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THE OCCURRENCE OF THE CILIARY REVERSION  
IN THE DILEPTUS FRAGMENTS

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*(Received August 19, 1961)*

The behaviour of the ciliate fragments became the subject of studies almost a century ago. Papers of Balbiani (1893), Jennings and Jamieson (1902), Alverdes (1922), Koehler (1934) and Worley (1934) are to be mentioned in the first place. These investigations have had a great importance as opening a field to further researches and raising many unsolved problems.

The necessity for the modern approach to these questions by means of quantitative methods becomes evident. The general problem to be solved, the problem of localization of the reactivity, its compensation and restoration is undoubtedly worthy of more modern researches.

The experiments to be described in this paper aim to establish which fragments of the ciliate are capable to reverse, and to show the return of the polarity of the ciliary movement to its norm. The general description of behaviour and reactivity of the *Dileptus* fragments was published elsewhere (Doroszewski 1961b). Some illustrations to these studies and a description of the photomicrographic method applied may be also found in a separate note (Doroszewski 1961a). Additionally, the purpose of the present investigation is to describe the time duration and the frequency of occurrence of the ciliary reversion in the *Dileptus* fragments after the transection of the individual.

The occurrence of the reversion is but one of the numerous aspects of the complicated behaviour of the ciliate fragments. This response was chosen by the author because of its remarkable importance in the whole behaviour of ciliates and its usefulness for the quantitative studies. In the course of experiments no additional stimulation except the operation

itself were applied, the movement being investigated under the constant conditions in given medium.

#### MATERIAL AND METHODS

In order to obtain the comparable data care was taken to secure the standardization of material. The experiments were made upon the stock of *Dileptus anser* O. F. M. taken from the pond in the neighbourhood of Warsaw.

The equal portions of Colpidium were given each day as the food to *Dileptus*. Colpidia were in turn cultivated upon the standard medium of the powdered milk. The desirable quantity of normally looking individuals of *Dileptus* was chosen from the culture, and washed. After that the ciliates were placed in the experimental solution for a period of 24 hours. By this procedure the possible differences between the individuals originating of the unequal state of nutrition were practically eliminated, all the ciliates being slightly underfed.

The transections of the individuals were made by means of the specially prepared steel spear-point needles. Observations were made in the widely spread ( $4 \times 4$  m.) drops of water upon large slides. The low power dissecting microscope was used for the observations. The time duration of the backward and forward movement of the ciliate was recorded by means of a stop-watch. The possible error of the measurement could range up to 0.5 sec. The purpose of the studies here described was to observe the ciliate or its fragments as a whole. The direct observations of ciliary movement with the use of photomicrography and the movie camera technique are now in course.

#### RESULTS

##### The post-operational reversion

The experiments consisted of the momentary transection of the individual. The immediate effects of cutting the animal were observed, the section being made upon the ciliate swimming forward. The observations and operations were made upon the ciliates being placed in the Dryl's buffer solution (Dryl 1959). In the series of experiments here described the posterior fragment of the ciliate after the cutting was observed. The five series of operations were performed with the transections being made at five defined levels of the ciliate cell. The transection marked by No. 1 was made through the proboscis in the middle of its length; section No. 2-near its basis, No. 3-immediately behind the cytostome; transection No. 4 was performed near the middle of the cell body (the proboscis was not counted), and No. 5-just before the basis of the posterior sprout. Each series of experiments consisted of 100 transections at the given level.

In response to the transection all the ciliates deprived of the part of proboscis reacted with the immediate withdrawal, i. e. they reversed the



direction of their movement without turning. During that backward movement the rotations around the long axis of the body were observed. The general pattern of this reaction was also similar in the cases of the next levels of the cuts, i. e. that after transections. No. 2 and No. 3 the fragments exhibited the immediate post-operational reversion of the movement. In series No. 4 the posterior fragment maintained its forward movement only in one case. The time durations of reversions were

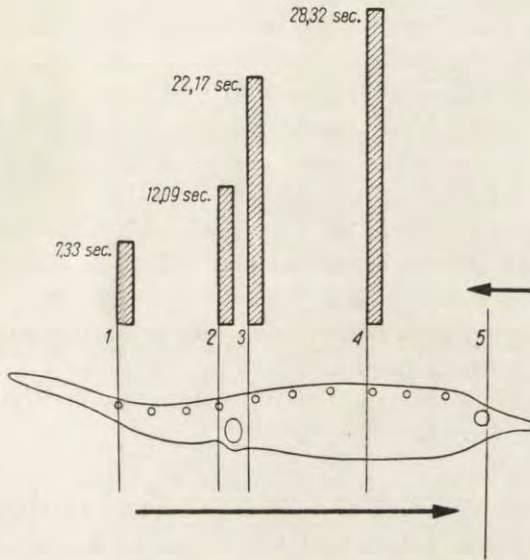


Fig. 1. Scheme represents the occurrence of the post-operational reversion in the *Dileptus* fragments.

Lines Nos. 1, 2, 3, 4, and 5 indicate the levels of the transections. The corresponding diagrams represent the time duration of the post-operational reversion occurring on the fragment situated posteriorly to each transection. Fragment No. 5 maintains its forward motion as indicated by the arrow.

remarkably longer when the level of transections was situated more posteriorly. The following arithmetical means for duration of the reversion were obtained in each series of experiments:

For the posterior fragment obtained by the transection	No. 1—	7.33
" " " " " " " "	No. 2—	12.09
" " " " " " " "	No. 3—	22.17
" " " " " " " "	No. 4—	28.32

The results of series No. 5, when the very posterior part of the ciliate

was cut off, were surprisingly different. In all these cases the post-operational reversion did not occur. The absence of the immediate response was, however, not the result of the general inability for reversion of the fragment. The first reversion occurred after a while, the fragment continued forward motion lasting several seconds (the arithmetical mean  $\bar{x} = 5.15$ ). The additional experiments were made to determine the exact border line between the fragment responding to cutting and the non responding one. Some experiments were made to obtain the posterior fragment by means of crushing the anterior part of the ciliate instead of cutting it. The duration of the reversion was apparently longer as compared to the results described above. The main tendency of the anterior fragment after cutting was to maintain its forward movement. Although the backward movement seldom occurred after the operation, it lasted much shorter. The movements of the proboscis have also its locomotory effect after cutting.

The results here described, as compared with those previously reported (Doroszewski 1961b), show the more clear uniformity following the standardization of the experimental conditions. As the occurrence of the outflows of the cytoplasm was avoided, the appearance of the post-operational reversion became more stable. The unexpected effect of the lack of the immediate response to the operation upon the very posterior fragment was not found before.

#### The occurrence of the reversion in the subsequent period

Further observations were conducted to follow behaviour of the fragments during the subsequent period after operation. The observation began before the operation to secure the control data concerning the movement at a given medium. The time durations of the backward swimming and that of the normal movement were continuously recorded. After a lapse of time (3—4 min.) the transection was done, and the observation was carried out upon the posterior fragment. In the experiments described the ciliates were sectioned near the middle of the cell body (the proboscis was not counted) as in the case of the transection No. 4 in the previous experiments. Two media were chosen for the experiments: 1) the Dryl's buffer solution, and 2) the 1 mM solution of the calcium chloride. In the solution of calcium chloride the general pattern of the movement of *Paramecium* is rather stable and the forward movement is rarely disturbed (Golińska, unpublished).

Upon the diagram (Fig. 2) a special mode of representing the reversion was applied.

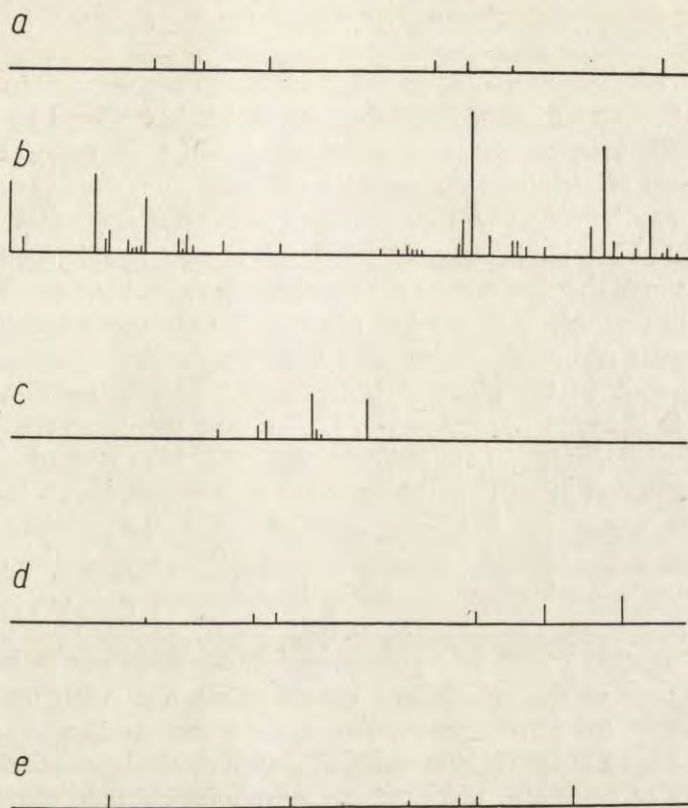


Fig. 2. Diagram illustrating the appearance of reversions on the posterior fragment of *Dileptus* in the period following the operation.

The horizontal lines represent the forward movement, the perpendicular ones — the backward movement. 0,66 millimeter on the diagram corresponds to one second of the duration of the movement. a — the control observation of the movement of the individual before the operation; b — the movement of the fragment after the operation. It begins with the post-operational reversion; c and d — the restoration period of the movement of the fragment; e — the movement 15 minutes after the operation.

Sometimes, the observed fragment touched the border of the drop of the medium. In these cases the fragment reacted by reversion or began circling near the border of the drop. Sometimes, the fragment stopped without any visible obstacle, yet this occurred rarely and without any regularity.

The tendency of reversion to occur in groups was observed, i. e. several reversions can appear one after another followed by the longer period of the normal forward movement. No regular resting stage during the regeneration was observed.

## Discussion

Briefly, we can say that after the transection of the individual the post-operational reversion immediately occurs, and it is followed by the period of the oscillations of the movement after which it restores its norm characteristic for a given medium. It is likely that the post-operational reversion may be regarded as a response to the traumatic stimulus of the transection. This gains support in the fact of the longer duration of reversion upon the fragments obtained by the crushing of the anterior part. The differences between the times of the reversion duration in various fragments of the ciliate can be due to the greater traumatic effect of the transection in the place of the greater diameter (section No. 4) as compared to the case of section No. 1. The capacity of reversion upon all fragments of *Dileptus* may indicate that the presence of neuromotor apparatus (Vissher 1927) is not the necessary condition for its evoking. The lack of the immediate response after the operation on the very last fragment may, however, be considered to be the proof of the differential localization of some kinds of reactivity in the ciliate cell. The transection disturbs the general state of the motor polarity of the ciliate cell, as it results in creation of the two autonomous individuals out of one. During the appearance of the subsequent reversion we deal with the return of the polarity of the ciliary movement to its norm. As it was said earlier (Doroszewski 1961b) the state of equilibrium is established, when the location of the motor pole and the morphological pole conform.

The recovery of the movement for a given medium is performed before the end of the morphological regeneration (vide Sokoloff 1924) of the fragment.

## SUMMARY

The observations carried out by the author concerned the movement of the posterior fragments of *Dileptus* after the transections. The continuous registration of duration of the forward and backward motion leads to the following conclusions:

1. Every posterior fragment of *Dileptus* is capable to reverse.
2. The posterior fragments respond to the transection with the ciliary reversion, except for the most posterior ones.
3. The spontaneous ciliary reversions occurring upon the fragment after the transection are more frequent than upon the intact individual.
4. The restoration of the normal polarity of the ciliary movement takes place before the end of the regeneration process.

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## ABSOLUTE VERSUS RELATIVE CUES IN DIFFERENTIATION OF TONES IN DOGS

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*(Received September 15, 1961)*

Innumerable studies carried out by various methods have been devoted to the problem of differentiation of frequencies of tones in animals. The classical method introduced by Kalischer (1907) and Pavlov (1909) consisted in applying two particular tones, of which one was always followed by presentation of food, while the other one was not. After a number of such trials the conditioned alimentary reaction was performed by the animal only to the "positive" but not to the "negative" tone. This reaction was measured in Pavlovian experiments by conditioned salivation and in Kalischer's experiments by a simple instrumental act — running towards food. Although a number of other methods has been introduced since then, their general character has remained the same: definite tones or their compounds have been used for differentiation, each of them being a signal of a different event.

The routine procedure of differentiation usually consisted in applying the positive and the negative stimulus in a more or less random order within intervals of seconds or minutes. In particular, according to the tradition of the Pavlovian laboratories the negative stimulus was applied rather rarely among the positive stimuli separated by intervals of several minutes.

It may be observed that such a procedure allowed the animal to make use in the differentiation training of two different principles: on the one hand, the animal could learn that one definite pitch signalled reinforcement, while the other did not; on the other hand, the animal could learn that, provided the first tone was always positive, all subsequent tones identical with it are also positive, while those which

were different were negative. Obviously, while the way of establishing differentiation based on absolute frequencies of the applied tones involves stable memory, the way based on comparison of the positive and negative tone, after the very principle of the task has been acquired, requires only recent memory.

We have so far no clear evidence which way the animals learn to differentiate two frequencies of tones under the usual experimental conditions. It seems certain that after a prolonged training the animal does memorize the absolute frequencies of tones, but we do not know whether in the initial period of training the animal makes use of the seemingly much easier way, namely that of reacting to the mere disparity of tones instead of their absolute values.

The aim of this paper is to establish whether the dog learns at once to differentiate the absolute frequencies of the applied tones, or whether he learns primarily, and may be more easily, to discriminate their conditional meaning by mere comparison.

Two different kinds of experiments were performed. In the first set-up the differentiation was carried out by interposing the negative tone amidst the series of positive tones. In the second set-up, on the contrary, the positive tone was applied after a series of negative tones.

These two kinds of experiments will be described separately.

## EXPERIMENTAL SET-UP I

### MATERIAL AND METHODS

The experiments were performed on 6 mongrel dogs in the sound-proof conditioned-reflex chamber. In the preliminary training the animals were taught, by method of passive movements, to raise the right foreleg and to put it onto the platform in response to a tone produced by an electronic generator. The performance of this movement was immediately reinforced by food presented in successively moving bowls. 6 to 8 reinforced trials were given in each experiment.

When the instrumental conditioned reflex was firmly established and intertrial responses had disappeared, the proper experiments began. The animals were trained in differentiation of pairs of tones differing in frequency, the negative tone being always higher than the positive by two octaves. The negative tone lasted 4 seconds and was not reinforced. It was applied only once in each experimental session, usually after 4th, 5th, or 6th application of the positive tone. The intertrial intervals were in initial experiments of 1½ to 3 min., but later they were reduced to 35 to 50 sec., and in some series to 20 sec. In experiments with shorter intervals the portions of food were very small to enable the animal to eat them within a few seconds.

The detailed design of the experiments will be given in the next section.



## RESULTS

First variety. In dog No. 1, after the preliminary training had been completed, 5 tones, viz. 60, 120, 240, 480 and 960 cps were used as positive conditioned stimuli. These tones were changed randomly from day to day, but in a single experimental session only the same positive tone was applied. The negative tone was by two octaves higher than the positive tone applied on the given day, i. e. its frequency was 240, 480, 960, 1920 and 3840 cps respectively. Thus, tones of 240, 480 and 960 cps were positive in some sessions and negative in others, while tones of 60 and 120 cps were always positive, and tones of 1920 and 3840 cps were always negative. A negative tone was applied once daily after several positive trials.

The course of experiments with this dog is presented in Fig. 1. It is seen that during the first 22 sessions the inhibitory conditioned reflex did not develop. During that time the animal became increasingly restless,



Fig. 1. The course of differentiation of tones in dog No. 1.

Abscissae, successive experimental days. Ordinates, frequencies of tones. Crosses, the frequency of positive tone applied on a given day. Circles, frequency of a negative tone applied on a given day. Crosses with squares denote that on the given day the conditioned reaction to the positive tone failed to occur in some trials. In such sessions the negative tone was not applied. Empty circles denote that the negative tone produced a positive (incorrect) response. Full circles denote that the negative tone produced inhibitory (correct) response. Note the failure to obtain differentiation with changing the tone frequencies each day, the easy solution of the task when the same pair of tones is applied on successive days, and experimental neurosis in the end of the experimental series.

whined and tried to run away. When on the 22nd day we began to apply an identical pair of tones, 120 vs. 480 cps in several successive sessions, the inhibitory reflex was established almost immediately. After shifting to the pair 240 vs. 960 cps (exp. 28) the conditioned response to the negative stimulus reappeared, but after a few repetitions it was inhibited (exps. 30—33). When, however, in the next session (34) the tone 480 cps was applied as a positive stimulus, there was no positive response to it; this

had never happened before. On the following days a heavy neurosis developed. The conditioned reaction to positive stimuli was often absent, and the animal refused to take food in the experimental situation.

In order to make the task easier, in the next experiments we returned to the lower positive tone, 240 cps, ceased to apply the negative tone at all, and gave the animal bromides. Gradually, the condition of the dog improved, and on the 5th day (exp. 38) all 8 positive reactions were present, although the dog was still restless. In view of this, in the next session the negative tone 960 cps was again introduced (exp. 39). It produced a correct inhibitory reaction, however in the following session it was disinhibited and heavy neurotic symptoms reappeared. In this situation the experiments were discontinued.

*C o m m e n t.* This series of experiments shows that when the dog had to differentiate two tones by means of their comparison, he failed completely to solve this task. On the other hand, he displayed a strong tendency to base differentiation on the absolute frequency of tones, which he remembered from one session to another. This tendency was manifested by mastering differentiation when the same pair of tones was repeated in several sessions, and by the nervous breakdown, which followed the change of the conditioned meaning of the applied stimulus.

In view of this negative result it was decided to use initially only one pair of tones until a criterion of three successive correct responses to the negative stimulus was reached, and only then to introduce other pairs.

*S e c o n d v a r i e t y.* Dog No. 2 was first trained in differentiation of tones 240 vs. 960 cps. When he mastered this task, the pair of tones 120 vs. 480 cps was applied without any disorder of the inhibitory reflex to the negative stimulus. When, however, the pair 60 vs. 240 cps was presented, the tone 240 cps elicited the positive (incorrect) response. In the further course of experiments the condition of the animal deteriorated precisely as in dog No. 1: he became exceedingly restless, whined and barked through experimental sessions trying to get loose from the stand, and eventually refused to take food. In this situation the experiments were discontinued.

In dog No. 3 after establishing differentiation 480 vs. 1920 cps both tones were lowered gradually by steps of about 1/6 of octave, while the original tone was also applied time and again. The dog reacted more or less correctly to the negative tone till the pair 140 vs. 560 cps was introduced. Then, similarly to the previous dogs, he began to manifest neurotic symptoms.

In the following experiments the procedure was changed by applying much shorter intertrial intervals, namely 35—50 sec., and then 20 sec. In this situation the dog was able to solve correctly the task, even when

the pair 120 vs. 480 cps was applied. But differentiation of these last tones was very unstable and often the inhibitory reaction to the tone 480 cps failed to appear. It was noticed that whenever the pair 120 vs. 480 cps was applied in a session following that in which the pair 480 vs. 1920 cps was used, the animal failed to react properly to the negative tone. In spite of more than 300 experimental sessions the animal's reactions did not improve: while differentiation of tones 480 vs. 1920 cps and also 240 vs. 960 cps was quite stable, the differentiation 120 vs. 480 cps remained most irregular.

The results obtained in three other dogs of this group (Nos. 4, 5, 6), which were trained from the very beginning with shortened intervals, did not differ from those described above. Dog No. 4 ended his experimental career by heavy experimental neurosis when the conflicting differentiation was introduced. Dog No. 5 was also not able to react correctly to differentiation 120 vs. 480 cps when experiments were interspersed with those involving differentiation 480 vs. 1920 cps. Dog No. 6 succeeded in doing so, but again his correct reactions were unstable.

**Comment.** In the experiments just described it was attempted to make differentiation by comparison easier, by gradual changing the pitch of tones and by shortening the intertrial intervals. Under such condition some animals were able to solve the problem, but its utmost difficulty was evidenced by the fact that correct responses were very unstable and neurotic disorders were often observed.

#### EXPERIMENTAL SET-UP II

The results of the first kind of experiments have shown that differentiation of tones by comparison is in dogs either very difficult or impossible. However, one objection could be raised against our previous experimental procedure. The negative tone, which should be compared with the positive one, was always separated from it by presentation of food and the act of eating. This event could interfere with the preservation of recent memory traces of the positive tone, and thus make the comparison of the two tones more difficult. Therefore, it was thought that if the experimental procedure is reversed, i. e. the positive tone is given after a series of negative tones, this handicap will obviously not take place. Accordingly, the following experimental program was designed.

#### MATERIAL AND METHODS

Experiments were performed on 2 mongrel dogs under the same general conditions as those in set-up. I. However, from the very beginning two tones differing from one another by two octaves were selected, the lower one being negative and

the higher positive. In each experiment the negative tone was presented 13 times, the positive one 5 times. The positive tone was always applied after 2, 3 or 4 applications of the negative tone. Either of the stimuli lasted 2 sec., intertrial intervals were 30 sec.

Initially, the positive tone was accompanied by passive placing the dog's foreleg on the platform, till the animal learnt to perform this movement actively. Then proper experiments began.

## RESULTS

Dog No. 7. Experiments began by differentiation of tones 480 cps (negative) and 1920 cps (positive). When the preliminary training was completed, the positive tone always elicited the conditioned response, while the negative tone was occasionally disinhibited.

After reaching fairly accurate responses to both tones, we passed by steps to the pair of tones 120 vs. 480 cps; and so the tone 480 cps, which was negative in the original training, became now positive. This did not produce any disorders in animal's behaviour, and the positive tone 480 cps gave always positive responses.

Then in a series of 60 experiments three pairs of tones were applied, namely 480 vs. 1920 cps, 240 vs. 960 cps and 120 vs. 480 cps.

As far as positive tones were concerned they elicited 100% of positive responses; this means that even in 120 vs. 480 cps sessions preceded by 480 vs. 1920 cps sessions the tone 480 cps did always give the positive response. This shows that the animal was quite capable to base his response to the positive tone on the comparison with the immediately preceding negative tones.

However, quite different were the responses to the negative tones. As seen in Fig. 2 and Table I, the animal made much more errors to the tone 480 cps (which was positive in other sessions) than to the tones 120 and 240 cps, which were always negative. Moreover, these errors were particularly numerous when a 480 vs. 120 cps session followed immediately a 120 vs. 480 cps session. If, however, a 480 vs. 1920 cps session was repeated, or preceded by a 240 vs. 960 cps session, the number of errors was smaller (Table I).

Closer examination of distribution of errors shows that it is quite different in different types of sessions. In 480 vs. 1920 cps sessions, especially when they were preceded by 120 vs. 480 cps sessions, disinhibition occurred, as a rule, in the very first inhibitory trials, while towards the end of the session the animal's performance was quite good. This means that in the course of a single session the animal learnt to inhibit the positive reaction to the negative tone 480 cps and then in 120 vs. 480 cps sessions he lost what he had learnt.

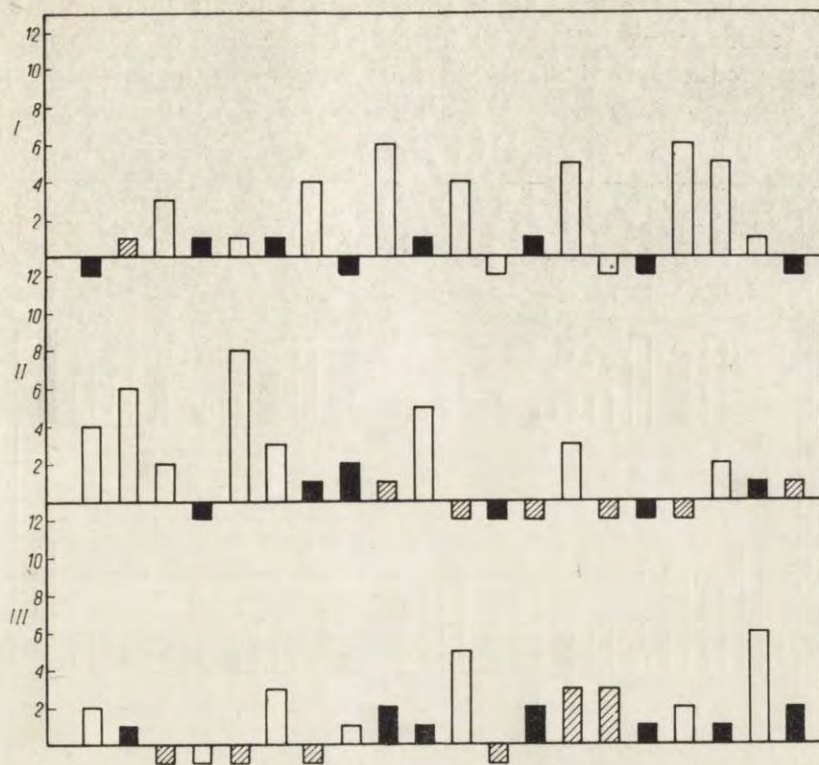


Fig. 2. Reactions to negative stimuli in dog No. 6 in 60 successive sessions.

Successive columns and rows from top to bottom denote successive sessions. Black columns denote negative tones 120 cps; striped columns, negative tones 240 cps; white columns, negative tones 480 cps. The height of each column denotes the number of errors (i.e. positive responses) in the given session in proportion to 13 negative tones applied each day. The small columns down the abscissa line means that no errors were performed in the given session. Note the much greater numbers of incorrect responses to the tone 480 cps. than to 240 cps. and 120 cps. Note also that particularly frequent errors occur when the session with the 480 cps. negative tone follows the session with the 120 cps. negative tone.

Table I

The percentage of incorrect reactions to various negative stimuli in dogs Nos. 6 and 7

Dog	120 cps	240 cps	480 cps	480 cps after 120 cps	480 cps after others
No. 6	6.6	5.3	25.7	37.0	18.4
No. 7	10.4	7.1	28.7	37.4	25.1

On the other hand, disinhibitory errors in 240 vs. 960 cps and 120 vs. 480 cps sessions were rather due to the predominance of excitation over inhibition produced, for instance, in those cases when the animal "expect-

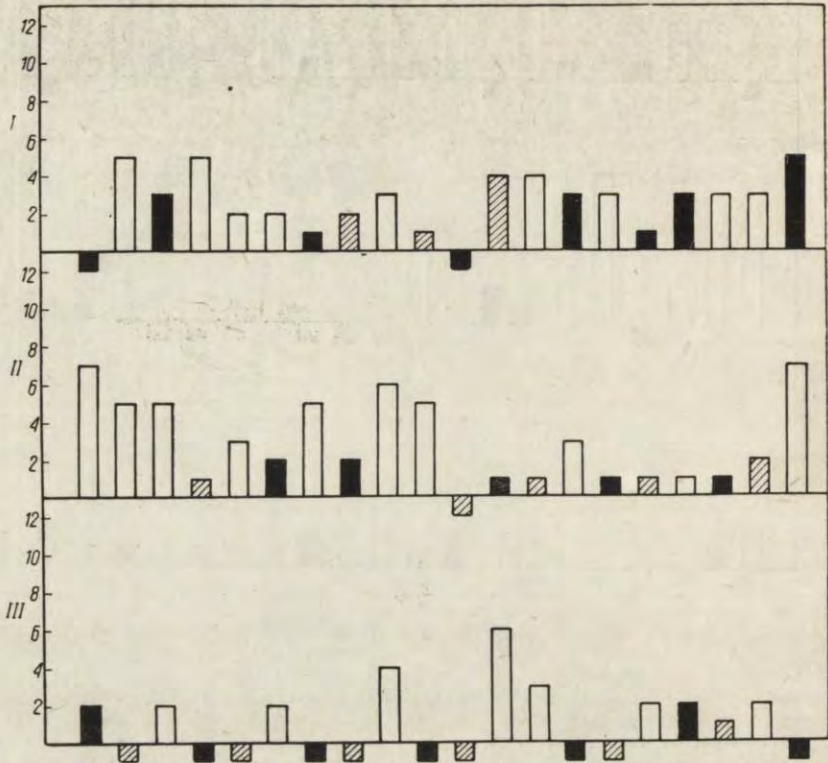


Fig. 3. Reactions to negative stimuli in dog No. 7 in 60 successive sessions.  
The same explanations as in Fig. 2.

ed" already the application of the positive stimulus (conditioned reflex to time).

The experiments with dog No. 7 followed exactly the same course as those with dog No. 6, and, as may be seen in Fig. 3, and in Table I, their results were quite similar.

Comment. As seen from these two experimental series, differentiation based on the comparison of two tones could be established relatively easily, if the negative tones preceded the positive one. Neither dog developed experimental neurosis, and both reacted correctly in 100% to positive tones, even when the conflicting tone 480 cps was used. However,

the reactions to negative tones were strongly affected by the absolute pitch of the applied tone, as evidenced by the fact that the negative tone 480 cps was frequently disinhibited, when on the preceding day it was used as a positive stimulus.

#### DISCUSSION

When a human observer attends regularly the experimental sessions, such as described in this paper, he is struck by the dog's incapacity to solve the problem presented to him. For human the disparity between two tones differing in pitch by two octaves applied within short intervals of time is so obvious that he has no difficulty at all to perceive it. On the other hand, he remembers very poorly, if at all, which pair of tones was used in the preceding session.

Quite a reverse situation was observed in dogs. As we have seen they are very poor in differentiation of tone frequencies by comparison, especially when the tones are separated one from another by the act of eating. On the other hand, they are very good in remembering the absolute frequencies of the applied tones and their respective conditioned significance, even when the presentation of these tones is separated from one another by an interval of a day or more.

All the data obtained in this paper explicitly point to this conclusion. When the pairs of tones separated by the act of eating in the first kind of experiments changed every day, the dog was totally lost, since the tone he had memorized one day as positive became negative on another day, or vice versa. Hence a strong conflicting situation, denoted by Pavlov (1926) as "clash" between excitatory and inhibitory processes, arises which leads to heavy experimental neuroses. If some animals were eventually able to catch the principle of the presented task, they were not capable to follow it consistently because of the strong tendency to base the differentiation on the absolute frequencies of tones. This tendency was particularly clearly seen when the same pair of tones was applied in several successive sessions, and then another pair was presented. Repetition of the same pair of tones led to differentiation based on absolute frequencies of tones, while application of a pair in which the positive tone became negative, and vice versa, led to a neurosis.

Shortening the intervals between trials, as well as shortening the act of eating, seems to have a favourable effect on differentiation, since the comparison of the two stimuli was thus facilitated. However, a much more considerable facilitation was provided by inverting experimental procedure so as to remove completely the distracting effect of food separating the tones to be compared in the second kind of experiments. Yet, even in

this much easier situation the tendency to develop differentiation of absolute frequencies of tones was manifested in disinhibition of inhibitory responses.

So far it is difficult to find an explanation for this striking difference between the perception of tone frequencies in dog and man. Two assumptions accounting for this difference seem to be equally plausible.

First, one may assume that the perception of particular frequencies of sounds is in dog much more "individualized" than in man and reminds, in this respect, of our perception of colours. Indeed, in human perception colours are not arranged along the qualitatively uniform range, progressing from lower to higher frequencies of waves, but constitute individual entities of totally different qualities. This fact is reflected, among other things, in our everyday (but not professional) language: we do have different names for different colours but not for different frequencies of tones. In consequence, if we had to master visual differentiation based on relative differences between frequencies of light waves, we might encounter the same difficulties as those encountered by our dogs in a similar acoustic test.

Another supposition explaining our results would be that whereas in dog the stable memory of tone frequencies is much better than in average human, his recent memory of tones is, on the contrary, poorer. In consequence when the dog and the man are confronted with the problem which may be solved both ways (as is the case with simple pitch differentiation) they utilize that faculty which they possess in higher degree. But if a differentiation requires only one of these faculties and not the other, the difference between these two species becomes apparent. Differentiation of frequencies involving immediate comparison of successive tones is very easy for us, but very difficult for dogs. On the contrary, if two definite tones are applied separately at intervals of a day or more, such a task is quite easy for the dog, but for the average human presents a considerable difficulty.

#### SUMMARY

1. The present study is concerned with the problem of the cues on which the differentiation of tone frequencies in dogs is based. For this aim two tones of different frequencies were given each day, one of them being positive and the other negative. The positive tone was consistently higher, or consistently lower, than the negative tone, but each day a different pair of tones was presented.

2. When the negative tone is always presented after a number of



positive tones, i. e. is separated from them by the act of eating, the differentiation task is exceedingly difficult for the dog. Some animals fail to solve this problem at all and develop heavy experimental neuroses, others solve it only partially, the correct responses being very unstable.

3. When the positive tone is presented after a number of negative tones, i. e. is not separated from them by any distracting agent, the task of differentiation becomes easier, however it cannot be solved by the animal wit 100% accuracy.

4. The results obtained show that in dogs differentiation of tone frequencies is based primarily on memorizing their absolute values, and not on the comparison of tones following one another in the same experimental session.

5. The reasons of this way of solving the differentiation task in dog in contrast to man is discussed.

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STUDIES ON THE AVERSIVE CLASSICAL CONDITIONING  
1. ACQUISITION AND DIFFERENTIATION OF MOTOR  
AND CARDIAC  
CONDITIONED CLASSICAL DEFENSIVE REFLEXES IN DOG

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In our previous papers we dealt with cardiac conditioned reflexes involved in the conditioning of avoidance reflexes (Sołtysik 1959, 1960a, 1960b, Sołtysik and Kowalska 1960). According to these data cardiac acceleration is a stable component of the avoidance conditioned reflex (CR) and probably represents a classically conditioned emotional background of the motor instrumental reaction. In an unpublished pilot experiment on one dog we found that the amplitude of the cardiac response accompanying both the classical and instrumental defensive CRs was exactly the same. On the other hand, neither classical motor nor salivary (in acid avoidance conditioning — Konorski and Miller 1936) reactions were preserved in the avoidance reflex. This raises the question as to the relationship between the cardiac and the specific local (motor or salivary) components of the classical defensive CR.

In this paper we present some data on the relationship between cardiac and motor classical CRs observed in the course of acquisition, acute extinction and differentiation.

MATERIAL AND METHOD

A. Subjects

The subjects were 7 mongrel male dogs between 2 and 7 years old, ranging in weight from 14 to 26 kg. They were well accustomed to the experimental situation (staying in the Pavlovian frame) and recording devices, but naive with regard to

all conditioning experiments. They were, however, previously given a number of external stimuli as they served as subjects in the study on cardiac responses accompanying orienting reflexes (Sołtysik et al. 1961). In all animals a carotid loop by van Leersum (1911) had previously been prepared.

## B. Apparatus

Experiments were performed in a light-shielded semi-sound-proof CR-chamber with one-way glass screen through which the experimenter made his observations. Recording apparatus consisted of two kymographs, pneumatic relays for registration of breathing and movements of the right hind- and foreleg, electro-magnetic system for measuring the latency of flexion of the right hindleg, and piezoelectric device for recording pulse waves from the carotid loop. Form and duration of the movements of the hindleg were recorded on a slow kymograph. Tracing movements of the foreleg served as an index of general restlessness of the animal. The fast kymograph was used for recording the latency of flexion, respiratory movements and pulse rate.

Conditioned stimulus (CS) was a buzzer-like sound delivered from a stimulator through the loud-speaker placed 1 m. in front of the animal. The intensity of sound, measured at the place of dog's head, was about 60 decibels; the background noise of about 40 db. was produced by recording and other electrical appliances masking thus any cue from outside. The same stimulus from another loud-speaker placed at the same distance but on the right side of the animal served as differential stimulus (DS).

As an unconditioned stimulus (US) a momentary shock from the condenser 1 or 2 $\mu$ F, fed from the anode battery with 70–120 volts, was used. The stimulating silver wet electrodes were fixed between toes of the right hindleg. Special attention was paid to secure a stable resistance between electrodes, which ranged from 4 to 6 kilohms, in order to eliminate any escape from the shock due to minor dislocations of electrodes during movements\*.

## C. Procedure

**Acquisition training.** During 27 days each dog was presented 10 times daily a CS-US trial in 1–2 min. intervals. Duration of the CS was 3 sec. and the shock was applied at the moment of its termination. The intensity of shock was adjusted in such a way that it produced a definite flexion reflex in the right hindleg.

Before starting this training, each animal was tested in one session (10 trials) by a tone (the future CS) in order to establish the cardiac response to it.

**Acute extinction.** On the 28th and 29th sessions the stimulus from the right loud-speaker was presented 50 times without shock. Intertrial intervals were 1–2 min.

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\* For the same purpose, i. e. to minimize the contamination of instrumental conditioning, the hindleg flexion was chosen because hindlegs are much less "instrumentalized" during life, and a brief shock as US was used to exclude eventual establishing the escape responses. In another paper an experimental verification of the classical nature of these reflexes is presented (Sołtysik and Jaworska 1962).

Chronic differentiation training. In the following 40 sessions the CS from loud-speaker placed in front of the animal and the DS from the loud-speaker placed on the right side of the dog were presented 5 times daily at random order. The CS was regularly reinforced with shock.

Statistics. Significance of differences between arithmetic means was calculated using the "t" test. Calculation of the coefficient of regression and its significance was the same as in our previous publication (Sołtysik et al. 1961). The random distribution of positive and inhibitory trials was ordered according to tables of random permutations (Cochran and Cox 1957).

## RESULTS

### Acquisition of conditioned motor and cardiac reflexes

Fig. 1 presents the course of acquisition of motor and cardiac conditioned reactions in our group of dogs. It is seen that both curves are not parallel; the curve of motor CR (black circles) rises steeply, reaches its maximum in the 5th session and afterwards declines slowly. On the other hand the curve of cardiac response continues to rise with negative acceleration throughout 15 sessions, i. e. it rises even when the motor CR

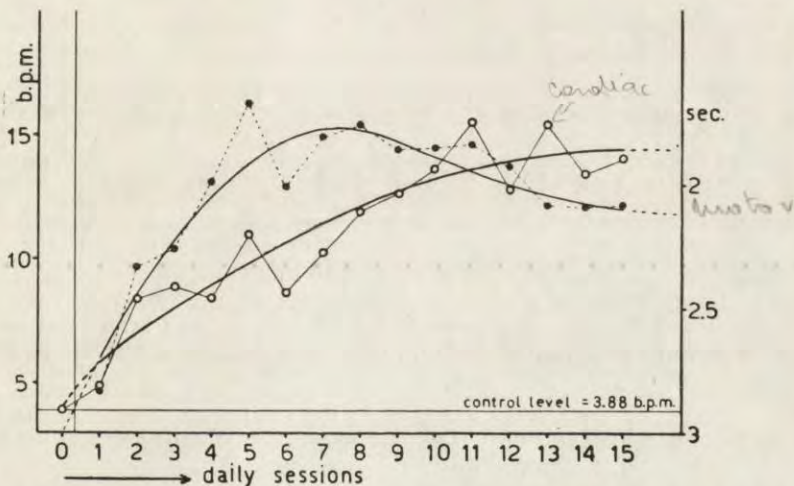


Fig. 1. Acquisition of motor (black circles) and cardiac (white circles) classical defensive conditioned reflex.

Motor CR (flexion of the right hindleg) was measured by its latency (scale on the right ordinates), and cardiac CR was measured as the difference between the mean pulse rate during the CS and the mean pre-stimulus pulse rate (the left ordinates). Data from 7 dogs are averaged.

begins to drop. Thus both curves, being parallel in few first sessions, soon become divergent exhibiting opposite tendencies. The first session denoted as "O", shows the size of the cardiac response to the tone prior to conditioning.

### Comparing the cardiac conditioned and unconditioned reflexes

Fig. 2 gives a comparison of changes in amplitude of cardiac CR and UR during the first 15 daily sessions. It is evident that while the CR increases forming a regular acquisition curve, the cardiac UR curve

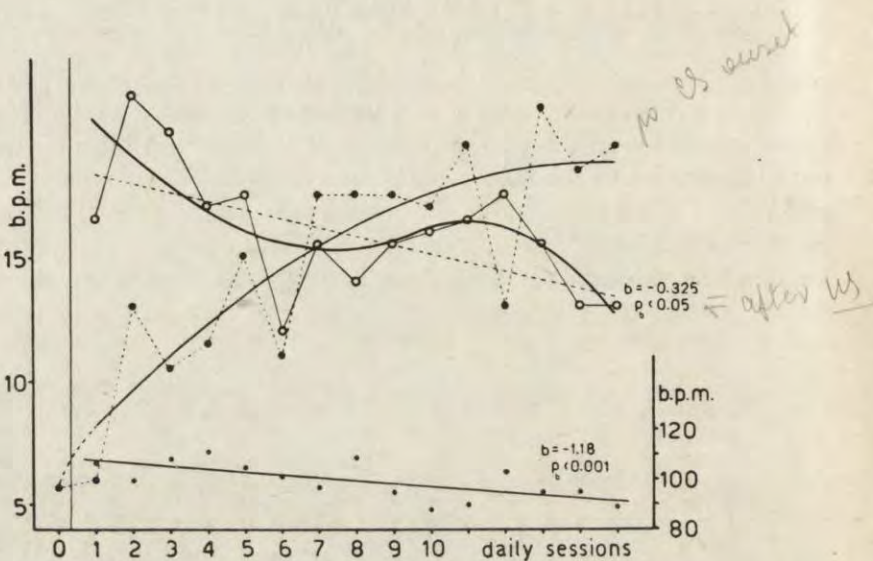


Fig. 2. Changes in cardiac CR, UR and the pre-stimulus level of the heart rate during acquisition of the defence CR.

Left ordinates show cardiac acceleration (in beats per minute) measured in the 2nd second after the onset of CS (black circles), or in the 2nd sec. after the presentation of US (white circles). It is seen that while the cardiac CR increases, the cardiac response to US gradually falls and becomes smaller than the CR. Linear (broken line) and curvilinear regression (S-shaped line) for the cardiac UR are shown. Right ordinates show the pulse rate during 10 sec. preceding the onset of CS and refer to the small black dots below the two former curves. There is evident extinction of this pre-stimulus (or intertrial) level of the heart rate.

decreases and finally drops below the CR curve. One can see that this diminution of cardiac UR is temporarily arrested at the moment when both curves cross. It means that the cardiac CR, becoming greater than the UR, prevents further decrease of UR. However, after a few sessions in which both the CR and UR run parallelly, the UR again decreases,

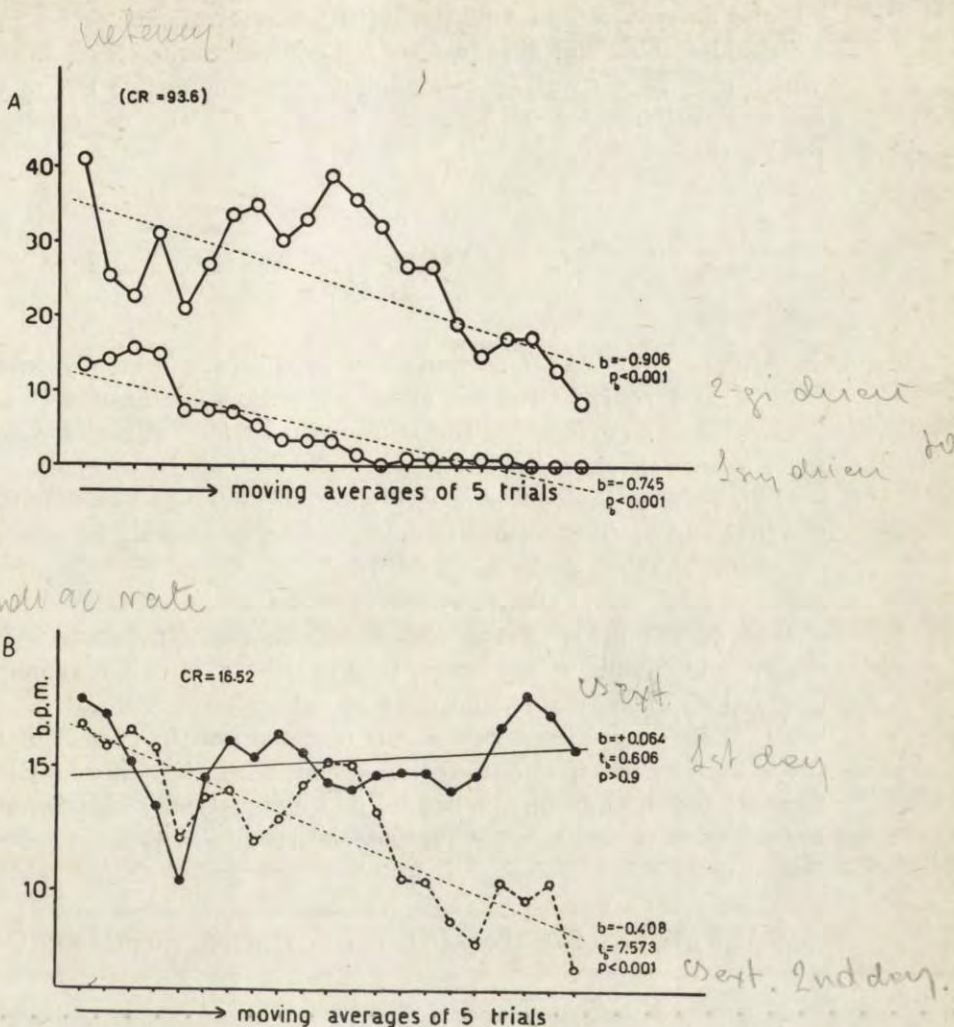


Fig. 3. Extinction of motor and cardiac classical defensive conditioned reflex.

A. First (upper curve) and second (lower curve) day of acute extinction of the classical motor defence reaction to DS. There is obvious extinction on the first day, and even in the first trials the latency of the conditioned flexion is much longer compared with the mean latency of this CR to the positive original CS. Ordinates are conventional units calculated from the latency by the formula:  $100(3-L)$ , where L is latency in seconds; the duration of stimulus was 3 sec. B. Cardiac response accompanying the motor defensive CR during the extinction shown above. The most striking fact is that cardiac responses in the first day of acute extinction do not decrease at all, and their size is exactly the same as to the positive CS (black circles). Only on the second day a clear extinction of cardiac responses is seen (white circles).

whereas the CR continues to rise. Both trends, i. e. increase of the cardiac CR and decrease of the UR, are statistically significant.

Besides this, Fig. 2 shows that the pre-stimulus level of heart rate, which may be considered as an index of the defensive CR to the experimental situation, or the intertrial level of anxiety, definitely decreased during this series of sessions.

#### Acute extinction of motor and cardiac defensive CRs

After completing 27 sessions and establishing a steady level of both cardiac and motor CRs, an acute extinction of these reflexes during 2 sessions was performed. The stimulus used for extinction was a buzzer from a loud-speaker placed on the right side of the animal.

Fig. 3 shows the course of extinction of motor and cardiac CRs during the first and second sessions of acute extinction. It may be seen that there are some striking differences between the course of extinction of both kinds of responses. First, the difference is seen in the immediate effect of change of the place of the source of stimulus. The motor CR is from the very beginning much lower, whereas the cardiac CR remains exactly unchanged. Furthermore, the evident extinction (cf. negative and statistically highly significant coefficient of regression in Fig. 3) of the motor CR is seen in the first extinction session, while the cardiac CR does not decrease at all. Only on the second day a statistically significant decrease of both cardiac and motor CRs was observed.

#### Differentiation of motor and cardiac CRs

The extinction described above was planned as a beginning of differentiation to the place — and in the following 40 experimental sessions we used two loud-speakers providing the same stimulus: tone from the front loud-speaker was the positive CS whereas that from the side loud-speaker was a DS. Each stimulus was presented 5 times daily at random order. Fig. 4 shows the course of this training. Introduction of the DS produced a decrease of motor CR, but at the same time the cardiac CR increased.

Numerical data concerning individual animals in the last 25 sessions are presented in Tables I and II. They show that in all dogs there was a perfect differentiation of motor CR and, with one exception (Tuz), all dogs reached very high level of cardiac differentiation.



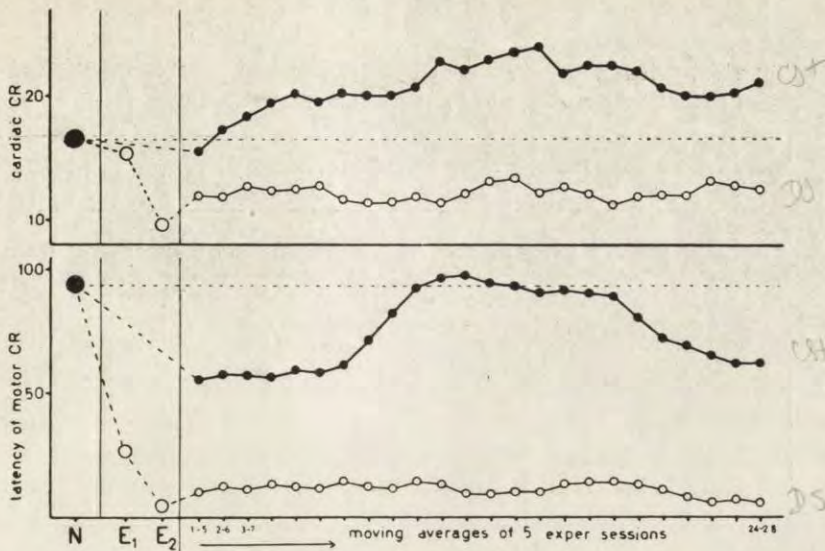


Fig. 4. Differentiation of motor and cardiac conditioned reflexes.

Cardiac and motor CRs to CS (black circles) and to DS (white circles) are shown in two sessions of acute extinction ( $E_1$  and  $E_2$ ) and in the following 28 days. First big circle denotes their size of the CR in the 5 preceding sessions. Ordinates for cardiac CR are cardioacceleration measured in the 2nd sec. of stimulus and expressed in beats per min. Ordinates for motor CR are the same units as in fig. 3 and table II.

Table I

Cardiac responses to conditioned, differential and unconditioned defensive stimuli in dogs.

Dog	Heart rate during				CS vs PS	DS vs PS	US vs PS	CS vs DS	US vs CS
	PS	CS	DS	US					
Ami	91.19	121.18	104.16	129.12	29.99****	12.97****	37.93****	17.02****	7.94*
Bar	67.42	82.52	70.74	79.06	15.10****	3.32°	11.64****	11.78****	-3.46°
Bob	92.18	118.21	102.32	113.01	26.03****	10.14****	20.83****	15.89****	-5.20°
Lal	70.52	85.62	78.13	92.48	15.10****	7.61****	21.96****	7.49****	6.86****
Max	103.44	123.73	113.54	110.59	20.29****	10.10****	7.15*	10.19**	-13.14****
Rud	92.11	106.69	96.09	108.05	14.58****	3.98°	15.94****	10.60**	1.36°
Tuz	97.05	130.21	126.14	127.96	33.16****	29.09****	30.91****	4.07°	-2.25°
All dogs	87.70	109.74	98.73	108.61	22.04****	11.03****	20.91****	11.01****	-1.13°

\*\*\*\* = significant at the .001 level

\* = significant at the .05 level

\*\*\* = " " " .01 "

° = not significant

\*\* = " " " .02 "

The Table shows frequencies of heart rate in the 10 sec. preceding the onset of the CS /column PS/ the heart rate in the 2nd sec. of CS /coll. CS/, in the 2nd sec. of DS /coll. DS/ and in the 2nd sec. after application of shock /coll. US/. In each dog data from 25 consecutive sessions /i.e. 125 applications of any stimulus/ are averaged. Amplitude and significance of the cardiac CR /coll. CS vs. PS/ the UR /coll. US vs. PS/ and the cardiac response to DS /coll. DS vs. PS/, as well as differences between cardiac reactions to CS, DS and US are shown.

Table II

Classical defensive motor conditioned response to positive conditioned stimulus and to the differential stimulus

Dog	Motor response to		Difference and its significance
	CS	DS	
Ami	41±4.1	0±0	41 p < 0.001
Bar	42±4.5	17±3.9	25 p 0.001
Bob	53±4.3	0±0	53 p 0.001
Lal	118±3.4	2±1.2	116 p 0.001
Max	77±6.7	9±2.9	68 p 0.001
Rud	67±5.3	1±1.0	66 p 0.001
Tuz	80±5.4	32±4.2	48 p 0.001
All dogs	68±2.2	9±1.0	59 p 0.001

The size of motor response was measured in arbitrary units calculated from the latency of the conditioned flexion using the formula:  $100(3-L)$ , where L is latency in seconds. The duration of CS (i.e. the maximal possible latency) was 3 sec. In each dog data from 25 consecutive sessions, i.e. 125 applications of CS and DS, are averaged. Arithmetic means and their standard errors are shown in two first columns. The third column shows differences between conditioned motor responses to CS and DS. It is seen that in all dogs differentiation reached high significance, and in two dogs it was „absolute”.

## DISCUSSION

Changes in heart rate occurring in the so-called stress situations were reported as early as in 1875 by Danilewsky, and in 1900 by Sherrington. The more systematic study of cardiac defensive CRs was done by Shipley in 1929, and afterwards by Gantt (1942, 1953) Dykman and Gantt (1956). More recently the cardiac defensive CR in dog was a subject of a number of papers (Black 1956, Froňkova and Ehrlich 1958, Fuller and Christake 1959, Pakovich 1960, Voznesenskii (1960). Although among them only Froňkova and Ehrlich presented statistically proved data, all authors agreed that the defensive cardiac CR in dog consisted in acceleration of heart rate. On the other hand, conflicting views were expressed as to the character of cardiac reaction to the DS. Voznesenskii (1960) described slowing the heart rate in response to DS. Also Strakhov (1951) and Havliček (1952) found antagonistic autonomic reactions to CS (increase of blood pressure) and to DS (drop in blood pressure). However, in these papers no statistically proved data were given. On the other hand, Froňkova and

Ehrlich (1958) presented convincing evidence that cardiac (and blood pressure) changes to positive CS and inhibitory DS did not differ in direction, the response to the DS being smaller than that to the CS.

Our results confirm the results of Froňkova and Ehrlich. In all our seven dogs the DS elicited acceleration of heart rate, and in all cases this acceleration was smaller than that produced by the CS. In none of the dogs we found a cardiac response to the DS bigger than that to the CS; such a case was described in Froňkova and Ehrlich's paper in one of their 3 dogs. Perhaps such cases are relatively infrequent or they are symptoms of neurotic states.

But the chief aim of this study was to observe the changes in motor and cardiac CRs during acquisition, extinction and differentiation. We have shown that cardiac and motor components in the classical defensive CRs develop rather independently, as if they were not related to one another. Similar facts were reported by Fuller and Christake (1959) who observed in puppies motor defensive CRs without constant cardiac responses.

Such a dissociation of cardiac and motor CRs was even more pronounced during the first extinction session. There are two important points we want to stress here. First, the motor CR was considerably diminished already in the first extinction trials, while the cardiac CR remained unchanged; and secondly, the motor CR extinguished easily during this session, whereas the cardiac CR did not. These two facts need some comment. As far as the absence of extinction of the cardiac CR during the first extinction session is concerned, it can be explained in terms of greater resistance to extinction in the cardiac CRs as compared with the motor CRs. This view was expressed by Gantt (1953). However, the fact that the "new" differential stimulus eliciting a much smaller motor CR evoked an unchanged cardiac CR suggests that neural structures mediating these two reflexes differ not only in plasticity, determined by different rate of acquisition and extinction, but also in their discriminatory function. Hence we may conclude that the CR arcs of cardiac and motor reactions divide already on the level of afferent pathway. One branch possessing a weak discriminatory power transmits impulses to neural structures establishing conditioned connections with "drive" (or governing the appetitive behaviour) centres; the other branch, possessing good discriminatory function activates, through other conditioned connections, specific centres of "consummatory responses" — such as a flexion in our case.

This assumption may account not only for the described dissociation of cardiac and motor CRs in classical defence conditioning, but it may also help, to explain some important features of defensive instrumental

CRs. For example, the resistance to extinction, so characteristic in the avoidance reflex, may be related to the sluggish extinction of the cardiac CR to any signal of danger or pain. The poor discriminatory power found in the cardiac CRs, being responsible for what may be called a generalization of conditioned drives, may account for the wide transfer of instrumental defensive responses. Finally, the relative independence of the cardiac and motor defensive CRs explains why in the avoidance CRs the cardiac response is fully preserved while the classical motor response is absent.

It is worthy to add that from the side of neuroanatomy such a division of afferent pathways, especially for distant signals, is well known. Usually the anatomical properties of the two branches arising from one receptor field are such that different discriminatory functions are supposed to be located in them. It is also suggested that the branch of lesser specificity has its destination in brain stem centres (directly related to the control of visceral activities), while the specific afferent fibres run to sensory thalamic and cortical structures.

And so, the old recognition of two kinds of unconditioned behaviour, usually denoted as appetitive and consummatory, may be extended to classical conditioned reflexes. In the defence reflexes the former is exemplified by the conditioned and unconditioned fear reaction, and the latter by the specific motor withdrawal response. Although no absolute distinction can be made between them (cf. *Hinde* 1953, *Thorpe* 1956) at the level of URs, differences become more evident when conditioning, especially the instrumental one, comes into play.

The last observation on which we would like to comment is that the cardiac response to the US decreased during acquisition of the CR (see Fig. 2), and finally became smaller than the cardiac response to the CS. Several explanations are possible, but their verification needs further experimentation; the only fact we already know from recent control experiments (unpublished) is that there is no such "extinction" of the cardiac UR to the shock when it is presented day-by-day without being preceded by the CS.

#### SUMMARY

In seven adult dogs classical defensive conditioned reflexes were studied. Special attention was paid to the mutual relationship between cardiac and motor conditioned responses during acquisition, acute extinction and differentiation.

In all the dogs a constant motor and cardiac conditioned reflexes were established. In all cases the conditioned stimulus as well as the unconditioned shock elicited a statistically significant rise in the pulse rate. The

differential stimulus also elicited in all the dogs cardioacceleration, however, it was much smaller and in two dogs did not even reach the 0.05 significance level.

During acquisition and extinction the cardiac and motor conditioned reflexes changed independently. This led to the conclusion that these two reactions, belonging to different types of behaviour (cardiac conditioned reflex being a representative of "appetitive behaviour", and motor flexion conditioned reflex a "consummatory response") are probably conditioned through different neural structures. Importance of these findings for understanding the mechanism of avoidance reflex is discussed.

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THE FORMATION OF INSTRUMENTAL CONDITIONED REFLEXES  
BY DIRECT STIMULATION  
OF SENSORI-MOTOR CORTEX IN CATS

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The problem of transformation of motor reaction produced by electrical stimulation of the sensori-motor cortex into instrumental conditioned reactions is almost unexplored. Only two previous papers devoted to this subject have appeared. L o u c k s (1936) carried out experiments in which he implanted electrodes on the sensori-motor cortex of a dog. These electrodes were interconnected by a small coil of great inductance. The skin over the coil was sutured, and when the wound healed, a primary coil was fixed on the skin in the vicinity of the implanted coil. An interrupted electric current was applied producing the inductive current in the implanted coil, and thus stimulating the cortex beneath the electrodes. L o u c k s found that if the movements of the hindleg elicited by stimulation were reinforced by food, the animal learnt to perform these movements spontaneously.

K o n o r s k i and L u b i ń s k a (1939) implanted electrodes according to the method of E w a l d and B a e r (cf. T r e n d e l e n b u r g 1923). The authors stimulated the sensori-motor cortex by rhythmic impulses from a neon stimulator. They found that if the movements of the foreleg or hindleg elicited by stimulation were reinforced by food, the animals quickly learnt to perform analogous movements.

Because of the small samples of subjects used in these experiments, it seemed advisable to reinvestigate this problem on a larger number of animals and by using more modern techniques both of implantation of electrodes and of stimulation. The principal aims of this study were:

1. to examine the rate of formation of an instrumental conditioned reflex (ICR) produced by cortical stimulation;
2. to determine whether and to what degree the conditioned movement is similar to that produced by cortical stimulation;
3. to determine if destruction of the site whose stimulation elicited a movement leads to the abolition of the particular ICR.

However, in the course of the experiments it turned out that food reinforcement of the cortically induced movement did not always lead to the formation of an ICR. Therefore, it was necessary to elucidate first this new, and rather unexpected, problem of the conditions in which the cortically induced movements can be "instrumentalized" under food reinforcement.

## MATERIAL AND METHODS

### Technique of implanting the electrodes

Since this technique will be described in detail elsewhere, only its most salient features are presented here.

Each cat was anaesthetized by Nembutal (25—30 mg./kg.) and placed in a stereotactic instrument. The skin was dissected and the bone over the sensorimotor cortex unilaterally removed. By exploratory stimulation, points whose stimulation produced the movements of either the contralateral foreleg or hindleg were found. Then a duracril plate was formed corresponding to the aperture in the skull, and in the appropriate points holes were drilled and electrodes were inserted. The other ends of electrodes were soldered to a small socket, to which the indifferent electrode, placed on the bone, was also soldered. The plate was then again placed in the aperture of the skull and the socket was fixed on the skull by small screws. Afterwards the skin was sewn together, so that the socket stood out above the suture.

### General course of experiments

The experiments were performed in a cage  $70 \times 50 \times 50$  cm. (Fig. 1). In the middle of the shorter wall a feeding apparatus was situated. Before the implantation of electrodes the cats were habituated to the cage and to the general procedure. Then the surgery was performed and when the wound healed the experiments began. Each cat was brought to the cage and the wires from a stimulator were connected by a plug to the socket. The wires hung down from the ceiling of the cage and allowed the cat free movements around the cage.

First, stimulation of various points was tested by pulses with a frequency of 50 cps and of 1 msec. duration. The amplitude of the pulses was gradually increased until high and virtually isolated movements of the leg were obtained without any after-effects.



When a definite electrode was chosen and the optimal amplitude of stimulation found, we proceeded to the establishment of an ICR. Every few minutes the stimulation of the cortex was applied during 0,5 to 1 sec., and when the animal performed the movement of the leg, a bowl containing a small piece of meat (1 cm<sup>3</sup>.) was immediately put into position. If in the course of the experiment the cat performed spontaneously a movement resembling that obtained by stimulation, it was also reinforced by food. After food intake we waited for several minutes to see whether the spontaneous movement will reappear and, if not, stimulation was again administered. In general, 24 reinforced trials were given in one experimental session.

When the animals started to perform the movement more or less regularly, the electrical stimulation was discontinued. Usually the animals performed then the

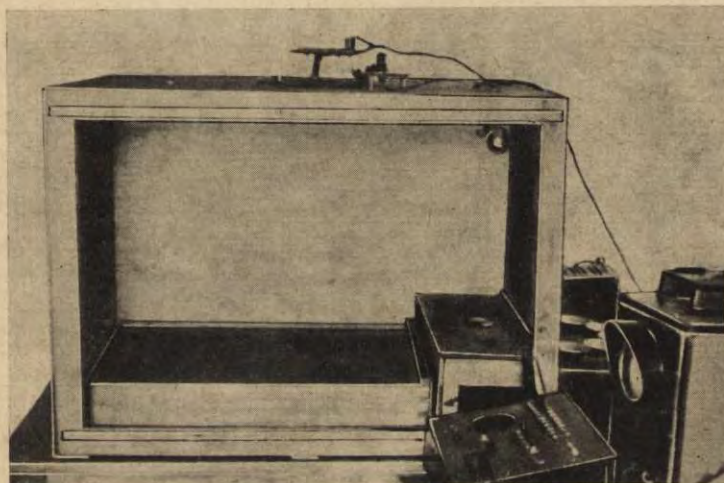


Fig. 1. Experimental cage.

movement with increasing frequency and eventually executed it very regularly with the inter-response intervals determined only by the duration of food intake.

In those cases in which the animals did not manifest any tendency to perform spontaneous movements, from 10 to 30 experiments (240—720 trials) were carried out and then a new series of experiments with stimulation of other cortical points was begun.

When the experiments were completed, in some of the cats the cortex beneath the electrode was destroyed in order to test the impairment of the ICR produced by this lesion. The results of these experiments will be described in another paper. In other cases the place of electrodes was determined by electrocoagulation of the cortex, and the animals were sacrificed.

## RESULTS

## I. Formation of ICR's by means of stimulation of cortical points evoking the foreleg movements

Experiments of this kind were performed on 21 cats. Subsequent verification of the position of the electrodes showed that in 12 cases they were located in the motor area, i. e. between the cruciate sulcus and the central sulcus (or its lateral prolongation) while in 9 cases they were between the central and the ansate sulcus (Fig. 2). In a few cats electrodes



Fig. 2. Location of electrodes in particular series of experiments.

Squares, electrodes situated on the hindleg area; triangles, electrodes on the hindleg sensory area; circles, electrodes on the foreleg motor and sensory area. Stimulation with electrodes denoted by squares did not produce any formation of ICR.

were situated in the motor area between the foreleg and the hindleg area. In these cases the movements evoked were irregular and complex, and with higher amplitudes of stimulation the movements of the hindleg appeared. These cats were rejected from further study.

a.) Experiments with stimulation of the motor area  
The characteristic feature of this area is that low voltages of stimulation are sufficient to evoke movements. As seen in Table I, these voltages were in most cases not higher than 2V. The movements obtained were in most cases very high, but they were often "unnatural", i. e. they never occurred in the behaviour of normal animals. For instance, flexion in shoulder and elbow joint was accompanied by a complete stiffness of wrist (Fig. 3a), or it was accompanied by abduction or twisting of the leg, etc. Very often flexors and extensors were simultaneously contracted, which made the movement appear "tense" and "strained". When stimulation was discontinued, the movement stopped immediately, provided that no Jacksonian seizure followed.

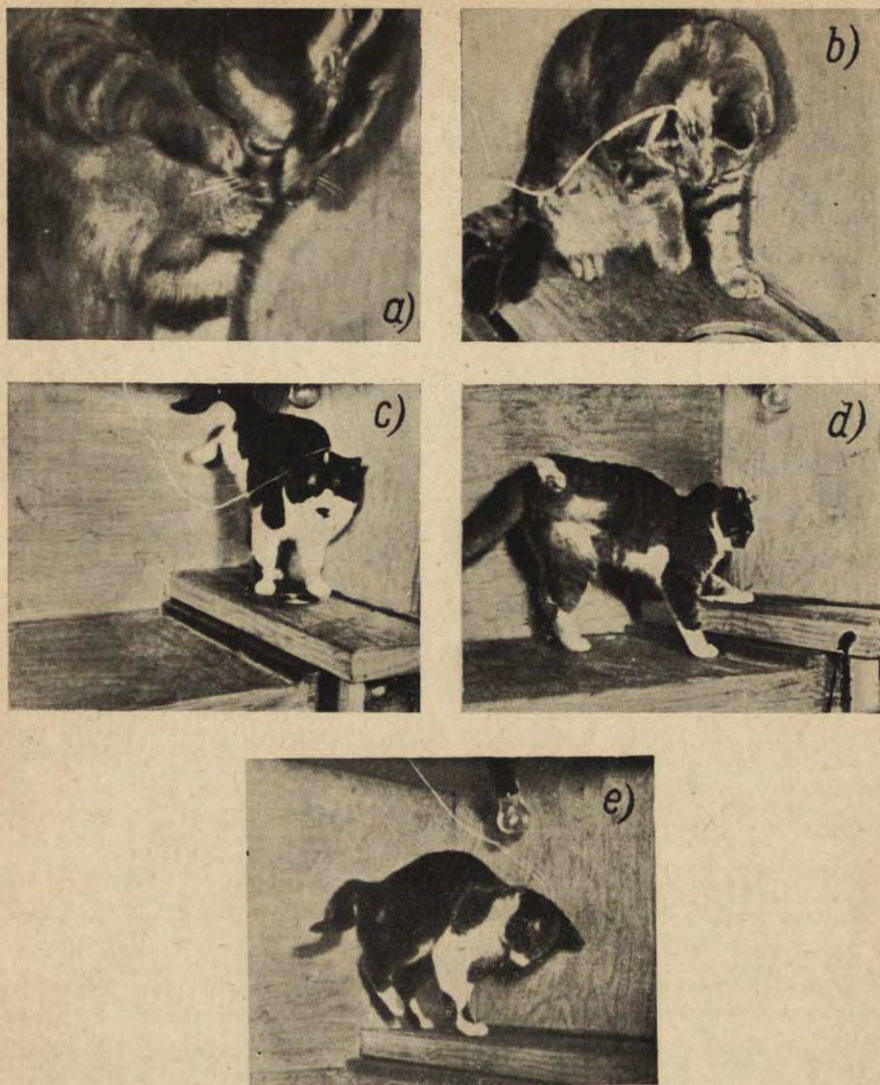


Fig. 3. Examples of motor reactions elicited by cortical stimulation.

a, c, d — stimulation of motor area; b, e — stimulation of sensory area. Note the high amplitude of movements elicited by stimulation of the motor area, and their „unnatural” character.

Usually, in the first experimental session the spontaneous movements of the same leg began to appear (Table II). They did not reproduce exactly the movements obtained by stimulation; in particular, no increased tension of muscles and no unnatural twisting was seen. In the following experiments the spontaneous movements became gradually even more natural.

Table I

Intensities of currents applied to the cortex,  
needed to elicit motor response

V	Numbers of animals			
	Foreleg		Hindleg	
	motor area	sensory area	motor area	sensory area
1	6		3	
1.5	4			
2	1		7	
2.5			6	
3			1	
3.5				2
4		2		8
4.5		3		7
5		4		7

Table II

The rate of „instrumentalization” of cortically  
induced motor reactions

Numbers of trials needed to establish ICR	Numbers of animals			
	Foreleg		Hindleg	
	motor area	sensory area	motor area	sensory area
5—10	7	6	0	4
10—20	5	3	0	10
20—30	0	1	0	3
30—40	0	0	0	6
40—50	0	0	0	1
Not established	0	0	17	0

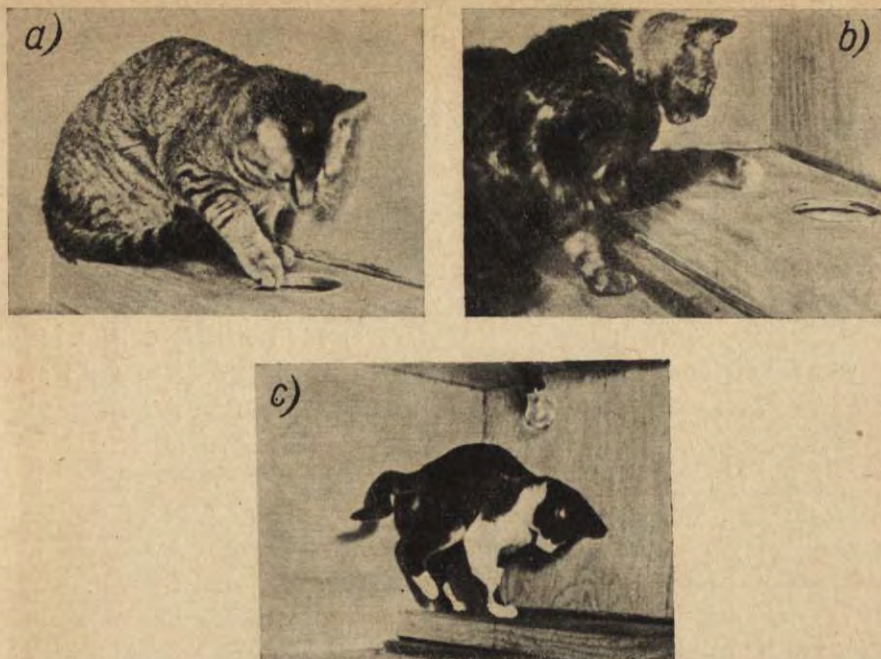


Fig. 4. Examples of ICR established by particular stimulation of the cortex.  
 a — ICR established by stimulation of the motor foreleg area; b — ICR established by stimulation of the sensory foreleg area; c — ICR established by stimulation of the sensory hindleg area.

Their amplitude lowered and they eventually consisted in putting the paw into the bowl or scratching its border (Fig. 4a).

b.) Experiments with stimulation of the sensory area. The voltage necessary for obtaining optimal movements by stimulation of the sensory area was approximately twice as high as that used in stimulation of the motor area (Table I). The movements obtained were not high, they were much slower than with stimulation of the motor area, and they subsided slowly after the cessation of stimulation. The character of movements was rather natural with flexion in all joints concerned and no contraction of antagonistic movements (Fig. 3b).

The spontaneous movements appeared as promptly as in the previous case (Table II). However, their character remained similar to that produced by stimulation. Sometimes both movements (elicited by stimulation and spontaneous) were practically undistinguishable (Fig. 4b).

## II. Formation of ICR's by means of stimulation of cortical points evoking the hindleg movements

Experiments with stimulation of this area were performed in 35 cats. In 17 cats the motor area was stimulated, and in 24 cases the sensory area

was stimulated. In 6 animals points in both areas were stimulated in different series of experiments.

a.) Experiments with stimulation of the motor area. Movements were obtained with stimulation ranging between 1V and 2.5V (Table I). In most cases the movements were very high, sometimes exceeding the body axis and even causing loss of balance of the animal. They were executed very rapidly and terminated immediately after cessation of stimulation. Usually they had an "unnatural" and "tense" character, both flexors and extensors being contracted. Most often the following movements were obtained: 1) high lifting of the hindleg with retraction and abduction (Fig. 3c), or 2) high flexion with strong abduction along the axis of the limb (Fig. 3d), or 3) flexion with strong protraction.

In all cases in which stimulation of the motor area for the hindleg was applied no spontaneous movements were obtained, even if we waited several hundreds of stimulations with reinforcement were applied, the established by means of this stimulation. In some cats in a number of trials it was noticed that in response to cortical stimulation, the animals not only lifted their legs very high, but also turned to the foodtray expecting food, or even jumped towards it on three legs.

After 8 to 10 experiments, and in some cases even more, in which several hundreds of stimulations with reinforcement were applied, the experiments were discontinued. In a few cats a new series of experiments with stimulation of the foreleg area was then conducted, but in most of them we applied stimulation of the sensory points for the hindleg.

b.) Experiments with stimulation of the sensory area. These experiments were performed both on those cats in which conditioning with stimulation of the motor area was unsuccessful, and on those cats in which stimulation of sensory area was applied from the very beginning. As seen in Table I, the voltage required to elicit the movement was twice as high as that used in stimulation of the motor area. The movements elicited consisted in slow and gradually increasing flexion, usually not ceasing at once with the end of stimulation. They had a smooth character and no tension of muscles of the leg was observed (Fig. 3e). Sometimes the flexion was accompanied by protraction or retraction of the leg, with or without abduction.

As seen in table II the formation of ICR occurred slower than the formation of this reflex with the foreleg movement. The character of the learnt movement did not generally differ from that obtained by stimulation. After several sessions the ICR was firmly established and the movement was performed with maximal frequency.

## DISCUSSION

The large body of evidence collected in experiments with ICR's indicates that movements provoked in any way (i. e. as an effect of unconditioned reflex, by passive displacement of the limb, or accidentally) become instrumentally conditioned if reinforced by food or by avoidance of nociceptive stimulus. Therefore, the fact that the movements produced by cortical stimulation can also become instrumentalized, if appropriately reinforced, had rather to be expected as a further illustration of this general principle. On the other hand, a quite unexpected result was that the movements elicited by stimulation of the hindleg motor area could not be instrumentalized in spite of their many repetitions with food reinforcement. Therefore, our discussion should be chiefly devoted to the explanation of this negative result.

The following hypotheses may be put forward to account for our findings.

1. There is some evidence to show that direct stimulation of the pyramidal cells produces suppression of the afferent impulses impinging on the cerebral cortex (Lindblum and Ottoson 1951, Jabbur and Towe 1960). Thus, one might assume that movements elicited by direct stimulation of the motor area are deprived of their sensory feedback, both central and peripheral. If we accept that at least a central feedback is necessary for the formation of ICR's (cf. Górska and Jankowska 1961), the failure of the formation of such a reflex would be understandable. The fact that the foreleg movements elicited by stimulation of the motor cortex can be instrumentally conditioned may be explained by assuming that here the visual feedback plays a decisive role.

This hypothesis could be verified by determining whether a classical conditioned reflex can be established to a movement of the hindleg elicited by stimulation of the motor cortex. However, the difficulty of such an experiment is that we must be sure that not the cortical stimulation, but the movement provoked by it becomes a conditioned stimulus.

2. Another hypothesis is that failure in instrumentalization of the hindleg movement obtained by stimulation of the motor cortex is due to the fact that this movement represents a quite unusual and unnatural excitatory pattern. Both the rhythm of stimulation and the pool of stimulated neurones are undoubtedly quite different from those which take place in normal movements. According to the observation of Penfield (1959) during the surgery on humans, stimulation of the motor cortex often prevents the patient from performing a voluntary movement; this shows that artificial stimulation of the motor cortex may block its normal activity.

One may assume that such unnatural excitatory patterns of movements as are induced by cortical stimulation cannot be elicited as an effect of reflex action, and, therefore, cannot become conditioned. On the other hand, stimulation of the sensory area produces movements which are analogous in character to the natural movements, and in consequence they can be instrumentally conditioned.

The fact that the movement elicited by stimulation of the foreleg motor area can be conditioned may be explained by supposing that the foreleg in cat takes part in many natural instrumental reflexes, and the animal during conditioning does not learn to perform a new movement, but simply learns to perform a movement belonging to its own motor repertory. This would explain the fact that the instrumental movement thus established becomes always "naturalized" and is never a replica of the movement obtained by stimulation of the motor cortex.

3. A third hypothesis presented in detail in another paper (T a r n e c k i and K o n o r s k i 1961), assumes that in instrumental conditioning not the motor cortical centres controlling a given movement are involved, but rather some other groups of neurones intermediary between the sensory and the motor centres. These intermediary centres are of course not excited when the pyramidal cells of the motor cortex are stimulated, while they are excited when the movement is elicited by stimulation of either the appropriate receptors, or the sensory centres. According to this hypothesis only "natural movements" as far as their origin is concerned can become instrumentalized. The formation of the ICR by means of stimulation of the foreleg motor area may be explained in the same way as in the previous hypothesis.

Which of these hypotheses, if any, proves to be correct, will perhaps be shown by further experimentation.

#### SUMMARY

This paper presents a study of instrumental conditioning obtained by means of food reinforcement of cortically induced movements in cats. Stimulation of the sensori-motor cortex by chronically implanted electrodes was used.

2. The character of movements produced by cortical stimulation depends on whether the electrodes are placed on the rostral ("motor"), or on the caudal ("sensory") part of the sensori-motor cortex. The movements produced by stimulation of the motor area require lower voltages, have higher amplitude, and are more "unnatural" than those produced by stimulation of the sensory area.



3. Movements of the foreleg produced by stimulation of both motor and sensory area are easily instrumentalized after 5 to 20 trials. Instrumental movements obtained by stimulation of the motor area lose their unnatural character.

4. Movements of the hindleg produced by stimulation of the sensory area are instrumentalized after 10 to 30 trials. Movements of the hindleg produced by stimulation of the motor area cannot be instrumentalized at all even after several hundreds of trials.

5. The failure of obtaining instrumental conditioning by stimulation of the motor area of the hindleg is discussed.

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## THE PROPERTIES OF DELAYED RESPONSES TO DOUBLE PREPARATORY SIGNALS IN NORMAL AND PREFRONTAL DOGS

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In one of the preceding papers on the properties of delayed responses (Ławicka 1959) it was noticed that if the triple choice method is used instead of double choice, there is a possibility of applying an additional test not quite clear in double choice conditions. If, instead of applying one preparatory signal indicating the foodtray the animal is required to approach, we apply two signals one after another, it is possible to see whether the animal when released, is able to approach correctly both foodtrays indicated by these signals. This experimental procedure has been called by us a double signal test. The present paper describes the properties of the double signal test both in normal and prefrontal dogs.

### MATERIAL AND METHODS

The experiments were performed on 7 dogs: 3 dogs tested only after prefrontal ablations, 3 dogs tested both before and after prefrontal ablation, and 1 dog on which the control parietal operation was made. All animals had past experience which was described in the earlier papers of this series (Ławicka 1959, Ławicka and Konorski 1959).

In a room 8 m. × 4 m. three foodtrays were placed near the middle of each of the three walls at a distance of 2 m. from the starting platform. A buzzer and a lamp used as preparatory stimuli were placed on each foodtray. In most experiments only buzzers were applied, but in some lighting was also used. During the delay period the dog was on a leash at the starting place. In intertrial intervals lasting about 2 min. he was allowed to move freely about the room. Further details of the experimental procedure were given elsewhere (Ławicka 1959).

The double signal trials were randomly distributed among the single signal trials. Both preparatory signals operated for 3 sec. each, and were separated

from one another by 1 sec. interval. Delays used in double signal trials were of 0 (minimal), 3, or 60 sec. If the animal approached both signalled foodtrays one after the other, both runs were reinforced independently of their sequence. If, however, the animal after approaching one correct foodtray went afterwards to the wrong one, and only then ran to the second correct foodtray, the last run was not reinforced.

The prefrontal ablation was performed by suction under aseptic conditions. The whole prefrontal pole rostrally to the presylvian sulcus was removed. The details of the surgical procedure were given elsewhere (Brutkowski et al. 1956).

## RESULTS

**Normal dogs.** In normal animals the double signal test was solved at its first or second application: when two preparatory stimuli were applied one after another, and the animal was released, he approached one of the signalled foodtrays, and then, after having eaten food there, ran immediately to the second one. The responses were correct quite independently of the delay used.

Generally, 130 double signal trials were given in all dogs. After release the animals approached in 80% the last signalled foodtray, and only then went on to the first one. However, it was observed that if in the double signal test, instead of two buzzers or two lamps, buzzer and lamp were applied, the animals had a tendency to approach first the foodtray signalled by the buzzer independently of the order of the signals.

Sometimes, especially in the initial sessions with double signal trials, it was observed that even in single signal trials the animals approached another foodtray after having eaten food in the signalled one. However, this tendency quickly disappeared in the course of testing.

The double signal test was always solved by the dogs in those cases where the buzzers signalled the middle foodtray and one of the side foodtrays. When, however, the two side foodtrays were signalled, not all the responses were correct: some of the animals after running to one correct foodtray and eating food there, instead of going directly to the opposite foodtray as it was required, looked into the middle one when passing. For this reason the trials in which two opposite foodtrays were signalled were not taken into account.

The performance of the dog subjected to parietal lesion did not change after the operation (Fig. 1d).

**Prefrontal dogs.** The double signal trials were introduced in prefrontal animals only after the dogs were able to react correctly in single signal trials with the delay of 1 min. None of the prefrontal dogs was able to solve correctly the double signal test (Fig. 1a, b, c). They exhibited very pronounced orienting reaction towards the first signal,

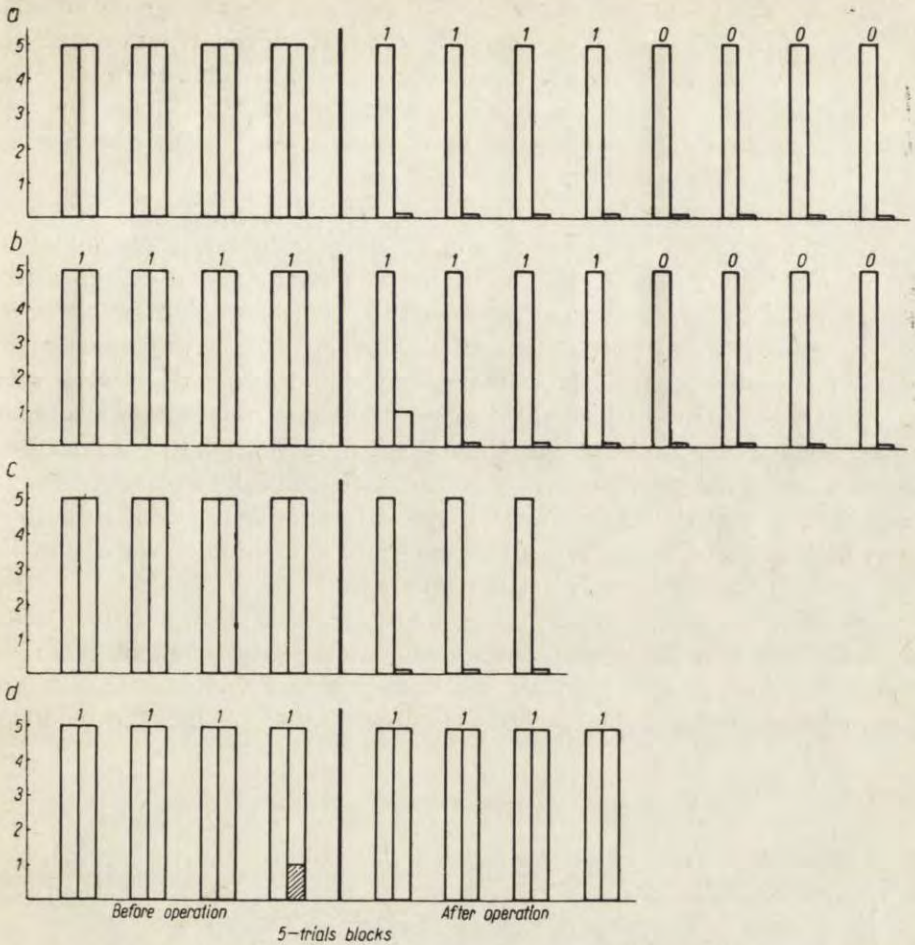


Fig. 1. Responses of animals in double signal test before and after prefrontal (a, b, c) and parietal (d) operation.

Double columns denote approaching both signalled foodtrays, single columns denote approaching one signalled foodtray. Striped part of the column indicates a wrong response.

then, to the second one they usually changed appropriately their bodily orientation, and after being released went only to this second foodtray. After having eaten the food there, they returned to the platform, or walked around the room, but did not approach the second signalled foodtray. Sometimes it was observed that during the delay period the animal oscillated between the directions of the two signalled foodtrays; in such cases it happened that after release he chose the first signalled foodtray. If instead of two buzzers a combination of buzzer-light was applied the animals went only to the foodtray signalled by the buzzer.

During the course of experimental sessions no change in animals' behaviour in double signal test was observed. However, it was found that by application of an appropriate procedure it was possible to improve the animal's performance. Such a procedure was applied in 4 dogs. Since the course of experiments was similar in all four cases, we describe here the experimental series performed in one of them.

The double signal test was resumed after the prefrontal operation, when the animal was able to solve in 95% the ordinary single signal test with 1 min. delay. The double signal trials were repeated 40 times (in 8 sessions) and in no case was an approach to the second foodtray observed.

The experimental procedure was changed in such a way that instead of giving double signal trials among one signal trials they were given several times in succession, and in each the same two signals (right and middle) were repeated. Already in the third such trial the animal went to both foodtrays. However, in the next two trials he approached again only the last signalled foodtray, but thereafter he again went to both. In the following trial other two foodtrays (left and middle) were signalled and the dog went correctly to both of them.

The double signal trials were then repeated 6 times in various combinations and the animal reacted quite correctly in all of them.

On the following day in all the double signal trials, except for the first, the dog reacted quite correctly running to the two signalled foodtrays.

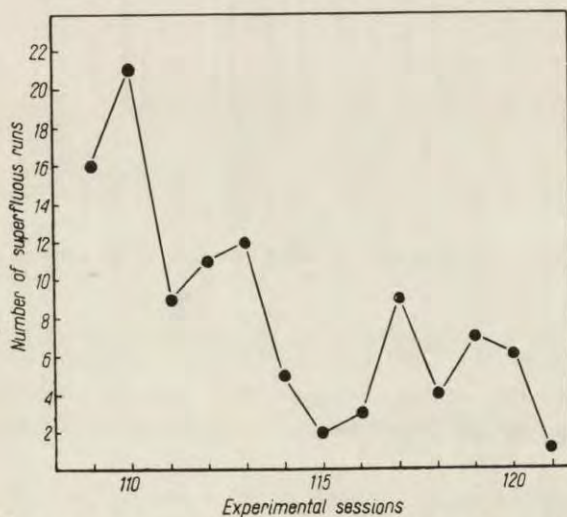


Fig. 2. Numbers of superfluous runs in single signal trials interspersed between double signal trials.

Three double-signal trials were given each day. Note the decrease of superfluous runs in the course of testing. See further explanation in text.

Simultaneously, however, a new type of behaviour was observed, namely, the animal approached persistently two foodtrays even in single signal trials.

In order to extinguish the additional runs in single signal trials the following procedure was adopted. After each successful double signal trial a series of single signal trials was given, till the dog performed no more double responses. Only then the next double signal trial was given which was followed again by a series of single signal trials, and so on. As seen in Fig. 2, the numbers of single signal trials given in successive sessions gradually decreased, because the dog more and more rarely approached the two foodtrays instead of one. Finally, the stage was reached where the animal reacted correctly both in double and in single signal trials. In other words, a kind of differentiation had been reached where the animal reacted to the single preparatory stimulus by a single response and to the double signal by a double response. It is worth mentioning that this kind of differentiation is established in normal dogs without any special training.

#### DISCUSSION

The fact that normal dogs are able to solve the double signal test without any difficulty might have been expected on the basis of earlier data. First, as pointed out in a previous paper (Ławicka 1959) these animals are able to solve the delayed response test without any training with 1 min. delay. Then, they are very resistant to distractions, such as presenting food on the starting platform in the delay period. If we consider approaching the one signalled foodtray and getting food there as nothing else but a distraction in relation to the run towards the second foodtray, it is not surprising that the animals are quite able to overcome this difficulty.

The main characteristic of the double signal test is, that the animal is deliberately provided by two cues represented by the two preparatory signals. We have seen that if both these signals are of the same modality the animal is directed at first by the second signal and only then by the first one; when however, buzzer and light are the preparatory stimuli, the animal is directed at first by the buzzer and only then by the light. Hence, we may deduce that if two stimuli of the same modality are applied, the second signal is physiologically stronger than the first one, and the buzzer is stronger than the light.

As far as prefrontal dogs are concerned, it has been shown that while their ability to solve the delayed response test, which had been severely impaired after the operation, gradually improves, they still fail to solve

this task when distractions are introduced (Ławicka and Konorski 1959, Ławicka and Konorski 1961b). According to these data it could be predicted that in double signal trials the prefrontal dogs would approach one of the signalled foodtrays (vis. the second one), but then they would choose the second one on the chance level. However, what actually happened was that after running correctly to the second signalled foodtray they did not try to approach a ny other foodtray.

How is this fact to be explained? First one could assume that when the second signal is applied, the first one is retroactively inhibited and the animal runs only to the second signalled foodtray. However, the observation that the animal's orientation oscillates between the direction of the two signalled foodtrays during the delay suggests that the first signal is not in fact inhibited by the second.

It is possible, however, that retroactive inhibition is produced not by the second signal itself but by the run to the foodtray and obtaining food there. The fact that the prefrontal dogs are able to solve the double signal test by the modified procedure would argue against this possibility.

The fact that after repeating several times the same pair of signals the animal did at last approach both signalled foodtrays and then began to react correctly to any pair of stimuli applied, seems to explain why the prefrontal animals failed at the double signal task. It seems likely that in the course of the experimental sessions, in which only single signal trials were given, the animals developed a strong motor stereotype to approach only one foodtray and then to return to the platform. The modified experimental procedure disrupted this stereotype and so the animal had no more obstacle to being directed by both preparatory stimuli. But now, as a result of runs to two foodtrays one after another, a new motor stereotype is formed consisting in running to a second foodtray irrespectively of whether one or two stimuli have been applied. In other words, a previous chain of motor reactions, namely, going to the signalled foodtray → eating there → returning to the platform, was substituted by a new chain of reactions: going to the signalled foodtray → eating there → going to another foodtray. This stereotyped behaviour was, in turn, so strong, that a number of single signal trials has to be applied until the animal extinguished his superfluous runs to a second foodtray. As seen from our results such an extinction is quite possible in the prefrontal animal and in consequence he is able to react adequately and discriminatively both in single signal and double signal trials.

The fact that the prefrontal animals tend to base their behaviour according to the cues provided by their earlier performance rather than in accordance with the cues provided by preparatory signals, was often observed in our earlier experiments (Ławicka and Konorski



1961a). It has been shown that in prefrontal cats errors in delayed response could be attributed to an increased perseverative tendency, as compared with normal animals. Moreover, after committing an error the animals very often attempted to correct themselves which indicates that the cue provided by a preparatory stimulus is not lost. Thus, when the animal is released after a delay period it is under the influence of two cues, one being provided by earlier performance and the other by the preparatory stimulus. As seen in our experiments the prefrontal animal has a tendency to be dominated by the first of these cues which, for some reasons, has become stronger.

Our present experiment suggests that the same deficit is present in prefrontal dogs in the performance of the double signal test, the inappropriate cue being provided by earlier established chains of animal's reactions.

The problem arises what is the cause of the increased strength of the cues provided by earlier performance, overcoming the action of preparatory stimuli. Here several explanations may be proposed.

One explanation is that the deficit of prefrontal animals lies in the impairment of the inhibitory process suppressing the inadequate motor acts (cf. also Stanley and Jaynes 1949, Warren et al. 1957, Brush et al. 1961, Ławicka and Konorski 1961a, b, Rosvold and Mishkin 1961). These acts are established by the instrumental learning necessarily involved in our experimental procedure. For instance, when the animal, within the delayed response testing, had approached a particular foodtray getting food there, he learnt to do so in response to simple release, not necessarily preceded by any preparatory stimulus. When after the act of eating in a foodtray he was used to returning to the platform due to repeated single signal trials, he tended to perform this motor act after the end of eating irrespective of whether the given response was adequate or not.

Obviously, these reactions, easily inhibited in normal animals, may interfere with the responses signalled by preparatory stimuli, being the main factor provoking errors. We have seen in our experiments that the inhibitory ability of prefrontal animals is not totally lost, since they are able after a number of appropriate trials, to suppress the inadequate instrumental reflexes and thus to respond correctly as indicated by preparatory stimulus. This is why, in prefrontal animals, we may observe, although with the help of an additional training, improvement in delayed response test (Ławicka and Konorski 1961a, b) due to the extinction of these inadequate instrumental reflexes.

Another hypothesis which may be put forward is that the prefrontal animals manifest an increased tendency to stereotyped behaviour not due

to impairment of suppressing ability but to the increased "conditionability" of motor acts and their chains. Since one can observe that these animals display an exaggerated orienting reaction towards the stimuli, it may be that the instrumental reactions performed against the excitatory background, are more readily fixated than in normal animals, and have some compulsory character. In fact, many of these stereotyped and ritual movements may be observed in prefrontal animals in the course of testing.

Finally, it may be assumed that the deficit consists in a decrease of the physiological strength of traces of the stimuli, as compared with the normal animals. And so we do not suppose that the traces of preparatory signals are lost in prefrontal animals, as claimed by Jacobsen (1936) and by us in previous papers (Ławicka and Konorski, 1959, Konorski 1961), but only that their relative potency as conditioned stimuli, is lowered. This would explain why in the competition of various factors, determining the animals behaviour, the reactions to actual stimuli established by instrumental conditioning would overcome the reactions controlled by traces of the preparatory signals. This hypothesis would easily account for the striking fact that, while the prefrontal animals are very poor in the performance of the delayed response test, they do not fail if they are released during the presentation of a preparatory stimulus.

Perhaps it is possible by further experimentation to find out which of these hypotheses, if any, will prove to be correct.

#### SUMMARY

1. A test for studying delayed responses in animals is described which consists in applying in the same trial two preparatory signals one after another. After release the animal is required to go to both signalled foodtrays. This test has been called double signal test.

2. Normal dogs are able to solve this test at its first or second application. When two preparatory signals of the same modality are given (two buzzers or two lights) the animal usually approaches at first the second signalled foodtray and only then runs to the first one. If however, buzzer and light are applied, the animal approaches first the foodtray signalled by the buzzer.

3. After prefrontal ablation the dogs are not able to solve the double signal test. They approach, as before, in most cases at first the second signalled foodtray but after having eaten food there, they return to the starting platform.

4. By repeating several times the same pair of signals it is possible to provoke the prefrontal animal to approaching both signalled foodtrays.

After that the ability is generalized over other pairs of signals. However, from that time on the animal tends to approach two foodtrays also in regular, single signal trials. This tendency may be suppressed by a special training.

5. The mechanism of the impairment in double signal test and its improvement by an appropriate procedure is discussed.

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