Studies of the Conditioned Reflex in the Lower Vertebrates

IV. Differentiation in Gold-fish [1] [2]

By

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In our previous paper, which is one of a series of studies on the conditioned reflexes in the lower vertebrates, we stated that the temporary connection between food and light is easily established, although the rate and form of its formation is quite variable among individual animals, while the extinctive inhibition is much more difficult to be worked out. It explicitly seemed to reveal the fact that the formation of the negative conditioned reflex, if at all possible, is not so easy as that of the positive one (Tuge and others, '55). Henceforth, it should bring forward the problem of how the varied types of internal inhibitions develop phylogenically as well as ontogenically.

Biryukov ('55) stated that it is possible to form differential inhibition in carps, but, generally, it is very unstable and hard to be retained to the day following experimentation. On the other hand, Voronin ('54, '55) and others did not find it dificult to work it out in fish. Our observations made upon sea bream, which possesses a most highly developed brain among fish, support their findings, but we were rather sceptical, in so far as other forms of fishes were concerned. The problem in consideration is not yet settled.

We are of the opinion that a disclosure of the mechanism of the internal inhibition is a matter of the greatest importance not only in the lower vertebrates, but as well as in the higher forms (Tuge, '56).

In the present paper we deal with the process of differentiation and the changing of signal denotations of stimuli, using the motor-food conditioned reflex in gold fish.

For the present study were used six gold-fish of 2 to 3 years of age. They were kept in our laboratory for about half a year before the experimentation. The appa-

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ratus and method by which the experiments were made almost the same as designed by Prazdnikova ('53 a, b). A fine thread with a bead, having been introduced in the experimental aquarium, 40 x 30 x 22 cm, is occasionally pulled by the fish. This pulling is coupled with bait. The bait, which is stiffened with a commercial foodpowder sold as a special feeding for fish, is poured into the aquarium through a funnel which is constructed at a place higher than the aquarium. The bait poured into the aquarium by means of jet water-flow drops into a feeder, which is made by enclosing the three sides with white celluloid plates.

As conditioned stimuli, both electric bulbs of red and green lights are used. This light illuminates the aquarium from the outside. The intensity of the light in both cases of red and green was about 7 luxes at the center of the aquarium and about 10 luxes at the feeder.

The present experiments were undertaken from October 1955 to March 1956. Temperature in the experimental aquarium throughout the experiments was kept at 7.5～17.0°C.

A thread with a bead was connected through an electric wire with a lever, so as to register mechanically the pecking reaction of the fish on the kymograph. However, in cases in which the pecking would not be strong enough to move the lever, it was necessary to supplement such slight reactions by means of another circuit, through which they were registered by automatical handling.

Prior to performing the conditioned connection between light and food, the pecking reactions were coupled with food. After several repetitions of this coupling, the fish were reinforced with food only when they pecked the bead, during stimulation of light.

The speed of a food-conditioned reflex thus formed in the gol -fish, is shown in Table 1. The formation of the temporary connection is shown in Fig. 3. As the Table shows, some of the fish failed to combine the light with food through the agency of pecking (*G. no. 112*, *G. no. 119*). In most cases they swam up to the feeder directly by an application of the conditioned stimulus, without pecking the bead. Others also failed to obtain the conditioned connection stable enough to make the experiment of differentiation (*G. no. 117*). As can be readily imagined, it appeared much more difficult to work out the food conditioned reflex by means of the so-called "food-getting" method (Voronin, 1954) than in the previous experiment. Here, we also may point out in our data, that, generally, many more repetitions of reinforcement were needed to establish a stable temporary connection, than those needed by Prazdnikova ('53 a), to obtain the same results.

In reality, it would not be easy to compare our data with those of Prazdnikova. Vatsuro ('54) suggested that there is an ambiguity in the expression of the speed of formation of the temporary connection by Prazdnikova.

For the experiment on differentiation, we used three fishes in which a stable

Table I

Speed of formation of the temporary connection and the differentiation observed in the gold-fish experimented upon

Cases	Formation of temporary connection			Formation of differentiation between two stimuli (red and green)					
	Number of stimuli required for primary formation of the temporary connection	Number of stimuli required for the establishment of stable temporary connection	Number of stimuli applied prior to differentiation	Number of stimuli applied prior to differentiation		Number of stimuli required for establishment of differentiation		Number of stimuli applied prior to differentiation	
				Positive	Negative	Positive	Negative	Positive	Negative
G. no. 111	38	66	308	17	22	35	46	235	349
G. no. 112	29	—	114						
G. no. 113	38	55	102	55	70	106*	128*	276	516
G. no. 114	13	75	96	18	24	38	54	77	106
G. no. 117	47	70	157						
G. no. 119	—	—	31						

Asterisks indicate that stable differentiation has not been worked out.

conditioned reflex had been established, with either red or green light. One out of three fishes (*G. no. 113*) generalized at a rate of 100% at the first application of a negative (differential) stimulus (Fig. 2), while the others did not do so entirely. But one fish, which did not show any generalization at all, was apparently effected by an intensive orientating inhibition, which caused an interruption of generalization. However, much to our regret, we have to diesist further disserting upon this matter, since our data are not conclusive enough for this purpose.

To work out the differential inhibition, the previously conditioned stimulus was continued with the accompaniment of food, while the differential stimulus was not reinforced with food. That is to say, the former is a positive stimulus, while the latter is a negative one. In connection with the afore-mentioned, we considered it as a formation of differentiation when a ratio of over 60 % in discrepancy between reactions to the negative and to the positive stimulus was established.

Among those experimented upon for differentiation, two fishes were found to have been relatively quickly and well established in the differentiation

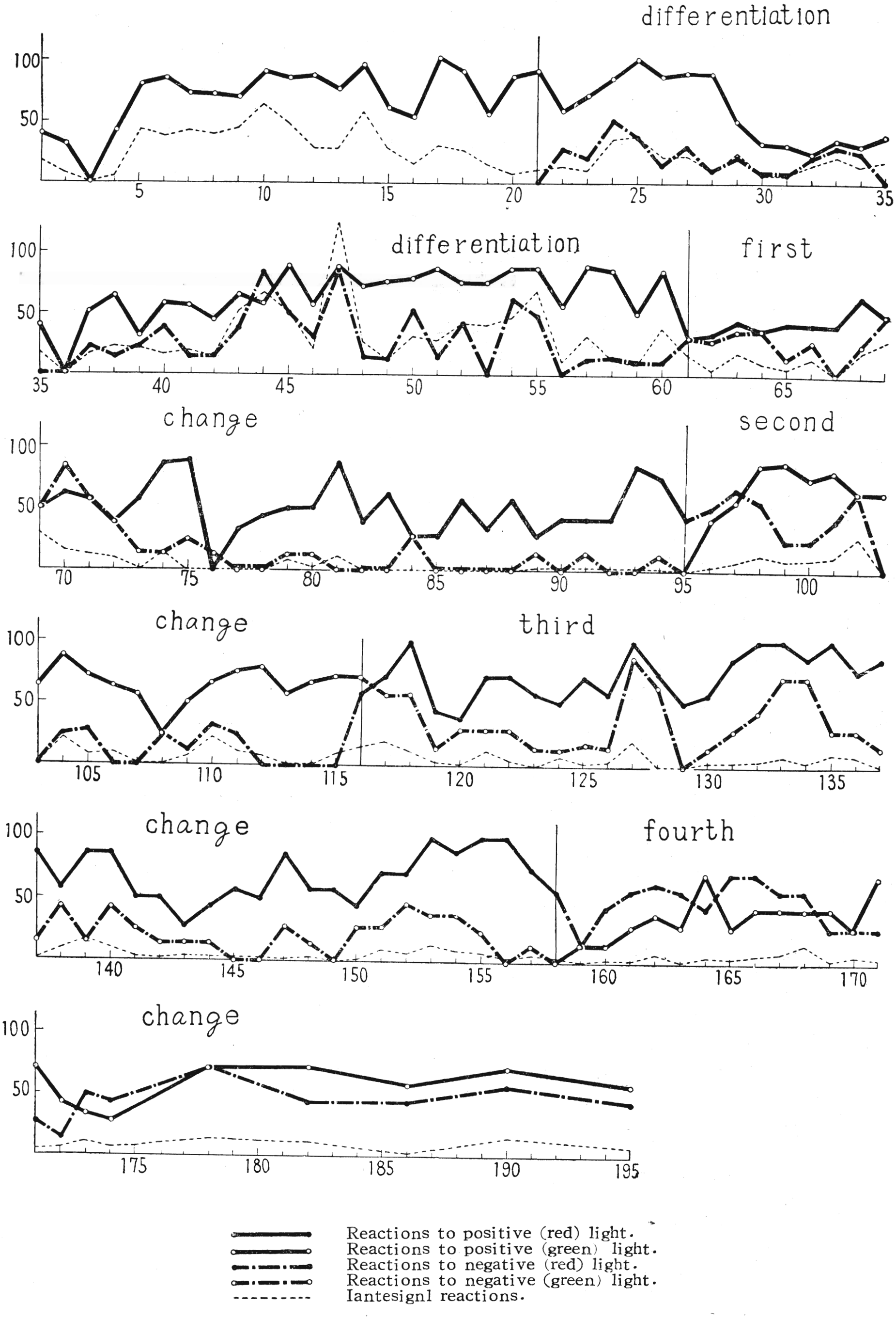

Fig. 1. Graph showing the entire course of experimentation in a gold-fish (*G. no. 111*). The differentiation was worked out by application of differential stimuli. However, in the course of differentiation, sometimes it was destroyed and sometimes re-established. Four changes of the signal denotations of stimuli were made. Ordinate indicates the number of peckings in per cent to the positive stimulus and the negative stimulus, and the number of intersignal reactions in one series of experiments; abscissa, days.

between colors. The course of differentiation in a fish (*G. no. 111*) is shown in Fig. 1. The differentiation in this fish was established by 46 repetitions of the differential negative stimulus (red) and by 35 repetitions of positive stimulus (with reinforcement of food). As is seen from the figure, differentiation, once formed, is destroyed and again re-established. To work out a stable differentiation, were required more than 251 repetitions of the negative stimulus. After 349 repetitions of the negative stimulus and 235 repetitions of the positive stimulus, the fish was experimented upon with a changing of the signal denotations of stimuli. Reference to this will be made later.

In an other case, (*G. no. 114*), differentiation was formed after 54 repetitions of the negative stimulus (green) and 38 repetitions of the positive stimulus (red). Afterwards this differentiation was not destroyed. After 106 repetitions of the negative stimulus and 77 repetitions of the positve one, the changing of the signal denotations was undertaken.

Concerning the last one, (*G. no. 113*), it formed the differentiation after 128 repetitions of the negative stimulus (green) and 106 repetitions of the positive one (Fig. 4), but, soon after, it failed to persist in the differentiation. The course of differentiation is shown in Fig. 2. Although 516 times of the negative stimulus and 276 times of the positive one were applied to it for about 70 days, no distinct differentiation was made. Accordingly, the experiment for changing the signal denotations proved to be impossible.

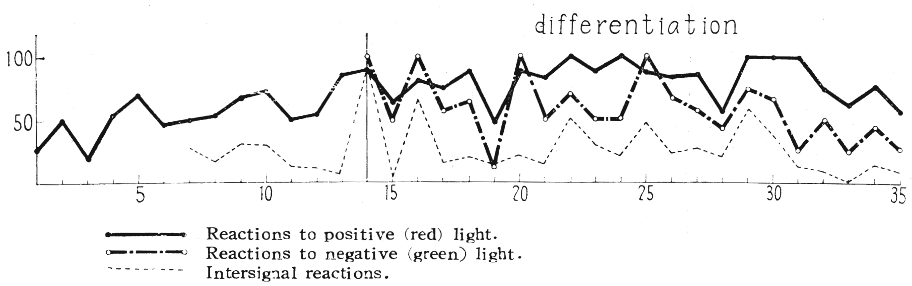

Fig. 2. Graph showing an example in which a total generalization was observed by the first application of differential stimulus (*G. no. 113*). Ordinate indicates the number of peckings in per cent to the positive stimulus and the negative stimulus, and the number of intersignal reactions in one series of experiments; abscissa, days.

As follows from the above mentioned, it appeared very difficult to work out a differentiation in gold-fish. This result, however, is quite different from that made Prazdnikova ('53 a). According to her results with *Carassius*, the differentiation is formed relatively quickly and stably. Why such a difference is found, requires further study.

The changing of the signal denotations of stimuli was made on the fish, (*G. no.*

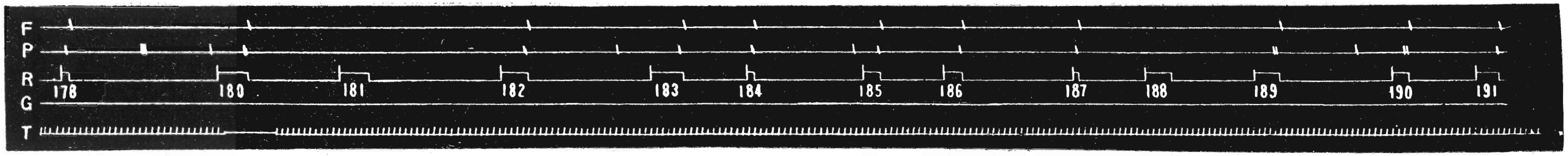

Fig. 3 Showing a formation of the temporary connection, 13 days after the beginning of experimentation. (*G. no. 113*) (20. XII, 1955) Abbreviations; F, reinforcement of food; P, pecking reactions; R, red light (conditiond stimulus); G, green light (conditioned negative stimulus); T, time (6 seconds). In the Fig. 3, numerals denoting on the line R indicate the number of application of conditioned stimuli (Positive) from the beginning of experimentation.

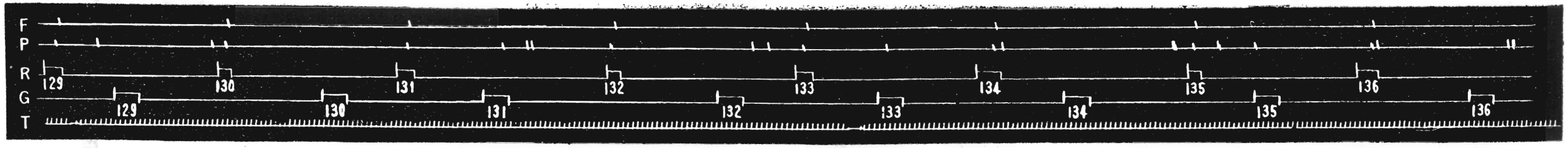

Fig. 4 Showing formation of differentiation, 18 days after introduction of the differential stimuli, (*G. no. 113*) (20. I. 1956). Numerals denoting on the line G indicate the number of differential stimuli (negative) and both the lines, G and R, give the number of stimuli from the beginning of differentiation. Other abbreviations are the same as in Fig. 3.

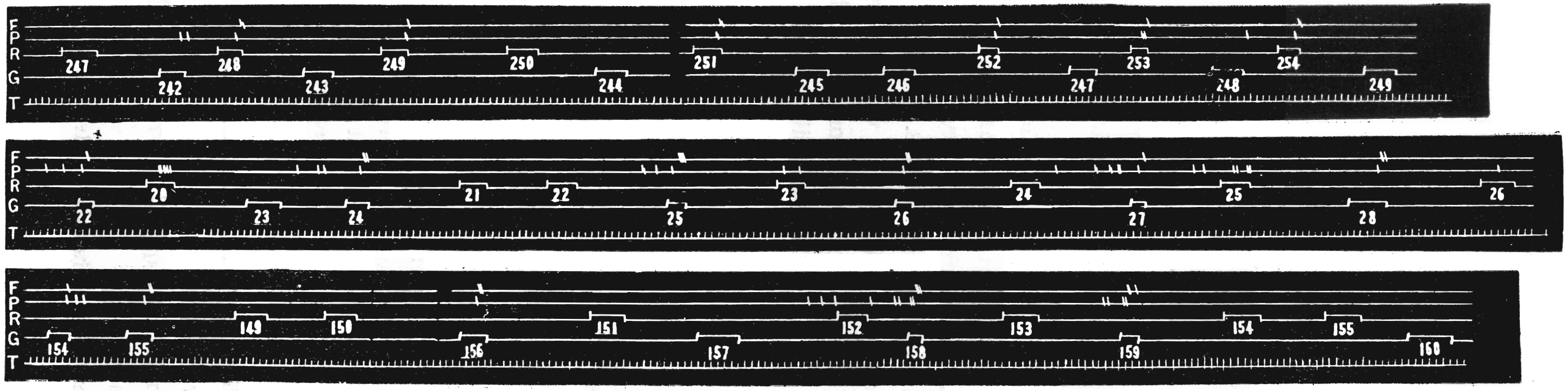

Fig. 5 Showing an accomplishment of the first change of the signal denotations of stimuli (a), the generalization at the second change (b), and an accomplisment of the second change (c), (*G. no. 111*); a, 34 days after the introduction of the first change. (14. II, 1956) b, 4 days after second change, but the differentiation was not established (18, II, 1956). c, Differentiation became complete 21 days after the second change. (6, III, 1956) In *a* of the figure, numerals denoting on the line R indicate the number of application of positive conditioned stimuli and the line G, that of negative conditioned stimuli applied from the beginning of the first change. In *b* and *c*, numerals denoting on the line R indicate the number of negative stimuli and the line G, that of positive ones in the second change.

111), as stated above. At the first change, the differentiation began to be appeared after 99 repetitions of the negative stimulus (green) and 47 repetitions of the positive stimulus (red), but it slightly decreased and then again increased to a certain extent (Fig. 5 *a*). After 249 repetitions of the negative stimulus and 124 repetitions of the positive stimulus, further experiments were made for the second change of the signal denotations. At the second change it was noticed that the changing appeared to be performed by a relatively smaller number of stimuli (Fig. 5 *b*), that is to say, by 26 repetitions of the negative stimulus (red) and 13 repetitions of the positive one (green.) But it required many more repetitions of stimuli to accomplish a sufficient change of signal denotations. In the course of the second change the changing was destroyed twice as shown in Fig. 1. After 155 repetitions of the negative stimulus and 101 repetitions of the positive stimulus it was transferred to the third change (Fig. 5 *c*). At the third change, the changing seemed to be accomplished after 65 repetitions of the negative stimulus (green) and 41 repetitions of the positive stimulus (red). However, it was once destroyed. After this the changing was settled by many repetitions of the positive and negative stimuli. The fish was transferred also to the fourth change after 304 repetitions of the negative stimulus and 217 repetitions of the positive stimulus throughout the third change. At the fourth change, although it was applied by 119 repetitions of the negative stimulus (red) and 42 repetitions of the positive stimulus (green), any indication of the possibiblity of the changing the signal denotations of stimuli was not found (Fig. 5 *c*). Further experimentation was not undertaken. Accordingly, we could not ascertain whether or not the changing was possible. According to our suggestion, however, further changing would not be impossible if a great deal of repetitions of the stimuli would be applied.[1)]

The experiment for changing the signal denotations for the fish (*G no. 114*), was quite difficult to make. In fact, it did not show any sign that the changing of the signal denotations had been established, in spite of the application of a large number of stimuli, that is, 370 times of the negative stimulus (green) and 83 times of the positive (red). Afterwards, both the negative and positive conditioned reflexes seemed to be destroyed, so that further experiments were not made.

According to our results, the intersignal reactions very much increased at the beginning of the experimentation and did not decrease to much even when the conditioned connection had been established. When the differential stimulus was introduced, the frequency of intersignal reaction did not reveal any apparent variations, although there appeared to be a slight indication for increase. Even when the changing of the signal denotations was made, a noticeable rise of the intersignal

1) In April 1956, Dr. Prazdnikova, at Koltsushi, Pavlov's Physiological Institute, U.S.S.R., told one of the authors (H. T.) that she succeded in changing the signal denotations of stimuli 8 times in the only one of gold-fishes experimented upon.

reactions was also not observed (Fig. 1.). It may be interesting to note that the intersignal reactions in the three fishes were extinguished after a great number of times, they were elicited as follows; 1870 times (76 days after experimentation) in *G. no. 111*, 910 (48 days) in *G. no. 113*, 450 (41 days) in *G. no. 114*.

Although the intersignal reactions of pecking were caused by the excitatory state of the food center, the above figures seem to indicate that the decrease of the reactions depends upon the formation of extinctive inhibition after a long period of time. Since the present experiments were made mostly in the winter time, further investigation may be needed to determine whether or not the decrease of the intersignal reactions is due to the lower temperature, and, or, to the bodily changes of the fishes.

According to the above mentioned results, generally speaking, the process of internal inhibition develops but in an unstable form. Even though differentiation between colors is possible, differential inhibition is only produced when the differential stimulus is applied a great number of times, otherwise it fails to develop into a distinct form.

As already stated, our results seem to be different from those obtained by Prazdnikova ('53 a) and Tretyakova ('53). According to Prazdnikova, stable differentiation between colors is formed by application of 20 to 40 times of differential stimuli, and according to Tretyakova, it requires also 15 to 76 times of stimulus. In both cases, gold-fish were used.

On the other hand, the same experiment with carps in Biryukov's Laboratory seems to be well in accordance with our own. That the process of internal inhibition is less well developed in gold-fish, has also been ascertained in our previous experiment on the extinctive inhibition in the same fish. However, it should be emphasized that one of the internal inhibitions, that is, the mechanism of differential inhibition, is not always the same in various kinds of fish. For instance, in sea breams the differentiation is established more quickly than in gold-fish, and is relatively stable.

Tretyakova ('53), studying the changing of the signal denotations of stimuli from a point of view of comparative physiology, reported that only one out of three gold-fishes was successful in being changed, while others failed to be so. At the first change, the change became stable after 29 repetitions of negative stimulus and at the second change after 150 repetitions. However, it was impossible to establish the third change, the conditioned reflex having been destroyed. In two other cases, even in the first change the changing was unsuccessful, in spite of 220 and 178 repetitions of negative stimulus, respectively. These results seem to be the same as ours. It shows that, although there are highly individual variations, mobility of the nervous activity is less well developed in fish. It may be added in this connection that with the sea bream, too, it was difficult to change the signal denotations. As to the problem of mobility, we observed that the gold fish, as well as the sea bream, displayed a somewhat neurotic state during the first change of signal denotations, and also a disturbance of the positive conditioned reflex, which makes matters worse as far as the development of the internal inhibition is concerned. This might be explained as a disturbance of the equilibrium of both processes of excitation and of inhibition, which would be caused by the imperfection of mobility in the developmental level, such as in fishes.

Studies of Voronin ('55) dealing with the mobility in the varied forms, from fish to apes, are very instructive. Judging from the speed required for the successive changes of signal denotations, he drew the conclusion that the mobility revealed by the first change does not run parallel to the developmental level of nervous systems in the different animals examined, even though a slight difference may exist. But, the degree of the disturbance of mobility caused by the successive changings is thought of as an index of the developmental level of animals. The data cited by Voronin, however, according to our suggestion, seem rather to conclude that the variations of mobility are appreciated throughout different forms of animals, that is to say, the mobilty in the fish is exceedingly lower than that in birds (Tuge and others, '56).

Frolov ('38) concludes, on the basis of his accumulated data dealing with fish, that it is very difficult to establish the internal inhibition and, at best an unstable one, the extinctive inhibition appearing in a wave-like form, which, as yet, indicates the weakness of the nervous process. Furthermore, he states that, from a point of view of animal phylogeny, strength of the nervous process is developed most quickly, while, among strength, equilibrium and mobility of the nervous process, mobility is the slowest to develop.

According to our results with fishes, in addition to those with birds, we strongly support the conclusion drawn by Frolov, and emphasize that the discrepancy of the results found among the investigators dealing with fish should be fully clarified in the near future.

SUMMARY

1) Motor-food conditioned reflex was studied with gold-fishes, in order to make a temporary connection between food and light (green and red), by means of the so-called "food-getting" method, in which the fish is conditioned to pull a bead and then to eat.

2) To form a temporary connection by using the food-getting method, seemed to be slightly difficult; a few of the fishes experimented upon, failed to establish the temporary connection through the agency of pulling. The speed of formation

of a stable temporary connection varied individually, but roughly 55 to 75 repetitions of reinforcement were necessary. It did not seem to according to the colors used.

3) Generalization after the first application of differential stimulus showed a remarkable difference in individual fishes. One exhibited 100 % of generalization, but the others did not at all. It may be suggested that this is due to the degree of orientating inhibition.

4) It was found to be extremely difficult to establish a stable differentiation.

5) In the successive changes of the signal denotations of stimuli, changing more than three times was successful in one case only. An alteration of the previously positive stimulus to negative one seemed much more difficult than the reverse. In each changing of signal denotations, either the positive or the negative conditioned reflex was difficult to establish, in comparison with the higher forms of animals. It indicates that the process of internal inhibition is remarkably weak in fishes.

6) The intersignal reactions take quite long time to be extinguished.

7) Mobility of the nervous system in fishes is discussed from the view point of the developmental level of animals.

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