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Studies of the Conditioned Reflex in the Lower Vertebrates

VIII. Conditioned Inhibition in Gold-Fish and Angel Fish

BY

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It is the established fact that the fish is capable of differentiation although its internal inhibition is not so well developed as in higher forms. In like manner, it is not without interest to find out that to what extent the synthetic function of higher nervous activity is developed in fishes which show, to a certain extent, the presence of analytic function.

To examine this in fishes, a certain number of workers have studied on the conditioned reflex, either positive or negative, by applying complex stimuli. Among them, Vedyaev (1956) and Tagiev (1957, 1958) found that the analytico-synthetic function is very inferior in fishes. Prazdnikova (1953a, 1955) observed that the conditioned inhibition is possible to be formed, but it is very difficult when complex stimuli are applied in a successive order in fishes. In view of comparative physiology of the higher nervous activity, Voronin (1953) concluded that the possibility of formation of conditioned inhibition by applications of chain-like stimuli depends upon the phylogenetic level of animals.

In the present paper, we intended to ascertain the possibility of conditioned inhibition and then to clarify the mechanism involved in fishes.

MATERIALS AND METHOD

For the present study were used six gold-fish and two angel fish (*Pterophyllum eimekei*). The aquarium for the experimentation was 19×35×27 cm.

In order to elaborate the food motor conditioned reflex, the food-getting method by Prazdnikova (1953) was used similar to that used in our previous study (Tuge, Kanayama and Ochiai, 1956). A bead tied by a fine thread was hung down in the aquarium. At first, the fishes were trained so as to pull the bead by their mouth whenever bait was furnished. After this combination became stable, a white light, 2 c.p. was introduced as a conditioned stimulus from the outside of the aquarium. As the complex stimuli, bubbling

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and light were employed. The bubbling as an additional agent was applied by blowing out the air from the air-stone fixed at the end of a glass tube that was led into the aquarium near the place where the bait was given. Before the experimentation, the orienting reaction of the fish to either light or bubbling was extinguished.

Pecking of the bead, food-eating, introduction of light and bubbling were registered on a kimograph, respectively.

The experiment was made in the following seasons: gold-fish no. 151, from November to January; gold-fish no. 153, no. 154 and no. 156, from August to December; gold-fish no. 159 and no. 160, from July to September; angel fish no. 115 and no. 117, from February to May.

RESULT OF OBSERVATIONS

In both the gold-fish and the angel fish employed for the experimentation of conditioned inhibition, the food motor conditioned reflex to a light was elaborated, being practically equal in the rate of its elaboration in both species, namely, 10 to 30 combinations.

As a first series of experiments, in the fish, in which the food motor conditioned reflex had been elaborated, was applied the simultaneous conditioned inhibitive stimuli (bubbling and light). As the second series, those in which the conditioned inhibition had been successfully established were subjected to the application of consecutive conditioned inhibitive stimuli. Finally, some of them succeeded in the formation of consecutive conditioned inhibition, were tested by one chain of stimuli with 5-second intervals between each stimulus, and the other with 10-second intervals.

I. *Simultaneous application of a conditioned inhibitive agent and a positive stimulus*

In order to examine the possibility of formation of conditioned inhibition, bubbling and light were applied simultaneously for 20 seconds as an inhibitor. In both the gold-fish and the angel fish, the conditioned inhibition of this kind was established. The rate of formation of conditioned inhibition is shown in Table 1 and 2.

Table 1. Speed of formation of a conditioned inhibition when the two stimuli were applied simultaneously

No. of animals	Number of applications of simultaneous conditioned inhibitive stimuli		
	Weak formation of conditioned inhibition	Stable formation of conditioned inhibition	Total number of applications
Gold-fish no. 151	18	30	42
Gold-fish no. 153	30	72	167
Gold-fish no. 154	2	2	36
Gold-fish no. 156	20	—	147
Gold-fish no. 159	14	84	111
Gold-fish no. 160	2	2	15
Angel fish no. 115	34	76	106
Angel fish no. 117	17	41	59

Table 2. Result of formation of a conditioned inhibition at various applications of complex stimuli

No. of animals	Simultaneous applications of complex stimuli	Consecutive applications of complex stimuli without intermission	Consecutive applications of complex stimuli with 5-sec. intervals	Consecutive applications of complex stimuli with 10-sec. intervals
Gold-fish no. 151	++(42)	++(72)		−(54)
Gold-fish no. 153	+++(167)	+++(66)		−(65)
Gold-fish no. 154	+++(36)	+++(18)		−(66)
Gold-fish no. 156	±(147)	±(60)		±(66)
Gold-fish no. 159	++(111)	++(100)	−(189)	
Gold-fish no. 160	+++(15)	+++(74)	±(121)	
Angel-fish no. 115	+++(106)	+++(48)	++(66)	−(54)
Angel-fish no. 117	+++(59)	+++(72)	−(48)	

+++ means stable formation of conditioned inhibition ; ++ means unstable formation; ± means that the formation was doubtful; − means that it was not formed.
Numerals in brackets indicates number of applications of complex stimuli.

In all of the gold-fish, except on G. no. 151, there appeared a generalisation in response to the first application of complex stimuli. During the course of several applications of the complex stimuli, the positive conditioned reflex began to be disturbed somewhat. In almost all of the fishes experimented on, weak conditioned inhibition was elaborated by some dozen applications of the complex stimuli, and it became stable after applications of 30 to 84 times. But, two of them (G. no. 154 and no. 160) began to differentiate very quickly and in that condition a stable differentiation was worked out. Figures 1 and 2 illustrate the process of the formation of conditioned inhibition. In only one case (G. no. 156) stable conditioned inhibition was not elaborated.

The two angel fish experimented on, also, generalised at the first application of complex stimuli. In the course of applications of complex stimuli, the positive conditioned reflex was disturbed. Frequently they reacted positively to the complex inhibitory stimuli. After this, the positive conditioned reflex restored and the conditioned inhibition was established (Figs. 3 and 5).

II. *Application in a chain order of the conditioned inhibitive agent and a conditioned stimulus*

Each element of the complex stimuli as inhibitor was consecutively applied on the gold-fishes in which the simultaneous conditioned inhibition had been elaborated. That is to say, after bubbling was introduced for 10 seconds, a light was applied without intermission for the same period. As is indicated in Table 2, five out of six fishes were successful to produce this kind of conditioned inhibition. One exceptional case (G. no. 156) was that in which a stable conditioned inhibition was not worked out even by the simultaneous application of complex stimuli.

In the fishes (G. no. 154 and no. 160) in which the inhibitory process was exceedingly developed, the conditioned inhibition was relatively quickly worked out (Fig. 1), but the conditioned reflex of the second order to bubbling was not easily formed. In the fishes in which the inhibitory process was not so

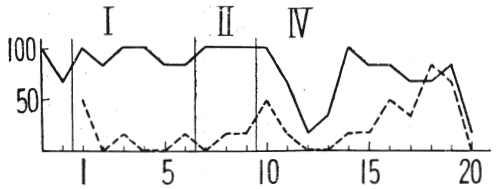

Fig. 1 Showing the process of formation of conditioned inhibition at which it was very quickly established in the simultaneous and consecutive applications of complex inhibitive stimuli. Gold-fish no. 154. Ordinate indicates the number of peckings in per cent to the positive stimulus and the complex inhibitive stimuli in one series of experiments; abscissa, days. Full line indicates reactions to the positive stimulus; broken line, reaction to the complex inhibitive stimuli; I, simultaneous applications of complex stimuli; II, consecutive applications of complex stimuli without intermission; III, consecutive applications of complex stimuli at 5-second intervals; IV, consecutive applications of complex stimuli at 10-second intervals.

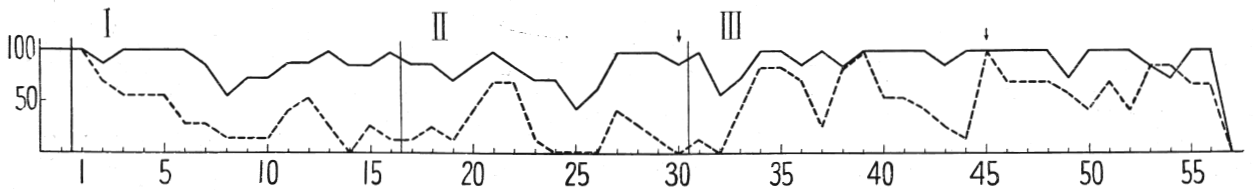

Fig. 2 Showing the process of formation of conditioned inhibition at which it was established in a wave-like process. Gold-fish no. 159. See figure 1 for the legends. Arrows indicate the expeimental days corresponding to the kimographs shown in figure 4a and 4b, respectively.

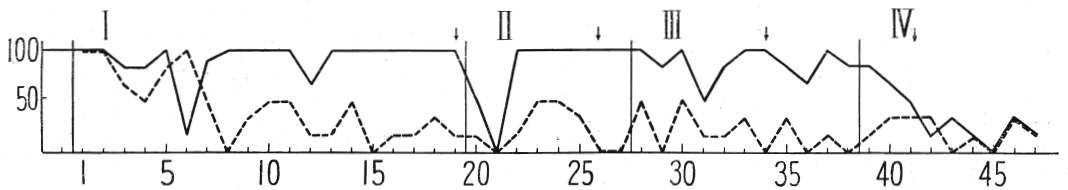

Fig. 3 Showing the process of formation of conditioned inhibition. Angel fish no. 115. See figure 1 for the legends. Arrows indicate the experimental days corresponding to the kimographs shown in figure 5a, 5b, 5c, and 5d, respectively.

strongly developed (G. no. 153 and no. 159), the differentiation was successful, but the progress of differentiation took a wave-like shape and the conditioned reflex of the second order occurred frequently. The positive conditioned reflex was not disturbed so much.

In those in which the inhibitory process was very weak (G. no. 151 and no. 156), the secondary conditioned reflex appeared and the positive conditioned reflex was broken down so prominently soon after the complex stimuli were introduced, that the differentiation was difficult to be stably established, taking a wave-like shape. Especially, one of them could not retain the differentiation over than 60 % for more than 2 days.

As has been clearly seen from the above, the phenomenon that the secondary conditioned reflex to bubbling is formed in the course of consecutive applicaltions of complex stimuli is largely dependent upon the individual differences. In other words, the secondary conditioned reflex is very easily formed in the fish in which the excitatory process is far more strong than the inhibitory process, and, on the other hand, in the reverse type, it is very difficult. Concerning the formation of the secondary conditioned reflex, however, it must be noted here that the fact that the secondary conditioned reflex was formed in the fish was based upon the observation that they proceeded towards the bubbles during bubbling. Really, it was on very rare occassions that the fish pecked the bead in response to bubbling stimulation.

The two angel fish in which the conditioned inhibition had been established by simultaneous applications of complex stimuli, too, were used to determine whether or not the conditioned inhibition would be brought about by the consecutive applications of complex stimuli. They were able to differentiate such complex stimuli as inhibitor, although they showed a wave-like process to attain a stable conditioned inhibition. Although the secondary conditioned reflex occurred very frequently in both cases, the positive conditioned reflex was not disturbed in the one, but in the another (Ang. no. 115) it was once completely destroyed immediately after introduction of complex stimuli and recovered soon (Fig. 3).

It may be said that the conditioned inhibition by means of consecutive applications of complex stimuli is just alike that found in the gold-fish whose inhibitory process is of medium quality (G. no. 153).

III. *Application in a chain order with 5-second intervals between the conditioned inhibitive agent and a conditioned stimulus*

In the two gold-fish (G. no. 159 and no. 160) and the two angel fish, in which the conditioned inhibition was worked out by consecutive applications of complex stimuli, complex stimuli with 5-second intervals in each were applied.

In gold-fish, the conditioned inhibition was effective during the first several applications of the complex stimuli, but then after the inhibition became very weak. During a long course of the above application, the conditioned inhibition over 50 % was observed only for one or two days of the whole experimental series. Notwithstanding that the positive conditioned reflex was not so much destroyed, the conditioned inhibition was lost. After 30 applications of complex stimuli, both the negative and positive conditioned reflexes were disappeared (Figs. 2 and 4).

In one of the two angel fish (Ang. no. 115), although the secondary condi-

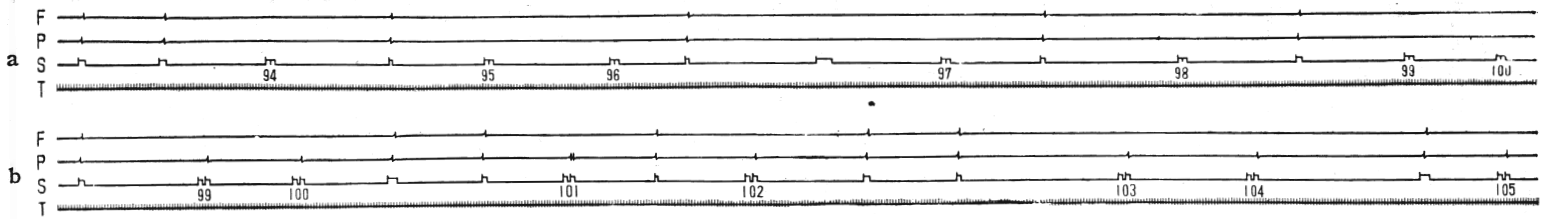

Fig. 4 The figure illustrating the formation, successful and unsuccessful, of conditioned inhibition. Gold-fish no. 159.
a, simultaneous applications of complex inhibitive stimuli. b, consecutive applications of complex inhibitive stimuli at 5-second intervals. F, food-eating; P, pecking; S, stimulations; T, time in 6 seconds. Signals without numerals denoted on the line S indicate positive stimuli and those with numerals, complex inhibitive stimuli. Numerals indicate the number of applications of complex inhibitive stimuli.

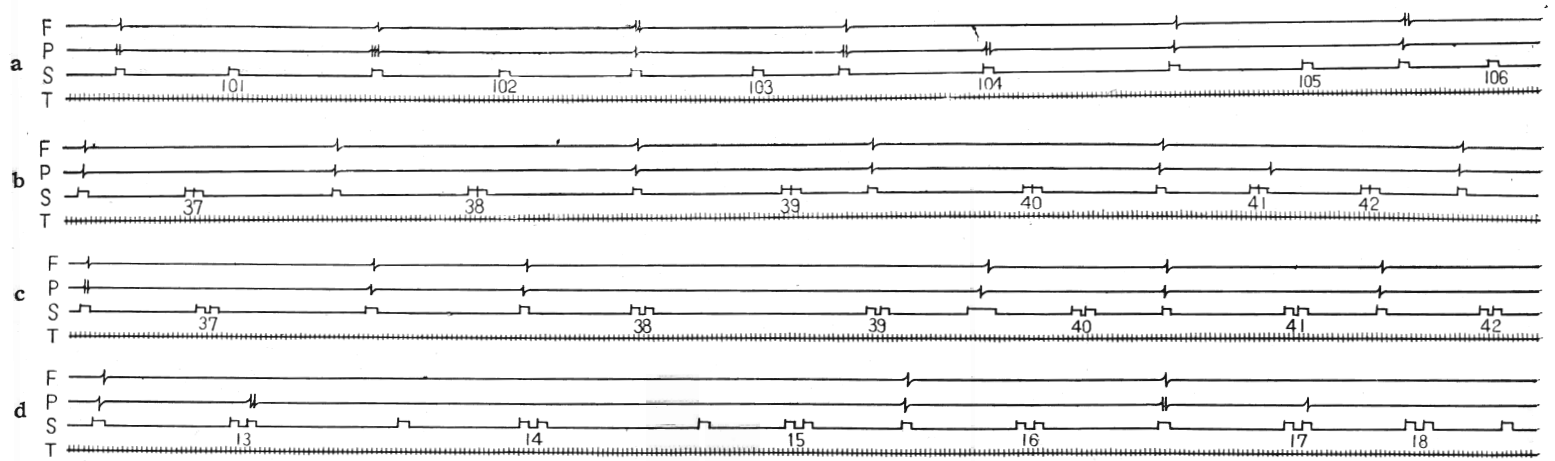

Fig. 5 The figure illustrating the conditioned inhibition at various applications of complex inhibitive stimuli, Angel fish no. 115.
a, simultaeous applications of complex inhibitive stimuli. b, consecutive applications of complex inhibitive stimuli without intermission. c, consecutive applications of complex inhibitive stimuli at 5-second intervals. d, consecutive applications of complex inhibitive stimuli at 10-second intervals. See figure 4 for the legends.

tioned reflex occurred very frequently and the positive conditioned reflex was slightly disturbed for some time after the introduction of complex stimuli, the differentiation was completed (Fig. 5). In the other one, however, the secondary conditioned reflex also occurred though less frequent and the positive conditioned reflex was abruptly destroyed after 50 applications of complex stimuli. Since this fish became so nervous that the further trials were not possible, the experiment was discontinued.

IV. *Application in a chain order with* 10-*second intervals between the conditioned inhibitive agent and a conditioned stimulus*

Further experiments with the three gold-fish in which the conditioned inhibition had been established by consecutive applications of complex stimuli and with the one angel fish succeeded at a 5-second interval (Ang. no. 115) were conducted by successive applications of complex stimuli with 10-second interval.

As regards the gold-fish, there appeared the secondary conditioned reflex very frequently and the positive conditioned reflex was severely destroyed. After all, all of the fish experimented on gradually became irresponsible to both the positive and negative conditioned stimuli. The occurrence of the secondary conditioned reflex, of course, was disappeared. The same was also true in the angel fish (Fig. 5).

DISCUSSION

According to Prazdnikova (1953a), who studied on the conditioned inhibition in fishes, the simultaneous conditioned inhibition is possible to be formed, but it is relatively difficult to obtain a stable conditioned inhibition when the inhibitory agent and the positive stimulus are given successively. If 5-second intervals between the two stimuli are placed, the conditioned inhibition is exceedingly unstable, and in cases of 10-second intervals it is completely impossible.

Our result seems to confirm that of Prazdnikova. Our observation showed that the simultaneous conditioned inhibition was successful to be stably worked out in all fishes examined but only one exception. Except this exception, the consecutive conditioned inhibition also was elaborated relatively stably, although Prazdnikova noted that it was not stable. However, when the two stimuli are given at 5-second intervals, in one out of four fishes the conditioned inhibition was elaborated. In all of the others, however, the positive conditioned reflex was destroyed and the conditioned inhibition was not formed. When applied at 10-second intervals between both stimuli, no conditioned inhibition was established.

As is seen from the above, the fact that the simultaneous conditioned inhibition is formed in fishes would reveal that they possess an ability, to some extent, to analyse the two stimuli. If the inhibitor be stronger than the excitator the conditioned inhibition will be elaborated.

We as well as other investigators find that in fishes, the process of internal inhibition is weak as compared with higher forms in general (Tuge, 1957). Accordingly, it requires much more time to develop the process of internal inhibition which is sufficient enough to surmount the excitatory process. Being different from higher forms, the conditioned inhibition is formed after 30 to 84 applications of complex stimuli as inhibitor. The result

agrees well with that obtained by Prazdnikova (1953a). It, however, is very peculiar that there were found a very few cases which showed a very quick formation of the conditioned inhibition, namely, G. no. 154 and no. 160. It is true that there are some fishes, in which rather intensive orienting reactions evoked by application of external stimulus are not readily extinguished, acting as inhibitory. In such cases, we presume that when applied both stimuli simultaneously, the external inhibition would annihilate the effect of the positive signal. Thus, the conditioned inhibition was elaborated very rapidly without passing through the generalisation phase. Vedyaev (1956) found in his experiment of complex stimuli with fishes that differentiation is formed without the generalisation phase, indicating that physiological mechanism concerning the analytico-synthesis for complex stimuli in fishes is not alike with that in higher forms. A few years ago, we found a similar phenomenon in chickens, in which there are two types of the process of inhibition in the course of formation of differentiation, that is, the property of orienting inhibition and that of differential inhibition (Tuge, Shima and Koga, 1956). The intensive orienting inhibition suppresses the process of generalisation and is then transformed into the differential inhibition.

Our experiment concerning the successive two stimuli without intermission between them, also, showed that the conditioned inhibition is worked out. This does not seem to be a very difficult task for fishes. The mechanism involved for the performance of this conditioned inhibition may be almost the same as shown in that applying both stimuli simultaneously. That is to say, in order to produce the conditioned inhibition, the synthetic function in the higher nervous activity would not participate in it, to a large extent. If, however, there is an interval of a few seconds between the two stimuli, the situation is somewhat different. In this case, it is dependent upon either an after-effect (traces) of the inhibiting stimulus, or the synthetic function between both stimuli, in order that the conditioned inhibition is elaborated. In reality, it was exceedingly difficult at 5-second intervals and furthermore it was impossible at 10-second intervals in the fishes we examined.

From our result, it may be said that in fishes, the analytic function is somewhat more developed than the synthetic function as well as that the excitatory process is more superior than the inhibitory one.

Voronin (1953, 1955) remarks in his comparative study of the higher nervous activity that the impossibility or extreme difficulty of forming a conditioned inhibition with a few second intervals between both stimuli in fishes may be explained by the fact that preservation of the traces of stimulations in the nervous system is weak. Thus, the formation of the conditioned inhibition with 5-second intervals is not possible in the lower vertebrates, but only in the higher forms, especially in chimpanzee, it is with 10-second intervals possible.

We observed frequently there occurred the positive reaction to the added agent when applied both stimuli successively. It must be a formation of the second order of the conditioned reflex, as observed by Prazdnikova and others. Voronin (1957) states that the secondary conditioned reflex is formed throughout the vertebrates series even in fishes, and, furthermore, in the animals in which the excitatory process is superimposed it tended to be formed more quickly than the conditioned inhibition is. Our observation would support his

hypothesis revealing that in those fishes which are far dominant in the excitatory process the secondary conditioned reflex is formed and then the conditioned inhibition is worked out, while in those which are superior in the inhibitory process the secondary conditioned reflex is not formed, if not at all, the conditioned inhibition is elaborated just as it is.

SUMMARY

1) Possibiiity of formation of the conditioned inhibition has been studied by applying the conditioned complex inhibitor in gold-fish and angel fish. As a conditioned inhibitive agent, bubbling was used and as a positive stimulus, a light. The observation was made by means of food motor conditioned reflex.

2) The conditioned inhibition by simultaneous applications of a conditioned inhibitive agent and a positive stimulus was formed relatively quickly.

3) The conditioned inhibition by successive applications of the two stimuli without intermission was also worked out without great difficulty. However, the conditioned inhibition was almost impossible when a 5-second interval between both stimuli was placed. If the interval was made 10-second, the conditioned inhibition was never formed.

4) Discussions concerning the mechanism of formation of the conditioned inhibition in lower vertebrates such as fish have come to the following conclusions: That the conditioned inhibition is quickly elaborated in fishes in which an additional agent acts as an external inhibition, and that the consecutive conditioned inhibition is hardly worked out, is due to the fact that the synthetic function of the higher nervous activity is poorly developed and the traces of stimulations are weak.

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下等脊椎動物の条件反射に関する研究
Ⅷ. 金魚とエンジエル・フィツシュの条件制止

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摘　　要

魚類においては，内制止過程が高等動物に比して充分に発達していないことは既に認められているが，さらに高次神経活動における分析と綜合の機能がどの程度まで発達しているかを明らかにすることは，魚類の行動を生理学的に分析する上で重要な課題である．

本実験では，金魚及びエンジエル・フィツシュを用い，食餌条件反射によつて条件制止の形成の可能性を追求した．まず Prazdnikova (1953) の食餌獲得法により陽性条件反射を形成し，その後，複合制止刺激を適用した．陽性条件刺激としては白色2燭光の電灯，複合刺激としては同上の白色光に水槽中への気泡を附加したものを用いた．

同時複合刺激を制止体として用いたときには，金魚においてもエンジエル・フィツシュにおいても，条件制止は比較的速かに形成される．また，複合刺激の両要素を継時的に適用した場合でも，金魚及びエンジエル・フィツシュにおいて，条件制止は大体容易に形成される．しかし，複合刺激の両要素の適用間隔を5秒にする連鎖複合刺激の場合には，この種の条件制止の形成は金魚でもエンジエル・フィツシュでも殆ど不可能であつた．さらに，両要素の適用間隔を10秒にする連鎖複合刺激の場合には，条件制止の形成は全く不可能であつた．

以上の結果からみて，魚類では複合刺激にたいする高次神経活動の綜合能力が著しく劣り，しかもそれは分析能力より劣つているし，また外部刺激にたいする痕跡機能も極めて弱いと考えられる．

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