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EFFECTS OF PREFRONTAL AND CAUDATE LESIONS ON DELAYED RESPONSE IN CATS [1]

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In 1956 Rosvold and Delgado published a paper in which they showed that in monkeys caudate nucleus stimulation or lesion caused the same impairment as that obtained by frontal lobe lesions. The implication of this and further related findings has been that neither the cortex nor subcortical formations have unique and independent functions but rather that they function in unison forming cortico-subcortical systems responsible for definable aspects of behavior (Rosvold and Szwarcbart 1964). Such reasoning, supported by data suggesting the functional heterogeneity of the caudate nucleus to be understood in terms of cortico-caudate connections (Divac et al. 1967), gives rise to many different problems. One such problem is whether in species other than the monkey, one can detect the existence of cortico-caudate systems and if so how to compare their properties with the properties of these systems in the monkey. This is the core problem of the present paper.

Comparing studies in which members of different species with frontal lobe lesions are subjected to delayed response testing, one finds marked quantitative species-dependent differences. The largest and apparently permanent deficit is observed in monkeys, whereas the deficits in species lying in either direction of the phylogenetic chain, e.g. in chimpanzees,

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on one hand (Rosvold et al. 1961), and in cats, on the other (Ławicka and Konorski 1961), are described as transient. In the cat, the compensation might occur through the involvement of a brain system which ordinarily does not play a role in delayed response type behavior. The other way in which the cat without prefrontal cortex can relearn the delayed response is through a mobilization of cortical or subcortical remnants of the „frontal lobe system". In the present paper only the second possibility will be considered.

The possibility that after prefrontal ablation the remaining parts of „the delayed response system" compensate for the loss, seems to be more probable but not easy to understand. In the monkey the sulcus principalis region of the prefrontal cortex is critical for delayed-response-type behavior and therefore is named the focus. The surrounding cortex also contributes to the deficit if removed; it may be called the field (Gross 1963a). Both focus and field receive projections from the parvocellular part of the medialis dorsalis nucleus of the thalamus (Akert 1964). According to Rose and Woolsey (1948), proreus-orbitalis cortex is the projection area of medialis dorsalis nucleus in the cat. They also claimed that if the orbitofrontal region and its connections remained intact, there was no degeneration observed in the medialis dorsalis. On the basis of this result it may be said that the lesions made by Ławicka and Konorski (1961) should have produced a considerable degeneration of the medialis dorsalis. Still, only a transient retention deficit was observed. Therefore if it is cortex which is responsible for the compensation, one must assume that in the cat the rest of the cortex responsible for the delayed response-type behavior merges with the surrounding frontal areas.

The investigations of Rosvold and his collaborators leading to the formulation of the concept of „the delayed rosponse system" (Rosvold and Szwarcbart 1964) suggested that in it several brain formations besides the prefrontal cortex must be included. The caudate nucleus was found to be most closely related, and therefore, besides the prefrontal cortical „field", was considered as another candidate for the compensating structure.

Such considerations, together with the meager existing literature on the delayed response behavior in cats with frontal lobe system lesions directed our attention toward an experimental analysis of the relative roles of the prefrontal cortex and caudate nucleus on the delayed response retention in cats.

Within such a broad task several particular questions were specified. The first and basic question was whether the prorealorbital ablation in the cat interferes with the delayed response performance. In this respect the conclusion of Warren et al. (1962) apparently contradicted that of

Ławicka and Konorski (1961). Next, we wanted to establish whether in the cat, as well as in the monkey (Rosvold and Delgado 1956), the caudate nucleus lesion produced a delayed response deficit. Furthermore, since it has been claimed that in the monkey a complete cortical lesion produces a larger delayed response impairment than a presumably insufficient caudate nucleus lesion (Rosvold et al. 1958, Battig et al. 1960), the cortex-caudate nucleus effects comparison has been attempted here. Further, in the light of the data suggesting the functional heterogeneity of the caudate nucleus (Divac et al. 1967, Divac 1968) it was hypothesized that the lesion of only that part of the caudate nucleus which is connected with the prefrontal cortex will impair the delayed response performance.

MATERIAL AND METHODS

Subjects. Twenty five mongrel, adult, male cats, experimentally naive, weighing 3—5 kg were used in this study. After a quarantine period the cats were caged individually and fed twice a day beginning after daily testing. Between the last, lighter, meal and testing there was an interval of at least twelve hours. The level of hunger was regulated by occasionally reducing the daily ration down to one third or two thirds of the ration. The cats were divided randomly into six groups according to the lesions they were going to receive:

1. Normal control group (Con) n = 6;
2. Prefrontal group (PF) n = 4;
3. Anterior caudate nucleus group (NCA) n = 5;
4. Prefrontal and anterior caudate group (NCA + PF) n = 6;
5. Posterior caudate nucleus group (NCP) n = 4.

The unmanageable cats were rejected during the shaping phase. Not included in this number are three cats from the frontal group. One of them died before the end of retention for unknown reasons. The other two were rejected because of large deformations discovered postmortem of the anterior part of the brain. Both of these cats, and only these two, exhibited rigidity while alive. The histological analysis showed in these two cats a massive fibre degeneration in the white mater of the frontal lobes.

Experimental design. The main experiment consists of the 2×2 design in which effects of prefrontal lesions vs. nonprefrontal, and anterior caudate lesions vs. nonanterior caudate are compared. In addition, the data obtained from the NCP group, treated in the same manner as the groups from the main experiment, are presented but not statistically analysed.

Apparatus and test procedure. The apparatus used is presented in Fig. 1 in exact proportions. Only the left and the right feeders were used, the opening of the third one being covered. The round cage in which the animal was confined during the delay had an opaque hood which could be lowered to cover completely the cage. The hood had an opening two cm in diameter facing the experimenter. The opening was kept closed with a rubber cork except when food distraction was given in the combined distractions training. Immediately above each feeder a small loudspeaker was mounted. Both of them were fed from the common noise-generator.

The whole testing can be divided into three phases: shaping, training and retention (Fig. 2). Except on the first day of shaping, when only six trials were given,

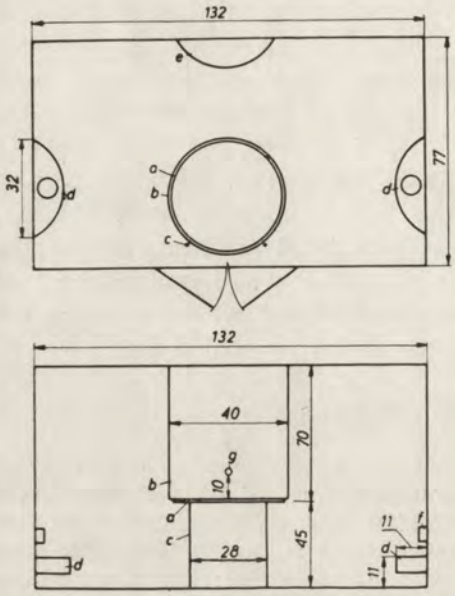

Fig. 1. Diagram of the apparatus used. The upper drawing presents it from above, and the lower one gives the front view. Dimensions are given in centimeters. Key: a, cage; b, hood; c, bars to guide both cage and hood; d, feeders used; e, covered feeder, not in use; f, loudspeaker; g, hole for food distraction

the daily training throughout the testing consisted of ten trials in which left and right positions were arranged according to Gellerman series. There were ten repeating sequences, of ten trials each. Shaping lasted seven days. On the first two days, the animals were trained to approach the feeders, to react to the click of the turning feeder, and to take the reinforcement, about 2 cm^3 of raw or boiled meat, depending on preference. On the third day the sound stimulus was introduced. It started when the animal was in the center of the apparatus and ended when the animal approached the corresponding feeder, at the moment of offering the reward. Such training lasted for three days. During the last two days of the seven day shaping phase the animals were put under the cage. One to four seconds afterwards, the noise stimulus generated either from the right or the left feeder was activated and at the end of the third second of its duration the animal was released, but the noise was kept on until the animal approached a feeder. Most often animals went straight to the signalled feeder. In the very rare cases of an error, a carrection trial was given. All animals performed with high proficiency at the end of shaping.

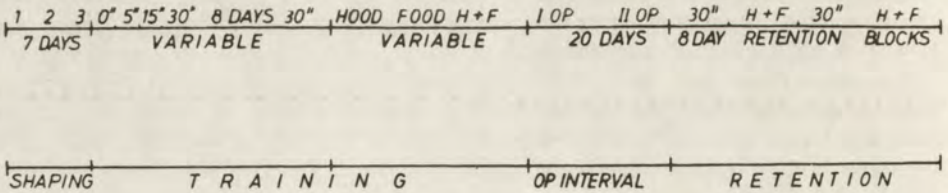

Fig. 2. Illustration of the course of training. The lower line gives the more global division of the sequence of events, whereas the upper line shows the subdivisions of each of them. 1,2 and 3 in the shaping period are the three phases described in the text. H + F represents the 8 day block of the combined distraction testing. For details see Method

The very next day after completing the shaping phase, animals were advanced to the training phase in which the delay, and later distractions, were applied. No corrections were given in this phase. At all delays the sound stimulus (preparatory stimulus, Konorski and Ławicka 1959) lasted for 3 sec after which the delay began. At the end of the delay the animal was released and, if correct, rewarded. The inter-trial interval was not fixed, the tendency being to have the animal return spontaneously to the starting position. On rare occasions, particulary when the longer interval had been introduced, it was necessary either to coax the animal or to push it gently under the cage. There were four steps of delay: zero, five seconds, fifteen seconds and thirty seconds. The animal was allowed to take the longer delay after reaching the criterion of a maximum of three errors in three days (90% correct) on the previous delay. Thus, the shortest time to complete all steps was twelve days. After reaching criterion on the 30 sec delay (30 sec DR), the animals were trained eight days on the same delay independently of the performance. The number of errors made in this period of eight days was used in the statistical analysis.

The next step was to introduce distractions in three stages. The animals had to reach the three-day 90% correct criterion on the two types of distraction: first, the hood, which prevented visual contact with the surrounding was lowered during the middle 20 sec of the unchanged thirty second delay; the second distraction involved giving minced meat from a wide-neck syringe from which the animal would lick for exactly 10 sec in the middle of the 30 sec delay. After successfully completing both tasks, the animals were given eight days of training on the combined hood and food distraction (Distraction DR) independently of proficiency. Only one cat from the NCA + PF group had the combined distractions training immediately after the block of eight days of the 30 sec DR. Number of errors collected during the eight days of Distraction DR testing was another number used in the statistical analysis. In this way the training phase was ended.

After the training phase, all animals went through a twenty days period without testing during which the relevant operations were performed. Animals from the NCA + PF group underwent the first operation, the caudate nucleus lesion, immediately after the training phase, and the prefrontal ablation was made 10 days later. Animals having only one operation were operated in the middle of the 20 days period. In this way all animals, including normal controls, waited for an equal amount of time between the training and retention phases, and all operated animals were operated ten days before the retention phase.

The retention phase consisted of four blocks of eight days each. 30 sec DR (A) and Distraction DR (B) sequence was repeated twice (ABAB). The number of errors in these blocks was used in the statistical analysis as described in the Results section. The following observations during both training and retention phases were made and recorded: (i) amount of circling during the delay (only in 30 sec DR), (ii) signs of cats' „paying attention" to the preparatory stimulus by any visible response during the 3 sec duration of the preparatory stimulus that could be related to its occurence, (iii) the body orientation at the moment of release.

Surgery. All lesions were bilateral, the operations being carried out under aseptic conditions, employing Nembutal (35 mg/kg intramuscularly injected) anesthesia. Animals which underwent only prefrontal or only caudate nucleus lesion were operated in one stage. The combined lesions were done in two stages separated by ten days.

Surgical procedure was described in detail (Divac 1967). Here it will be mentioned only that cortical ablations were performed by suction, and that both types of

caudate nucleus lesions were performed in a Kopf stereotaxic apparatus, using tungsten electrodes 0.4 mm thick, covered with three layers of an epoxyde — phenol laquer („Orex 1305") baked at 200°C, and with the 0.75 mm bare tip made conical by electrolysis. The lesion was made with 3 mA constant direct current, the anode being connected to the tungsten needle, and the cathode to the retractor. All points were coagulated during one minute unless otherwise stated. The coordinates for the anterior lesion were: A, 20.5; L, 3.0; H, +2.5 and +4.0. A, 19.5; L, 3.0; H, + 2.5; and +4.0. A, 18.5; L, 3.0; H, +1.5; +3.0; and +5.0 (30 sec). The coordinates for the posterior lesions were: A, 14.0; L, 5.0; H, +4.0; +5.0; and +7.0 (30 sec). A, 13.0; L, 5.0; H, 5.0; and +6.5. A, 12.0; L, 6.0; H, +6.0; and +7.5. The number of points, the distances between points and the total current passed were approximately identical in the anterior and posterior lesions. Each side was done with a separate electrode.

Histology. After completion of the retention phase, the animal was injected with a large dose of nembutal and perfused through the heart with saline and neutral 10% formaline. The brain was then removed, the anterior half of the brain embedded in paraffin, sectioned at 10 μ and every 20th section stained with Klüver and Nissl methods alternately. Every fortieth section was stained in the region of cortical lesion. In the region of the caudate nucleus lesion every twentieth section was stained. Reconstructions of the lesions were made for every brain (Divac 1967).

Histological findings. The illustration of the histological material is given here for each group separately (Fig. 3).

The lesions of the PF group showed that in all cats a part of the medial aspect of the prefrontal cortex, as well as the posterior part of the gyrus orbitalis remained undamaged. It turned out that in two cats of this group the anterior parts of their brains were deformed presumably due to a postoperative oedema, they had, however, no gliosis in the white matter of the frontal lobes. The latter two cats were not worse than the former ones in retention.

There are four cats in the NCA group in which there was an invasion of the white matter just rostral from the anterior end of the caudate nucleus. One of these lesions was placed more ventrally than the rest. There was one cat in which the lesions avoided not only the white matter but also the anterior tip of the caudate nucleus head. This finding is contrasted with the behavioral results further in the text.

The cortical lesions of the NCA + PF group have the same characteristics as those described for the PF group, except that in three cats medial lesions on one side are smaller than usually, and the lateral left side lesion in one cat is considerably smaller than the average. There was one cat which had a deformation of the anterior part of the brain. There were no additional cortical lesions or degenerations of fibre tracts in this animal.

The caudate lesions in the NCA + PF group in three out of six cases were approximately the same in size and localization as in the majority of animals from the NCA group. The lesions did not encroach upon the white matter in the other three animals. Lesions on the left side in two cats spared the rostral portion of the caudate nucleus. It is not possible to correlate these findings with the degree of impairment in the respective animals.

The lesions in the NCP group do not differ in size from most of the lesions in the NCA or NCA + PF groups. The only difference is that they are placed in the posterolateral and dorsal part of the caudate nucleus head.

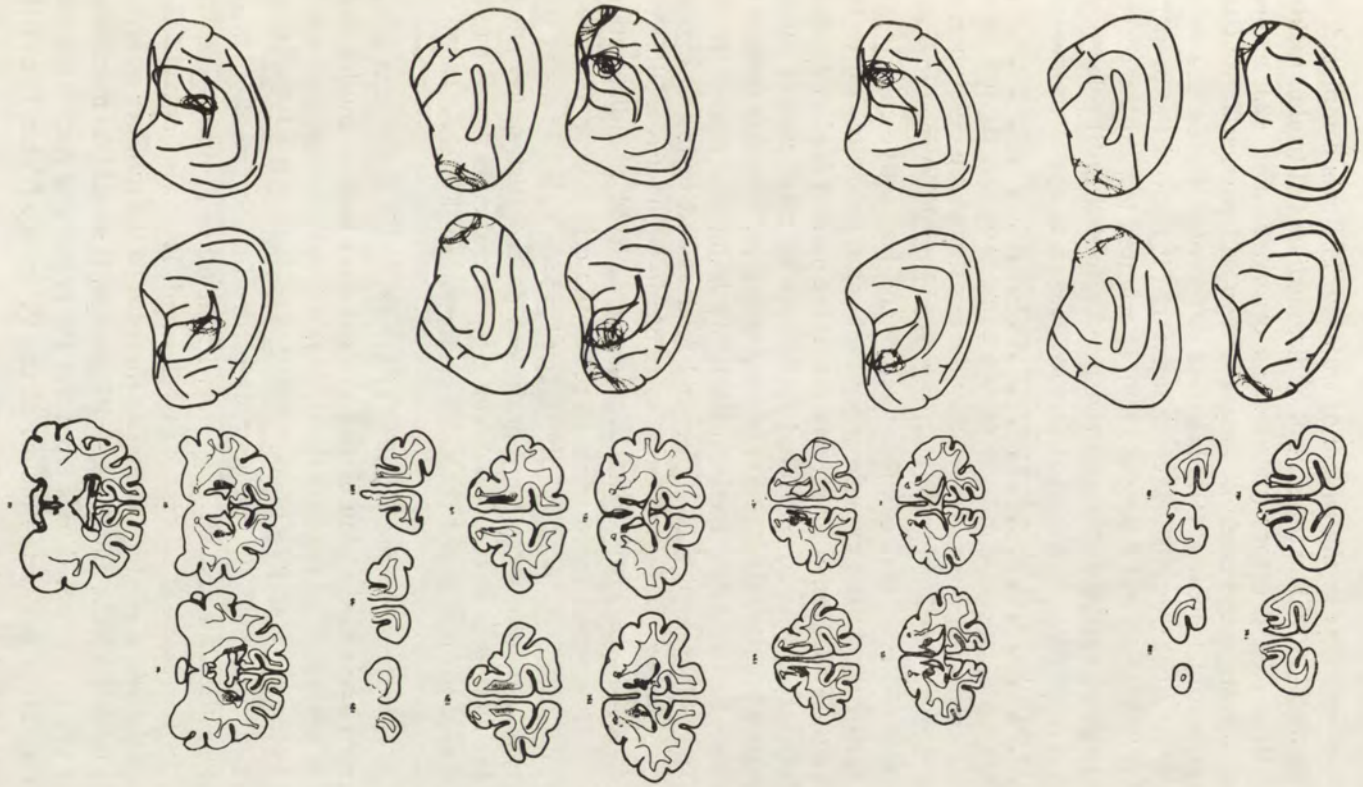

Fig. 3. Reconstructions of lesions for all four operated groups. On the left side the superimposed projections of all animals from a group are shown. On the right side only a typical lesion is presented. Lesions for each individual cat are shown in Divac (1967)

RESULTS

General observations in the course of training. The cats completed the training after 340—580 trials (mean 408 trials, median 400 trials); making during that time 15—78 errors (mean 42 · 4 errors, median 42.0 errors). Most of these errors were made at longer delays and particularly at the distraction phases of learning. Cats behaved differently during the delay time. Most often a cat would show clear signs that it noticed the preparatory stimulus (Konorski and Ławicka 1959). It oriented toward the correct feeder and at short delays many cats remained oriented until release. At longer delays, however, individual differences began to appear, some cats continuing to be quiet and keep the correct orientation, others slowly moving as a compass needle, and a considerable number of cats circled inside the round cage either in bursts or steadily during the whole delay period. It should be mentioned that in the learning period no difference in speed of learning was observed between more active and lass active cats. It was sometimes surprising to the experimenter that some cats after up to ten circles during the 30 sec delay invariably found the correct feeder. Another frequent observation was an increased reluctance of most cats to return under the cage with the introduction of longer delays. The cats tended to walk from one to the other feeder clearly avoiding the center of the apparatus. They often kept sniffing around feeders and trying to extract meat by manipulating the feeder in all sorts of ingenious ways. Different cats were disturbed to a different degree by each of the distractions applied. Some took much more trials to reach the criterion in the „hood distraction", others — in the „food distraction". It may also be noted that the vast majority of cats were very proficient on the Distraction DR in spite of its precluding both the visual and the postural cues thought to be the essential means for delayed response performance (Fletcher 1965).

Effect of operations on the number of errors. Table I presents group means of errors made during the blocks of eight days both in the learning and retention periods. The mathematical chance level performance would correspond to the score 40 in a block. The examination of the table shows that the groups in which the number of errors in the retention phase decreased were the Con and NCP groups. In all other groups there was an increase in the number of errors in retention. It is also notable that the PF group showed a relatively smaller increase in the number of errors as compared with the NCA and NCA + PF groups. It should be mentioned here that of the two rejected cats from the PF group one had the postoperative error scores below the means of the PF group (Table I) and the other had the scores above those means.

Table I

Mean errors in blocks of 80 trials

	Learning		Retention			
	30″	Distraction	30″ I	Distraction I	30″ II	Distraction II
	a	b	c	d	e	f
Con	8.5	14.3	6.7	10.7	3.7	5.2
PF	4.0	10.8	18.0	18.0	17.8	14.3
NCA	1.8	14.6	26.4	31.4	22.6	27.8
NCA + PF	3.5	9.2	28.8	31.1	22.1	29.5
NCP	4.5	10.0	8.0	6.2	2,5	4.0

Table II shows the statistical analysis [3] of the data from Table I excluding the NCP group. The left part of the table shows that both frontal and caudate nucleus lesions significantly increase the number of errors in the delayed response task. In addition it is clear that although both „F-s" are significant below the 0.01 level, the „F" for the caudate nucleus effect is almost twice as large as that for the frontal group. In an attempt to find out whether these two values of „F" are significantly different, the relation $t_v^2 = F_{(1, v)}$ was used. In our case $t' = 5.28$ and $t'' = 7.05$. In the tables for t it was found that alpha equals in the first case 0.00002 whereas in the second case is smaller than 0.000001. Since these values are of different orders of magnitude, it can be said that the effects of caudate nucleus lesion are larger than the effects of the prefrontal lesion [4]. Statistically significant interaction shows that the prefrontal lesion, if added to an existing caudate nucleus lesion does not further increase the impairment.

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[3] Analysis of variance, 2×2 table for disproportionate sub-class numbers, and Keuls method for comparison of all means (Snedecor 1957) were used as statistical tools. The original scores were transformed in the following way: The score in the analysis is the natural logarithm of the percentage obtained as follows: $\frac{c+d+e+f}{2(a+b)} \cdot 100\%$. (The letters correspond to the columns in the Table I). The combination of the 30 sec DR and Distraction DR is justified by a significant positive correlation between them.

[4] This procedure was suggested by Dr. K. Metelski.

The group differences, presented on the right side of Table II, only show that the Con group differs significantly from all operated groups (excluding the NCP group). No significant differences between the operated groups were found. It should be pointed out that this does not contradict the results of the 2 × 2 analysis of variance which is more powerful.

Table II

Analysis of variance and group comparisons

2 × 2 table				Group differences				
Source of variation	df	Mean square	$F_{(1.17)}$	Groups	X	$X-X_C$	$X-X_F$	$X-X_{NC}$
Frontal	1	5.798	27.929*	NCA+ PF	6.109	2.201*	0.596	0.339
Anterior caudate	1	9.608	49.778*	NCA	5.770	1.862*	0.257	
Interaction	1	0.927	4.465** ($p_{0.05}=4.45$)	PF	5.513	1.605*		
Individuals	18	0.2076		Con	3.908			

* Significant at 0.01. ** Significant at 0.05 level.

Another way of presenting the results from experiment is shown in Fig. 4 and 5. Fig. 4 shows the group means of daily performance in the 30 sec DR for the learning period and for the two retention blocks. Fig. 5 similarly compares the daily group means for the Distraction DR. Here again it can be seen that the Con group improves in the retention tests that the NCP group practically does not differ from the Con group, that the curve of the group PF is far below the curves of the NCA and NCA + PF groups and that the last two curves hardly differ from each other. One thing should be noted: on Retention II of the 30 sec DR (Fig. 4) there is an observable decrease of errors in both NCA and NCA + PF groups which thus overlap with the PF group. There is no such tendency, however, in the Distraction DR curves (Fig. 5).

Finally, the Wilcoxon test (Siegel 1956) showed a significant improvement in the course of postoperative testing of either the operated animals taken as a whole or of NCA and NCA + PF groups taken in isolation from PF group. What was compared were the c + d vs. e + f retention blocks. (Letters designate the columns from the Table I.) In other words, in the last two retention blocks the operated animals were better than in the first two retention blocks.

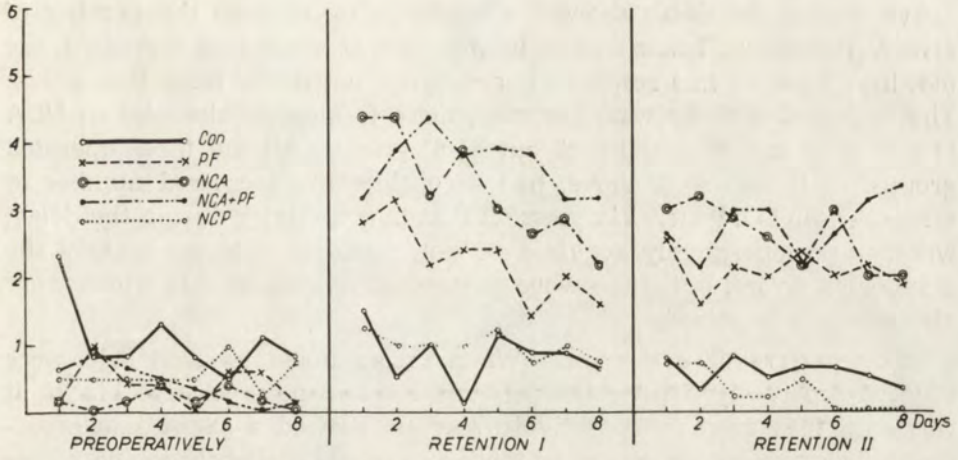

Fig. 4. Means of errors on the 30 sec DR. Ordinate: mean errors on the corresponding day

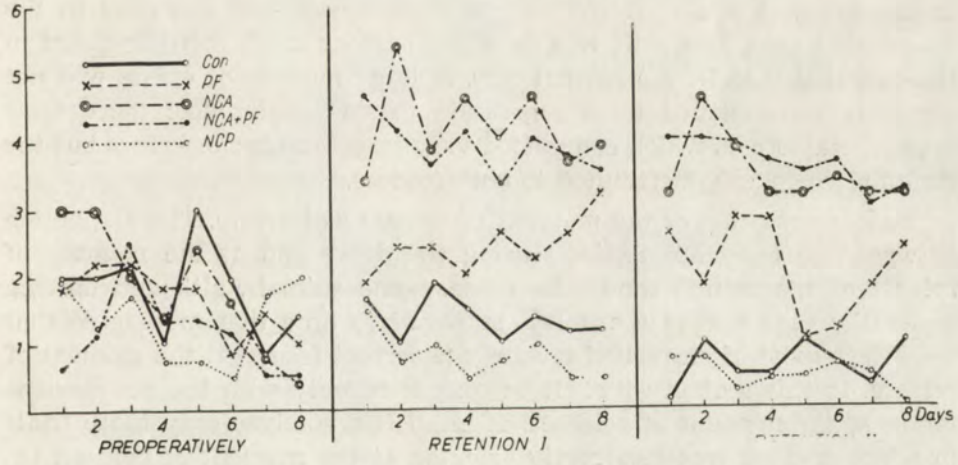

Fig. 5. Means of errors on the Distraction DR. Ordinate: mean errors on the corresponding day

Error analysis in the learning and retention periods

Hyperactivity and errors. At the beginning of the Results section it was mentioned that normal cats in the learning period could be divided into those which were quiet and those which were active during the delay. The measurement allowed only an approximate estimation of activity: „greater than" or „equal to". As estimated in that way, in only one out of six control cats was there an increase of locomotor activity in the retention period. Neither this cat nor the cats which continued to be

active during the delay showed a tendency to increase the number of errors. Prefrontal lesions only in one out of four cats increased the motility; thus, in this respect PF group did not differ from Con group. The increased activity was, however, more frequently observed in NCA (3 out of 5) and NCA + PF (3 out of 6) groups. All the three operated groups, as it was seen above, had a considerably increased number of errors. There is a general impression that hyperactivity during the delay, whether postoperatively acquired or not, tends to increase slightly the number of errors, but there were postoperatively quiet cats which were also strongly impaired.

Side preference and errors. When errors made to each side were counted separately both in the preoperative and postoperative blocks, it was seen that even preoperatively animals showed a slight side preference. This preference generally tended to increase slightly in the retention period in all the operated groups except PF. One cat from the Con group showed in the retention phase a clear side preference not apparent in the learning phase. A shift of side preference was observed in the unoperated cats. This shift was for some reasons much more frequent in the operated than in the normal cats. A large number of errors was not regularly accompanied by a regression to side preference: there were some operated cats which committed very large number of errors but the errors were equally distributed to both feeders.

Body orientation at the moment of release and errors. The distinction between the body orientation during the delay and at the moment of release of the animal should be made. Some animals, although moving under the cage during the delay, managed to time that moving so that they were most often turned toward the correct feeder at the moment of release. In this section we shall be concerned only with the body orientation at the moment of release and shall first analyze only those trials in which the cat was incorrectly oriented at the moment of release, i.e. oriented to any direction but not toward the correct feeder. Let us mention at once that the absolute number of incorrect body orientation at the moment of release increased in the retention periods for the operated groups, except the NCP group, mainly for the NCA and NCA + PF groups and mainly as a result of an increased mobility. The counting of the correct and incorrect outcomes of such trials showed that in all operated groups except NCP, the percentage of correct responses to the incorrect body orientation decreased postoperatively. The same, however, happened with the Con group.

Most often an error was made when the cat was incorrectly oriented at the moment of release, but it happened that even with a correct body orientation a trial ended with an error. The percentage of errors (total

errors taken as 100%) made with the correct body position was calculated. There was a general but slight tendency in the operated groups to increase the percentage of errors made after a correct body orientation. The reverse was seen in the Con group.

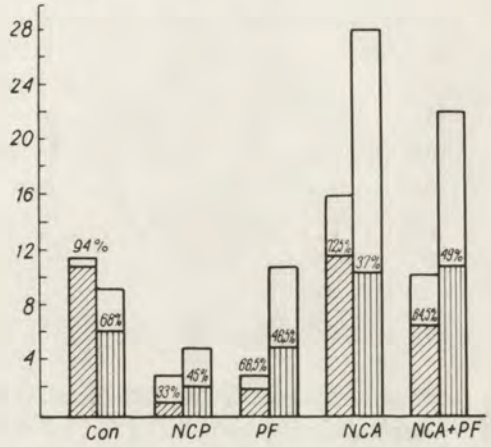

Fig. 6. Analysis of trials in which a notable orienting response to the sound stimulus was not observed. The height of a column represents the group mean of unattended trials. Each first column represents the learning phase, and the second the retention phase. Shaded area shows a percentage of those nonattended trials which ended with a correct response. The unshaded, complementary area shows the nonattended responses which ended with an error. An increase in the total number of nonattended responses in the operated groups is also shown

„Nonattention" and errors. Fig. 6 presents both the absolute mean of „nonattended" trials as well as the percentage of the correct responses made to such trials. It is striking that in the groups showing greater impairment (NCA and NCA + PF) there was a large increase in the number of trials in which no visible sign of the cat's paying attention to the preparatory stimulus was observed. In some cases this increase was due to the incessant locomotion which obliterated any possible existing signs of attending to the preperatory stimulus. If the NCP group is excluded from the comparison due to a very small absolute number of nonattended trials, it becomes clear that the Con group differs from the other operated groups by its decrease of the total number of „nonattended" trials. It is surprising that the percentage of correct responses to the nonattended trials decreased not only in the operated groups but also in the Con group. It may be stressed, however, that the outcome of the nonattended responses in the retention period of the Con group was more often correct than incorrect whereas in all operated groups it was about at the chance level.

DISCUSSION

This study demonstrates that in the cat either prefrontal cortical lesion or lesion in the *corresponding part* of the caudate nucleus produces an impairment in delayed response retention.

The effects of prefrontal lesions in cats on the delayed response behavior were reported several times: Warren et al. (1962) could not detect a deficit in the postoperative *learning* of the delayed response, Thompson (1965) also failed to find differences in delayed response learning after orbitofrontal lobectomy. On the other hand, both Ławicka and Konorski (1961) and Warren (1964) found a definite impairment of the *retention* of the delayed response. The present results support the latter effect, but the possibility remains that the prefrontal ablation in cats does not interfere with postoperative learning.

Surprisingly, in the decade after the discovery of the role of the caudate nucleus in the delayed response behavior of monkeys (Rosvold and Delgado 1956), only one paper (Ungher et al. 1966) described the effects of the caudate nucleus lesions on the delayed response in cats. The evidence presented in the latter paper is not entirely convincing but the results as presented showed that the lesioned animals performed at a chance level. Thus, the present results are in general agreement with the results of Ungher et al. (1966). The question of the localization of the lesion cannot be discussed because no reconstructions or serial setcion were shown.

When the results of the present NCA group were compared with the reconstructions of lesions (Divac 1967) it was seen that the only cat with a small deficit was that one in which the caudate lesion excluded both the tip of the caudate nucleus and the surrounding white matter. Thus, it was not possible to say whether the large impairment of the delayed response performance in the four remaining cats was due to the complete destruction of the rostrum of caudate nucleus or to the involvement of nearby white matter.

The comparison of the NCA and NCP groups shows that the placement of lesions in the different caudate nucleus areas results in different effects. The present results are in agreement with the more complete study done in monkeys (Divac et al. 1967). In the present study, as well as in the mentioned study in monkeys, the mapping of the caudate nucleus was done on the basis of the cortico-caudatal projections (Whitlock and Nauta 1956, Nauta 1964, Webster 1965). Thus, the notion of the functional heterogeneity of the caudate nucleus probably based on regional cortico-caudate connection received further support.

In a series of papers Rosvold, Mishkin and their callaborators (Rosvold

et al. 1958, Battig et al. 1960) claimed that cortical lesions in monkeys produced a larger delayed response or delayed alternation deficit than caudate nucleus lesions, but stressed that an increase of the caudate nucleus lesion size tended to increase the deficit. On the other hand, the present results in cats show that the effect of caudate nucleus lesions is stronger than the effect of prefrontal lesion. The question arises as to whether such results in cats were obtained because of the relatively larger caudate nucleus lesions and the relatively smaller prefrontal lesions than in the experiments on monkeys. This question is almost impossible to answer because of the difficulties in both morphological and functional comparisons of the frontal lobe systems in the monkey and cat. In order to discuss the problem we shall make three assumptions. The first is that there is an area of the frontal cortex and a *corresponding part,* or parts, of the caudate nucleus which together belong to a neural system responsible for delayed-response-type behavior (Divac et al. 1967, Divac 1968). The second assumption is that the critical frontal area for delayed response behavior is the area which receives the projection from the parvocellular part of the medialis dorsalis nucleus of the thalamus. Our third assumption is that the differences in the experimental setups between the experiments on monkeys and cats do not obscure the measuring of the same „delayed response type performance".

The discussion of the problem (Divac 1967) will only be summarized here. It should be pointed out that the ablation of the parvocellular medialis dorsalis projection area in the cortex of the monkey produces a large and long lasting deficit in delayed response-type behavior (Mishkin 1957, Gross 1963a). In the cat, however, the ablation of a corresponding cortical area produces a similar deficit but neither large one, as the present results showed, nor a long lasting one, as results of Ławicka and Konorski (1961) demonstrated. Thus, it was concluded that the prefrontal cortex plays a more important role in the monkey than in the cat (under our assumptions and for the delayed response retention). On the other hand, lesions of approximately that caudate region which is anatomically related to the prefrontal cortex [5] produce an impairment of a similar intensity in the monkey and cat. (The lesion of the critical caudate area in the monkey, not necessarily large, was found to be impairing delayed response-type behavior to a degree similar to that observed after the prefrontal cortical lesions Divac et al. 1967.)

In summary, the discrepancy between the data obtained in the monkey, showing that the cortical lesions produce a larger delayed response-type

[5] The exact prefrontal projection area in the caudate nucleus is not known either for the monkey or for the cat (Divac 1967).

deficit than the caudate lesions, and the data from this study which point to a relatively less important role of the prefrontal cortex in the cat may be explained in the following way: the caudate nucleus lesions in the older experiments on monkeys (Rosvold et al. 1958, Battig et al. 1960) had a nonprecise localization and thus the deficit was incomplete; the cause of the relatively larger effects of the caudate nucleus lesions over the prefrontal lesions in the cat should be attributed to a relatively less important role of the cortex in the delayed response behavior in that species.

One further point should be stressed. One might say that the present cortical lesions did not include the entire prefrontal cortex, particularly on the medial side and in the orbital gyrus, and that this may explain a smaller impairment than that obtained after the caudate nucleus lesion which destroyed more of the relevant tissue. The answer is that the comparison of Webster's (1965) summary figure with our histological findings showed that the present caudate nucleus lesions did not include the basal part of the anterior portion of the caudate nucleus head — that area which would presumably receive fibers from the gyrus orbitalis. Thus neither the cortical nor the caudate lesion included all the tissue which could be considered relevant for the delayed response behavior. Still, the effects of the caudate nucleus lesions were larger.

Although the present study was not designed to test the capacity of the impaired animals to relearn, there are ways to detect at least an idication of such a tendency, e.g., there is an improvement from the first to the second pairs of retention tests as detected by the Wilcoxon's test. This can be seen in the Fig. 4 where there is a strong tendency of NCA and NCA + PF groups to reduce the number of errors in the second 30 sec DR block.

The animals from the NCA group showed more often a postoperative hypermobility than the animals from the PF group. Thus, again we see a relatively larger influence of the caudate nucleus lesion than the cortical lesion on behavior of the cat.

It is not possible to relate the delayed response deficit in the cat to the positional habit. In that respect our results are in agreement with the analysis of errors made by Gross and Weiskrantz (1964) who came to the conclusion that the position habit might be a consequence rather than a cause of the prefrontal animal's inability to solve the delayed response task. The same view is shared by Konorski and Ławicka (1964).

The present observations suggest a decisive role of the body orientation at the moment of release but not during the delay for delayed response solving either in normal or in the operated cats. Even very active normal sats, which almost incessantly circle and thus are not oriented toward

the correct feeder during the delay, can solve the 30 sec DR with a very high degree of proficiency. Moreover, normal cats were able to perform successfully in a situation in which they *had to* disturb their body orientation, as in the Distraction DR. In that respect our observations agree with those of Adams (1929) and Ławicka (1959), but not with the earlier observations of Yarborough (1917).

On the other hand, the present results suggest that practically every incorrect body orientation at the moment of release ends with an incorrect response. The instances in which a correction occurs may be those cases in which an animal goes by chance to the correct feeder from the body orientation which was „neutral" in respect to the feeders (either towards the experimenter or towards the unused feeder *e* shown on Fig. 3). It is not an error-producing ablation which causes the postoperative fall of the percentage of correct responses to the incorrect body position since the same phenomenon is seen in retention of the Con group.

Our results showed that errors do occur even after a correct body orientation and that such errors became even more frequent postoperatively, contradicting the observation made in dogs by Ławicka and Konorski (1959). These authors suggested that the operated animals adopted the body orientation as an aid to solve the delayed response task.

SUMMARY

Twenty five cats were divided into six groups and tested for retention in two versions of the delayed response task: 30 sec delay and Distraction. The groups were: normal control, prefrontal, anterior caudatus, anterior caudatus *and* prefrontal, and posterior caudatus.

Results showed that:

1. Prefrontal or anterior caudate lesions or their combination impaired retention of the delayed response task.

2. The effect of an anterior caudate lesion appeared larger than that of a prefrontal lesion.

3. The combined anterior caudatal *and* prefrontal lesion did not produce a significantly larger impairment than that produced by the anterior caudate lesion alone.

4. A posterior caudate lesion of the same size as the anterior caudate lesion did not produce an impairment of the delayed response retention.

5. In all postoperatively impaired groups the number of trials in which the animals showed no signs of attending to the preparatory stimulus increased considerably, and only in these groups the outcome of such trials was guided by chance.

6. The distinction has been made between the body orientation during the delay, apparently unimportant for the correctness of response, and the body orientation at the moment of release which seems to be of a great influence for the correct or incorrect outcome of the trial.

7. Postoperatively impaired performance of the delayed response task was not paralleled by a pronounced side preference except in some cats.

It was concluded that in the cat the prefrontal cortex and the corresponding part of the caudate nucleus subserve the same function and that the cortex seems to be relatively less important than the caudate nucleus. The notion of the functional heterogeneity of the caudate nucleus was supported by the results.

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REFERENCES

ADAMS, D. K. 1929. Experimental studies of adaptive behavior in cats. Comp. Psychol. Monogr. 6: (27) 1—168.

AKERT, K. 1964. Comparative anatomy of frontal cortex and thalamo-frontal connections. In: Warren, J. M. and Akert, K. (ed.), The frontal granular cortex and behavior. McGraw-Hill Book Co., New York, p. 372—394.

BATTIG, K., ROSVOLD, H. E. and MISHKIN, M. 1960. Comparison of the effects of frontal and caudate lesions on delayed response and alternation in monkeys. J. Comp. Physiol. Psychol. 53: 400—404.

DIVAC, I., ROSVOLD, H. E. and SZWARCBART, M. K. 1967. Behavioral effects of selective ablation of the caudate nucleus. J. Comp. Physiol. Psychol. 63: 184—190.

DIVAC, I. 1967. The role of the prefrontal cortex and the caudate nucleus in delayed response. Unpublished doctoral dissertation. Nencki Institute of Experimental Biology. Warsaw.

DIVAC, I. 1968. Functions of the caudate nucleus. Acta Biol. Exp. (Warsaw) 28 (in press).

FLETCHER, H. J. 1965. The delayed-response problem. In: Schier, A. M., Harlow, H. F. and Stollnitz, F. (ed.), Behavior of nonhuman primates. Academic Press, New York, p. 129—165.

GROSS, Ch. G. 1963. A comparison of the effects of partial and total lateral frontal lesions on the test performance by monkeys. J. Comp. Physiol. Psychol. 56: 41—47.

GROSS, Ch. G. and WEISKRANTZ, L. 1964. Some changes in behavior produced by lateral frontal lesions in the macaque. In: Warren, J. M. and Akert, K. (ed.), The frontal granular cortex and behavior. McGraw-Hill Book Co., New York, p. 74—101.

KONORSKI, J. and ŁAWICKA, W. 1959. Physiological mechanism of delayed reactions I. The analysis and classification of delayed reactions. Acta Biol. Exp. (Warsaw) 19: 175—197.

KONORSKI, J. and ŁAWICKA, W. 1964. Analysis of errors by prefrontal animals on the delayed-response test. In: Warren, J. M. and Akert, K. (ed.), The frontal granular cortex and behavior. McGraw-Hill Book Co., New York, p. 271—286.

ŁAWICKA, W. 1959. Physiological mechanism of delayed reactions. II. Delayed reaction in dogs and cats to directional stimuli. Acta Biol. Exp. (Warsaw) 19: 199—219.

ŁAWICKA, W. and KONORSKI, J. 1959. Physiological mechanism of delayed reactions. III. The effects of prefrontal ablations on delayed reactions in dogs. Acta Biol. Exp. (Warsaw) 19: 221—231.

ŁAWICKA, W. and KONORSKI, J. 1961. The effects of prefrontal lobectomies on the delayed responses in cats. Acta Biol. Exp. (Warsaw) 21: 141—156.

MISHKIN, M. 1957. Effects of small frontal lesions on delayed alternation in monkeys. J. Neurophysiol. 20: 615—622.

NAUTA, W. J. H. 1964. Some efferent connections of the prefrontal cortex in the monkey. In: Warren, J. M. and Akert, K. (ed.), The frontal granular cortex and behavior. McGraw-Hill Book Co., New York, p. 397—407.

ROSE, J. E. and WOOLSEY, C. N. 1948. The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep and cat. Res. Publ. Ass. Nerv. Ment. Dis. 27: 210—232.

ROSVOLD, H. E. and DELGADO, J. M. R. 1956. The effect on delayed alternation performance of stimulating or destroying electrically structures whitin the frontal lobes of the monkey's brain. J. Comp. Physiol. Psychol. 49: 365—372.

ROSVOLD, H. E., MISHKIN, M. and SZWARCBART, M. K. 1958. Effects of subcortical lesions in monkeys on visual-discrimination and single-alternation performance. J. Comp. Physiol. Psychol. 51: 437—444.

ROSVOLD, H. E. and SZWARCBART, M. K. 1964. Neural structures involved in delayed response performance. In: Warren, J. M. and Akert, K. (ed.), The frontal granular cortex and behavior. McGraw-Hill Book Co., New York, p. 1—15.

ROSVOLD, H. E., SZWARCBART, M. K., MIRSKY, A. F. and MISHKIN, M. 1961. The effect of frontal lobe damage on delayed-response performance in chimpanzees. J. Comp. Physiol. Psychol. 54: 368—374.

SNEDECOR, G. W. 1957. Statistical methods. Iowa State Univ. Press. Ames.

SIEGEL, S. 1956. Nonparametric statistics. McGraw-Hill Book Co., New York.

THOMPSON, V. E. 1959. Neonatal orbitofrontal lobectomies and delayed response behavior in cats. Unpublished doctoral dissertation, Ohio State Univ.

UNGHER, J., APPEL, E. and ŞIRIANU, S. 1966. Delayed responses in cat with bilateral caudate nucleus lesions. Rev. Roum. Neurol. 3: 349—359.

WARREN, J. M. 1964. The behavior of carnivores and primates with lesions in the prefrontal cortex. In: Warren, J. M. and Akert, K. (ed.), The frontal granular cortex and behavior. McGraw-Hill Book Co., New York, p. 168—188.

WARREN, J. M., WARREN, H. B. and AKERT, K. 1962. Orbitofrontal cortical lesions and learning in cats. J. Comp. Neurol. 118: 17—41.

WEBSTER, K. E. 1965. The cortico-striatal projection in the cat. J. Anat. (London) 99: 329—337.

WHITLOCK, D. G. and NAUTA, W. J. H. 1956. Subcortical projections from the temporal neocortex in macaca mulatta. J. Comp. Neurol. 106: 183—212.

YARBOROUGH, J. U. 1917. Delayed reaction with sound and light in cats. J. Anim. Behav. 7: 87—110.

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ELECTRICAL HIPPOCAMPAL ACTIVITY AND HEART RATE IN CLASSICAL AND INSTRUMENTAL CONDITIONING [1]

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In spite of the fact that both the anatomical and electrophysiological research works on hippocampus have been much advanced in recent years, the functional role of this structure is still poorly understood. The behavioral data obtained after hippocampal lesions are most contradictory and uncertain and cannot be so far organized along a definite hypothesis, which would throw some light upon its function.

One of the important approaches to the physiology of the hippocampus has been recently developed by Grastyan et al. (1966). These authors, by employing in cats the instrumental response of pressing a pedal which switched off electrical stimulation of the hypothalamus, could ascertain that those stimulations which produced a slow theta rhythm in the hippocampus (4—5 c/sec) resulted in the animals avoiding touching the pedal, whereas those producing the fast theta rhythm (more than 6 c/sec) led to its immediately being pressed. Similarly, a CS producing an instrumental response, either alimentary or defensive (avoidance), gave rise to the increase of the rate of hippocampal activity, whereas the termination of the CS produced the slow theta rhythm. To sum up it could be con-

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cluded from these experiments that all drive producing factors generate a fast rhythm and/or desynchronization in the hippocampus, whereas the factors producing a state of drive reduction generate a slow theta rhythm.

The problem of the mechanism of drive CRs and their relation to instrumental conditioning has been in recent years the subject of thorough investigations in this laboratory. First it was hypothesized by Sołtysik (1960) that the hunger drive, being a *spiritus movens* of alimentary instrumental responding, is inhibited by the consummatory food reflex; in other words the drive reduction theory of instrumental conditioning has been replaced by him by the drive inhibition theory. Konorski (1967) further developed this theory by assuming that the states of satisfaction of various drives, denoted by him as antidrives, depend on special centers of the emotive brain reciprocally related to the drive centers.

In experiments by Ellison and Konorski (1965) a chain alimentary CR was established in which a CS eliciting an instrumental response and no salivation was followed by another CS eliciting salivation and no instrumental response. Konorski accepted that the first CS produces a hunger drive, while the second one, being the immediate signal of food, gives rise to the hunger antidrive CR.

It was thought that the Ellison and Konorski experimental procedure, clearly separating particular phases of feeding behavior, might be a good method of testing Grastyan's et al. findings by utilizing hippocampal activity as a reliable indicator of the states of drive and antidrive. The aim of this paper was to follow this line of research.

MATERIAL AND METHODS

Experimental procedure. The present experiments were performed on five mongrel dogs in a standard CR soundproof chamber. These dogs were originally trained by Miyata and Sołtysik (1968) according to a technique recently proposed by Ellison and Konorski (1965). Briefly, the dogs were first trained in classical alimentary conditioning, the sound of a buzzer being followed by presentation of food (minced bread and meat) in a bowl put into position by an automatic device. In the second phase of training the dogs were taught to press a pedal situated in front and at the right side of the stand with the right foreleg, each press signalling the classical CS (buzzer) followed by food. Finally, the whole sequence was made conditional upon another stimulus (metronome), the animal being required in its presence to make a definite number (14) of presses; thereupon the metronome was turned off and the buzzer turned on, after 10 sec of its operation food being presented.

After this chain CR was established, the sequence of events during each trial was as follows. As soon as the metronome was presented the animal started to hit vehemently at the pedal, this being accompanied by an increase of heart rate and moderate salivation. After the required number of movements was performed, the

metronome was discontinued and the buzzer presented. The behavior of the animal changed immediately: the instrumental responses stopped almost abruptly, the animal became immobile with his gaze fixed at the feeder, heart rate was slightly reduced (remaining, however, above the pretrial level) and salivation became copious (Fig. 1). At the end of the CS-US interval food was presented, the act of eating lasting about 30 sec. Altogether, about 200 to 300 trials were given for each dog.

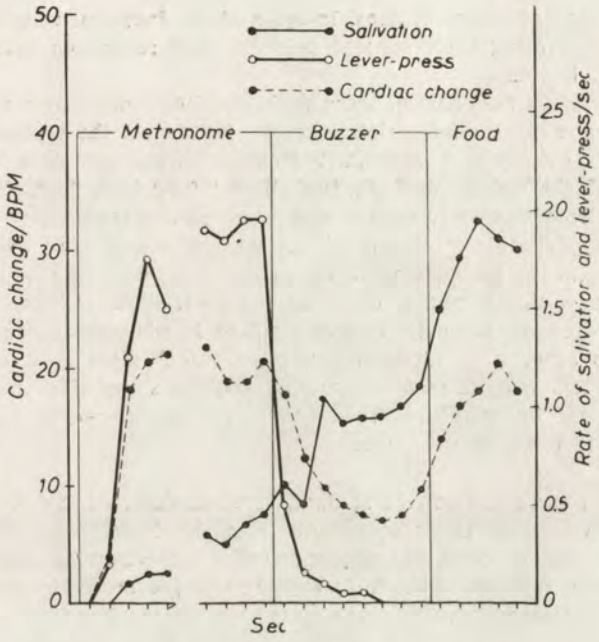


Fig. 1. The average course of trials in Hideki in Miyata's experiments. All explanations in the text (Miyata and Sołtysik 1968)

The present series of experiments ran in exactly the same way, except that salivation was, for the sake of simplicity, not recorded, this having been explored in Miyata's and Sołtysik's experiments. Five trials were given daily separated by 3—4 min intervals.

In all the dogs the electrodes were implanted under Nembutal anaesthesia in aseptic conditions. Stainless bipolar electrodes, 0.5 mm wide were implanted stereotaxically into the dorsal hippocampus, and monopolar silver wire electrodes were placed epidurally on the parietal cortex. The electrodes were connected to a socket without soldering and the whole was fixed to the skull with acrylic cement.

Two weeks after surgery the routine CR experiments were resumed and the hippocampal and cortical electrical activity was systematically recorded with an Offner electroencephalograph. The heart rate was recorded on the same apparatus. In each dog about 50 sessions were given.

After this series of experiments was terminated, in one dog a classical defensive CR was established in which a light of 5 sec duration was associated with an electric

shock administered to the left hind leg. The training was carried out in the same chamber, but the animal was placed on the stand in the reverse direction to that with the food training.

When a sufficient amount of data was collected the dogs were sacrificed, their brains perfused with saline and formalin and fixed in formalin. The histological verification showed that all the electrodes from which records were taken were localized in the dorsal hippocampus.

Individual characteristics of dogs. In spite of the fact that each dog was subjected to the same training their general behavior and responses during the sessions were markedly different.

Two dogs (Hideki and Kunio) were both excellent experimental animals. They were quiet but alert in the intertrial intervals, performed the trained movements to the metronome regularly and with short latency, became immobile but tense during the operation of the buzzer and ate the offered food voraciously. Their behavior was stereotype from session to session and from trial to trial.

A third dog (Takeshi) was atypical. He was very quiet but exceedingly timid. His performance to the metronome was poor, since he pressed the pedal irregularly and touched it very lightly and as if cautiously. Even slight changes in experimental procedure destroyed his CR activity and resulted in his refusing to take food.

Finally, two other dogs (Susumu and Kyu-chian) were less reliable than the first two dogs. Their instrumental responding was not so regular, they were occasionally excited in the intertrial intervals, which was manifested by barking, climbing the food-tray and pressing the lever.

Handling of the data. Two sets of data were carefully analysed, namely the rate of the theta rhythms in the hippocampus and the heart rate. In some sessions respiration was also recorded, but no quantitative analysis was carried out.

The analysis of the rate of the theta rhythm was performed in the following way. From all the hippocampal records those fragments were taken into account in which ten successive theta waves without any desynchronization disturbances were clearly and unmistakably seen. The length of each such fragment was measured and hence the rate of the theta rhythm in each ten waves spell was calculated.

Then, on the heart rate record the same measuring and calculating was made taking into account those fragments which closely corresponded to the analysed theta waves fragments. Five heart beats were taken as one spell.

All the data were classified into five groups, according to the periods in which they were collected. These periods were: (i) pretrial periods (Pre), preceding a trial for about one minute; (ii) metronome periods (M); (iii) buzzer periods (B); (iv) food-intake periods (F); (v) posttrial periods (Post) lasting about 1 min after the consumption of food.

More than 3000 measurements of theta waves were made altogether that is an average of 600 measurements for each dog. The data were distributed into classes according to the duration of spells, each class differing from the next by 0.066 sec. The cumulative curves for theta waves and heart rate were constructed for each of the five periods in each dog separately. For estimation of the significance of the differences between distribution the Smirnov-Kolmogorov test was used. Since distribution of the data might be considered roughly normal, the parametrical method of Pearson correlation coefficient was used in order to esteem the correlation between the rate of the theta waves and that of the heart beats.

RESULTS

The first thing that deserves our attention is that in our experimental condition (well overtrained animals subjected to stereotyped schedule and highly alimentarily motivated) the clear theta rhythm was observed throughout nearly all experimental sessions. This rhythm was nearly identical in all the dogs and amounted to an average of 4.3 c/sec. Time and again the rhythm became disordered, without any visible cause, either for a short time (one or a few seconds) or for longer periods. There were dogs in which the theta rhythm was very distinct and present in all periods of the session (Fig. 2, Hideki) and others in which it was occasionally disordered (Fig. 3, Kunio and Fig. 4, Takeshi). We could not find any general rule concerning these irregularities, except that occasionally during the act of eating muscular artefacts masked the theta performance. We also could not detect, any regular increase of the amplitude of the theta waves in response to the CSs.

The rates of the theta rhythm in particular periods of sessions are presented in Fig. 5A—7A. Fig. 5A and 6A represent the distribution of theta waves frequencies for Hideki and Kunio, who were characterized by very regular CR activity (see also Fig. 2 and 3 respectively). Fig. 7A represents the performance of Takeshi who behaved in an atypical manner, being exceedingly slow and timid (see also Fig. 4). Fig. 8, higher graphs, represents the medians of the frequencies of theta waves for each period in each dog in percentage of the pretrial periods.

It is seen from these Figures that there are two clearly separated classes of the theta waves frequencies. Fast theta waves are characteristic for the metronome and the buzzer, slow ones are manifested in the pretrial period, posttrial period and during the food intake. These differences are statistically highly significant.

Both in Hideki and Kunio the rate of the theta waves was slightly higher (the difference being statistically significant) during the operation of the metronome, when the animal was motorically excited, than during the operation of the buzzer — when he was immobile but tense, gazing attentively at the food-tray. This difference was not obtained, however, in three other dogs. In Susumu and Kyu-Chian the theta wave rate was almost exactly the same during the operation of the metronome and the buzzer, and in Takeshi the relations were even reverse.

The distribution of slow theta waves was also not identical for all the dogs. In Hideki and Kunio the rate of the theta waves was identical for the food-intake period and the posttrial period, being slightly higher than during the pretrial period. In Takeshi and Kyu-Chian the theta rate during the food-intake period was slightly higher than during the inter-

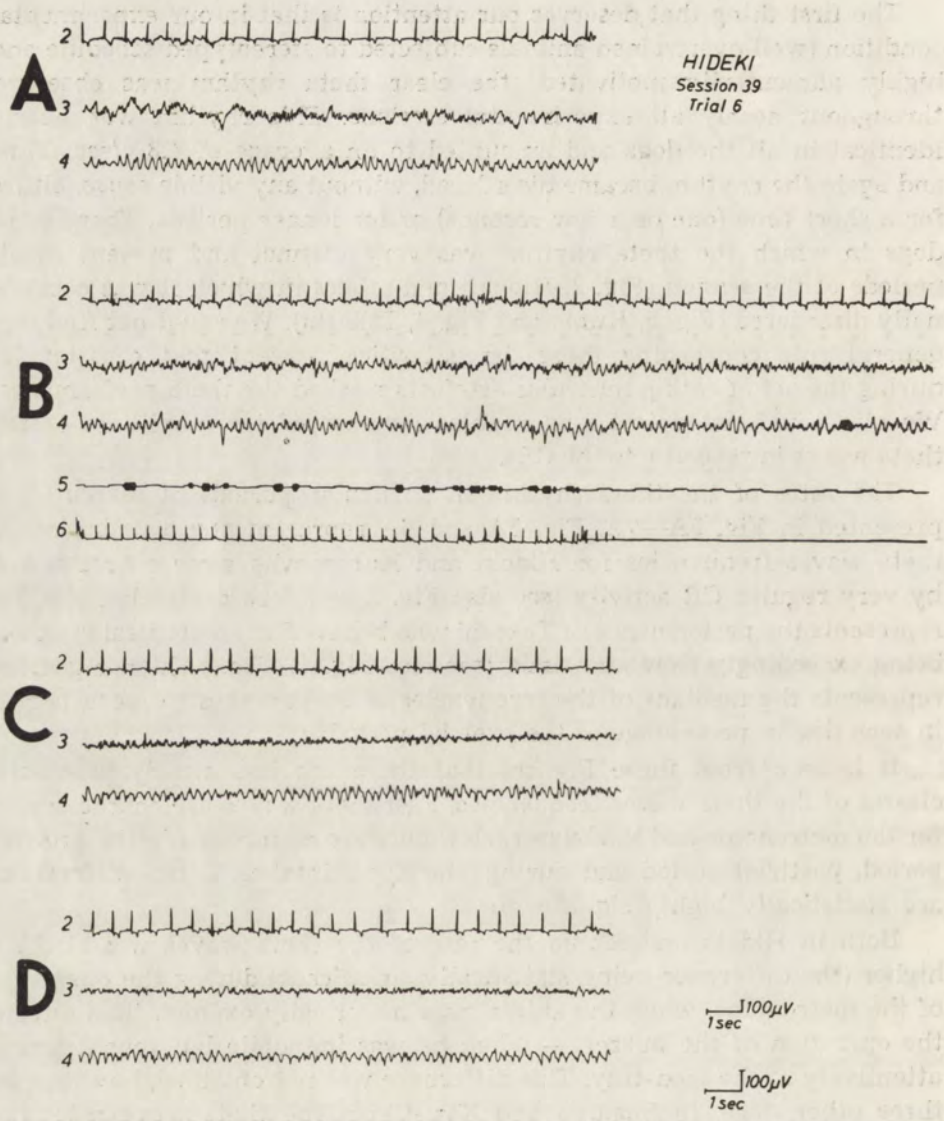


Fig. 2. Typical record of a trial in Hideki. A, pretrial period; B, metronome period (on the left) and buzzer period (on the right); C, food-intake; D, posttrial period. 2, heart rate; 3, EEG record from the parietal cortex; 4, record from the hippocampus; 5, lever presses; 6, beats of the metronome. Note the regular theta rhythm in the hippocampus almost throughout the record

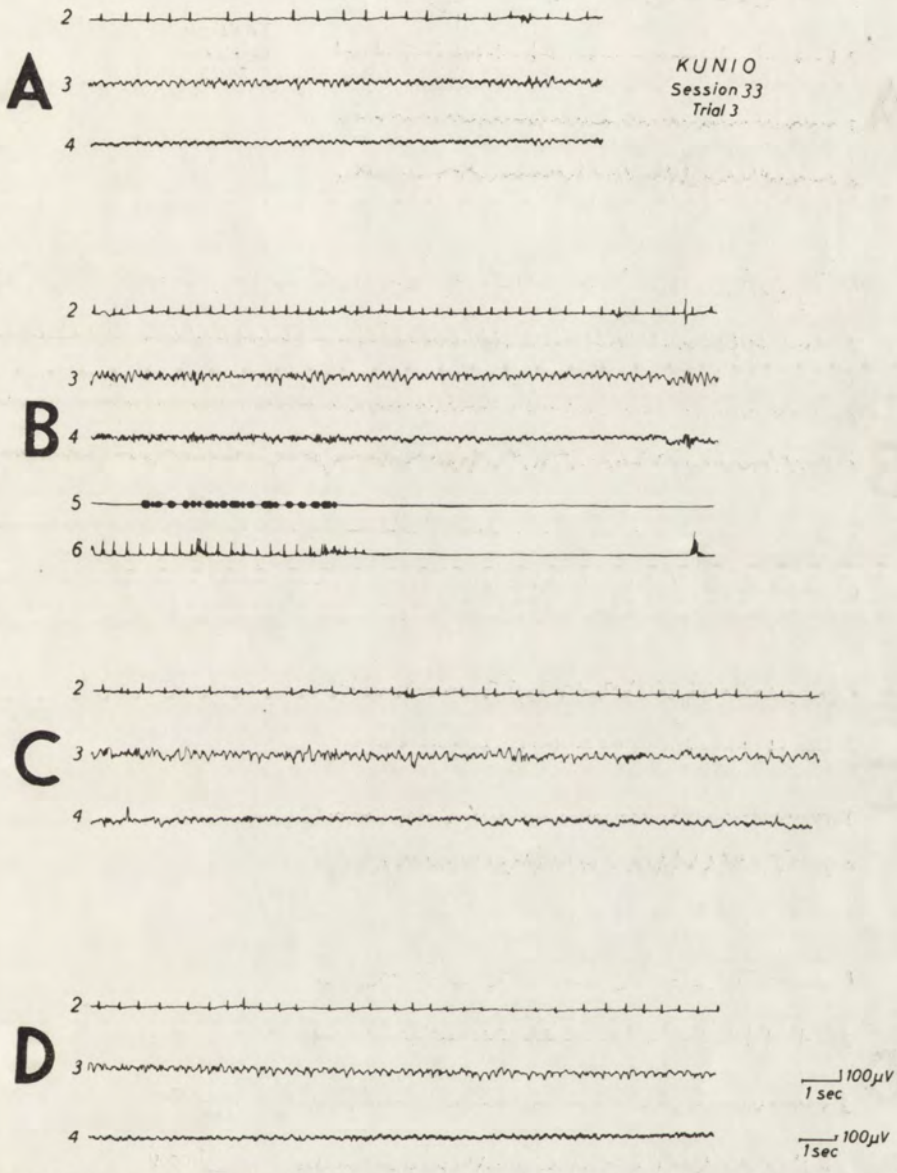


Fig. 3. Typical record of a trial in Kunio. A, pretrial period; B, metronome period (on the left) and buzzer period (on the right); C, food-intake period; D, posttrial period. 2, heart rate; 3, EEG record from the hippocampus; 4, record from the parietal cortex; 5, lever presses, 6, beats of the metronome (on the right an artefact of moving of the bowl into position.) Note the occasional desynchronization of the hippocampal rhythm in all records

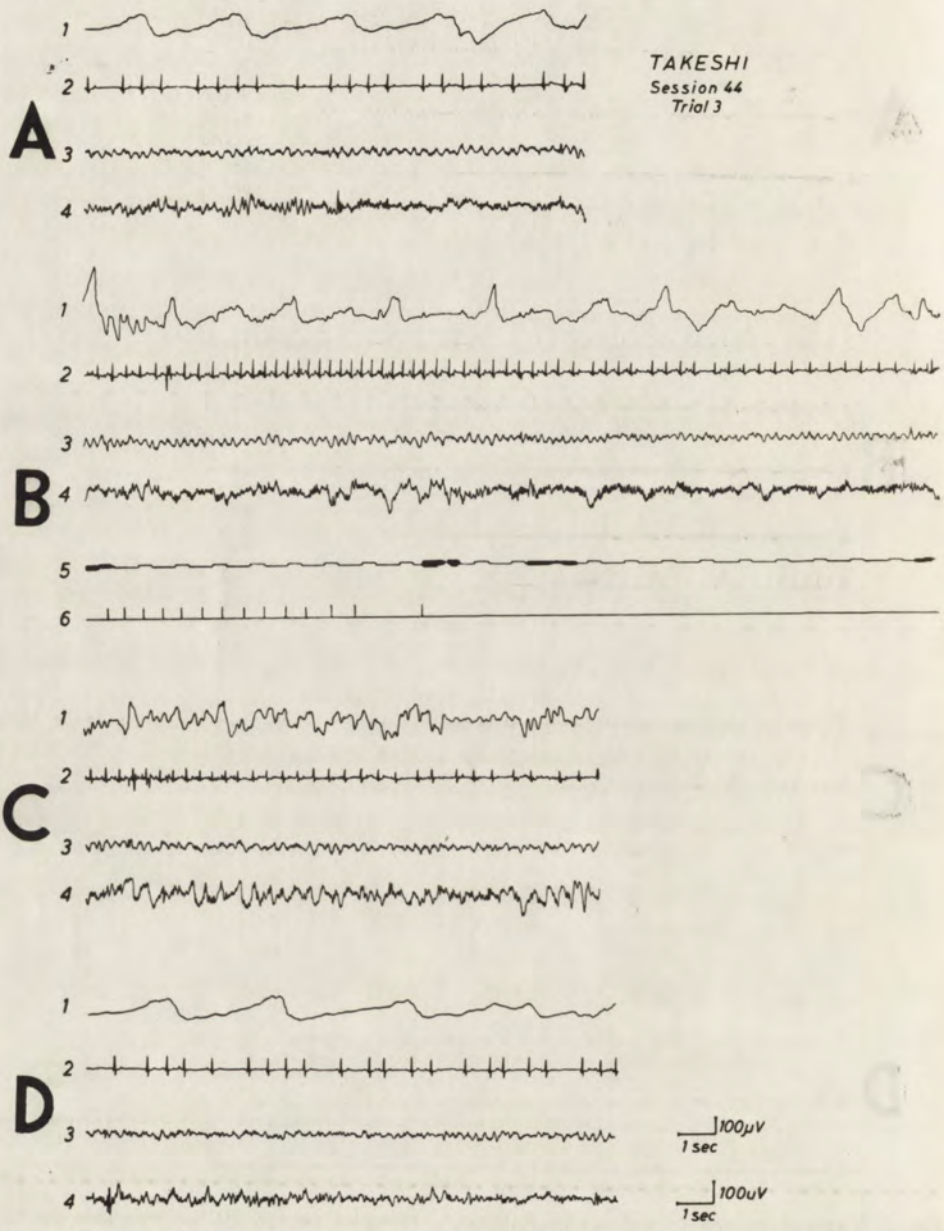


Fig. 4. Typical record of a trial in Takeshi. A, pretrial period; B, metronome period (on the left) and buzzer period (on the right); C, food-intake period; D, posttrial period. 1, respiration; 2, heart rate; 3, hippocampus record; 4, parietal cortex record; 5, lever presses (not all are recorded, because the animal usually touched the pedal without pressing it); 6, beats of the metronome. Note the desynchronization of the hippocampal rhythm particularly in D. Also note the occasional alpha rhythm in parietal record in A and D

vals, while in Susumu on the contrary, the theta waves during the food intake were the slowest of the whole cycle.

As far as the ECoG is concerned, all the dogs manifested a clear and permanent desynchronization. This is in keeping with the fact that throughout the sessions they were always alert and did not display any somnolence. The only episodes of synchronized slow rhythms were seen in Takeshi (see Fig. 4A and D), a dog who was inactive and indifferent in the intertrial intervals.

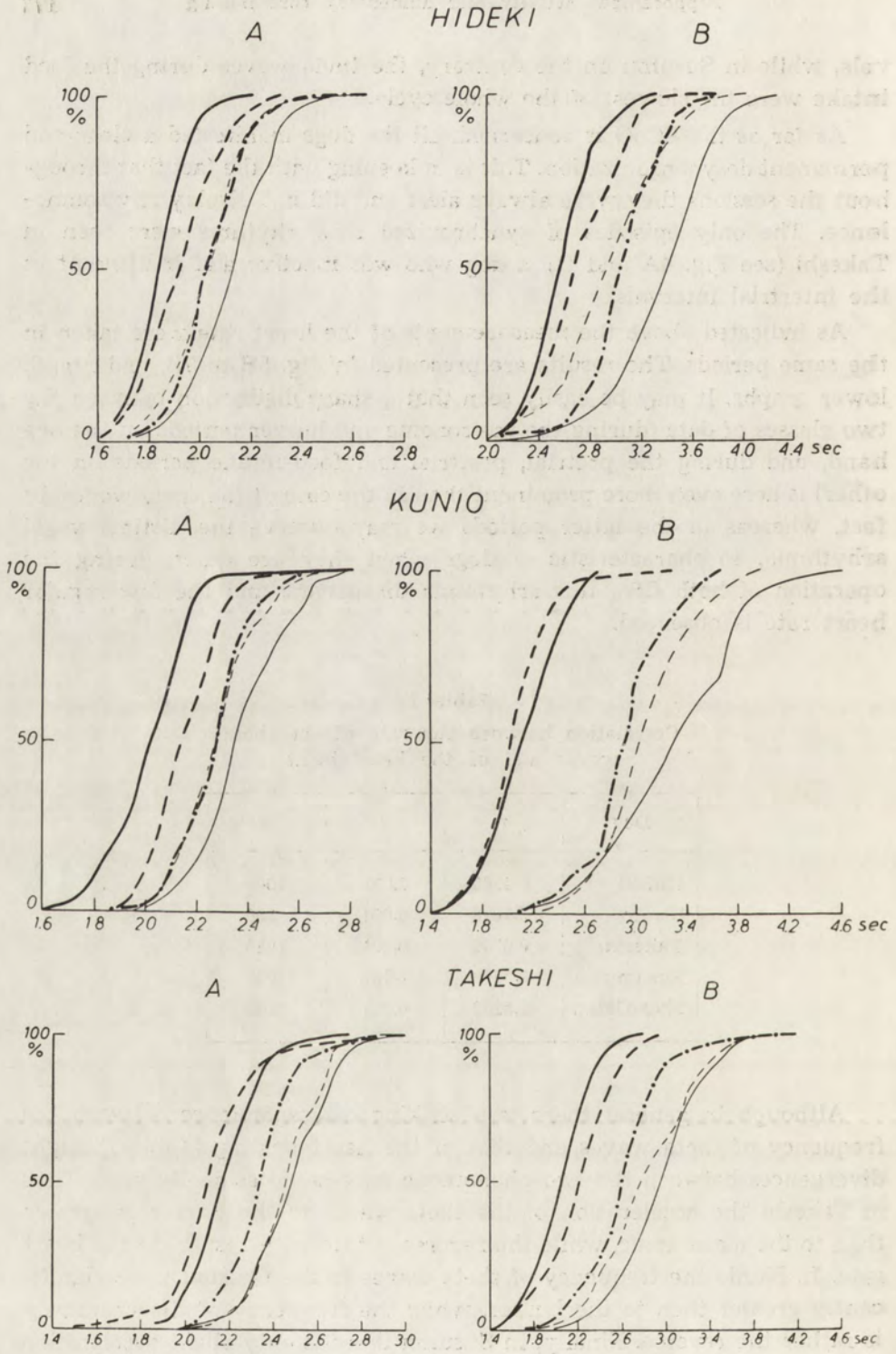
As indicated above the measurements of the heart rate were taken in the same periods. The results are presented in Fig. 5B to 7B, and Fig. 8, lower graphs. It may be easily seen that a sharp distinction between the two classes of data (during the metronome and buzzer periods on the one hand, and during the pretrial, posttrial and food-intake periods on the other) is here even more prominent than in the case of the theta waves. In fact, whereas in the latter periods we may observe the distinct vagal arhythmia, so characteristic of dogs when they are quiet, during the operation of both CSs, this arhythmia disappears, and the fast regular heart rate is observed.

Table I

Correlation between the rate of the theta waves and of the heart beats

Dog	r	P	N
Hideki	0.4263	0.001	1008
Kunio	0.5098	0.001	355
Takeshi	0.6396	0.001	1045
Susumu	0.5679	0.001	1031
Kyu-Chian	0.5299	0.001	223

Although in general there is a striking correspondence between the frequency of theta waves and that of the heart beating (Table I) slight divergences between the two phenomena may be occasionally seen. Thus in Takeshi the acceleration of the theta waves to the buzzer is greater than to the metronome, while the reverse is true with respect to the heart rate. In Kunio the frequency of theta waves to the metronome is significantly greater than to the buzzer, while the frequency of the heart rate is rather the reverse. Finally, in Susumu there is only slight acceleration



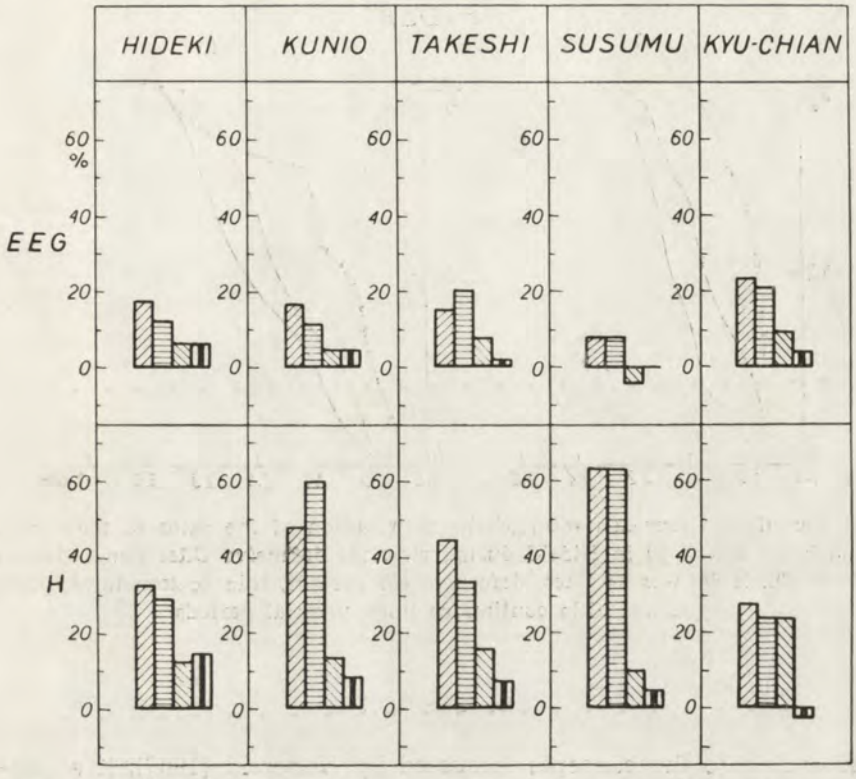


Fig. 8. The changes of the rate of theta waves (EEG) and heart beats (H) in comparison to the pretrial periods (denoted as zero level) in percent. In each graph first column denotes metronome periods, second column, buzzer periods, third column, food-intake periods, fourth column, posttrial periods

of the theta waves to the metronome and buzzer, while the acceleration of the heart rate to those stimuli is very conspicuous.

Pilot experiments performed on Hideki with classical defensive CRs gave quite similar results. As seen in Fig. 9, both the theta rhythms and the heart rate are dramatically accelerated to the defensive CS (light) in comparison to the pretrial and posttrial periods. The animal was exceedingly excited during the CS, becoming relatively calm in the intervals.

Fig. 5—7. Cumulative representation of the distribution of the rates of theta rhythm (A) and heart beats (B) in Hideki, Kunio and Takeshi. Abscissae, durations of 10 theta wave spells (in A) and 5 heart beat spells (in B) in sec. Ordinates, cumulative record of the number of occurrence of particular spells in percent. Thick continuous lines, metronome periods; thick broken lines, buzzer periods; thick dash-point lines, food-intake periods; thin broken lines, posttrial periods; thin continuous lines, pretrial periods

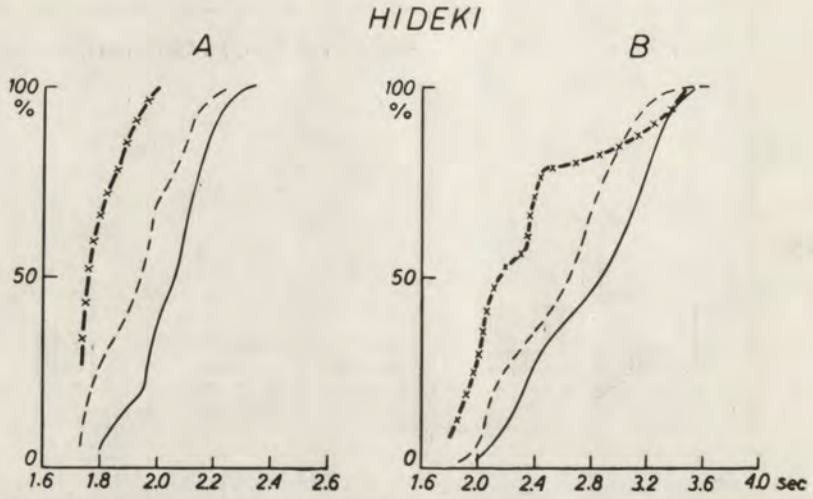


Fig. 9. Cumulative representation of the distribution of the rates of theta rhythm (A) and heart beats (B) in Hideki during classical defensive CRs. Denotation as in Fig. 5—8. Thick dash-cross lines, defensive CS periods, thin broken lines, posttrial periods, thin continuous lines, pretrial periods

DISCUSSION

According to the concepts discussed by Konorski (1967), if a non-satiated animal is repeatedly brought into a situation where he receives food, he developes a hunger CR to that situation. If presentation of food is preceded by a definite motor act, the animal learns to perform that act in the given situtation. If food is presented to the animal only when the movement is performed in the presence of a certain sporadic stimulus, the hunger CR becomes partially inhibited in the intertrial intervals and is released when the stimulus is given. Thus the performance of the instrumental movement is a behavioral indicator of the hunger drive. On the other hand, when the food is in the mouth the hunger drive is inhibited by the hunger antidrive elicited by the taste of food.

The results obtained in this paper show that the rate of the theta waves in hippocampus is correlated with the hunger drive, since it is increased during the operation of the hunger CR and decreased during the act of eating. The heart rate behaves in exactly the same way as the theta rhythm, being therefore another indicator of the hunger drive.

In our experimental schedule the presentation of food is preceded by the classical CS in response to which the animal salivates copiously, but does not perform the trained movement. According to Sołtysik's concept, this stimulus, being a signal of the food in the mouth, produces a consum-

matory CR reproducing the consummatory UR. Thus it should also produce decelaration both of the theta waves and of the heart rate.

According to Miyata's results the classical CS following the instrumental CS did produce the deceleration of the heart rate, this effect was, however, not general and not very strong (cf. Fig. 1). Exactly the same result was obtained by Ellison et al. (1968). In our experiments both the heart rate and the theta waves were also occasionally slowed down, but again this effect was not conspicuous. Accordingly, the conclusion should be drawn that in our experimental conditions the classical CR, although not eliciting the instrumental response, did not significantly inhibit the hunger drive. This result is in agreement with recent experiments by Ellison et al. (1968), who showed that the EMG level is very high during the classical CS, the evidence to show that the animal remains tense and not relaxed in its presence.

This fact seems to be explained in the following way. In the usual alimentary CR method the food is not directly injected into the mouth, but is presented in a bowl; both the sight of food and its smell are very strong natural hunger producing stimuli, as documented by the fact that they elicit a strong rise of the heart rate (cf. Fig. 1). In consequence, a stimulus closely preceding the presentation of food becomes, not only a consummatory food CS, but also a hunger-drive CS. Depending on the details of training and the character of the animal, either this or that aspect of the CS may prevail. If the hunger drive CR takes the upper hand, both the heart rate and the theta wave rate are high, if the consummatory CR plays a dominant role the heart rate and the theta waves should be slowed down. The fact that both these rates may be high when the animal is immobile, and may be slowed down when he performs some movements (the act of eating) shows that they do not depend, in our experimental condition, on the physical work.

The question may be asked as to whether only the arousal connected with the hunger drive produces the acceleration of the theta rhythm, or whether arousal produced by other drives gives the same effect. As seen from our pilot experiment the second alternative seems to be correct. In fact, in defensive CRs, *pari passu* with the acceleration of the heart rate, we may observe a clear increase of the rate of the theta rhythm.

Results almost identical with ours were obtained by Grastyan et al. (1966), by Elazar and Adey (1967, see also Adey et al. 1960) and by Klingberg and Pickenhain (1965ab).

The experiments of Grastyan et al. were reported above.

Elazar and Adey trained their cats in light-dark discrimination, and by using a precise spectral analysis method they have found that during the „prestimulus epoch" the slow theta hippocampal rhythm of 4 c/sec was

observed; during the „stimulus epoch", when the animal was still in the starting box, but the door to food was closed, the theta rhythm increased to 5 c/sec, whereas in the first phase of approach (i. e. locomotor instrumental response) it rose to 6 c/sec. During the act of eating the theta rhythm dropped again to 4 c/sec. It is interesting to note that in the second phase of approach, when the animal was close to food the rate of the theta rhythm dropped to 5 c/sec, thus showing that the consummatory CR was already in operation. When a well trained animal committed errors, thus showing that his hunger drive was temporarily decreased, the theta rhythm was slow. It increased, however, momentarily when the animal reached the goal and found no food there. As explained by Konorski (1967), this is indeed the most reliable means to increase a diminished hunger drive. Similarly, when the CR was extinguished by non-reinforcement and consequently the hunger drive CR to the situation became inhibited, the slowing down of the theta rhythm was also manifest.

Klingberg and Pickenhain trained the rats in defensive CRs and they clearly stated that the conditioned fear drive, particularly well seen in the avoidance procedure, leads to an acceleration of the hippocampal theta rhythm. On the contrary, the firmly established classical defensive CR does not produce this acceleration because the „active" fear is replaced by the „passive" fear. In our experiments this stage was not reached, and the trained animal was strongly aroused during the action of the CS.

To sum up, it follows from all these results that the arousal produced by drives, whether alimentary or defensive, is accompanied, among many other behavioral and electrophysiological manifestations of arousal, by acceleration of the theta rhythm. This statement does not of course imply that this acceleration is the cause of drive; we have in fact a great body of evidence to show that it is not so, since hippocampal lesions do not abolish drives. What is the cause of the acceleration of the theta rhythm in drives and arousal remains to be elucidated.

The idea of connecting the theta rhythms with arousal is not new. As a matter of fact, it was already put forward by Green and Arduini (1954) in their classic paper. However, those authors claimed that, not the acceleration, but the very occurrence of the theta waves depends on arousal and that when the animal is drowsy the rhythmic hippocampal activity is not present. The dependence of theta activity on arousal (due to fear drive) was also shown by Bremner (1964).

The possibility that any theta activity of the hippocampus, whether slow or fast, depends on some emotive states deserves a close attention. In the paper of Green and Arduini the rabbits, when somnolent, manifested a slow irregular activity in the hippocampus which became syn-

chronized by external stimuli. This activity was occasionally present also in the intervals. On the other hand, new external stimuli failed to produce the theta rhythm in monkeys. It may be supposed that the stimuli presented provoked fear drive in rabbits, but failed to do so in monkeys. It should be added that according to Grastyan et al. very strong arousal, produced either by hypothalamic stimulation or by natural CSs, gives rise to a fast rhythm in the hippocampus, easily turning to full desynchronization. We have occasionally seen the same effect in our dogs during the operation of the instrumental CS.

All these facts allow us to propose a tentative hypothesis to the effect that any emotional states, both of the drive and antidrive character, produce the theta rhythm, its rate depending on the degree of arousal produced by the given state.

This hypothesis is in good agreement with the classic concept of Papez (1937) who claimed that the activity of the hippocampal closed self re-exciting circuits is connected with emotional states. It is also supported by the fact that the theta rhythm can be traced in the fornix, septum and mammilary body, whereas cutting the fornix or destroying septum causes its abolition (Green and Arduini 1954). The increase of the frequency of the theta rhythm with the increase of arousal may be easily explained by the fact that unspecific impulses facilitating the particular links of the closed circuit of neurons shorten their latencies and thus improve their transmissibility. The fact that in our experimental sessions the theta rhythm, although slow, was almost permanent is also in agreement with this hypothesis. The point is that in our animals the whole experimental situation was a firmly established hunger CS which, although partially inhibited, was present throughout the session. It should also be reminded that cortical EEG presented a permanent desynchronization.

Whether this speculation is right or wrong, it is clear that acceleration of the theta rhythm has nothing to do with „recapitulation of past experience" determining the correct choice in discrimination learning, as claimed by Elazar and Adey. In our experiments, as well as in experiments of other authors quoted above, no discrimination learning was given, and even (in the case of classical defensive conditioning) no learned goal directed behavior was present. On the other hand, all the available data show that this acceleration, which rums *pari passu* with the acceleration of the heart rate, is a manifestation (but certainly not a cause) of arousal due to the activity quite independent of any „storage of information" due to learning.

SUMMARY

1. In five dogs well overtrained in an alimentary chain CR, consisting of the instrumental and classical segments, the rate of the hippocampal waves and the heart rate were measured in the pretrial period, in the instrumental CS period, classical CS period, food-intake period and post-trial period.

2. Slow theta rhythm was observed permanently throughout the experimental sessions. It was significantly accelerated during the operation of the instrumental and classical CSs; and decelerated during the food-intake, approximating the levels of the pretrial and posttrial periods.

3. The heart rate was highly correlated with the rate of the theta rhythm: both were strongly accelerated during the instrumental and classical CSs and decelerated during the food-intake.

4. The ECoG manifested desynchronization throughout the experimental sessions.

5. The increase of the theta wave and the heart rate was also observed in one dog during the operation of the aversive CS.

6. The role of the hippocampus in emotional states is discussed.

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REFERENCES

ADEY, W. R., DUNLOP, C. W. and HENDRIX, C. E., 1960. Hippocampal slow waves. Arch. Neurol. 3: 74—90.

BREMNER, F. J. 1964. Hippocampal activity during avoidance behavior in the rat. J. Comp. Physiol. Psychol. 58: 10—15.

ELAZAR, Z. and ADEY, W. R. 1967. Spectral analysis of low frequency components in the electrical activity of the hippocampus during learning. Electroenceph. Clin. Neurophysiol. 23: 225—240.

ELLISON, P. D., HUMPHREY, G. L. and FEENEY, D. 1968. Some electrophysiological correlates of two behavioral states. J. Comp. Physiol. Psychol. (in press).

ELLISON, P. D. and KONORSKI, J. 1965. An investigation of the relations between salivary and motor responses during instrumental performances. Acta Biol. Exp. (Warsaw) 25: 297—316.

GRASTYAN, E., KARMOS, G., VERECZKEY, L. and KELLENYI, L. 1966. The hippocampal electrical correlates of the homeostatic regulation of motivation. Electroenceph. Clin. Neurophysiol. 21: 28—33.

GREEN, J. D. and ARDUINI, A. A. 1954. Hippocampal electrical activity in arousal. J. Neurophysiol. 17: 533—557.

KLINGBERG, F. and PICKENHAIN, L. 1965a. Electrophysiologische und Verhaltungsuntersuchungen über die Ausarbeitung eines bedingten Abwehrref-

lexes auf elektrische Reize ohne Fluchtmöglichteit bei Ratten. Acta Biol. Med. Germ. 14: 749—763.

KLINGBERG, F. PICKENHAIN, L., 1965b. Uber die Beteiligung des Hippokampus an der Ausarbeitung eines bedingten Fluchtreflexes bei der Ratte. Acta Physiol. Acad. Sci. Hung. 27: 359—374.

KONORSKI, J. 1967. Integrative activity of the brain. Univ. Chicago Press, Chicago.

MIYATA, Y. and SOŁTYSIK, S. 1968. The relations between salivary, cardiac and motor responses during instrumental performance. Acta Biol. Exp. 28 (in press).

PAPEZ, J. W., 1937. A proposed mechanism of emotion. Arch. Neurol. Psychiatr. 38: 725—743.

SOŁTYSIK, S. 1960. The problem of intercentral relations in instrumental conditioning (in Russian). In: Gutmann, E., Asratian, E. A. and Konorski, J. (ed.), Centralnyie i periferichеskiie mechanismy dvigatelnoi deiatelnostii. Acad. Nauk, Moskva, p. 58—60.

Acta Biol. Exp. (Warsaw) vol. 28, no. 3, p. 187—196, 1968

HIPPOCAMPAL ELECTRICAL ACTIVITY DURING ACUTE EXTINCTION OF DEFENSIVE CONDITIONED REFLEX IN RABBITS [1]

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The work of Green and Arduini (1954) demonstrated that the hippocampus, in contrast to the neocortex, responds to afferent stimulation by synchronization of bioelectrical activity (theta rhythm). Further research provided evidence that the theta rhythm depends on activity of single neurons of the pyramidal layer of the hippocampus (von Euler and Green 1960ab; Andersen 1960ab; Andersen et al. 1964 ab; Fujita and Sato 1964; Yokota and Fujimori 1964; Vinogradova 1965). However, the physiological significance of this reaction remains unknown. Pharmacological (Bradley and Nicholson 1962) and electrophysiological (Yokota and Fujimori 1964) studies indicate the existence of a connection between the hippocampus and the brain stem reticular formation. Research based on the conditioned reflex technique (Grastyán et al. 1959, Sadowski and Longo 1962) showed that the hippocampus takes part in the development and consolidation of conditioned reflexes.

This work is concerned with changes in electrical activity of the hippocampus during acute extinction of a defensive conditioned reflex. Klingberg and Pickenhain (1965) observed extinction of the theta rhythm, i. e. hippocampal arousal, after repeated acoustic stimuli. Hence, it was considered of interest to investigate whether extinction of hippocampal arousal under the influence of an acoustic defensive conditioned stimulus is possible.

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MATERIAL AND METHODS

Experiments were carried out with eight female pure-breed chinchilla rabbits weighing 2.5—4.5 kg with chronically implanted subcortical electrodes in the dorsal hippocampus and other sites in the brain. For operative implantation of the electrodes, the rabbits were anesthetized with hexobarbital (80 mg/kg body weight). Implantation was performed by the method previously described (Traczyk 1962), except that instead of plugs of miniature radio valves, standard miniature valve sockets or rectangular subminiature socket connectors (SMRE — 7S, product of Ether Ltd.) were fastened directly to the bone of the cranial vault by means of Duracril dental cement.

The experiments were begun several months after the operation, after the reaction of the cerebral tissue elicited by the electrodes had subsided (Alexandrovskaya 1962). In the first phase, defensive conditioned reflexes were established in the rabbits by the method described by Volochov and Obrastsova (1953). The sound of an electric bell served as conditioned stimulus. The CS—US interval was 10 sec. CS was always reinforced by an unconditioned stimulus, in the form of an electric shock to the ear. Stimulation was performed with an electronic stimulator connected with electrodes on the ear of the rabbits. Before each session, the threshold was determined on the basis of the minimal stimulus eliciting the defense reaction, i.e. shaking of the ears and head. During the session, the rabbits were held in a special grounded metal cage covered with a lid. At each session, 10 CSs were applied at one-minute intervals.

Establishing and consolidation of the conditioned reflex was continued until the animal attained a criterion of 50% positive conditioned reactions in the course of one session.

After the reflex had been formed, its acute extinction was begun while simultaneously recording electrical activity. The rabbits were held in the same cage in which the conditioned reflex had been established. The electrodes were connected with an „Ediswan" eight-channel electroencephalograph, by means of which electrical activity was recorded during repeated, unreinforced application of the conditioned stimulus. Unipolar leads were used; the indifferent electrode was implanted on the frontal bones of the rabbit's cranium. Extinction of conditioned reflexes was not interrupted when the somatic reaction disappeared, but was continued until 100 unreinforced conditioned stimuli at one-minute intervals had been applied. Thereafter, the conditioned stimulus was repeated a number of times at irregular intervals.

Electrical activity during the action of the conditioned stimulus was assessed mainly on the basis of the shape of the electrohippocampogram. The conditioned reaction was also assessed on the basis of frequency of electrical potentials measured separately betwen the first and fifth seconds and sixth to tenth seconds of the conditioned stimulus as compared with the frequency of potentials during the five seconds immediately preceding the conditioned stimulus.

After the experiments, the rabbits were killed by a lethal dose of barbiturates, and the head was perfused first with physiological saline and then with 10% formalin solution. After fixation in formalin and dehydration in alcohol, the brains were imbedded in celloidin and cut on a microtome into coronal sections 60 μ thick. The sections were stained for myelin by the method of Weil (Fig. 1).

Localization of the electrodes in the hippocampus was checked on the basis of

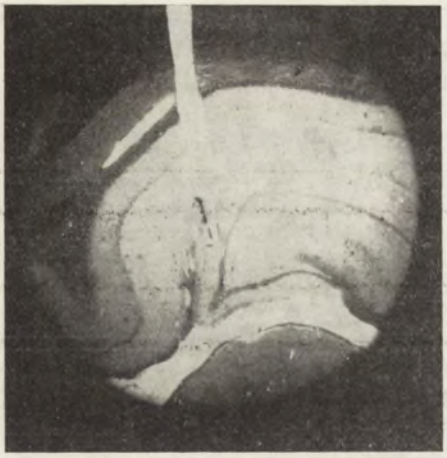

Fig. 1. Photomicrograph of a coronal section of the rabbit's no. 291 brain with traces of the electrode implanted in the dorsal hippocampus. Histological section 60μ thick, stained for myelin by the method of Weil

these preparation. The histological verification confirmed that in all eight rabbits the electrodes were implanted within the dorsal hippocampus (Fig. 2).

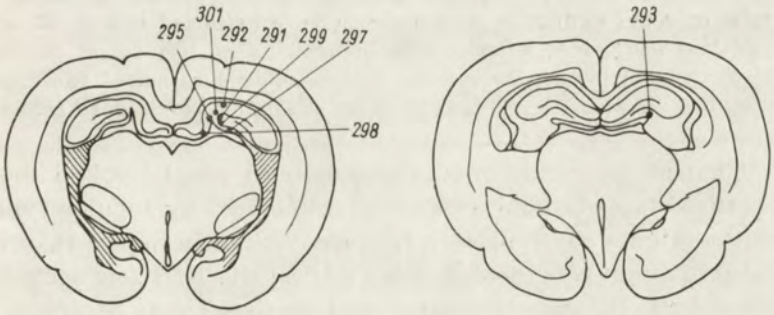

Fig. 2. Schematic coronal sections of rabbit brain showing localization of electrodes implanted in the dorsal hippocampus. The figures denote sites of implantation of the electrodes in different rabbits.

RESULTS

Conditioned reflexes developed in different rabbits after various times; in individual animals after 8—39 sessions. The degree of consolidation of the reflex was also variable (Table). Extinction of the motor conditioned reaction, assessed on the basis of disappearance of the motor artefact in the electroencephalogram (Fig. 3), occurred in different animals after 2—19 unreinforced applications of the conditioned stimulus (Table). Du-

ring further trials, the somatic component of the conditioned reflex reappeared many times, but irregularly. After several dozens of applications of the unreinforced conditioned stimulus, the motor reaction ceased to appear.

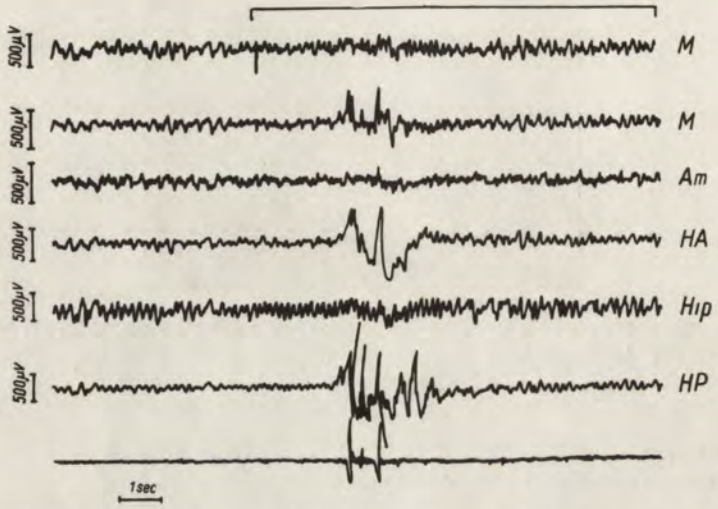

Fig. 3. Bioelectrical activity recorded from subcortical centers in rabbit no. 291 in 7 th trial of acute extinction of the defensive conditioned reflex: M, midbrain; Am, amygdaloid body; HA, anterior hypothalamus; Hip, left dorsal hippocampus; HP, posterior hypothalamus. During the 4—5 seconds duration of the conditioned stimulus, a motor artefact was recorded indicating conditioned reflex

The action of the conditioned stimulus (CS) which evoked the motor reaction caused hippocampal arousal, characterized by regular synchronized potentials (theta rhythm) with frequency slightly higher than resting. Increased frequency was most distinct during the first few seconds after starting the bell. Between the sixth and tenth seconds of action of the conditioned stimulus (CS) frequency decreased, and toward the end of the bell stimulus attained valeus lower than before its starting (Fig. 4A). Amplitude of the electrical potentials during the hippocampal arousal was not appreciably changed.

During extinction of hippocampal arousal, changes in the frequency of hippocampal potentials were observed. These consisted in an increase of frequency in the period preceding the bell signal, and decrease during its ringing, especially between the sixth and tenth seconds (Fig. 4B). Toward the end of extinction the differences in frequency described above were slight, or absent.

During the experiments, synchronization of electrical activity of the hippocampus was often observed between applications of the conditioned

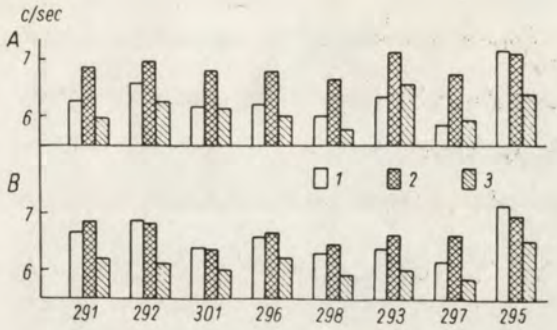

Fig. 4. Frequency of electrical activity from the hippocampus: A, before extinction; B, after extinction of the somatic component and hippocampal arousal. The height of the columns shows the frequencies of electrical potentials in eight rabbits: no. 291, 292, 301, 296, 298, 293, 297 and 295, calculated during three 5 sec intervals. The left column represents frequency of potentials before action of the conditioned stimulus; the middle column, between the first and fifth seconds; and the right column, between the sixth and tenth seconds of action of the conditioned stimulus 1, frequency of potentials before action of the CS; 2, frequency between 1st and 5th sec of action of CS; 3, frequency between 6th and 10th sec of action of CS

stimulus. In rabbit No. 297 this phenomenon was observed regularly immediately before application of the conditioned stimulus. Against this background, the conditioned stimulus applied failed to produce distinct changes in electrical activity.

In a majority of the animals, hippocampal arousal was extinguished gradually. At first, theta rhythm appeared at once after application of the conditioned stimulus. As the number of unreinforced conditioned sti-

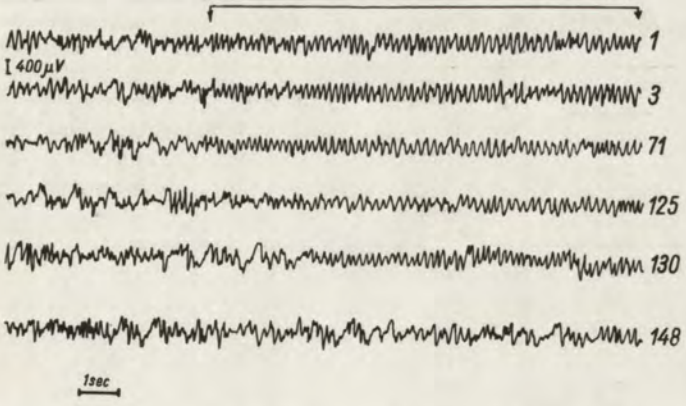

Fig. 5. Electrohippocampogram of rabbit no. 291, recorded during the action of 1, 3, 71, 125, 130, and 148th sequential unreinforced conditioned stimuli

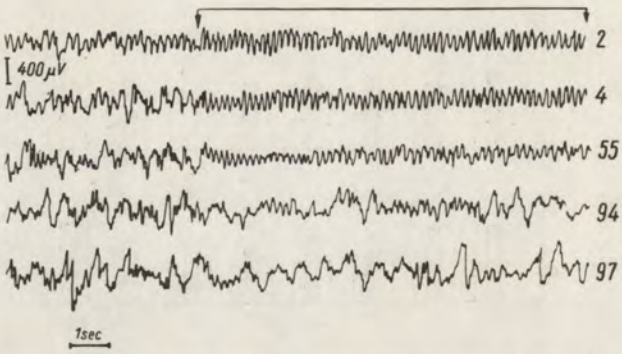

Fig. 6. Electrohippocampogram of rabbit no. 301, recorded during the action of 2, 4, 55, 94 and 97th unreinforced conditioned stimuli

muli increased, the hippocampal arousal was delayed, until it finally disappeared altogether from the EHG tracing (Fig. 5 and 6). Delay of hippocampal arousal did not appear until the motor component of the conditioned reflex was completely extinguished.

Hippocampal arousal was extinguished only if the experimental regime was rigid. Changes in the duration of intervals produced reappearence of the previously extinguished arousal.

The electrical activity of the hippocampus recorded during the action of the conditioned stimulus was much more permanent than the motor component of the conditioned reflex. It was not extinguished until 60—130 unreinforced conditioned stimuli had been applied (Table).

Table

Elaboration and acute extinction of defensive conditioned reflex

Rabbit no	Elaboratior of defensive CR		Acute extinction of defensive CR Number of trials after which:		
	number of trials before extinction	percentages of CR in 5 sessions before extinction	somatic reaction did not appear for the first time	somatic reaction was entirely extinguished	theta rhythm did not appear
291	80	20	3	103	130
292	390	95	19	76	80
293	120	36	2	62	73
295	110	40	18	60	75
296	280	34	11	74	56
297	240	28	10	60	94
298	220	30	3	93	80
301	100	22	6	85	70

The typical EHG pattern characteristic of extinction of the arousal reaction consisted of irregular potentials with variable amplitude and relatively low frequency. Extinction of hippocampal arousal was not permanent, being abolished by the smallest change in the intervals between conditioned stimuli or weak extra acoustic stimuli.

DISCUSSION

In the experiments described above, the course of changes in hippocampal electrical activity during acute extinction of the defense conditioned reflex was studied. The conditioned stimulus produced besides the conditioned motor reaction also hippocampal arousal (Grastyán et al. 1959; Sadowski and Longo 1962). Both reactions could be extinguished by means of many repetitive unreinforced stimuli.

The fact that the motor reaction conditioned was extinguished much more quickly that the hippocampal arousal reaction indicates that the two components of the same conditioned reflex are brought about by different nervous mechanisms. Our observations suggest a similarity between hippocampal arousal and a vegetative reaction, the cardiac conditioned reflex. Dykman and Gantt (1956), Jaworska et al. (1962) and Jaworska and Sołtysik (1962) found that the somatic conditioned reflex is extinguished more rapidly than the cardiac reaction accompanying it. Jaworska et al. (1962) supposed that the reflex arcs of the cardiac and motor reactions, for the somatic and cardiac reaction, run through centers with different properties. The motor reaction, associated with specific centers, is consummatory response, whereas the cardiac reaction, like hippocampal arousal, is elicited by nonspecific drive centers.

According to the hypothesis of Gastaut (1958), the reflex arcs of conditioned reflexes are closed at various levels of the central nervous system. Quicker extinction of the motor reaction indicates that the neurons converging in the centers of the somatic system are more easily inhibited. This process takes place much more slowly in neurons eliciting nonspecific reactions, e. g. hippocampal arousal.

Theta rhythm has been evoked by stimulating the midbrain reticular formation (Yokota and Fujimori 1964; Stumpf 1965); or lateral hypothalamus and septum (von Euler and Green 1960a, b; Sager and Butkhuzi 1962; Elul 1964 a, b), i.e. centers lying in the pathway of impulses running from the reticular formation to the hippocampus. The rhythm was observed during the action of stimuli on receptors (Gloor et al. 1964; Vinogradova 1965; Klingberg and Pickenhain 1965).

In our experiments, hippocampal arousal failed to appear only after several dozens of unreinforced acoustic stimuli had been applied, indicating active inhibition of nonspecific midbrain neurons conducting impulses from the organ of Corti, or suppression of conduction between the midbrain and hippocampus. That this process represents active inhibition is evidenced by the fact that slight changes in the lenght of intervals between applications of acoustic stimuli caused reappearance of theta rhythm in the hippocampus.

Our results are not altogether concordant with the observations of Grastyán et al. (1965), who noted desynchronization of hippocampal electrical activity evoked by hypothalamic stimulation. Species differences may be the explantation for the discrepancy, since Grastyán et al. (1965) performed their experiments on cats, in which theta rhythm is less pronounced than in rabbits; or their results were an effect of strong excitability of hypothalamic motivational centers.

SUMMARY

1. During acute extinction of defensive conditioned reflex the somatic component is extinguished first.

2. Hippocampal arousal, which occurs at first immediately to the unreinforced conditioned stimuli, in the course of extinction is increasingly delayed.

3. Hippocampal arousal is entirely extinguished after 60—130 unreinforced stimuli presented at regular intervals, in different animals.

4. After complete extinction, hippocampal arousal reappears at once if the acoustic stimulus is applied at irregular intervals.

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REFERENCES

ALEXANDROVSKAYA, M. M. 1962. Morfological data on the state of tissue structural elements of the brain after implanting elektrodes (in Russian). Zhurn. Wyssh. Niervn. Dieiat. 12: 727.

ANDERSEN, P. 1960a. Interhippocampal Impulses. II Apical dendritic of CA_1 neurons. Acta Physiol. Scand. 48: 178.

ANDERSEN, P. 1960b. Interhippocampal Impulses. III Basal dendritic activation of CA_3 neurons. Acta Physiol. Scand. 48: 209.

ANDERSEN, P., ECCLES, J. C. and LOYNING, Y. 1964a. Location of postsynaptic inhibitory synapses on hippocampal pyramids. J. Neurophysiol. 27: 592.

ANDERSEN, P., ECCLES, J. C. and LOYNING, Y. 1964b. Pathway of postsynaptic inhibition in the hippocampus. J. Neurophysiol. 27: 608.

BRADLEY, P. B. and NICHOLSON, A. N. 1962. The effect of some drugs on hippocampal arousal. Electroenceph. Clin. Neurophysiol. 14: 824.

DYKMAN, H. A. and GANTT, W. H. 1956. Relation of experimental tachycardia to amplitude of motor activity and intensivity of the motivating stimulus. Amer. J. Physiol. 185: 495.

ELUL, R. 1964a. Regional differences in the hippocampus of the cat. I. Specyfic discharge patterne of the dorsal and ventral hippocampus and their role in generalized seizures. Electroenceph. Clin. Neurophysiol. 16: 470.

ELUL, R. 1964b. Regional differences in the hippocampus of the cat. II. Projections of the dorsal and ventral hippocampus. Electroenceph. Clin. Neurophysiol. 16: 489.

VON EULER, C. and GREEN, J. D. 1960a. Excitation, inhibition and rhythmical activity in hippocampal pyramidal cells in rabbit. Acta Physiol. Scand. 48: 95.

VON EULER, C. and GREEN, J. D. 1960b. Activity in single hippocampal pyramids. Acta Physiol. Scand. 48: 110.

FUJITA, Y. and SATO, T. 1964. Intracellular record from hippocampal pyramidal cells in rabbit during theta rhythm. J. Neurophysiol. 27: 1011.

GASTAUT, H. 1958. Some aspects of the neurophysiological basis of conditioned reflexes and behaviour. Ciba foundation symposium an the neurological basis of behaviour, London, p. 255.

GLOOR, P., VERA, C. L. and SPERTI, L. 1964. Electrophysiological studies of hippocampal neurons. III. Responses of hippocampal neurons to repetitive perforant path volleys. Electroenceph. Clin. Neurophysiol. 17: 353.

GRASTYÁN, E., LISSÁK, K., MADRÁSZ, I. and DONHOFFER, H. 1959. Hippocampal electrical activity during the development of conditioned reflexes. Electroenceph. Clin. Neurophysiol. 11: 409.

GRASTYÁN, E., KARMOS, G., VORECZKEY, L., MARTIN J., and KELLENYI, L. 1965. Hypothalamic motivational processes as reflected by their hippocampal electrical correlates. Science 10: 91.

GREEN, J. D. and ARDUINI, A. A. 1954. Hippocampal electrical activity in arousal. J. Neurophysiol. 17: 533.

JAWORSKA, K., KOWALSKA, M., and SOŁTYSIK, S. 1962. Studies on the aversive classical conditioning 1. Acquisition and differentiation of motor and cardiac conditioned classical defensive reflex in dog. Acta Biol. Exp. (Warsaw) 22:23.

JAWORSKA, K. and SOŁTYSIK, S. 1962. Studies on the aversive classical conditioning. 3. Cardiac responses to conditioned and unconditioned defensive (aversive) stimuli. Acta Biol. Exp. 22: 193.

KLINGBERG, F. and PICKENHAIN, L. 1965. Über die Beteiligung des Hippokampus an der Ausarbeitung eines Bedingten Fluchtreflexes bei der Ratte. Acta Physiol. Acad. Sci. Hung. 27: 359.

SADOWSKI, B. and LONGO, V. G. 1962. Relationship EEG and behavioural of on instrumental reward conditioned respons in rabbit. Electroenceph. Clin. Neurophysiol. 12: 465.

SAGER, O. and BUTKHUZI, S. 1962. Electrografical study of the relationship between the dorsomedian nucleus of the thalamus and the rhinencephalon (hippocampus and amygdala). Electroenceph. Clin. Neurophysiol. 14: 835.

STUMPF, C. 1965. The fast component in the electrical activity of rabbits hippocampus. Electroenceph. Clin. Neurophysiol. 18: 477.

TRACZYK, W. 1962. Feeding and flight reactions evoked in rabbits by hypothalamic stimulation. Acta Physiol. Polon. 13:239.

VINOGRADOVA, O. S. 1965. Dynamic classification of responses to sensory stimuli in the hippocampal neurons of the rabbit (in Russian). Zhurn. Vyssh. Nervn. Dieiat. 15: 500.

VOLOCHOV, A. A. and OBRASTSOVA, G. A. 1953. Quated after SADOWSKI B. 1958. A trial of characteristic conditioned reflex activity in rabbits based on the conditioned defensive reflexes (in Polish). Acta Physiol. Pol. 9: 451.

YOKOTA, T. and FUJIMORI, B. 1964. Effect of brain-stem stimulation upon hippocampal electrical activity, somatomotor reflex and autonomic function. Electroenceph. Clin. Neurophysiol. 14: 375.

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EFFECTS OF FRONTAL LESIONS IN BLACK-WHITE DISCRIMINATION TEST IN WHITE RATS

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Dąbrowska's data (1962, 1963, 1964) concerning reversal learning in the four-unit-quadruple-choice apparatus showed specific differences between animals with frontal lesions and normal ones in reversal learning. To explain these results the hypothesis was given that the saving in the number of runs observed in normal animals during reversal learning is due to the integrating process which takes place during the elaboration of chain reflexes. The animals solved such a complex task as one unit. The next results (Dąbrowska 1967) showed that this integration disappeared in rats with frontal lesions and that they elaborated every unit of such complex task separately.

On the other hand, experiments performed by Srebro and Dąbrowska (unpublished data) showed that the performance in the normal animals and rats with frontal lesions is similar in reversal learning if additional visual cues are used in every compartment of the maze. These results permited us to suppose that rats with frontal lesions use predominantly visual cues during maze learning whereas normal rats use predominantly kinesthetic cues. Normal animals can learn the same maze using visual cues but in this case the course of reversal learning is different.

If the hypothesis is correct, the rats with frontal lesions should be as good as normal ones or better in black-white discrimination test.

MATERIALS AND METHOD

Experiments were perfomed in 20 white Wistar rats 2,5 months old. During the training period the animals were deprived of food 22 hours before testing.

The apparatus used in experiments was a modified Lashley jumping apparatus (Fig. 1). The starting platform in this apparatus consited of two arms connected to each other and the distal part of each arm was situated in front of one of two doors. The distance between arms and doors could be regulated. Usually this distance was such that an animal could touch the door by forelegs without jumping. If an animal made a wrong choice he could withdraw and make a second choice. Correction method and food reward were used. Before begining the experiments the animals have given preliminary training for four days to adapt them to the experimental situation.

Fig. 1. The modified Lashley jumping apparatus

After the preliminary training was over the animals were divided into two groups: Group I, consisting of 10 normal animals, Group II, consisting of 10 animals with frontal lesions.

Each animal of the second group was subjected to an operation in which the rostro-dorsal parts of the cortex in front of the motor area (Zubek 1951) were bilateraly removed (Fig. 2). The cortex was removed by suction under Nembutal anesthesia. The recovery of the animals was unevenful.

One month after the operation the main training was started. During this training an animal could obtain food by stepping through the correct white door. The other door, the incorrect one, was black. The position of the white and black doors was changed randomly (predetermined rand order of Gellerman 1933). Three hundred trials of 10 trials a day were given with the white door correct. Afterwards, the reversal learning was begun 10/day for 300 trials with black correct.

After the termination of the reversal learning the animals were sacrificed, the brains removed, fixed in 10% formalin, embedded in parafine and cut serially. The sections were stained with Nissl technique and reconstructions of the cortical lesions were done as described by Lashley (1931). The black areas in Fig. 2 denote the parts of the brain in which both gray and white matter were removed. Striped parts show the parts in which only gray matter was removed.

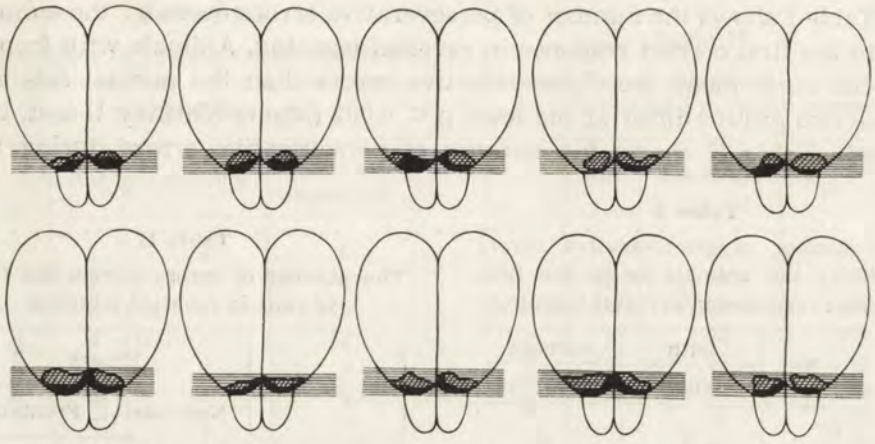

Fig. 2. Brains of the rats with lesions in frontal area. Explanation in text

RESULTS

The number of correct responses in consecutive 50 trial blocks were analysed using analysis of variance mixed design, type VI method (Lindquist 1953). It was found that total number of errors committed by frontal rats was smaller than that in normal animals ($p<0,05$). Frontal animals showed higher level of accuracy than normal Ss during original and reversal learning (the interaction between these two factors was not significant). During 300 trials of the original learning all animals performed more correct responses than during the reversal learning ($p < 0\ 001$). The course of learning and reversal learning in frontal animals differs from the course of learning and reversal learning of normal animals and these differences are statistically highly significant ($p < 0.001$). Fig. 3 shows the average number of correct responses made by 10 animals in each group in blocks consisting of 50 consecutive trials.

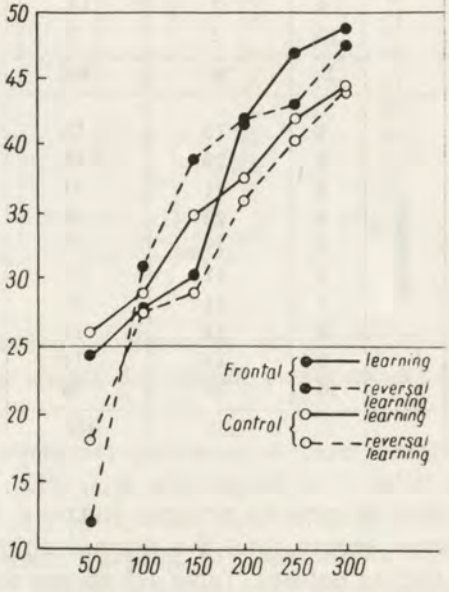

Fig. 3. The average number of correct responses made by 10 animals in each group in blocks consisting of 50 consecutive trials. The horizontal line on the level 25 shows the probability by 50% correct responses

Table I shows the number of perseverative errors made by the animals up to the first correct response in reversal learning. Animals with frontal lesions made meny more perseverative errors than the normal rats and these two groups differ at the level $p < 0.002$ (Mann-Whitney U-test, two tailed). Table II shows the number of perseverative errors during the

Table I

The number of perseverative errors made by the animals up to the first correct response in reversal learning

	Rat	with incomplete	without responses
Normals	1	8	8
	2	4	9
	3	0	11
	4	0	16
	5	5	8
	6	0	8
	7	0	0
	8	0	6
	9	2	13
	10	1	1
	Σ	20	80
Frontals	1	15	15
	2	29	26
	3	11	11
	4	25	25
	5	17	17
	6	11	11
	7	14	14
	8	14	14
	9	14	14
	10	19	19
	Σ	169	166

Table II

The number of errors during the first 20 runs in reversal learning

Rat	Errors	
	Normals	Frontals
1	16	18
2	18	20
3	14	17
4	18	20
5	15	19
6	13	17
7	18	19
8	14	19
9	16	19
10	16	20
Σ	158	188

first 20 runs in reversal learning. Frontal rats made a greater number of such errors than the normal animals (difference $p < 0.002$ — the same test as before). Table III shows the number of incomplete responses[1] in the first 20 runs in learning and reversal learning. Normal animals made many more incomplete responses than animals with frontal lesions in

[1] An incomplete response is, when an animal comes near to the door but does not touch it.

Table III

The number of incomplete responses in the first 20 runs in learning and reversal learning

	Rat	Learning	Reversal learning
Normals	1	4	0
	2	3	5
	3	1	2
	4	1	2
	5	2	3
	6	1	2
	7	1	4
	8	0	1
	9	1	1
	10	1	1
	Σ	15	21
Frontals	1	0	0
	2	2	0
	3	0	0
	4	2	0
	5	0	0
	6	0	0
	7	0	0
	8	0	0
	9	0	0
	10	0	0
	Σ	4	0

Table IV

The number of directional preferences in learning and reversal learning

	Rat	Learning	Reversal learning
Normals	1	98	217
	2	96	68
	3	81	139
	4	93	184
	5	75	45
	6	94	112
	7	47	132
	8	86	80
	9	43	27
	10	111	74
	Σ	824	1078
Frontals	1	33	29
	2	44	46
	3	101	138
	4	76	31
	5	35	46
	6	58	61
	7	80	38
	8	41	55
	9	138	22
	10	51	48
	Σ	657	514

learning as well as in reversal learning (differences are statistically highly significant $p < 0.002$). Table IV shows directional preferences [2] in learning and reversal learning.

Normal animals showed more directional preferences than frontal rats but in learning this difference is not statistically significant while in reversal learning it is ($p < 0.05$). The normal animals number of directional preferences is increased in reversal learning in comparison with learning while in the rats with frontal lesions a decrease of these preferences can be observed.

[2] The term directional preference is used when an animal makes at least 5 consecutive responses to the same direction.

DISCUSSION

Results of the present paper showed that the learning as well as reversal learning of the rats with frontal lesions are better than in normal animals in a black-white discrimination test. Comparison of the directional preferences during the training in both groups of animals explains the way in which normal animals have difficulty in performing these tasks. This data permits us to suppose that preliminary tendency of the normal rats is to solve such a task on the basis of kinesthetic cues and only when the corresponding responses appear to be inadequate during the training course the visual stimuli begin to play a dominant role in these animals. In reversal learning better fixation of the established habits was manifested by the fact that frontal animals made many more perseverative errors than normal animals. However, when the previously correct response is inhibited in frontal animals, the switching of the response to the new stimulus is rapid and the level of perfomance is high. On the other hand normal animals inhibit previously correct response much quicker than frontal ones, but their performance is low because they return to their tendency of directional preferences which is even stronger than during the original learning. Incomplete responses also give evidence that visual cues play a stronger role in the animals with frontal lesions, because normal rats make many more insomplete responses than frontal Ss.

The guestion arises, do frontal lesions change only the blance between kinesthetic and visual cues used by subjects, or do they impair other functions which are required during learning and reversal learning?

Experiments performed by Srebro and Dąbrowska (in preparation) in normal rats using four-unit-quadruple-choice apparatus in which every compartment was marked by different visual cues showed that if we take under consideration the number of runs, performance of the normal rats is similar to the performance of the Ss with frontal lesions as reported by Dąbrowska (1967). But if we compare number of errors and places in the maze in which these errors were made normal animals differ from frontal Ss.

On the basis of our experiments it can be concluded that two symptoms are observed in the rats with frontal lesions which distinguish them from normal animals. The first, the change of the balance between kinesthetic and visual stimuli used by animals. The second, impairment in integrative processes involved in reversal learning of the chain reflexes.

The present paper shows clearly the first symptom. Consequently, if the task can be solved only on the basis of visual stimuli, performance of the frontal rats is much better than normal ones.

SUMMARY

Frontal and normal rats were trained in learning and reversal learning in black-white discrimination test using food reinforcement. Three measures were used to show differences between the animals: (i) number of correct responses in blocks consisting of 50 trials in learning and reversal learning; (ii) number of perseverative responses in reversal learning; (iii) number of directional preferences in learning and reversal learning. Statistical analysis of variance showed significantly high differences between frontal and normal animals in all measurements. Rats with frontal lesions solved black-white discrimination test better and quicker than normals. The mechanism of this improvemment is discussed.

REFERENCES

DĄBROWSKA, J. 1962. An analysis of reversal learning in relation to the complexity of task in white rats. Acta Biol. Exp. (Warsaw) 22: 139—145.

DĄBROWSKA, J. 1963. An analysis of reversal learning in relation to the pattern of reversal in rats. Acta Biol. Exp. (Warsaw) 23: 11—24.

DĄBROWSKA, J. 1964. Reversal learning in frontal rats. Acta Biol. Exp. (Warsaw) 24: 19—26.

DĄBROWSKA, J. 1964. Multiple reversal learning in frontal rats. Acta Biol. Exp. (Warsaw) 24: 99—102.

DĄBROWSKA, J. 1967. Reversal learning in relation to the pattern maze alterations in frontal rats. Acta Biol. Exp. (Warsaw) 27: 421—428.

LASHLEY, K. S. 1931. The mechanism of vision: IV. The cerebral areas necessary for pattern vision in the rats. J. Comp. Neurol. 53: 419—478.

LINDQUIST, E. F. 1953. Design and analysis of experiments in psychology and education. Riverside Press.

ZUBEK, J. P. 1951. Studies in somesthesis. I. Role of the somesthetic cortex in roughness discrimination in the rat. J. Comp. Physiol. Psychol. 44: 339—353.

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RETURNING BEHAVIOR IN FRONTAL RATS

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In a previous series of experiments the returning behavior of white rats was extensively investigated (Łukaszewska 1959, 1961, 1963a 1963b). Generally the S was required to go for food to some place and then to return to the start by the same route. From the start to the food the S was guided visually by the sight of the cup with food; on the return way the S was confronted with a choice of two paths. As has repeatedly been found, normal Ss responded correctly in about 90% of trials in a given testing period. The high percentage of correct responses was observed from the very beginning of testing which suggests that this type of behavior is unlearned, or learned in the pre-experimental period of life in the home-cage. While returning to the starting point rats do not respond to actual stimuli but to the traces of stimuli acting several seconds earlier. There is strong evidence that the most important cue is the trace of proprioceptive stimuli elicited by turns on the way to food (Łukaszewska 1963a).

Since the returning behavior can be counted as a modification of a delayed response test it seemed interesting to examine frontal animals which have been reported deficient in the problems involving the delay of reaction.

METHOD

Subjects. Fifty-one male albino rats of Wistar strain 3—4 mo old at the time of experiment were used. Thirty Ss underwent bilateral removal of the frontal poles under nembutal anaesthesia. In six Ss a lesion of comparable size was placed in

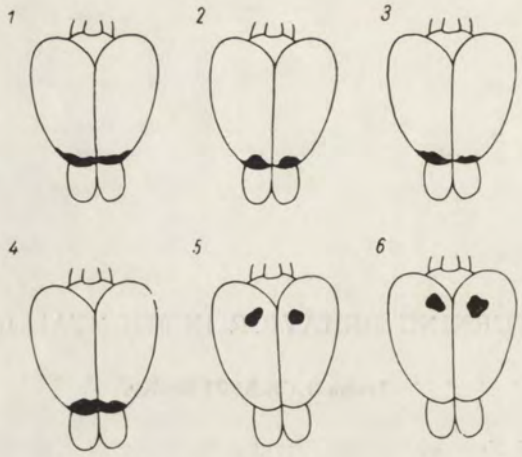

Fig. 1. Representative lesions in frontal (1—4) and occipital (5,6) cortex

occipital cortex. Typical examples of frontal and occipital lesions are presented in Fig. 1. The remaining 15 served as unoperated controls.

Apparatus and procedure. Experiments were carried out in a modified T maze (Fig. 2). The S was required to leave the small cage which was placed on one of the two starting platforms (S_1 or S_2), reach the cup on the maze stem, take the food (small pieces of cookie) and return to the cage where he was allowed to eat. The intertial intervals were controlled by the behavior of S: generally, after finishing one food portion the S immediately went for another one (30—40 sec). The Ss were permitted to correct an error in the same trial. Full description of the method has been given previously (Łukaszewska 1961).

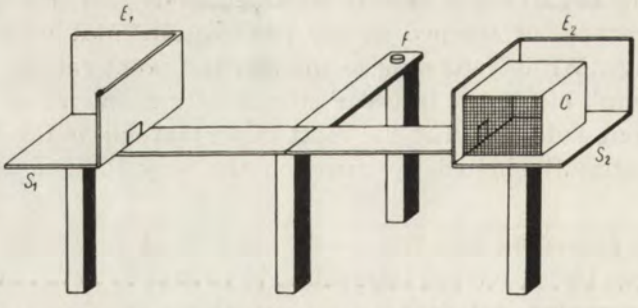

Fig. 2. Elevated T maze. S_1, S_2, starting platforms; C, cage; E_1, E_2, wooden screens; F, cup with food

The returning behavior of all Ss was tested in 10 sessions. Each session consisted of 3 trials in which the cage remained on the same starting platform. The position of the cage was changed from day to day either in the alternation sequence (L, R, L, R.....) or in a double alternation sequence (L, L, R, R,....).

Three experiments were performed with the differences presented in Table I.

Table I

Experimental design

	Group	No of Ss	Operation	testing		starting platform position
				before operation	after operation	
Experiment I	CN	15	unoperated	+	−	L, R, L, R...
	F_1	10	frontal	−	+	L, R, L, R...
Experiment II	CO	6	occipital	+	+	L, R, L, R...
	F_2	9	frontal	+	+	L, R, L, R...
Experiment III	F_3	10	frontal	−	+	L, L, R, R...

RESULTS

The results will be reported in terms of the percentage of correct responses of all Ss in a given group, scored separately for each trial in a session during 10 days.

Experiment I

Returning ability of control unoperated Ss and frontal Ss tested after the operation

The Group CN performed very well; in trial no I above 90 per cent of correct responses were observed and this number increased in trial no II and no III up to the level of 100%. In contrast, the Group F_1 achieved only 70% of correct responses in trial no I and though it improved significantly in trial no II ($p < 0.05$, Wilcoxon matched-pairs, signed-ranks test, two tailed), it failed to reach the level of normal Ss (Fig. 3). The difference in performance between normals and frontals is statistically confirmed for all three trials ($p < 0.05$ Mann Whitney U test, two tailed).

It should be noticed that individual differences in frontal Ss were large. Half of the animals performed poorly but the scores of remaining Ss were indistinguishable from the scores of normal Ss (Fig. 4). As we see, two frontal Ss made no errors, the other three — only two errors. These rats seemed to be not affected by the operation and it could not be attributed

to the size of the lesion. However, since we studied only postoperative performance, it is not possible to judge whether moderately inferior performance reflects the moderate effect of the frontal damage or only individual lower returning ability. This problem will be tested in Experiment II.

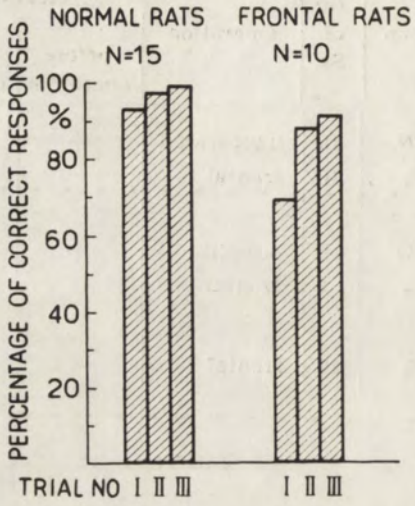

Fig. 3. Percentage of correct responses in normal and frontal rats. I—III successive trials of every experimental session. Each column represents the percentage of correct responses by all Ss during 10 days' testing

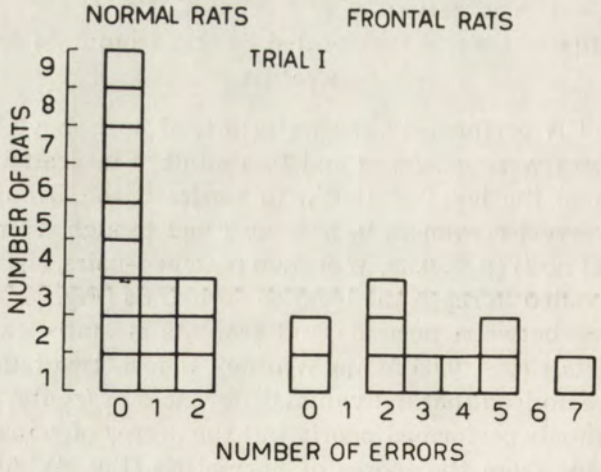

Fig. 4. The diagram showing number of rats which made different number of errors in trial no I during 10 days' testing

Experiment II

Pre- and postoperative performance in frontal and control operated Ss

In trial no I the performance of Group F_2 clearly decreased after the frontal operation, whereas no changes were observed in the performance of Group CO subjected to the occipital operation. In trial no II and no III the Ss of both groups performed on the same level before and after the operation (Fig. 5). Comparing the performance of individual animals in

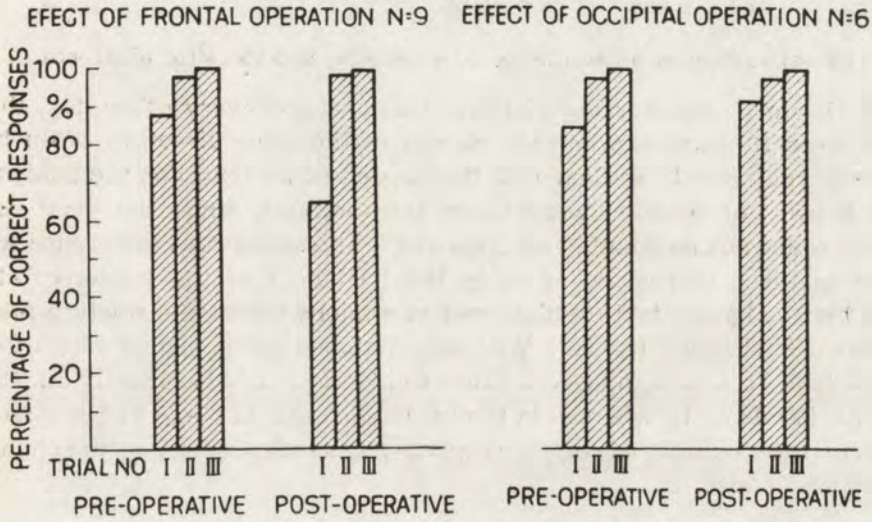

Fig. 5. Pre- and postoperative performance by frontal and occipital rats. Denotations as in Fig. 3

trial no I one can notice that all but one frontal Ss earned poorer scores after the operation with differences as large as 4—5 scores in four Ss. Even the scores of two Ss which performed considerably well after the operation (1—2 errors) were somewhat better before the operation; only one animal out of 9 „improved" after the operation (one error less). This result suggests that frontal damage affects all subjects, but the degree of deficit is different.

Out of six occipital Ss four Ss performed 1—2 errors less than before the operation and the remaining two Ss performed one error more. The difference between the Group F_2 and Group CO is statistically significant at the level of $p<0.05$ (the Fisher exact probability test, two tailed). Thus the effect of frontal lesion is specific and cannot be attributed to any cortical damage.

It should be emphasized that no improvement in returning behavior was observed during successive sessions. Both Groups F_1 and F_2 showed about the same degree of deficit in the first half of the series as in the second one, except the very first experimental session where only two Ss committed errors in trial no I. The responses of the remaining 17 Ss were correct. Therefore it seemed interesting to test how much the frontal animals were disturbed by the experimental schedule, i. e. alternation sequences of changing the starting place.

Experiment III

Double alternation sequence of changing the starting platform

In Group F_3 the starting platform was changed every other day, thus there were 10 sessions when the Ss ran in the same direction as on the preceding day and 10 sesions with the opposite direction. For the analysis, only 9 pairs of sessions were taken into account, since the very first session could not be counted as „changed". The significant difference was found in the performance of Ss in the trial no I of the sessions following the change of starting place and in sessions where the starting place remained unchanged ($p<0.01$ Wilcoxon matched-pairs, signed ranks test, two tailed). In the changed condition the Ss responded correctly in 71% of trials (trials no I), whereas in the unchanged one in 85%. In trials no II and no III the number of correct responses was independent on the change of starting place.

DISCUSSION

Impairment of delayed responses and delayed alternation has been repetitively found in higher mammals subjected to frontal operations. Similarly frontal rats showed the deficit in alternation (Loucks 1931, Hunter and Hall 1941, Morgan and Wood 1943, Pickett 1952, Gross and al. 1965); delayed responses, however, have hitherto not been tested. The present paper presents the method comparable to delayed response test used in experiments with higher mammals.

Contrary to the delayed response situation for higher mammals the nature of the stimulus trace in returning behavior ist defined. As was mentioned before the correct response is based on the proprioception of turns performed by the animal on the way to food.

The turns on the return way and on the way to food are opposite, although it does not mean that the correct response is elicited automatically by the sequence of turns (LR or RL).

Our frontal rats showed clear, although not dramatic, decrease of performance, mainly in the first trial of every session. Since the correct response requires the ability to use recent information it seems reasonable to conclude that the deficit observed in our test is produced by the impairment of immediate memory. However, this hypothesis is weakened by the finding that in the very first session 17 out of 19 rats responded correctly. Obviously when the animals have no previous experience in the relevant behaviour they are able to solve the problem as in session I, but in subsequent sessions performance is poorer. Thus the version proposed by Konorski and Ławicka (1964) fits our results better. According to these authors, the essential factor producing the deficit in delayed responses of frontal animals is the weakening of the reflexogenic strength of the trace stimuli. It can easily be imagined that even the weak trace stimulus can elicit the correct response when it does not have to compete with other intervening stimuli.

On the other hand, since the performance is better in all trials in which the starting platform remains unchanged and deteriorates after the change of the starting platform, one can suppose also that the deficit is produced by a tendency to repeat the response made on the preceding trial. The only difficulty is that in the trials no I the previous response was made on the preceding day, thus it is not the perseverative tendency in the ordinary sense. This problem will be elaborated in more detail in further experiments.

It should be mentioned that few animals were not handicapped by the frontal lesion and this fact cannot be explained in terms of the size of the lesion. It rather points to the possibility of the existence of more than one mechanism in solving the delayed response test. Similar findings with similar interpretation were reported by Olds (1966) for frontal stimulation in the monkey.

SUMMARY

Returning ability of 30 frontal Ss was tested in a modification of a simple T maze. Control groups consisted of 15 unoperated Ss and 6 Ss lesioned in occipital cortex. The S had to go for food and to return to the start point by the same route. The correct return response required the immediate memory of turn performed on the way to food. Frontal Ss showed significantly larger number of errors than control unoperated or occipital Ss. The performance of frontal Ss improved in trials in which the direction of runs remained unchanged. Impairment of immediate memory and a tendency to perseveration are discussed as the factors responsible for the observed deficit in frontal Ss.

REFERENCES

GROSS, C. G., CHOROVER, S. L. and COHEN, S. M. 1965. Caudate, cortical, hippocampal and dorsal thalamic lesions in rats: alternation and Hebb-Williams maze performance. Neuropsychologia 3: 53—68.

HUNTER, W. S. and HALL, B. E. 1941. Double alternation behavior of white rat in a spatial maze. J. Comp. Physiol. Psychol. 32: 253—266.

KONORSKI, J, and ŁAWICKA, W. 1964. Analysis of errors by prefrontal animals on the delayed-response test. In: Warren, J. M. and Akert, K. (ed.), The frontal granular cortex and behavior. McGraw-Hill Book Co., New York, p. 271—294.

LOUCKS, R. B. 1931. Efficiency of the rat's motor cortex in delayed alternation. J. Comp. Neurol. 53: 511—567.

ŁUKASZEWSKA, I. 1959. Return reaction as a test of the space orientation of white rats in the horizontal and perpendicular plane. Acta Biol. Exp. (Warsaw) 19: 273—280.

ŁUKASZEWSKA, I. 1961. A study of returning behavior of white rat on elevated maze. Acta Biol. Exp. (Warsaw) 21: 253—265.

ŁUKASZEWSKA, I. 1963a. Sensory cues in return reaction. Acta Biol. Exp. (Warsaw) 23: 249—256.

ŁUKASZEWSKA, I. 1963b. Delay in return reactions in rats. Acta Biol. Exp. (Warsaw) 23: 257—262.

MORGAN, C. T. and WOOD, W. M. 1943. Cortical localization of symbolic processes in the rat. II. Effect of cortical lesions upon delayed alternation in the rat. J. Neurophysiol. 6: 173—180.

OLDS, M. E. 1966. Effects of electrical stimulation and electrocoagulation in cortex and thalamus on the delayed response in monkeys. Exptl. Neurol. 15: 37—53.

PICKETT, J. M. 1952. Non equipotential cortical function in maze learning. Amer. J. Psychol. 65: 177—195.

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TWO CASES OF EXPERIMENTAL NEUROSES IN DOGS CURED BY A TEMPORARY CHANGE OF REINFORCEMENT

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The treatment of neuroses is a difficult problem for both the animal experimenter and clinician. The systematic investigations of experimental neuroses in dogs by the Pavlovian School showed the good therapeutic effect of the change of the CSs, especially those connected directly with the genesis of neurosis (Ivanov-Smolenski 1949). On the other hand, the effect of the change of the US on the neurotic state is much less known. In the present paper two cases will be described, in which a temporary replacement of one kind of reinforcement by another caused the elimination of the neurotic symptoms.

RESULTS

Bekas. This dog (castrated) was for two years an excellent experimental animal. He behaved very quietly in the experimental chamber, and his salivary alimentary CRs were very regular (bread moistened with broth was used as the US, and a buzzer and an intermittent light as the CSs).

During one session an electric shock was applied twice to the leg of the dog. This produced a neurotic state. The dog became very disquiet in the experimental chamber, his CRs became small and irregular and he often refused to eat. During several weeks these neurotic symptoms gradually disappeared but from this time onwards the dog was always less quiet during experiments and his CRs less regular.

After about one year the dog fell off experimental table on the floor and hung for a while in the harness. Next day the same neurotic symptoms

as he exhibited one year previously (anxiety, irregularity of CRs and disinclination for food) appeared. In the days following, the dog became more and more neurotic and finally he refused to eat at all in the experimental chamber although he ate voraciously in the pre-chamber. Such treatment procedures as starving him in the animal house and the discontinuation of the CSs (during the experiment only food was given) did not produce any improvement.

After about one month of the neurotic state, the food was changed and instead of bread moistened with broth the dog obtained pure meat which was not preceded by the CS. Now gradually the neurotic symptoms disappeared. After about two weeks we returned to the old reinforcement and this change produced only a very transient neurotic disturbance. Next we began to reapply the old CSs with the same good result. However, the dog continued to be a little unquiet in the experimental chamber and his CRs were not quite regular.

About six months later, the food reinforcement was discontinued (the dog came satiated to the experimental chamber); and the defensive US, namely the weak solution of acetic acid introduced into the mouth, was applied. With this reinforcement, the CRs to new stimuli (bubbling and metronome) were elaborated. Under these experimental conditions the dog worked very well for the following three years. His acid salivary CRs were now again as regular as the alimentary CRs in the beginning of his CR career.

Chudzik. This dog was a naive experimental animal. In the very beginning of the experiments he behaved very timidly in the CR chamber. His first task was to learn the simple instrumental CR (lifting of the right foreleg) which was reinforced by water (during experiments the dog was thirsty because he received a portion of sodium chloride in his morning meal in the animal house). In the beginning of training, the instrumental CR was elaborated to the whole experimental situation: i. e. immediately after the dog had finished drinking water given as reinforcement, he performed the next movement which was also reinforced by water.

At this stage the sporadic CS (metronome) was introduced, i. e. only the movements performed during its action were reinforced. Already on the second day of this new training the dog began to exhibit strong neurotic symptoms. During the experiment he was anxious, growled, defecated, and refused to drink water although after the experiment he drank it willingly in the animal house.

After four days we stopped applying the metronome and again the instrumental CR of the dog was always reinforced. Then during a few days the dog slowly improved. But when the metronome was introduced

once more, all neurotic symptoms reappeared. In the weeks following the metronome was used several times and always with the same bad result.

During this time we observed that although the dog refused to drink, he nevertheless ate willingly in the experimental chamber. Therefore, it was decided to give as a reinforcement the food (bread cubes) instead of water. The morning meal of the dog was eliminated (he came unthirsty but hungry to the experimental chamber), and during the experiment his instrumental CR was always reinforced by food (the metronome was not presented). As a consequence of this change, which was done in the period of severe neurotic symptoms, the dog improved immediately. It is also worth noting, that the CR was not disturbed at all by the change of reinforcement. This is in accordance with observations of Żernicki and Ekel (1959) who showed that there is a considerable generalization of instrumental CRs to thirst and hunger stimuli in a dog.

After a few days the metronome was again introduced and the intertrial responses not reinforced. This time this procedure produced only very temporary anxiety of the dog. After about three weeks of training the intertrial responses were extinguished almost completely.

At this stage we returned again to the water reinforcement. This produced no neurotic symptoms at all. The dog continued to perform the instrumental CR only to the metronome and behaved quietly in the intertrial intervals.

DISCUSSION

The essential outcome of our observations is the therapeutic effect of the temporary change of reinforcement during the acute stage of neuroses (with Bekas meat was used instead of bread for 2 weeks, and with Chudzik bread instead of water for 3 weeks). Masserman (1946) showed that the essential factor in the treatment of a neurosis in a cat is forcing it to eat again in the experimental chamber. Among other things he achieved this by seasoning the food with catnip. It seems to be probable that in our cases the rapid relief of the neurotic state was obtained also due to the acceptance of the new positive reinforcement. It should be considered, however, why it was accepted by our dogs.

In the case of Bekas the new reinforcement (meat) was both different and more attractive. Probably both factors helped in its acceptance in the experimental chamber. It should be noted, however, that due to the new reinforcement the appetite of Bekas was increased but not his hunger; the latter depends on food deprivation which remained unchanged. This distinction is important because it is well known that simple starvation

does not cure the neurosis (it may even have an opposite effect, Masserman 1946), and in fact during starvation Bekas did not accept the food in the experimental chamber.

In the case of Chudzik the reinforcement and the drive stimulus were changed: water into food and thirst into hunger respectively. Again it seems to be probable that both these changes played a role in the curing of the neurosis. The introduction of the thirst drive was obviously the necessary condition for acceptance of the water reinforcement. It is interesting, however, to speculate whether or not Chudzik would accept it equally willingly when he remained also hungry during the „water experiments".

The disappearence of the residual neurotic symptoms in Bekas after the elimiation of the alimentary drive and reinforcement and introduction of the aversive reinforcement (acetic acid) should be also discussed. It may be noted that a similar observation was made by Gantt (1944): in a dog the long-lasting neurotic state, which appeared as a consequence of a difficult differentiation, was reliefed by the change of alimentary reinforcement into the aversive one (electric shock). In Bekas, however, both the stimuli producing neurosis (electric shock, falling off the table) and the stimulus curing it were aversive. We see, therefore, that in this dog in the alimentary situation the defensive element connected with the previous single application of the aversive stimulus was neurogenic, but in the defensive situation the alimentary element connected with previous alimentary training was not. In other words, in the alimentary-defensive „clash" the alimentary and defensive factors did not play a symmetrical role.

SUMMARY

The temporary change of reinforcement (from one food to another, and from water to food) produced rapid relief from neurotic symptoms.

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REFERENCES

GANTT, W. H. 1944. Experimental basis for neurotic behavior. Psychosom. Med. Monogr. 3, no 3 and 4.

IVANOV-SMOLENSKI, A. G. 1949. Ocherki patofiziologii vysshei nervnoi deyatelnosti. Medgiz, Moskva.

MASSERMAN, J. H. 1946. Behavior and neurosis. Univ. Chicago Press, Chicago.

ŻERNICKI, B. and EKEL, J. 1959. Elaboration and mutual relations between alimentary and water instrumental conditioned reflexes in dogs. Acta Biol. Exp. (Warsaw) 19: 313—325.

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STUDIES ON THE PERCEPTION OF COLOURS IN SOME *PAPILIONIDAE (LEPIDOPTERA)*

I. DISCRIMINATION BETWEEN COLOURED AND NEUTRAL SURFACES WITH SPECIAL REFERENCE TO RED SENSITIVITY

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In the year 1913, the well known oculist von Hess brought forward some evidence to support the claim that insects are totally colour blind. He did not deny the fact that they can distinguish between papers of different colours, but maintained that this discrimination involved, not the colour quality of the papers concerned, but their degree of brightness, texture, odour, etc. Further work by numerous distinguished workes not only disproved the above supposition, but also brought to light the fact that among insects probably no two species possess exactly the same type of colour vision. Moreover, it was observed that a large number of insects are either „short" in red or are completely red-blind, with the result that the perception of his colour by an insect attained a special significance.

In this paper the results of experiments on the colour discrimination in three species of *Papilionidae, Papilio demoleus, Polydorus aristolochiae* and *Graphium agamemnon* are described. Special attention was paid to the study of sensitivity of these insects to the colour red.

MATERIAL AND METHOD

Papilio demoleus is a pest on *Citrus* plants and other *Rutaceae* in the larval form. The larvae of *Polydorus aristolochiae* commonly feed on the leaves of *Aristolochia indica* while those of *Graphium agamemnon* attack *Anona, Polyalthia, Michelia* and other *Anonaceae.* All three species have a very wide distribution in south and south-east Asia (Talbot 1939).

The experiments with *Papilio demoleus* were performed in a special experi-

mental part of a large insect observation cage measuring 4m in lenugth, 4m in breadth and 2m in height. Those with other species were conducted in another slightly smaller cage. The imagines of insects used in the experiments were reared from eggs previously collected on the respective host plants. This assured that they had no previous experience with colour, and consequently, all complications arising due to previous training to certain colour were avoided very effectively. Natural feeding was replaced by feeding with dilute sugar sulution with a camel-hair brush.

The coloured papers used in these experiments belonged to the standardized Ostwald series, which consists of 24 hues or colours, ranging from yellow to yellow-green via orange, red, purple, blue, blue-green and green. Each of the papers is indicated by a numeral, e. g., numbers 7 and 14 represent spectrum-red and spectrum-blue colours respectively. Added to the numbers are letters indicating the degree of saturation of the colours. The suffix „pa" indicates the Saturated Ostwald series. Thus „pa7" means spectrum-red colour of the Saturated Ostwald series. Series „ia" and „ea" are obtained by successively increasing dilutions of a colour with white. In addition, papers of the Bauman Grey series were used. This series consists of 30 steps of grey shades ranging from withe to dark grey or almost black, in fine gradation.

Each Ostwald paper reflects a broad but specific wave-length band. The wave-lengths reflected by some of the Ostwald papers have been mentioned by Hertz and Imms (1937). Thus, the spectral area they reflect is selective in contrast to that reflected by a grey paper, which reflects all the wave-lengths of our own visible spectrum in their normal proportion (as found in „white" light). In the following experiments therefore, in Ostwald paper represents a coloured surface and a Bauman paper represents a neutral surface.

Artificial flowers measuring about 5 cm in diameter were prepared from the coloured and grey papers. Each flower was provided with a central glass test-tube about 1 cm in diameter. With the help of the test-tubes the flowers were arranged on a piece of wire-netting held horizontally on a wooden frame. When artifical flowers are thus offered to the hungry insects, they show a characteristic feeding response; each one approaches the artifical flower in flight, lands on it, unrolls its tongue and performs probing and sucking movements with it on the flower surface. Such responses on each of the papers were counted separately and conclusions were drawn therefrom.

EXPERIMENTS AND OBSERVATIONS

The investigations carried out can be broadly divided into the following two series of experiments:

1. *Experiments on the fundamental ability to perceive colours, i. e., on the discrimination between coloured and neutral surfaces.*

The aim of these experiments was to determine whether these insects possess the faculty of perceiving different colours. Twenty artificial flowers were offered to a batch of freshly emerged hungry insects. Out of these twenty artificial flowers, two belonged to a certain Ostwald Saturated coloured paper and the remaining eighteen belonged to the different shades of the Bauman Grey papers. This procedure was repeated

separately for each of the Ostwald Saturated colours (omitting the spectrum-red no. 7, which is dealt with later) and for each of the three species of Papilionids.

In a certain experiment, one of the series of the Bauman Grey papers is bound to match the Ostwald Colour paper under consideration in its „luminosity value" to the insect concerned. Therefore, it is logical to expect that an insect blind to the colour under consideration will confuse the coloured paper with the grey paper on account of their equal luminosities. Results of all experiments are shown in Table I.

Table I *

Results of experiment series 1

Ostwald paper	Colour	Total number of feeding responses			Number of feeding responses on colour			Percentage of colour vision		
		PD	PA	GA	PD	PA	GA	PD	PA	GA
pa 1	Yellow	28	25	25	20	21	18	71.4	84	72
pa 2	Yellow	25	25	25	21	22	17	84.0	88	68
pa 3	Orange yellow	20	25	25	16	20	17	80.0	80	68
pa 4	Orange	34	25	25	32	22	20	94.1	88	80
pa 5	Orange	22	25	25	22	25	23	100.0	100	92
pa 6	Red	24	25	25	24	25	24	100.0	100	96
pa 7**	Red	—	—	—	—	—	—	—	—	—
pa 8	Redish purple	26	25	25	26	25	22	100.0	100	88
pa 9	Purple	34	25	25	32	25	20	94.1	100	80
pa 10	Purple	21	25	25	14	25	22	66.6	100	88
pa 11	Purple	38	25	25	38	25	25	100.0	100	100
pa 12	Blue	23	25	25	23	25	24	100.0	100	96
pa 13	Blue	25	25	25	24	25	25	96.0	100	100
pa 14	Blue	21	25	25	21	23	18	100.0	92	72
pa 15	Blue	22	25	25	18	20	19	81.8	80	76
pa 16	Blue green	20	25	25	14	19	19	70.0	76	76
pa 17	Blue green	00	00	00	00	00	00	00	00	00
pa 18	Blue green	00	00	00	00	00	00	00	00	00
pa 19	Blue green	00	00	00	00	00	00	00	00	00
pa 20	Green	00	00	00	00	00	00	00	00	00
pa 21	Green	00	00	00	00	00	00	00	00	00
pa 22	Yellow green	00	00	00	00	00	00	00	00	00
pa 23	Yellow green	20	25	25	14	20	17	70,0	80	68
pa 24	Yellow green	21	25	25	14	19	17	66,6	76	68

* In this, as well as in all the remaining tables, following short forms have been used to denote the different species: PD = *Papilio demoleus;* PA = *Polydorus aristolochiae* and GA = *Graphium agamemnon.*

** Responses to Ostwald paper pa 7 (Spectrum red colour) have been investigated separately.

What sort of results would be obtained in the absence of the fundamental ability of the perception of colour? The proportion of the coloured flowers to the total number of flowers offered in each sub-experiment being 2 : 20, the coloured flowers should receive only 10% of the total number of visits in that sub-experiment. Let us consider visits between 10% and 40% as being within the range of error. In that case, a frequency of more than 40% of the total visits to coloured flowers is definitely an indication of a decided preference to that colour. That means that the colour under consideration is spontaneously differentiated by the insect from amongst all the neutral grey stages.

In the present series of experiments, however, the percentage of visits by each of the three species is appreciably higher (i. e., above 66%). It will be noted from the results that colours between pa 1 and pa 16, and those from pa 23 to pa 24 are easily distinguished by the imagines of *Papilio demoleus, Polydorus aristolochiae* and *Graphium agamemnon* from the neutral grey shades. The blue-green and the green colours (pa 17 to pa 22) were not responded to, showing thereby that they are not proper feeding colours for them.

2. *Experiments on the sensitivity to red colour.*

The response of the imagines of the three species to this colour was worked out in some details as follows.

i) In this experiment, artificial flowers prepared from the Saturated red Ostwald popers (pa 6 and pa 7) together with the same hues successively diluted with white in two different steps (ea 6, ia 6, ea 7 and ia 7) were offered. Thus, six types of red Ostwald papers were used which differed from each other in saturation and brightness. Each flower was offered in duplicate. To these twelve red flowers were added thirteen flowers prepared from the different Bauman Grey shades. This group of twenty five flowers was then offered to the hungry insects after arranging the flowers in five rows of five flowers each. The effect due to a particular flower occupying a certain advantageous or disadvantageous position was minimised initially by using two flowers of each red paper and then almost excluded by turning the whole arrangement through an angle of 90 degrees very often during the course of the experiment.

In the absence of red perception, the grey papers comparable in luminosity to the respective red papers should have received an appreciable number of visits. However, the results of these experiments carried out separately for the three species (Table II) show that the red papers received all the visits and there were none on the greys. This indicates that the red colour is easily distinguished by all of them from the neutral grey shades of the corresponding degree of brightness.

Table II

Results of experiment series 2a

Ostwald (red) paper	Distribution of feeding responses on different red papers (in percentages)			Total number of feeding responses			Total number of feeding responses on grey papers		
	PD	PA	GA	PD	PA	GA	PD	PA	GA
pa 6	4.3	5	7	184	100	100	00	00	00
pa 7	13.0	27	18						
ia 6	34.7	30	28						
ia 7	24.0	24	25						
ea 6	15.2	9	11						
ea 7	8.6	5	11						

ii) This interesting experiment was a crucial test which confirmed the presence of distinct red vision in these insects. Only five flowers prepared from the Ostwald Saturated spectrum-red paper (pa 7) were offered together with 20 flowers of the different shades of the Bauman Grey papers. The results for all three species are shown in Table III. In the absence

Table III

Results of experiment series 2b

Number of feeding responses on Ostwald paper pa 7 (saturated spectrum red colour)			Number of feeding responses on Bauman grey papers			Total number of feeding responses		
PD	PA	GA	PD	PA	GA	PD	PA	GA
69 (98.5)	50 (100)	48 (96)	1 (1.5)	0 (0)	2 (4)	70	50	50

Figures in brackets represent percentages.

of red vision, and only taking into consideration the number of flowers offered, it is evident that each of the twenty five flowers should have received 4% of the total number of visits. Thus, the expected result would have been — 20% visits on the red colour and 80% visits on the grey shades. The actual result showed that there were 96% to 100% visits on red flowers and only 4% to nil on the grey flowers.

Thus, it now becomes absolutely convincing that the imagines of these theree species of Papilionids were attracted to the saturated red colour of the articial paper flower because of its colour quality and not because of its particular degree of brightness.

DISCUSSION

It must be noted first of all that the results obtained with these Ostwald coloured papers cannot be interpreted directly in terms of specific spectral regions, since each of these coloured papers reflects a more or less broad spectral band. Moreover, each one differs in the degree of saturation of the colour from the rest of the series. However, the work done on *Daphnia* by means of such pigment colours has been fully confirm-ed by similar work done with spectral colours (Köhler 1924). Also, Kühn's training of bees with pure spectral colours (1927) has yielded results which are in good agreement with those obtained by von Frisch (1914) with the help of the Hering series of coloured papers. The analysis of light reflected by certain Ostwald coloured papres (Hertz and Imms 1937) has revealed that there is, in each paper, a single predominant colour, while the other colours are reflected in such small quantities as to be safely termed „slight impurities".

As already stated at the outset, insects differ very widely as regards the extent of their visible spectrum towards the red end. Many insects are already shown to be red blind, of which the following are some of the examples. The European hive bee *Apis mellifera* has been shown to confuse the Hering paper no. 1 (red, which according to Knoll's measurement reflects light beyond 648 mμ) with dark grey (von Frisch 1914). The experiment with spectral colours has shown that for them the actual visible spectrum ends at about 650 mμ (Kühn 1924 and 1927; Berthoff 1928). *Vespa rufa* is another hymenopteran which is proved to be red blind (Schremmer 1941). Among *Diptera, Fannia canicularis* has also been found to be red blind (Wasmann 1918).

One of the earliest experiments in which the red vision in *Lepidoptera* was studied was performed by Eltringham (1919). In his experiments, he used a dye transmitting only the red rays with which he painted the eyes of different species. He came to the conclusion that *Vanessa urticae (Vanessidae)* has perfect red vision and that *Pieris brassicae, Pieris napi* and *Pieris rapae (Pieridae)* were distinctly „shorter" in red than *Vanessa urticae*. Subsequently however, Ilse's more refined experiments (1928) using the Hering series of coloured papers have shown that for the Vanessids the limit of the perception is somewhat curtailed at the red end, and that Pierids possess well developed red vision. The hawkmoth

Macroglossum stellatarum has been shown to be red blind. It almost mistakes Hering no. 1 (red) for dark grey (Knoll 1922). It was also found that the feeding reactions on the Hering no. 1 and 2 (red) followed only training on dark grey papers.

The present series of experiments has clearly shown the presence of distinct red vision in *Papilio demoleus, Polydorus aristolochiae* and *Graphium agamemnon*. They thus belong to that small group of insects which possess this faculty.

About vision in other colours it can be said that as far as the regions of the regions of the Ostwald papers pa 1 to pa 16 and pa 23 to pa 24 are concerned, these insects are capable of differentiat-ing the colours concerned from the neutral greys. However, it may not be supposed that they are blind to the intermediate blue-green and green colours represented by the Ostwald papers pa 17 to pa 22. In fact it has already been proved, at least in case of *Papilio demoleus,* that this whole range which is neglected in the feeding state is chosen by it in preference to all other colours during oviposition (Vaidya 1959). This indicates that *Papilio demoleus* (and most probably, the remaining two species investigated here) can distinguish the green and the blue-green areas from the adjoining regions on either side of it within the spectrum visible to them.

The present series of experiments bring out clearly a very striking similarity in the colour perception of the three species of *Papilionidae*. Therefore, the results obtained here would probably serve to illustrate the type of colour vision of this family in general and would be helpful in comparing such studies on various sub-groups of *Lepidoptera*.

SUMMARY

1. Freshly emerged imagines of *Papilio demoleus, Polydorus aristolochiae* and *Graphium agamemnon (Papilionidae, Lepidoptera),* which had no previous experience with colour, were kept in a large cage devoid of any coloured objects apart from those specially provided for the experiments.

2. The hungry insects were offered artificial flowers prepared from papers of the Ostwald colour series and the Bauman grey series. In these experiments, an Ostwald paper represented a coloured surface while a Bauman paper represented a neutral surface.

3. The insects showed on these artificial flowers a characteristic feeding response.

4. The number of feeding responses on each of the coloured and grey surfaces were counted separately; from these, the inferences were drawn regarding their ability for colour discrimination.

5. It was found that all the three species showed an ability for colour discrimination (though no inferences could be drawn about their perception of blue-green and green colours with the help of feeding responses). They were remarkably sensitive to red colour.

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REFERENCES

BERTHOLF, L. 1928. Reactions of the honeybee to light. J. Agricult. Res. 42: 379—419.

ELTRINGHAM, H. 1919. Butterfly vision. Trans. Entomol. Soc. London, p. 1—49.

FRISCH, K. von 1914. Der Farbensinn und Formensinn der Biene. Zool. Jb. Abt. Allg. Zool. Physiol. 35: 1—188.

HERTZ, M. and IMMS, A. D. 1937. On the responses of the African migratory locust to different types of background. Proc. Roy. Soc. London, Series B, 122: 281—297.

HESS, C. von 1913. Gesichtssinn. In: Winterstein, Handbuch der vergleichenden Physiologie, B. 4, Fischer Verlag, Jena.

ILSE, D. 1928. Über den Farbensinn der Tagfalter. Z. Vergl. Physial. 8: 658—692.

KNOLL, F. 1922. Insekten und Blumen. II. Lichtsinn und Blumenbesuch des Falters von *Macroglossum stellatarum*. Abh. Zool. Bot. Ges. Wien, 12.

KÖHLER, O. 1924. Über das Farbensehen von *Daphnia magna*, Z. Vergl. Physiol. 1: 84—174.

KÜHN, A. 1924. Versuche über das Unterscheidungsvermögen der Bienen und Fische für Spektrallichter, Nachr. Ges. Wiss. Göttingen, math.-phys. Kl.

KÜHN, A. 1927. Über den Farbensinn der Bienen, Z. Vergl. Physiol. 5: 762—800.

SCHREMMER, F. 1941. Versuche zum Nachweis der Rotblindheit von *Vespa rufa* L. Z. Vergl. Physiol. 28: 457—466.

TALBOT, G. 1939. Fauna of British India, Butterflies. Vol. I. Taylor and Francis, Ltd, London.

VAIDYA, V. G. 1959. Observations and experiments on the behaviour of the egg-laying female of *Papilio demoleus* L. Abstr., Proc. Ind. Sci. Congr. 3: 381.

WASMANN, E. S. J. 1918. Totale Rotblindheit der kleinen Stubenfliege *Homalomyia canicularis*. Biol. Zol. 38.

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MAMMILLARY COMPLEX IN THE DOG'S BRAIN

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Many authors (Craigie 1925, Gurdjian 1927, Kuhlenbeck 1954, Bleier 1961, Diepen 1962, Ban 1964, Welento 1964) worked on the mammillary bodies of several animals. Their observations were based on cytoarchitectonic criteria. Mammillary bodies in the dog were doscribed by Rioch (1929, 1931) in his paper on the diencephalon in Carnivora. He was the first to take into consideration the course of fibres and he employed cytoarchitectonic as well as myeloarchitectonic criteria. The aim of this paper is to verify and to complete Rioch's observations.

As in the previous investigations, 10 uninterrupted series of sections of dog brains were studied. After fixation, the brains were embedded in celloidin or paraffin, sectioned in one of the three main planes and stained by the Weigert-Wolters method or alternately by the Nissl, Klüver and the Schultze methods.

OBSERVATIONS

It is easy to find the mammillary complex on the ventral surface of the dog's brain. It is a roundish elevation between the tuber cinereum (anteriorly) and fossa interpeduncularis (caudally). Orally the mammillary bodies are bounded by the nuclei of the tuber cinereum area and caudally reache the dorsal part of the supramammillary commissure (decussatio ventralis tegmenti, Singer 1962) and dorsocaudal hypothalamic nucleus. Laterally they neighbour on the fornix and the lateral hypothalamic nucleus.

The main nuclei of the mammillary complex of the dog are the nuclei medialis, which are surrounded by the fibrous capsule (Fig. 1, 3, MM, ML). In the dorsal direction this nucleus is separated by fasciculus mammillaris princeps which collect the fibres from the medial and lateral mammillary nucleus. Between the right and left medial mammillary nuclei is squeezed orodorsally median nucleus (Fig. 1, MN). Ventrally along the median line lies the magnocellular nucleus which borders on the floor of the brain. Dorsally the medial nucleus is covered by the supramammillary nucleus (in this line run the fibres of the ventral part of the supramammillary deccussation) and more laterally lies nucleus intercalatus II (between the main trunk of fasciculus mammillaris princeps and lateral hypothalamic nucleus. Laterally to the medial nucleus appears the fornix, and below lies the lateral nucleus (Fig. 1, F, L). Both nuclei are penetrated by the fornix fibres and mammillary peduncle fibres. There is also a small nucleus intercalatus I comma shaped, which lies laterocaudally to the lateral mammillary nucleus (Fig. 3, I 1).

Medial mammillary nucleus (Fig. 1, 3, 6, 7, MM, ML)

Topography. The medial mammillary nucleus is situated inside the fibre capsule of the mammillary body. It is spherical and its measurements in the frontal sections are about 1500 μ in height and 1200 μ in width. This is the largest nucleus of he mammillary bodies of the dog.

Inside the medial nucleus of the dog we distinguish two differentiated parts. These are the larger medial part and the much smaller lateral part, which is a little slab lying laterally close to the pars medialis (Fig. 1, 3, ML).

Architectonics. 1. Medial part of the medial mammillary nucleus. In myelin sections it is filled by a loose network of fibres. This network occupies nearly the whole medial area of the nucleus except its ventral and caudal portion, where the bow shape fibres exhibit a radial set and run towards the centre of the nucleus (Fig. 1, 3, MM). Nearer the centre these single fibres connect and form size bundles of different sizes. These bundles annex also fibres running from the network. They are directed dorsally and in the top part of the nucleus form a trunk named fasciculus mammillaris princeps, which runs outside the boundaries of the medial nucleus (Fig. 1, FMP).

There are many connections between the medial part of the medial nucleus and median nucleus. These connections consist of two systems of fibres. The first runs outside the medial and ventromedial portions of the medial nucleus and is directed dorsomedially. Inside the median nucleus the larger portion of the fibres of this system disperse, but some

reach the fasciculus mammillaris princeps. The second system consists of the horizontal fibres. They run out medially from the medial nucleus and disperse inside the median nucleus or, after passing through it, they disperse inside the opposite medial nucleus (Fig. 1, 6).

2. Lateral part of the medial mammillary nucleus. This nucleus is situated between the medial part of the nucleus and the fornix. This part of the nucleus is penetrated by the terminations of fornix fibres. The lateral part of the medial nucleus contains a characteristic tract of fibres, which reaches its area through the lateral boundary of the lateral mammillary nucleus. Subsequently, the fibres run dorsally in compact, well myelinated bundles, through the dorsal boundary of the nucleus towards the main trunk of fasciculus mammillaris princeps (Fig. 1, 6). This area is mainly penetrated by fibres which join the nucleus lateralis with the fasciculus mammillaris princeps. In silver sections the precise connections between this area and the medial part have been ascertained, so there was no reason to distinguish this area as an especial portion.

3. Fibre capsule of the medial nucleus. This forms a distinct element of architecture. It reaches the best development in the ventral and caudal portion of the nucleus, where it is composed of the fibres running from the external part of the medial nucleus (from among its cells) (Fig. 1, 3, 5—7, C). These fibres have a mediolateral course and they also sink into the lateral nucleus, the tuberomammillary nucleus and the mammillary peduncle. A small number of these fibres turn in a dorsal direction forming a thin layer between the medial and lateral nucleus. This part of the capsule joins a small number of fibres from fornix bundles. In the medioventral part the capsule is entered by fibres of nucleus magnocellularis. This thin system of fibres is directed anterolaterally. Moreover, in the ventral part, anteriorly to the nucleus magnocellularis we can find in Weigert sections a system of commissural fibres (commissura mammillaris ventralis, Śmiałowski 1967) connecting the fibre capsules on each side.

In the medial and dorsal part, the mammillary capsule has a different construction. It takes its origin caudally to this part where the fasciculus mammillaris princeps leaves the corpora mammillaria. On the medial side the capsule consists of fibres vertically running with a slight orodorsal deviation. They join the fibres of fasciculus mammillaris princeps in the medial part of the nucleus. In the mediodorsal and dorsal part of the capsule there are fibres running perpendicularly to the plane of the frontal section. They also reach the fasciculus mammillaris princeps.

The cells of the medial mammillary nucleus are round in shape and their measurements are about 14 to 18 μ in diameter. Sometimes especially

in the dorsal part of the nucleus larger cells are found (about 12 × 25 μ), oval in shape, well stained by Nissl method. In the lateral part of the nucleus there are many cells as on the other part.

Median mammillary nucleus (Fig. 1, 2, 6, MN)

Topography. It is an unpaired nucleus situated along the median line between the medial nuclei of the right and left mammillary bodies. In frontal sections in the anterior region of the mammillary complex of the dog, the nucleus is similar in shape to an isosceles triangle with its vertex direct downwards. The measurments of this nucleus are 1300 μ in height and 400 μ in length of the base. The upright sides of the nucleus are slightly curved towards the centre. The nucleus terminates in the central portion of the mammillary complex and at first it narrows being compressed between the medial nuclei growing in a medial direction.

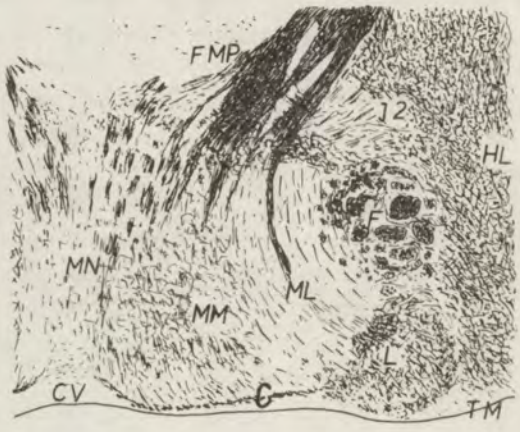

Fig. 1. Frontal section through the mammillary complex of the dog. Stained according to Weigert-Wolters

Architectonics. Inside the median nucleus appears a set of thin fibres running singly, or in small bundles (containing only 2—4 fibres) directed dorsoventrally (Fig. 2). In the dorsal part of the nucleus we can see more abundant bundles sinking into the right (from the right part of the nucleus) or left (from the left part of the nucleus) fasciculus mammillaris princeps (Fig. 6). This is the main connection of the median nucleus. The second system of fibres connects the nucleus with the capsule of the medial nucleus. It takes its origin from the fibres of the first set. The third set of fibres is the horizontal fibres penetrating mainly the ventral part of the nucleus connecting the right and left medial nuclei. The fourth system

Fig. 2. Myeloarchitectonics of the median nucleus. Weigert stain

consist of fine scattered fibres running in loose bundles from the median nucleus throughout its dorsal boundary to the supramammillary nucleus.

The median nucleus contains small cells, oval in shape, poorly stainable with the Nissl method (diameter 10—12 μ) and bigger cells better stainable (dimensions 25 × 15 μ), shaped like a drop of water scattered in the dorsal portion of the nucleus.

Lateral mammillary nucleus (Fig. 1, 6, L)

This nucleus has the maximum density of fibres of the nuclei of the mammillary complex. In frontal sections the shape of this nucleus is similar to an isosceles triangle with its base running paralelly to the floor the brain and bordering ventrally upon it. The height of the nucleus is about 700 μ, and the width of its base is about 600 μ. The medial leg of the triangle lies close to the lateral part of the medial nucleus (in a dorsal portion) and to the medial part (in a ventral portion). Dorsally and dorsolaterally it extends as far as the fornix fibres system. Laterally to the nucleus lie the posterolateral hypothalamic nucleus, mammillary peduncle and tuberomammillary nucleus. Orally the nucleus is bounded by the caudal wall of the ventrolateral hypothalamic nucleus and caudally it is bounded by the wall of the brain.

Architectonics. Inside the lateral mammillary nucleus there is dense network of well myelinated fibres. At first they run dorsomedially but subsequently they form bundles containing a small number of fibres, and after passing through the medial boundary of the nucleus, they reach the lateral part of the medial mammillary nucleus. Then they run dorsally,

and join to the fibres of fasciculus mammillaris princeps. This is the main fibres system (Fig. 1, 6, L).

Anteriorly the nucleus area is entered by the fibres of the medial forebrain bundle. They are noticeable especially in Weigert sections of horizontal series where they appear like a single fibre tract longitudinal in the section plane.

The next system of fibres reaches the nucleus anteriorly running from the ventral portion of the fornix. These fibres may be found in the dorsal part. Laterally the lateral nucleus is entered by fibres from the posterolateral hypothalamic nucleus. Some fibres of this system disperse, but the rest of them mingles with the fibres running to the fasciculus mammillaris princeps. The nucleus is also reached (through its lateral boundary) by the fibres running from the tuberomammillary nucleus in a mediocaudal direction. In the lateroventral portion of the lateral nucleus the fibres which form the mammillary peduncle are concentrated. They have a dorsocaudal course and contain the fibres from the lateral mammillary nucleus as well as a small number of fibres running from the lateroventral part of the capsule. From the dorsolateral part of the lateral nucleus a small bundle of fibres leaves and is directed dorsally with a caudal deviation. Above the fornix the fibres turn medially and sink into the supramammillary nucleus area, where the majority of them disperse though some reach the mammillary peduncle on the other side.

Inside the lateral nucleus we can find a small number of cells, sparsely distributed, mostly oval in shape, and less frequently triangle or polygonal. Their diameter is about 20 to 25 μ.

Intercalate nucleus I (Fig. 3, 7, I 1)

Topography. This nucleus can be found in frontal sections of the middle portion of the mammillary complex. It is situated laterocaudally to the lateral nucleus. In Weigert-Wolters sections this nucleus appears as a bright area surrounded by areas filled with fibres. Medially and orally it borders on the lateral mammillary nucleus, in its caudal portion it neighbours the fornix, while ventrally and caudally it touches the mammillary peduncle.

The anterior region of the intercalate nucleus I is shaped approximately like a right angle triangle (Fig. 3). The shorter leg of this triangle (450 μ in length) is directed vertically, but the longer horizontal leg (ca 600 μ in length) is directed laterally. A hypotenuse forms an arch, drawn between the ends of these two lines. The concavity of this arch is directed towards the right angle. Afterwards the fibres of the mammillary peduncle run upwards along the lateral side of the nucleus, compressing its lateral

part. In this segment the nucleus looks approximate like a suspended drop of water and further it dwindles away.

Architectonics. The main system of nucleus fibres connected with the mammillary peduncle, consist of well myelinated fibres. They run parallel to the mammillary peduncle singly or in small bundles. These fibres penetrate the whole nucleus but their density is poor. In silver sections (Schultze method) another two systems of fibres can be distinguished. The first system reaches the nucleus medially from the lateral part of the medial nucleus and from the bundles of fornix fibres and has a laterodorsal course. The second system is formed by small fibres running horizontally as well as vertically, penetrate the whole nucleus disorderly. A part of this set is connected with the fornix fibres, lateral part of the medial nucleus and lateral part of the capsule (in the caudal portion of the nucleus).

The cells of intercalate nucleus I are small (diameter about 12 μ), usually oval in shape, though sometimes triangle or polygonal. They are poor stainable by the cresyl violet method.

Supramammillary nucleus (Fig. 3, 4, 7, NSM)

The supramammillary nucleus is an unpaired region in the dog's brain lying along the median line and covering the mammillary complex. It is rectangular in shape with measurements of 2000 × 500 μ in frontal sections.

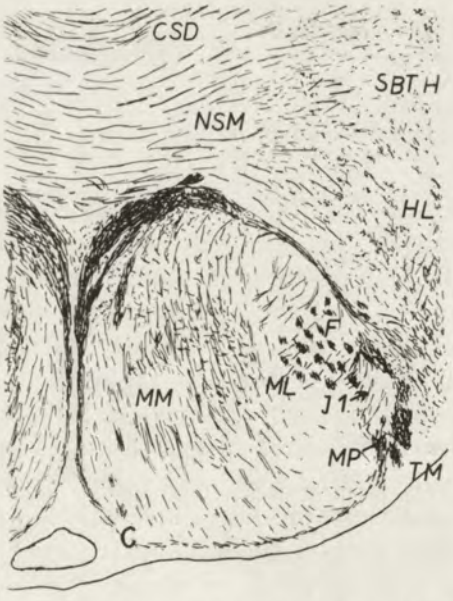

Fig. 3. Posterior sector of the mammillary complex in the dog. Frontal section

Architectonics. The set of fibres in the supramammillary nucleus is principally composed of the system of the ventral part of the supramammillary commissure. These well myelinated fibres pass through the nucleus forming a loose plexus (Fig. 4). Cell bodies also located in this area. The commissural fibres consist of fine horizontally running fibres, poorly stainable and of somewhat thicker (2—3 μ in diameter) fibres, intensely stainable which appear less frequently. In the median part of the mammillary complex the fibres run through the supramammillary nucleus in the frontal plane. The caudal portion of the nucleus is penetrated by fibres with a slight laterocaudal deviation.

The area of the supramammillary nucleus is penetrated through its lateral boundary by fibres running from the posterolateral hypothalamic nucleus (Fig. 3, 7) and through its lateroventral boundary by fibres from the mammillary peduncle bundle. They mix with the main system of

Fig. 4. Fiber system of the supramammillary nucleus in the dog. Weigert stain

commissural fibres. In horizontal sections a laterooral penetration of the medial forebrain bundle fibres into the supramammillary nucleus area has been visualised. After passing through the nucleus the fibres turn medially to the opposite side, where they come laterоorally again to the posterolateral hypothalamic nucleus. Ventrally the supramammillary nucleus receives a small number of fine fibres from the median nucleus. They can be found in a ventral region of the nucleus. It has been also ascertained that the fibres of the commissural system sink into the dorsal part of the medial nucleus capsule and mix with its fibres.

The cells of the supramammillary nucleus area are small and poorly stainable with cresyl violet method. They are oval (about 10—15 μ in diameter) though sometimes they are bigger (25 μ in diameter) mainly in the ventral part of the nucleus.

Intercalate nucleus II (Fig. 1, 7, I 2)

This nucleus is one of the smaller nuclei in the mammillary complex of the dog. In frontal sections it approximates in shape to an isosceles triangle with its legs about 500 μ long. Laterodorsally it borders on the posterolateral hypothalamic nucleus, ventrally on the fornix and the medial mammillary nucleus (separated from it by the capsule). Mediodorsally the nucleus is bordered by the fasciculus mammillaris princeps fibres.

Inside the nucleus a set of mediolaterally running fibres can be seen, which turn laterodorsally in the lateral part of this nucleus. This set is made up of two types of fibres. There are single fibres as well as bundles, contained of various number of fibres (few to several) which are about 40—60 μ long and 10—15 μ in diameter. The single fibres run towards the fasciculus mammillaris princeps and, penetrating through its fascicles, enter the dorsocaudal hypothalamic nucleus. The bundles come through the laterodorsal boundary to the posterolateral hypothalamic nucleus and mix with its system of fibres.

The cells of intercalate nucleus II are oval and stain poorly with Nissl method.

Magnocellular mammillary nucleus (Fig. 5, MG)

The magnocellular nucleus can be seen in the ventral region of the mammillary bodies. This unpaired nucleus occupies an area limited on the sides by the right and left capsules of the mammillary bodies.

Topography. In horizontal sections it approximates in shape to an isosceles triangle with its vertex direct to the front. The measurements of this nucleus are 300 μ in width (in its widest caudal portion), 150—200 μ in height (dorsoventrally) and up to 500 μ in length (orocaudally). The size of the magnocellular nucleus of the dog is various. The measurements given above should be treated as maximum ones.

Ventrally the magnocellular nucleus touches the floor of the brain, and laterally it is bordered by the capsule of the fibres of the medial (right and left) mammillary nuclei, which capsules approach nearer together. The rostral portion of the nucleus extends as far as the inframammillary recess and the ventral mammillary commissure, whereas caudally the nucleus is limited by the wall of the brain.

Architectonics. Weigert-Wolters sections exhibit a set of slightly myelinated thin fibres within the magnocellular mammillary nucleus. This set is made up of 3 systems of fibres, an orocaudal (1), a lateromedial (2) and a mediolateral (3), which penetrate loosely throughout the nucleus in

the horizontal plane. The two components of system 2, which intersect at an acute angle, from a loose network, though rendered more dense in the ventral portion of the nucleus by system 1, which is less abundant here, and by system 3. On the other hand, in the dorsal portion of the nucleus system 2 windles and system 1 becomes predominant. The fibres of all these systems deviate slightly caudodorsally.

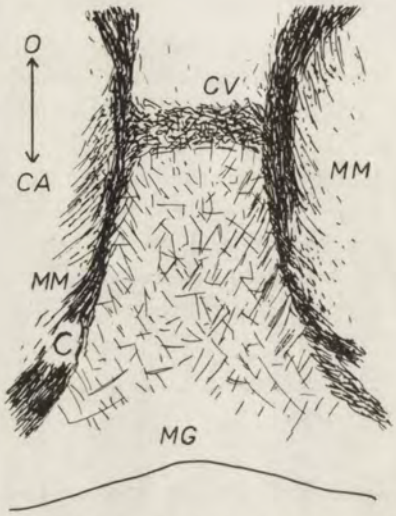

Fig. 5. Horizontal section at the level of the magnocellular mammillary nucleus in the dog. Weigert stain

System 1 penetrates through the rostral portion of the nucleus and connects it with the plexus of fibres of the ventral mammillary commissure. System 2, having passed across the lateral boundary of the nucleus (on its right or left side), terminates, among the fibres of the capsule of the medial mammillary nucleus, with which it mingles so thoroughly that it becomes impossible to trace its further course by anatomical methods.

The fibres of system 3, which have a lateromedial direction, penetrate through the nucleus transversely. After passing through the lateral boundary of the nucleus, not unlike system 2, they sink into the capsule of the medial nucleus, but enter it perpendicularly and not at a slant, as system 2 does. System 3 consists of a small numbers of fibres.

The magnocellular mammillary nucleus has nerve cells of large size. These have a multipolar or, occasionally, a fusiform shape. Their measurements are about 15 × 25—30 μ in multipolar cels and about 10 × 40 μ in fusiform cells. They stain intensely the Nissl method, which manifests their large cellular nucleus and large granules of tigroid. Inside the

nucleus these cells lie near each other and from a compact group. Occasional single cells which correspond morphologically to those inside the nucleus are present between the nucleus and the fibrous capsule. They do not penetrate deeply among the fibres of the capsule but are only slightly entangled with them on the outer (medial) side of the capsule.

CONNECTIONS OF THE MAMMILLARY COMPLEX

Here only the main tracts entering the mammillary complex or leaving will be noted here, and without distinction of their course inside the nuclei since this has been described above.

Fornix (Fig. 1, 3, 6, 7, F).

This an important outflow from is the hippocampal formation. It reaches the mammillary body anteriorly scattering in the lateral mammillary nucleus and in the lateral part of the medial mammillary nucleus. Some of the fibres disperse inside the fibre capsule in its lateral part.

There are similar connections in the degeneration material of the rabbit's brain described by Ban (1964). The majority of the fibres of the rabbit's fornix terminates in the lateral part of the medial nucleus, some in the lateral nucleus. The rest of them running throughout the lateral nucleus enter the mammillary peduncle (homolaterally) and, after getting outside it, they sink into the red nucleus area. According to Ban (1964) the fornix is ontogenetically younger than the mammillothalamic tract. In the 3 month old human embryo the mammillothalamic tract has myelinated already, whereas the fornix does not myelinate the 8th or 9th month.

The compact connection between the fornix and the tegmentum in the elephant's brain has been decribed by Diepen et al. (1956). In the elephant's brain the majority of fornix fibres passing dorsally by the mammillary bodies run to the tegmentum. Only some of them terminate in the bodies.

This connection has not been found in the dog's brain. All its fornix fibres terminate inside the nuclei of the mammillary complex. Also Rioch (1931) has not found the tegmental component in the fornix in the dog's and cat's brain.

Medial forebrain bundle

This tract enters chiefly the lateral mammillary nucleus and also the supramammillary nucleus where it reaches the contralateral side with the fibres of ventral supramammillary commissure. This tract conduct impulses from the olfactory area, septum, area preoptica and from the lateral hypothalamic nucleus.

Ban (1964) found connections of the medial forebrain bundle with the lateral and medial nuclei of the rabbit's mammillary body. The fibres of the medial forebrain bundle terminate there. These fibres transfer impulses from the lateral hypothalamic nucleus, the lateral preoptic area and from septal nuclei (nucleus septalis medialis, the nucleus septalis lateralis, and the nucleus septohippocampalis). But only few medial forebrain bundle fibres terminate in the mammillary bodies, the majority of them passing by the bodies to enter the tegmentum.

Fasciculus mammillaris princeps (Fig. 1, 6, FMP)

In dog's brain this tract takes its origin in the nuclei of the mammillary complex, as a loose bundle of fibres forming a compact trunk above the bodies. This trunk is made up of the median, medial and lateral mammillary nucleus. Above the mammillary complex the fasciculus mammillaris princeps forks into two branches. These are the mammillothalamic tract and the mammillotegmental tract. According to Koelliker (1896), Ramon y Cajal (1904) and Gurdjian (1927) the mammillothalamic tract is a collateral of the fibres of mammillotegmental tract. Ban (1964) suggested that this tracts consist of different neurons. According to degenerations studies, this tract leads out of the mammillary bodies.

Mammillothalamic tract. It direct orodorsally to the anterior thalamic nuclei, where its fibres disperse.

Guillery (1956) observed a degeneration in the medial part of the medial mammillary nucleus after injury of the anterodorsal and antero-

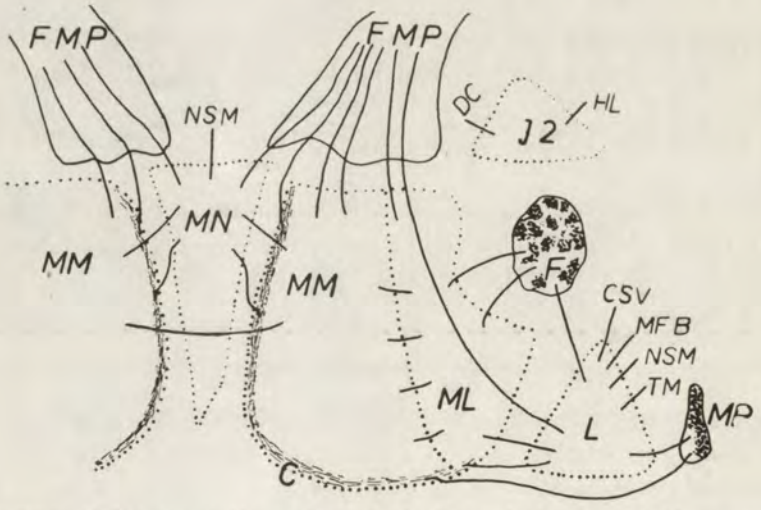

Fig. 6. Diagram of the connections of nuclei in the anterior part of the mammillary body in the dog

ventral thalamic nucleus. On the basis of the degeneration studies in the rabbit's brain Ban (1964) gave the termination of this tract in anteromedial nucleus and in anteroventral, anterodorsal and paraventricular anterior thalamic nuclei. The connection between the mammillary complex and the gyrus cinguli comes by means of the anterior thalamic nuclei.

In degeneration studies of this tract in the cat it was ascertained by Fry et al. (1963) that the medial mammillary nucleus has more compact connection with the thalamus than the lateral nucleus. From the medial nucleus ca 70% of neurons send their fibres to the mammillothalamic tract and it forms 50% of the total number of fibres in this tract. But only 40% of neurons in the lateral nucleus send their fibres to this tract. In the cat, the fibres of the lateral nucleus are thicker (above 1,5 μ in diameter). In the dog fibres of both nuclei have similar diameters.

Mammillotegmental tract. This is the second tract arising from the fasciculus mammillaris princeps. It is directed caudally and according to our observations it disperses soon in the caudal area, behind the mammillary bodies. In studies of rabbit's brain Ban (1964) found terminations of this tract in the red nucleus, in the mediodorsal part of tegmentum, in the nucleus ventralis tegmenti, in the nucleus dorsalis tegmenti and in the gray substance of the fourth ventricle floor.

According to Stehr (1963) the fasciculus mammillothalamicus of the dog reaches the anteromedial and ventral thalamic nucleus. According to Rioch (1931) and Stehr (1963) the fibres of the mammillothalamic tract have not been found in the dog's anterodorsal thalamic nucleus. According to recent studies (Fry et al. 1963) this tract in the cat is connected with the anterodorsal thalamic nucleus also, but this connection is poor. It may be that more accurate studies will show this connection in the dog's brain.

Mammillary peduncle (Fig. 3, 7, MP)

In the dog's brain this tract has a connections with the lateral mammillary nucleus, the medial mammillary nucleus, the nucleus intercalatus I and with the ventral portion of the fibre capsule. Some of the mammillary peduncle fibres reach the supramammillary nucleus. According to Diepen (1962), this is the tract conducting to the medial and lateral mammillary nuclei. New investigations by Ban (1964) show the presence of afferent fibres as well as efferent in the mammillary peduncle of the rabbit. Ban differentiated in these afferent fibres the tract from the nucleus ventralis tegmenti, from the ventral part of the nucleus dorsalis tegmenti and from the lateroventral part of the midbrain. The majority of these fibres terminate in the lateral and in the medial mammillary

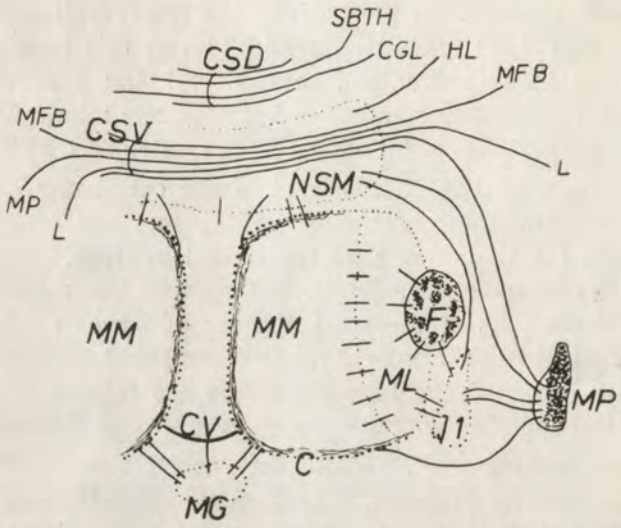

Fig. 7. Diagram of the connections of nuclei in the posterior part of the mammillary body in the dog

nuclei but some reach the medial and the lateral nucleus on the opposite side, by means of the fibre capsule. According to Ban (1964) the efferent fibres of the mammillary peduncle consist of 3 components (1) a fornix component leading the fibres out from fornix, (2) a hypothalamotegmental and hypothalamonigral tract and (3) a lateral mammillotegmental tract.

Supramammillary commissure (Fig. 3, CSD)

On the basis of the myelin sections it has been ascertained that the supramammillary commissure in the dog's brain is made up of dorsal and ventral parts which differ in architecture and in connections. The dorsal part is formed as a compact bundle of fibres connecting the right portion of the subthalamus with the left one. A ventral part runs through the supramammillary nucleus as a loose system of parallel fibres joining together the right and left posterior hypothalamus and both sides of the mammillary bodies (see at the supramammillary nucleus).

In the attainable papers about the mammillary complex of the other animals the supramammillary commissure was treated as a uniform one.

Diepen (1962) affirms that the supramammillary commissure connects subthalamic centres (corpus Luysi, zona incerta, globus pallidus) with the nucleus ruber and substantia nigra in the opposite side. On the contrary Kuhlenbeck (1954) suggests that this commissure joins the subthalamic centres with the motor centres of the tegmentum contralaterally.

Another opinion was given by Gurdjian (1927), who divided the supramammillary commissure into tree components; (1) the intersubthalamic component which joins both sides of the nucleus subthalamicus; (2) rubrocommissural fibres and (3) deccusation of the fornix fibres. The first and the second components correspond to our dorsal part, the third to the ventral part.

Ventral mammillary commissure (Fig. 5, 7, CV)

There is a small system of commissural fibres in the frontal section below the mammillary complex (connecting the right capsule with the left one). In horizontal sections this set becomes better visible. Analysing this system we found that the commissural fibres show no typical commissural course (from one structure to another) because the ventral commissure is made up of a compact braid of fibres. The majority of these fibres connecting the capsule of the right or left mammillary bodies, may exhibit a commissural set. There are also confused fibres in this braid running in several directions and a system getting caudally outside the braid in the horizontal plane and running to the magnocellular nucleus.

In the available papers only Gurdjian (1927) is the one who mentioned the commissural fibres in the ventral portion of the mammillary complex in the rat's brain. According to him the ventral mammillary commissure fibres of the rat run through the medial mammillary nucleus pars commissuralis ventralis. In the dog the ventral mammillary commissure runs independently anteriorly to the magnocellular nucleus and is connected with it by only a poor system of fibres.

DISCUSSION

There are many descriptions of the mammillary complex of several animals in the neuroanatomical literature. But only some of the authors worked out the physiology of this area.

The nuclei differentiated in the complex by all authors are: the medial nucleus, the lateral nucleus and the supramammillary nucleus.

The lateral mammillary nucleus may be easily found in various animals because it contains a large, well stainable nervous cells, so that it may be located without any difficulty. The nucleus described in the present paper as a lateral mammillary nucleus corresponds to that referred to be Bleier (1961) and in the papers of Rioch (1929, 1931), Kuhlenbeck (1954), Diepen (1962) and Maršala (1963).

The supramammillary nucleus has been found in the dog as an unpaired nucleus, covering the mammillary complex. It is frequently

described as two nuclei (for instance in the cat, Bleier 1961). In regard to topography our description of supramammillary nucleus corresponds to the same in the dog and the cat (Rioch 1929, 1931) and also in a pig (Welento 1964) and in man (Kuhlenbeck 1954). These authors are also consistent in their opinions about the connection between the supramammillary nucleus and the supramammillary commissure. In the dog this connection is strong because the main system of supramammillary nucleus fibres is formed by the fibres of the ventral part of the supramammillary commisssure.

There are great differences in the descriptions of the medial mammillary nucleus between the authors. In the cat Bleier (1961) distinguishes the basal division, medial, central, intermediate, posterior and mediobasal division. According to Gurdjian (1927) there are only medial, median, lateral and posterior components in the mammillary complex of the rat. Our description of the medial nucleus resembles that by Kuhlenbeck (1954) which distinguishes the medial and lateral part. Our lateral part of the nucleus corresponds also to the Gurdjian (1927) lateral part. According to him in this part of the nucleus terminates the fornix fibres, so that also corresponds to our observations in the dog's brain. Remaining parts of the medial nucleus separated by Bleier (1961) and Gurdjian (1927) correspond to our medial part, except the median described by us as a distinct nucleus.

We can find the median nucleus in the dog, the cat (Bleier 1961, nucleus medialis central division) and the rat (Gurdjian 1927, medial mammillary nucleus pars medianus), but not in man's brain (Kuhlenbeck 1954). It is associated with the complete division of both mammillary bodies by a deep furrow, reaching as for as the dorsal portion of these bodies. In animals this furrow is shallow or absent. Rioch (1929) has not distinguished the median nucleus in his description of the mammillary complex in the dog and the cat.

In some studies we can find a description of intercalate nucleus I (like ours in the dog). This nucleus appears also in a man (Kuhlenbeck 1954, Diepen 1962) and in a pig (Welento 1964). It has not been described in a cat (Bleier 1961), a rabbit (Ban 1964), a rat (Gurdjian 1927) and also in a dog and cat (Rioch 1929).

Nuclei gemini described by Lundberg (1962) in the rabbits brain topographically and anatomically correspond to the intercalate nucleus I and the intercalate nucleus II in the dog's brain. According to him they are two nuclei connected with the mammillary complex. The anterior nucleus lies near to the mammillothalamic tract and to the mammillotegmental tract, whereas the posterior nucleus extends to the superior part of the pedunculus corporis mammillaris (mammillary peduncle). Therefore

the anterior nucleus corresponds to intercalate nucleus II and the posterior nucleus to the intercalate nucleus I. The nucleus gemini posterior was described by Kuhlenbeck (1954) in the mammillary complex of the man brain. He named it the intercalate nucleus. The anterior gemini nucleus has not hitherto been described. In the present paper is the first description of this nucleus in the dog brain. It is named the nucleus intercalatus II. We can not agree with Lundberg's terminology because in the dog the nuclei intercalati I and II are distant, so that they can not be denominated as a „twin nuclei".

The magnocellular mammillary nucleus has not hitherto been described in the dog brain. Owing to its small size it may have been overlooked in the series of sections cue at rather large intervals. Topographically, the magnocellular nucleus of the dog corresponds to the nucleus medialis pars commissuralis ventralis of the rat (Gurdjian 1927). In the dog, however, this nucleus has not the nature of a commissural nucleus, because the ventral mammillary commissure runs independently anteriorly to it and is connected with it by only a poor system of fibres. Commissural fibres are also absent inside the nucleus. Having found such a nucleus in the hedgehog that it corresponded in its topography to the nucleus described above, Diepen (1962) denominated it the caudal part of the tuberomammillary nucleus. Similarly, he found a group of cells along the median line in the anterior region of the mammillary bodies of man and described it as „a certain number of cells of the tuberomammillary nucleus". In the dog the cells of the tuberomammillary nucleus and the magnocellulary nucleus show some similarity but the lack of connections between them makes it impossible to treat these structures as a single nucleus.

The nucleus magnocellularis praefascicularis mammillaris in a guinea pig has been described by Mühlen (1966). It is situated in premammillary region bordering on the fasciculus mammillaris princeps and fornix. This nucleus in the guinea pig has nerve cells of large size (similarly to the supraoptic and paraventricular nucleus). In the dog this nucleus is absent, but inside the median nucleus, the dorsal area of the medial nucleus and in the ventral area of the supramammillary nucleus there are the intensely stainable large nervous cells. Probably these scattered cells correspond to the same in nucleus mammillaris praefascicularis magnocellularis.

SUMMARY

Using the myeloarchitectonics criterion of division, the following parts have been differentiated in the mammillary complex of the dog: the medial nucleus, the median nucleus, the lateral nucleus, the intercalate

nucleus I, the intercalate nucleus II, the supramammillary nucleus and the magnocellular nucleus.

It was for the first time that the magnocellular mammillary nucleus, the nucleus intercalatus II and commissura mammillaris ventralis have been described. Also the nucleus intercalatus I of the mammillary complex belongs to the area which have been infrequently described in the literature.

ABBREVIATIONS

C	Mammillary capsule	I 1	intercalate nucleus 1
CA	caudalis	I 2	intercalate nucleus 2
CGL	corpus geniculatus lateralis	L	lateral mammillary nucleus
CSV	ventral supramammillary commissure	MFB	medial forebrain bundle
CSD	dorsal supramammillary commissure	MG	magnocellular mammillary nucleus
CV	ventral mammillary commissure	MM	medial part of the medial mammillary nucleus
DC	dorsocaudal hypothalamic nucleus	MN	medial mammillary nucleus
F	fornix	ML	lateral part of the medial mammillary nucleus
FMP	fasciculus mammillaris princeps	MP	mammillary peduncle
HL	posterolateral hypothalamic nucleus	NSM	supramammillary nucleus
		SBTH	subthalamic area
		TM	tuberomammillary nucleus

REFERENCES

BAN, T. 1964. The hypothalamus, especially on its fibres connections and the septo-preoptico-hypothalamic system. Med. J. Osaka Univ., 15: 1—83.

BLEIER, R. 1961. The hypothalamus of the cat. Johns Hopkins Press, Baltimore.

CRAIGIE, E. H. 1925. Central nervous system of the albino rat. P. Blakiston's Son and Co., Philadelphia.

DIEPEN, R. 1962. Der Hypothalamus. Handbuch der Mikroskopische Anatomie des Menschen. Springer Verl., Berlin, Göetingen, Heidelberg.

DIEPEN, R., JANSEN, P., ENGELHARDT, Fr. and SPATZ, H. 1956. Reserches sur le cerveau de l'elephant d'Afrique (Loxodonta africana Blum.). Acta Neurol. Psychiat. Belg. 11: 759—788.

FRY, W. J., KRUMINS, R., FRY, J. F., GARTH, T., BORBELY, S. and ADES, H. 1963. Origins and distributions of some efferent pathways from the mammillary nuclei of the cat. J. Comp. Neurol. 120: 195—210.

GUILLERY, R. W. 1956. Degeneration of the post-commissural fornix and the mammillary peduncle of the rat. J. Anat. (London) 90: 350—360.

GURDJIAN, E. S. 1927. The diencephalon of the albino rat. J. Comp. Neurol. 43: 1—114.

KOELLIKER, A. 1896. Handbuch der Gewebelehre des Menschen. Band 2: Nervensystem des Menschen und der Tiere. Vrl. von W. Engelman, Leipzig.

KUHLENBECK, H. 1954. The human diencephalon, a summery, developement, structure and pathology. Conf. Neurol., Suppl. vol. 14.

LIM, R. K. S., LIU, C. N. and MOFFIT, R. L. 1960. A stereotaxic atlas of the dog's brain. Charles C. Thomas Publ., Springfield I11.

LUNDBERG, P. O. 1962. New hypothalamic nuclei. J. Comp. Neurol. 119: 311—316.

MARŠALA, J. 1963. Frontohypothalamicke drahy mozgu kočky. Českoslov. Morf. 11: 266—277.

MÜHLEN, K. aus der 1966. Der Hypothalamus des Meerschweinchens. S. Karger, Basel, New York.

RAMON Y CAJAL, S. 1904. Textura del sistema nervioso del hombre y de los vertebrados. Vol. II. Nicolas Moya, Madrit.

RIOCH, D. M. 1929. Studies on the diencephalon of Carnivora. Part I. J. Comp. Neurol. 49: 1—119.

RIOCH, D. M. 1931. Studies on the diencephalon of Carnivora. Part III. J. Comp. Neurol. 53: 319—387.

SINGER, M. 1962. The brain of the dog in sections. Saunders Comp., Philadelphia, London.

ŚMIAŁOWSKI, A. 1967. Magnocellular mammillary nucleus. Bull. de l'Academie Polonaise des Sci. 17 (in press).

STEHR, F. 1963. Fasciculus mammillaris princeps and its branches in the dog. Acta Biol. Exp. (Warsaw) 23: 221—237.

WELENTO, J. 1964. Structure and topography of diencephalic nuclei in pig (in Polish). Ann. Univ. Mariae Curie Skłodowska (Lublin) 19: 125—187.

Acta Biol. Exp. (Warsaw) vol. 28, no. 3, p. 245—251, 1968

A HIGH FREQUENCY GENERATOR FOR INTRACEREBRAL THERMAL STIMULATION [1]

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(Received January 12, 1968)

The homeothermic animals are equipped with an efficiently working thermoregulatory mechanism, containing peripheral, cutaneous thermosensitive detectors as well as receptors located within the brain. Of special interest to the authors are those intracerebral centers having thermodetectory and thermoregulatory properties, that can trigger — according to necessity — an increase of heat production or heat exchange.

To carry out experiments in this sphere, an apparatus with thermostimulating properties is necessary.

The thermosensitive brain centers identified so far, are located in the hypothalamus (Magoun et al. 1938, Hardy 1961) and in the bulb (Holmes et al. 1960). These are small structures and thus the thermode, being the output electrode of the thermo-stimulator must have a small active surface of about 1 mm^2.

For selective heating of the suspected thermosensitive brain centers three kinds of apparatus are employed:

In the first the thermode is one „U flexed" tube, or a joined complex with circulating water, having the desired temperature (Dondey et al. 1962, Nakayama et al. 1963, Adams 1964).

There is also another type in use made of metal wire and specially constructed to allow conduction of heating or cooling by circulating water (Folkow et al. 1949). The devices of this kind have numerous advantages but are really difficult to make in small dimensions.

[1] Supported by a grant from the Physiological Committee of the Polish Academy of Sciences.

In the second the thermode has inside it a fine heating coil supplied with direct current (Carlisle 1966).

In the third the „point" heating is performed by high frequency current through two needle thermodes implanted into the brain (Magoun et al. 1938, Folkow et al. 1949, Nakayama et al. 1963), or by one „active" implanted thermode, and by the other one with a large surface placed on the skin of the limb or trunk (Holmes et al. 1960). This kind is very convenient as it is possible to use a really minute thermode. It is of crucial significance in chronic experiments on central, intracerebal thermosensitive centers.

RESULTS

A high frequency generator suitable for thermal stimulation of the brain was constructed. (See diagram, Fig 1.)

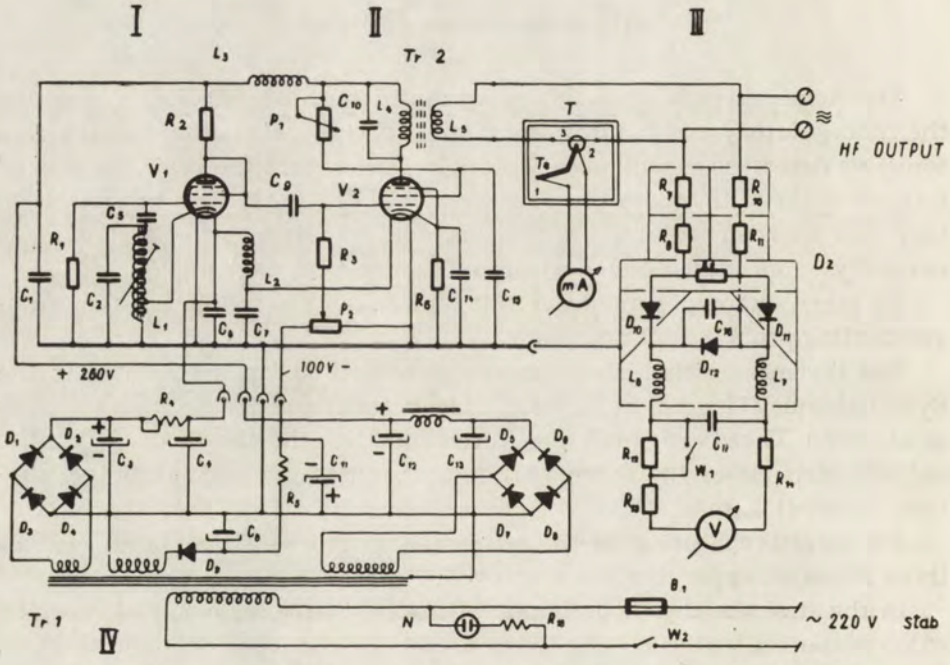

Fig. 1. Diagram of HF generator for intracerebral thermal stimulation

Resistors

R_1 — 30 kΩ	R_6 — 200 Ω	R_{11} — 10 kΩ
R_2 — 5 kΩ	R_7 — 47 kΩ	R_{12} — 300 kΩ
R_3 — 100 kΩ	R_8 — 10 kΩ	R_{13} — 10 kΩ
R_4 — 4 Ω	R_9 — 30 kΩ	R_{14} — 10 kΩ
R_5 — 4 kΩ	R_{10} — 47 kΩ	R_{15} — 150 kΩ

Capacitors

C_1 — 2000 pF	C_7 — 5000 pF	C_{13} — 50 μF
C_2 — 60 pF	C_8 — 50 μF	C_{14} — 5000 pF
C_3 — 200 μF	C_9 — 600 pF	C_{15} — 2000 pF
C_4 — 200 μF	C_{10} — 160 pF	C_{16} — 2000 pF
C_5 — 150 pF	C_{11} — 16 μF	C_{17} — 1000 pF
C_6 — 5000 pF	C_{12} — 50 μF	

Germanium diodes

D_1, D_2, D_3, D_4 — DMG 2 D_5, D_6, D_7, D_8, D_9 — DZG 7
D_{10}, D_{11}, D_{12} — DOG 58

Valves

V_1 — EF 80, V_2 — EL 81

HF chokes

L_2, L_3, L_6, L_7

The constructed thermo-stimulator consists of the following electric systems: (i) HF generator (I); (ii) power amplifier (II); (iii) measurement system (III); (iv) power pack (IV).

The HF generator works as a self-excited HF generator (Hartley oscillator) on the EF 80 valve (V_1). Its frequency is 3 MHz. The HF power amplifier working on the EL 81 valve (V_2) is capacitively coupled to the HF generator by a C_9 capacitor. This amplifier contains an HF output fitting transformer (Tr 2). To avoid the stimulation artifacts during simultaneous brain activity recording, the L_4 coil has not a capacitance coupling with the L_5 coil. The output power is regulated by the two potentiometers P_1 and P_2. Its maximum value is 2.5 watt. The advantage of this amplifier lies in the possibility of adjusting the output power from zero. The measurement system consists of a thermo-ammeter (mA) and a voltmeter (V). The thermo-ammeter of the HF current has two copper-constantan thermocouples (Te — 1; Te — 2); one a compensating and the other active, separated galvanically from the heater (3), (Lebson 1965). To increase the stability of the measurement system thermocouples and heater are placed in the thermos bottle (T). The voltometer (V) has a HF voltage attenuator (Dz) with an rectifying system (D_{10}, D_{11}, D_{12}) together with a switch (W_1). The rectifying system of the power pack (IV) works on germanium diodes DMG 2 (D_1, D_2, D_3, D_4) and DZG 7 (D_5, D_6, D_7, D_8). The Tr 1 transformer is supplied from electric mains by a magnetic stabilizer.

The high frequency alternating current is conducted to the thermostimulated animal by the electrodes (thermodes) presented in Fig. 2.

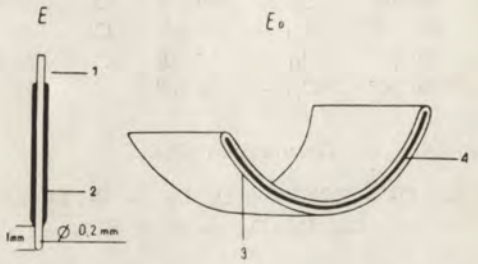

Fig. 2. Electrodes for intracerebral thermal stimulation. E, thermode: 1, platinum wire; 2, glass insulation; E_0, plate electrode: 3, rubber insulation; 4, lead plate 150×100 mm

The thermode (E) is made of platinum wire 0.2 mm in diameter, insulated with a glass tube of 0.4 mm outside diameter. The bare tip is 1 mm long. The large plate electrode (E_0) consists of a flexible lead plate 150×100 mm placed in rubber insulation. Because of the great size difference between the active (E) and passive (E_0) thermodes, the tissue surrounding the first will become heated (Börner 1966).

To control the behavior of the HF generator described and to measure the temperature increase of the brain near to the thermode in relation to the power heating HF current, experiments were performed with a chronically implanted thermode (E) and with a copper-constantan thermocouple (Te), (Fig. 3). They were both introduced into the brain according to stereotaxic coordinates of Monnier and Gangloff aB, 13 mm deep. The thermode was 1 mm distant from the thermocouple. The intracerebral thermal stimulation was performed with simultaneous measurements of brain temperature and HF current power, according to the schematic drawing in Fig. 3. The intracerebral temperature was measured

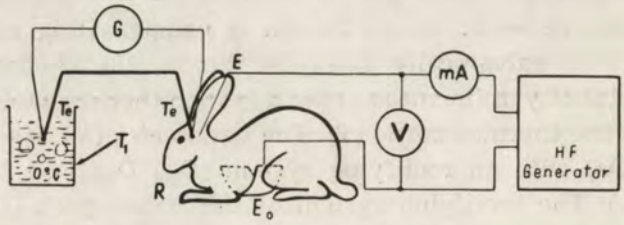

Fig. 3. Intracerebral thermal stimulation with simultaneous measurement of brain temperature. R, rabbit; E, thermode; E_0, plate electrode; T_e, thermocouple; T_1, insulated container with melting ice; G, galvanometer

by means of two copper-constantan thermocouples (Te). One of them was implanted 1 mm from the thermode and the other inside the thermos bottle, filled with melting ice (Delgado and Hanai 1966, Kawamura et al. 1966). The temperature of the brain was read from the galvanometer, calibrated in degrees Centigrade (G). The galvanometer was additionally connected with the compensative setting to adjust its scale to zero for the desired temperature. The thermocouples were insulated with a glass tube of external diametr 0.5 mm and fixed inside with epoxy resin.

The measurement of the intracerebral temperature in relation to the constant temperature of melting ice assures correct measurement regardless of room temperature.

As a result of the performed measurements a diagram illustrating the intracerebral temperature increase in relation to the power of the heating HF current was obtained (Fig. 4).

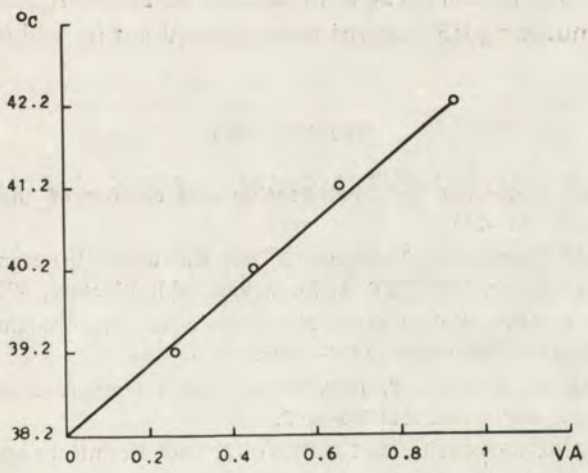

Fig. 4. Diagram illustrating intracerebral temperature increase in relation to power of HF current. Rabbit no. 2

On the abscissa there are values of the heating HF current power in volt-amperes (VA) and on the ordinate the temperature obtained a distance 1 mm from the thermode tip in degrees Centigrade (°C). The intracerebral temperature increase in relation to HF current power is approximately linear. To increase the temperature of the hypothalamus by 1°C, 1 mm from the thermode, a HF current power from 190 to 276 millivolt-amperes was necessary.

CONCLUSIONS

The experiments performed demonstrated that the HF generator described can be applied to intracerebral thermal stimulation. It is convenient and advantageous because of the small dimensions of the thermode, the possibility of a continual regulation of the output power from zero, and the control of thermal stimulation intensity during experiments. The latter is necessary because a thermal injury of the brain tissue during such stimulation is possible.

SUMMARY

A HF generator (3 MHz) for intracerebral thermal stimulation was constructed. Its maximum power is 2.5 watts, regulated from zero, and it contains a thermo-ammeter and voltmeter for the stimulation current. Measurements of intracerebral temperature increase in relation to power of thermal stimulating HF current were carried out in rabbit.

REFERENCES

ADAMS, T. 1964. A method for local heating and cooling of the brain. J. Appl. Physiol. 19: 338—340.

BÖRNER, H. 1966. Elektrische Methoden in der Chirurgie. In: Handbuch medizinischer Elektronik. FRUCHT A. M. (trans. ed.), Warsaw: WNT, 6: 1—20.

CARLISLE, H. J. 1966. Behavioural significance of hypothalamic temperature-sensitive cells. Nature 209: 1323—1325.

DELGADO, J. M. R., HANAI, T. 1966. Intracerebral temperatures in free-moving cats. Amer. J. Physiol. 211: 755—769.

DONDEY, M., ALBE-FESSARD, D., LE BEAU, J. 1962. Premières applications neurophysiologiques d'une méthode permettant le blocage électif et réversible de structures centrales par réfrigération localisée. Electroenceph. Clin. Neurophysiol. 14: 758—763.

FOLKOW, B., STRÖM, G., UVNÄS, B. 1949. Cutaneous vasodilation elicited by local heating of the anterior hypothalamus in cats and dogs. Acta Physiol. Scand. 17: 317—326.

HARDY, D. 1961. Physiology of temperature regulation. Physiol. Rev. 41: 521—606.

HOLMES, R. L., NEWMAN, P. P., WOLSTENCROFT, A. 1960. A heat-sensitive region in the medulla. J. Physiol. 152: 93—98.

KAWAMURA, H., WHITMOYER, D. I., SAWYER, CH. H. 1966. Temperature changes in the rabbit brain during paradoxical sleep. Electroenceph. Clin. Neurophysiol. 21: 469—477.

LEBSON, S. 1965. Electrical measuring instruments. WNT, Warsaw.

MAGOUN, H. W., HARRISON, F., BROBECK, J. R., RANSON, S. W. 1938. Activation of head loss mechanisms by local heating of the brain. J. Nieurophysiol. 1: 101—114.

MONNIER, M., GANGLOFF, H. 1961. Atlas for stereotaxic research on the conscious rabbit. Elsevier, Amsterdam.

NAKAYAMA, T., HAMMEL, H. T., HARDY, J. D., EISENMAN, J. S. 1963. Thermal stimulation electrical activity of single units of the preoptic region. Amer. J. Physiol. 204: 1122—1126.

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Book reviews

Neurosciences Research Symposium Summaries. An Anthology from the Neurosciences Research Program Bulletin. Vol. 1. M. I. T. Press, Cambridge, Massachusetts. 570 p.

From Foreword:

Any comprehensive understanding of the nervous system will include both holistic and molecular concepts, will derive from both the „systems" and the „components" aproaches. Molecular neurology and molecular neuropsychology, now being established, need to be integrated with the findings of the classical neural and behavioral sciences. Committed to this vision, a group of „systems" and „components" scientists joined four years ago to from an international, interdisciplinary, interuniversity association called the Neurosciences Research Program (NRP). As the „faculty" of this „invisible college", the Associates have pursued an active course of investigation, synthesis, and communication. The NRP Work Session series helps to fuel such theoretical and experimental labors.

Francis O. Schmitt, Chairman

This volume of Neurosciences Research Symposium Summaries reports nine of the twenty Work Sessions held in the last three years by the Neurosciences Research Program-one from 1963 and the eight held in 1964.

Beneath we give reviews of some parts of the volume.

Cell membranes

The first NRP Work Session has been devoted to basic aspects of cell membranes, dealing with both their morphological and functional aspects. There are several reasons for which cell membranes are considered as one of the most important form of the organization of the living matter. First, they probably represent the highest order of molecular organization of this matter; second, they constitute a large percentage of the cell; and third, they play a key role in all vitally important processes, like protein synthesis (endoplasmic reticulum and ribosomes) and energy production (mitochondria); they also separate intracellular milieu from the external medium and mediate all kinds of external stimuli. It is therefore obvious that the function and the structure of cell membranes are of considerable importance in understanding of many problems of neurosciences.

The summary of the session, presented by A. L. Lehninger, with supplementary remarks by D. E. Green and J. D. Robertson, and a separate discussion article by A. L. Lehninger on the supramollecular organization of enzymes give an excellent and comprehensive review of the actual knowledge on various kinds of membraneous cell structures. The following problems are described and discussed in more detail: membrane ultrastructure as revealed by electron microscopy, chemical and physical properties of cell membranes and artificial lipid bilayers, and enzyme organization of cell membranes. Various aspects of multi-enzyme systems, as exemplified by mitochondrial electron-and energy-transfer enzyme assembly, are also discussed.

L. Wojtczak, Warsaw, Poland

Mathematical Concepts of Central Nervous System Function

The chapter has a descriptive character and presents the possibilities and the sphere of applications of some mathematical methods in neurophysiological research. The great part of presented reports and discussions were concerned with rather technical then pure mathematical methods because the term „mathematical methods" was used in a broader sense by the organisers of the session. For example, the modeling of the nervous functions and the application of mathematical computers for that purpose were included.

Here are some problems subjected to discussion:

a) mathematical description and modeling of molecular processes of the nervous system (particulary interesting are trials of modeling intracellular processes with the help of mathematical computer performed by W. R. Stahl);

b) mathematical description and modeling of neural structures (works of Mc Culloch, Arbib, MacKay and others);

c) the dynamics of neural systems with the emphasis on the importance of problems connected with temporal and spatial description of neurophysiological processes;

d) technical suggestions and concepts taken from biological systems with special emphasis on rules of operation of mathematical computers;

e) the need of formulation of „meta-language" with help of which we could describe the rules of the activity of the CNS.

A part of discussion was devoted to the concept of a „model" of biological system. The term „model" may be understood either in a broader sense when we give only general principles of functioning of a system, or in a narrower sense when we strictly formulate the rules which control the functioning of the system. The necessity of the search for the new mathematical methods for proper description of the processes in the CNS.

Among many subjects which ran throughout the whole chapter we must mention the problems of learning, plasticity, redundancy and sensitivity to damage. Especially interesting is here the conclusion concerning the methods of investigations of redundancy of elements in the CNS.

In the last part of this chapter some mathematical descriptions of particular processes occurring in the CNS are shortly presented. The first concerns the visuomotor reactions of the beatle chlorophanus and other insects. The seconds concerns several types of oscillations at the pupillary system after light stimulus.

R. Gawroński, Warsaw, Poland

Immuno-neurology

Although immunological pathogenesis had been proposed for some neurological diseases much earlier, the term „immuno-neurology" was coined in 1964 to name a much broader set of phenomena dealing with immunology and nervous system, and not only pathological ones.

There is ground to believe that the deciding factors promoting the birth of the new science were first, the hypothesis that memory could be stored in nucleic acids and/or proteins and second, the entirely new experimental approach of Mihailović and Janković, which supposedly allows the interference with functions of specific brain proteins. While the problems of relationships between hypothetical macromolecular coding of memory and even more hypothetical „immunoidal" readout mechanism were the subjects of an earlier symposium, this one was devoted to the topic of the protein build in the brain and the possibilities to investigate it by means of injected specific antibodies.

The symposium on immuno-neurology features three papers and an introductory article. The central paper is that of Mihailović and Janković in which summarized results of work done in their laboratories have been presented. The essential finding is that an injection of antibrain antibodies, avoiding the blood-brain barrier, affects biochemical and physiological processes in the brain resulting also in behavioral changes. Much more important is the suggestion that there is a significant degree of structural antigenic specificity so that e. g. the anticaudate antibody affects mainly the caudate nucleus. Despite the dangers in putting much weight in the results which the authors sensibly call „preliminary", it is worth pointing out that the main promise of this approach is the virtual possibility to dissect brain along a new dimension, that of protein build. In other words, the functional meaning of the existing brain structure has been studied by means of electrophysiology, neuropharmacology and lesion making. The immuno-neurological approach resembles mostly the last one but its applications are potentially different, since it does not act according to the localization but according to protein constitution of the brain structures.

The next two papers deal with protein build of the brain. Levine and Moore report discovery of a protein which is common for the gray matter of the central nervous systems of a wide array of species, the characteristic shared with only one other protein, that of lens. The supposition that the protein might be intracellular because the circulating antibodies in the acceptor organism do not apparently attack its brain is less likely than that Rappens because of the blood brain barrier. Authors do not report the CSF antibody content.

Rubin and Stenzel describe in vitro synthesis of protein in the suspension of brain cell organelles. In the far future this may be the way of obtaining antigenes for the immuno-neurological experiments.

Summarizing, this symposium presents first steps into an area which may supply otherwise unobtainable data once we overcome the difficulties inherent in any pioneering work.

L. Divac, Warsaw, Poland

ANNOUNCEMENT

PROPOSED EUROPEAN BRAIN AND BEHAVIOUR SOCIETY

We, the undersigned, wish to propose the formation of a EUROPEAN BRAIN AND BEHAVIOUR SOCIETY. The objects of the Society shall be the furtherance of scientific enquiry within those fields that bear on the interrelationships between brain and behaviour by holding periodic meetings at which papers are read and discussions held, by the dissemination of information and education materials made available as a consequence of research in the interrelationship of brain and behaviour, and by such other activities as may be decided upon by the Society.

A study group will meet in Rotterdam in the Spring of 1969 in order to continue our discussions in detail of the aims of the proposed Society and the direction in which it shall move, to consider and adopt its constitution, and to select its first membership. All persons interested in becoming a member of the Society are invited to write as soon as possible to Dr. A. Cowey (Institute of Experimental Psychology, South Parks Road, Oxford, England) stating their qualifications, experience, present work and interests. In addition, it would be appreciated if any opinions and suggestions about the general aims of the proposed Society could be expressed at the same time, and if applicants would state whether they are willing to deliver a paper at the first meeting of the Society, reporting scientific work that is considered to be in line with the aims of the proposed Society.

K. Akert, Zurich
A. Cowey, Oxford
M. Frankenhaeuser, Stockholm
H. G. J. M. Kuypers, Rotterdam
J. Paillard, Marseille
D. Ploog, Munich
J. Scherrer, Paris
L. Weiskrantz, Oxford

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ACTA BIOLOGIAE EXPERIMENTALIS

Zamówienia i przedpłaty na publikację przyjmują: od instytucji i wszelkiego rodzaju zakładów pracy Oddziały i Delegatury „Ruchu", od prenumeratorów indywidualnych: Centrala Kolportażu Prasy i Wydawnictw „Ruch", Warszawa, ul. Wronia 23, konto PKO Nr 1-6-100020.

Na odwrocie środkowego odcinka blankietu wpłaty należy podać zamówienie: tytuły zamówionych publikacji, liczbę egzemplarzy każdego tytułu.

Zamówienia bez pobierania przedpłat przyjmują księgarnie naukowe „Domu Książki" (adresy poniżej) na podstawie zgłoszenia i zobowiązania do odbierania poszczególnych tomów bezpośrednio w księgarniach lub za zaliczeniem pocztowym.

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