned inhibition than in differentiation; d) the general level of defensive activity, as manifested by records of respiration and intertrial defensive reactions was increased.

3. The above described changes in the animal's behaviour after prefrontal ablations tend to be attenuated with the lapse of time.

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REFERENCES

AFANASEV N. I. 1913 — Materialy k izutsheniju funkcji lobnykh doley. Diss., S. Petersburg.

ALLEN W. F. 1949 — Effect of prefrontal brain lesions on correct conditioned differential responses in dogs. Amer. J. Physiol. *159*, 525.

BRUTKOWSKI S., KONORSKI J., ŁAWICKA W., STĘPIEŃ I. and STĘPIEŃ L. 1956 — The effect of the removal of frontal poles of the cerebral cortex on motor conditioned reflexes. Acta Biol. Exper. *17*, 167.

BRUTKOWSKI S. 1957 — The effect of prefrontal lobectomies on salivary conditioned reflexes in dogs. Acta Biol. Exper. *17*, 327.

BRUTKOWSKI S. 1959a — Effects of prefrontal ablations on salivation during the alimentary unconditioned reflex and after its cessation. Acta Biol. Exper. *19*, 281.

BRUTKOWSKI S. 1959b — Comparison of classical and instrumental alimentary conditioned reflexes following bilateral prefrontal lobectomies in dogs. Acta Biol. Exper. *19*, 291.

KOZAK W. 1950 — Method of registration of salivary secretion. Acta Biol. Exper. *15*, 185.

LICHTENSTEIN P. E. 1950 — Studies of anxiety: II. The effects of lobotomy on a feeding inhibition in dogs. J. Comp. Physiol. Psychol. *43*, 419.

ŁAWICKA W. 1957 — The effect of the removal of the prefrontal areas on the vocal conditioned reflexes in dogs. Acta Biol. Exper. *17*, 319.

ŁAWICKA W. — unpublished experiments.

PRIBRAM K. H. and WEISKRANTZ L. 1957 — A comparison of the effects of medial and lateral cerebral resections on conditioned avoidance behavior in monkeys. J. comp. physiol. Psychol. *50*, 74.

SOŁTYSIK S. and ZBROŻYNA A. 1957 — The chronic fistula of shortened Stensen's duct in dogs. Acta Biol. Exper. *17*, 339.

STREB J. M. and SMITH K. 1955 — Frontal lobotomy and the elimination of conditioned anxiety in the rat. J. comp. physiol. Psychol. *48*, 126.

WATERHOUSE I. K. 1957 — Effects of prefrontal lobotomy on conditioned fear and food responses in monkeys. J. comp. physiol. Psychol. *50*, 81.

YEROFEEVA M. N. 1912 — Elektriceskoe razdrashenie koshi sobak kak uslovnyj vozbuditel raboty slyunnykh shelez. Diss. S. Petersburg. Cit. after Pavlov, I. P. 1951 — Wykłady o czynności mózgu. Warszawa.

ŻERNICKI B. 1960 — The effect of prefrontal lobectomy on the water instrumental conditioned reflexes in dogs. In preparation.

Acta Biologiae Experimentalis, Vol. XX, 1960

ALIMENTARY TYPE II (INSTRUMENTAL) CONDITIONED REFLEXES IN AMYGDALA DOGS

S. BRUTKOWSKI, E. FONBERG, and **E. MEMPEL**

Department of Neurophysiology, Nencki Institute of
Experimental Biology, Warsaw

Department of Neurosurgery, Polish Academy of Sciences, Warsaw

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Recent evidence from the work of Bard and Mountcastle (1948), Schreiner and Kling, (1953, 1956), Rosvold, Mirsky and Pribram (1954), Weiskrantz (1956), Fuller, Rosvold and Pribram (1957), Green et al. (1958) and others indicate that ablation of the temporal lobe and subjacent amygdala--pyriform area produces changes in emotional behaviour. Careful observations by these workes have shown that this part of the brain contributes to the mechanisms of at least sexual, alimentary and fear behaviour. These findings seem to be in agreement with anatomical data indicating that the periamygdaloid areas project to the hypothalamus. It has been particularly well established that fibres can be traced from the amygdaloid nucleus into the ventromedial nucleus of the hypothalamus (Adey and Meyer, 1952). Consequently, there is universal agreement that the amygdaloid region exerts a marked influence on the hypothalamus and in some way regulates its activity.

Despite the many reports on the functioning of the periamygdaloid area, however, there still remains a large amount of incomplete data concerning this problem and some of the reports appear to be conflicting. The general plan of a series of studies undertaken in this laboratory has been to determine the deficit produced in various kinds of conditioned reflexes (alimentary, defensive, classical and instrumental) following amygdala lesions in dogs. The present paper deals with the effects of amygdala lesions on excitatory and inhibitory type II (instrumental) alimentary conditioned reflexes (Konorski and Miller, 1933).

MATERIAL AND METHODS

The experiments were performed on 2 experimentally naive male dogs (D-11 and D-21) and on 2 dogs (D-8 and D-20) already used in other experiments and described elsewhere (Fonberg, 1958, Brutkowski, 1959b). All animals were trained in a soundproof conditioned-reflex chamber. The training involved the following steps:

1. According to the usual procedure of this laboratory, in the preliminary training each animal was taught by a passive movement to put its right foreleg (D-20 and D-21) or hindleg (D-8 and D-11) on a small platform to acoustic stimuli. The movement was reinforced originally by immediate presentation of food, but later only after a 10-second delay during which the response was usually maintained or repeated. The CS overlapped the act of eating for about 3 sec.
2. When this instrumental CR had been established so that the animal performed the trained movement to every presentation of the CS, all the dogs were trained in differentiation and D-8, D-11 and D-21, in conditioned inhibition additionally. The differentiation test consisted in the presentation for 10 sec. of a negative stimulus similar to the positive CS (a tone of a different intensity). The conditioned-inhibition test consited in presenting a combination of two stimuli in which the conditioned inhibitor (Rattle) presented for 10 sec. was followed after 10 seconds by the positive (Buzzer). This combination of stimuli was never reinforced by food while the Buzzer itself was always reinforced.

The experiments were carried out 3—4 times a week. In each experimental session there were 5—7 positive trials and 1—3 negative trials (the latter including both differentiation and conditioned inhibition).

The motor reactions were recorded on a kymograph. In 2 dogs (D-8 and D-21) salivation from glandula parotis was also recorded.

An attempt was made to train the animals to a criterion of at least 85% correct inhibitory responses out of 25 inhibitory trials on each test. In spite of extended training, however, D-8 and D-20 were not able to master the inhibitory tasks in more than 60 per cent of such trials; neurotic states were often observed in them.

All operations were carried out with aseptic precautions under Nembutal anesthesia. The removals were performed in a manner similar to that described by Fuller et al. (1957). Thus, a skin incision was made in the temporal region above the zygoma, and the temporal muscle was split, some parts of it being removed. The zygoma itself was removed only in its upper part.

By this procedure it was possible to approach the lateral and basal parts of the dog's brain and to remove the entire amygdaloid complex. Neural tissue was resected by subpial suction. The extirpations were performed in two stages, first on the right and then on the left side, separated by an interval of about 2—3 weeks, except in D-8 where the second operation followed the first one after 6 months. Postoperative testing was started a week after each operation.

While histological examination of the operated brains has not been accomplished, gross verification of the lesions in three of the dogs reveals that the removal was restricted mostly to the lower portions of the amygdaloid complex and the ventral pyriform cortex. Presumably, however, there was also some slight damage to the ventral putamen and the lateral portion of globus pallidus, though the mass of the hippocampal cortex and the optic tracts appear to have been spared. In the fourth dog, D-8, the excision was extended into the depths to remove the dorsal portion of amygdaloid complex where it approaches the internal capsule. The removal was particularly extensive on the left side where the internat capsule was damaged.

RESULTS

Although the postoperative examination of the CRs was begun after the unilateral amygdalectomy, in three of the dogs (D-11, D-20, and D-21) the period of observation between the first and second operations was too short to draw conclusions about possible changes.

However, the results after bilateral amygdalectomy in these three dogs present a clear picture:

1. There were no visible changes in excitatory CRs. As in the preoperative period the animals reacted soon after the presentation of the positive CSs and kept the leg that they placed on the platform or repeated the placing response until the food was presented.

2. The responses to the inhibitory CSs often were disinhibited. The animals reacted in the inhibitory trials either in the same manner as they did to the postitive CSs (complete disinhibition) or in an attenuated form (partial disinhibition). In many instances the animals responded to the inhibitory CS immediately after its presentation and kept their legs on the platform long after the cessation of the stimulus. Then, since the reinforcement did not appear, they often whined, barked and repeated the instrumental reaction, or violently pressed on the platform revealing a marked "disappointment", a form of behaviour that had never been observed before the amygdalectomy. They also frequently looked into the empty bowl and licked it out when the inhibitory CS was presented. The

positive instrumental reaction to the inhibitory CS was accompanied by an increase in salivation. However, despite their obvious impairments, it should be noted that in some trials, even in the immediate postoperative period, the inhibitory response to the negative CS was fully preserved (Fig. 1).

The disinhibition of differentiation and conditioned inhibition was not persistent. In D-11 and D-21 it lasted for about 2 months

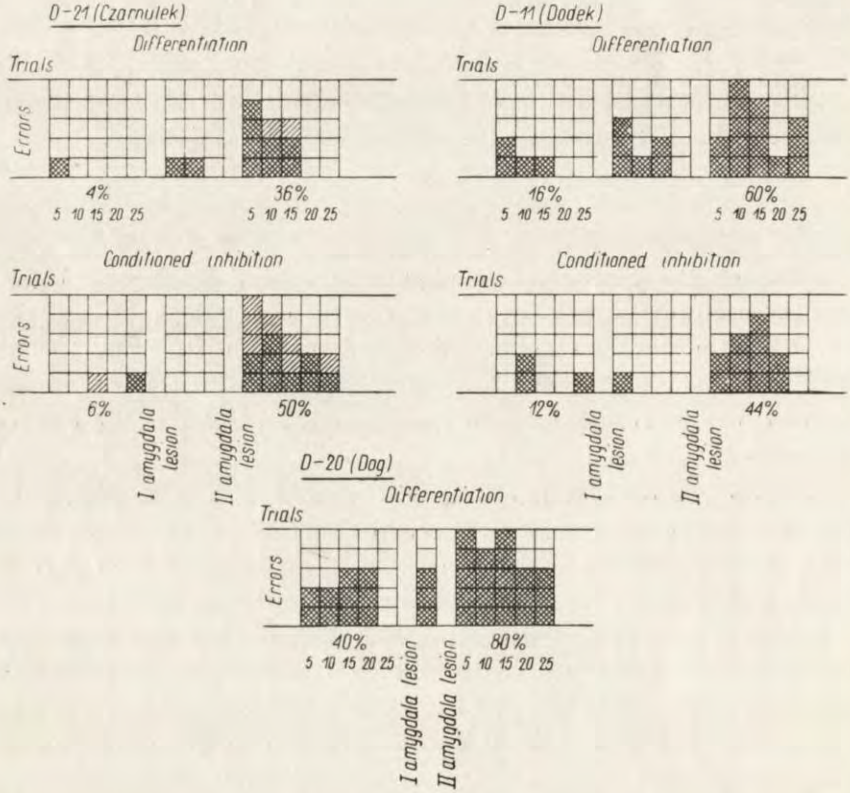

Fig. 1. The impairment of inhibitory conditioned reflexes in dogs after amygdaloid lesions

Each square denotes one inhibitory trial. Every column of five squares represents a bloc of five successive inhibitory trials. Checked squares denote full disinhibition of the inhibitory reflex, striped squares partial disinhibition (abortive movement), white squares — normal inhibitory reaction. In each column from bottom to top are shown fully disinhibited reactions, partially disinhibited reactions and normal reactions. Percentage of errors is shown in each series of experiments.

postoperatively. However, in D-20 it persisted very much longer. Even five months after the amygdalectomy, when this dog was sacrified, it still failed to differentiate at the preoperative level. In the two other dogs the subsequent recovery was more rapid but was also never complete.

In the fourth dog, D-8 in which a complete destruction of the amygdaloid nuclei had been made, quite different changes occurred. Although the unilateral lesion did not result in any impairment, the second operation (on the left side) produced complete aphagia which persisted for about half a year. During this period the dog was fed by placing food in its mouth. Put on the stand in the conditioned-reflex chamber one week after the operation D-8 reacted correctly to the CS in spite of the absence of active eating. He looked into the bowl and even attempted to take food, but unsuccessfully. Then, further testing became impossible since the experimental situation, in which food was presented but not taken by the dog, made the animal highly irritable. Three months after the amydgalectomy salivary reflexes could be conditioned by reinforcement with milk introduced directly into the dog's mouth. Since the animal was so reductant to test, no inhibitory stimuli were presented.

DISCUSSION

The findings of this study indicate that bilateral lesions in the lower portions of the amygdaloid complex in dogs resulted in the temporary impairment of the alimentary inhibitory type II CRs to acoustic stimuli while the excitatory CRs remained unaffected. The disinhibition of the inhibitory CRs persisted for a period of several months although during this time there were occasionally correct inhibitory responses even in the immediate postoperative period.

These results closely resemble those obtained on prefrontal dogs (Brutkowski et al., 1956, Brutkowski, 1957). Thus, it has been found that dogs with lesions in the proreal and antero-orbital regions of the frontal lobes exhibited marked impairment in the performance on inhibitory tasks (differentiation, conditioned inhibition, alternation) which persisted for varying periods depending on the extent of the lesion, the "amount" of inhibition involved in the test, and the individual properties of the animals. The suggestion has been put forward (Konorski, 1958, Brutkowski, 1959a) that the impairment in inhibitory CRs in frontal animals is due to partial sectioning of the descending fibres which originate in the orbital and premotor cortex and terminate in the ventro-medial nucleus of the hypothalamus (Le Gros Clark and Meyer, 1950), a nucleus which has been considered to be an inhibitory "feeding centre" (Anand and Brobeck, 1951).

A large body of evidence has recently been collected to show that the posterior part of the frontal lobes and the temporal poles including the periamygdaloid region and amygdala form an anatomically integrated system (Pribram, Lennox and Dunsmore, 1950, Pribram and Kruger, 1954, MacLean, 1955) associated with the hypothalamus. This phylogenetically old fronto-orbito-temporal region ("visceral brain") has recently become the focus of great interest since it has been purposed that in connection with many other rhinencephalic structures it forms the highest level of autonomic and emotional integration (Papez, 1937, 1958, Fulton, 1953, MacLean, 1954, 1955). From the description of several authors it appears that this area is concerned, among others, with alimentary functions. Thus, Pribram and Bagshaw (1953) found the temporal polar-amygdaloid formation to be related to food intake. Furthermore, Fuller, Rosvold and Pribram (1957), Morgane and Kosman (1957) and more recently, Green, Clemente and de Groot (1958) demonstrated that hyperphagia can be produced by lesions placed in the amygdaloid complex region. The evidence of Green and coworkers points to an association between hyperphagia and lesions of the amygdala near the junction of the lateral and basal nuclei, a region which was removed in our dogs. Our results do not permit any conclusions regarding hyperphagia due to the fact that the amount of food given was exactly the same before and after the operation. However, from the description of their general behaviour in the alimentary situation it seems to follow that there was an increase in their alimentary activities. Consequently, it is possible that an enhancement of alimentary drive, i.e. an increase in the excitability of the alimentary centre following amygdalectomy, may have influenced the perbormance of CRs. Assuming some hyperphagic tendencies in the amygdala or frontal dogs, these tendencies could cause difficulty with suppression of the alimentary response to the inhibitory CS, thereby resulting in a disinhibition of inhibitory CRs. The temporary nature of this impairment in frontal as well as in amygdala dogs may be due to the incomplete removal of the areas concerned with alimentary functions.

On the other hand, there is some evidence indicating that damage to the fronto-orbito-temporal region does not always result in an increase in alimentary response. A reduction or even a complete absence of feeding patterns is sometimes observed. Recently, Bell and Lawn (1957) found that orbito-frontal lobectomy in 5 goats produced a decrease of food and water intake for about 2 months, while Green et al. (1958) observed hypophagia in some amygdala cats. These results, together with those obtained in our dog D-8 (to be analyzed in detail elsewhere) in which a more complete amygdalectomy resulted in aphagia, seem to suggest that in the fronto-temporal region there are two antagonistic mechanisms regulating alimentary activity.

SUMMARY

1. The aim of this paper was to study the effects of bilateral amygdala lesions on alimentary conditioned reflexes in dogs.

2. It was found that positive conditioned reflexes are not changed after amygdala lesions. On the other hand inhibitory conditioned reflexes were strongly impaired and did not return to normal even after a long postoperative training.

3. In one dog the bilateral amygdala lesion resulted in a complete longlasting aphagia.

4. The present results are discussed in the light of the possible functional role of the amygdala complex.

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REFERENCES

ADEY W. R. and MEYER M. 1952 — Hippocampal and hypothalamic connexions of the temporal lobe in the monkey. Brain, 17, 358.

ANAND B. K. and BROBECK J. R. 1951 — Localization of a "feeding centre" in the hypothalamus of the rat. Proc. Soc. exp. Biol. N. Y. 77, 323.

BARD P. and MOUNTCASTLE V. B. 1948 — Some forebrain mechanisms involved in expression of rage with special reference to suppression of angry behaviour. Res. Publ. Ass. Nerv. Ment Dis. *27*, 362.

BELL F. R. and LAWN A. M. 1956 — The effects of orbito-frontal lobectomy on the behaviour of goats. XXth Intern. Physiol. Congress, Bruxelles, *79*.

BRUTKOWSKI S., KONORSKI J., ŁAWICKA W., STĘPIEŃ I. and STĘPIEŃ L. 1956 — The effect of the removal of frontal poles of the cerebral cortex on motor conditioned reflexes. Acta Biol. Exper. *17*, 167.

BRUTKOWSKI S. 1957 — The effect of prefrontal lobectomies on salivary conditioned reflexes in dogs. Acta Biol. Exper. *17*, 327.

BRUTKOWSKI S. 1959a — Effects of prefrontal ablations on salivations during the alimentary unconditioned reflex and after its cessation, Acta Biol. Exper. *19*, 281.

BRUTKOWSKI S. 1959b — The solution of a difficult inhibitory task (alternation) by normal and prefrontal dogs. Acta Biol. Exper. *19*, 291.

CLARK LE GROS W. E. and MEYER M. 1950 — Anatomical relationships between the cerebral cortex and the hypothalamus. Brit. med. Bull. *6*, 341.

FONBERG E. 1958 — The manifestation of the defensive reactions in neurotic states. Acta Biol. Exper. *18*, 89.

FULLER J. L., ROSVOLD H. E. and PRIBRAM K. H. 1957 — The effect on affective and congenitive behavior in the dog of lesions of the pyriform-amygdala-hippocampal complex. J. comp. physiol. Psychol. *50*, 89.

FULTON J. F. 1953 — The limbic system: a study of the visceral brain in primates and man. Yale J. Biol. Med. *26*, 107.

GREEN J. D., CLEMENTE C. D. and GROOT J. de 1957 — Rhinencephalic lesions and behavior in cats. J. comp. Neurol. *108*, 505.

KONORSKI J. i MILLER S. 1933 — Podstawy fizjologicznej teorii ruchów nabytych. Warszawa.

KONORSKI J. 1958 — Procesy pobudzenia i hamowania w korze mózgowej. Acta Physiol. Polon. *9*, 17.

MACLEAN P. D. 1954 — The limbic system and its hippocampal formation. J. Neurosurg. *11*, 29.

MACLEAN P. D. 1955 — The limbic system ("visceral brain") and emotional behavior. Arch. Neurol. Psychiat. *73*, 130.

MORGANE P. J. and KOSMAN A. J. 1957 — Alterations in feline behaviour following bilateral amygdalectomy. Nature, *180*, 598.

PAPEZ J. W. 1937 — A proposed mechanism of emotion. Arch. Neurol. Psychiat. *38*, 725.

PAPEZ J. W. 1958 — Visceral brain, its component parts and their connections. J. nerv. ment. Dis. *126*, 40.

PRIBRAM K. H., LENNOX M. A. and DUNSMORE R. H. 1950 — Some connections of the orbito-fronto-temporal, limbic and hippocampal areas of *Macaca mulatta*. J. Neurophysiol. *13*, 127.

PRIBRAM K. H. and KRUGER L. 1954 — Functions of the "olfactory brain". Ann. N. Y. Acad. Sci. *58*, 109.

PRIBRAM K. H. and BAGSHAW M. 1953 — Further analysis of the temporal lobe syndrome utilizing frontotemporal ablations. J. Comp. Neurol. *99*, 347.

ROSVOLD H. E., MIRSKY A. F. and PRIBRAM K. H. 1954 — Influence of amygdalectomy on social behavior in monkeys. J. comp. physiol. Psychol. *47*, 173.

SCHREINER L. and KLING A. 1953 — Behavioral changes following rhinencephalic injury in cat. J. Neurophysiol. *16*, 634.

SCHREINER L. and KLING A. 1956 — Rhinencephalon and behavior. Amer. J. Physiol. *184*, 486.

WEISKRANTZ L. 1956 — Behavioral changes associated with ablation of the amygdaloid complex in monkeys. J. comp. physiol. Psychol. *49*, 381.