RELATIONSHIP BETWEEN BEHAVIORAL RESPONSE AND METABOLIC RESPONSE OF NITROGEN UNDER AVOIDANCE CONDITIONING: I. AN EXPERI- MENTAL VERIFICATION OF THE CRITERION OF EXTINCTION

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For 100 days in the rat, fluctuations in behavior and urinary excretion of nitrogen were determined before, during and after the conditioning, extinction and test period. Avoidance conditioning was held 20 trials per day for 10 days. Behavioral effects of the conditioning were elicited and disappeared rapidly, whereas metabolic effects were gradually. Excretion per intake ratio of nitrogen significantly increased during the conditioning and restored completely long after the extinction. According to the test which was held after the metabolic response disappeared completely, behavioral responses were not elicited again, and metabolic response also did not result. That is, spontaneous recovery of the learned emotional response did not result. The metabolic response indicated the completion of extinction, whereas behavioral responses did not.

Writer's previous investigations presented evidence for a relation between behavioral response and the response of protein selection under the emotional learning, such that, in the rat, protein selection increases whereas glucide selection decreases in a free selecting situation coincidentally with an increase in the learned emotional behavior under the fear-conditioning and the presentation of shocks (Shigehisa, 1962, 1963). It was suggested that protein selection increased according to the metabolic deficiencies of protein based on the emotional learning. In addition, increased selection of protein was a specific fluctuation as a learned emotional response in the experimental treatment used in the previous studies.

Avoidance conditioning was used for the investigation of a detailed behavior-metabolism relationship. Changes in the urinary excretion of nitrogen correlate with those in protein metabolism under the emotional learning.

In accordance with former results, in

case that urinary excretion of nitrogen increases, it will be suggested that catabolic process of the protein metabolism did occur in relation to emotional learning. On the contrary, in case of decrease in urinary nitrogen, occurrence of anabolic processes will be suggested. Thus, the possible cause mechanism of the previous results will be elucidated.

A behavior-metabolism relationship that is delayed parallelism suggested in the previous and observed in the present study is fully suggestive of the nature of experimental extinction. Considering experimental extinction of a conditioned-fear (learned emotional response) in the conventional psychology of learning, the criterion of extinction under the behavioral level and therefore the standardized clue for the accomplishment of the extinction is somewhat inconsistent with the complete extinction. The occurrence of spontaneous recovery and the like indicate the fact of this sort. It may be conceivable that the determinations of indices on a behavior-

al level alone and the vascular, muscular, respiratory and visceral moving responses etc. hold the critical point of problem of this sort. The acquisition and extinction of an intense emotional response seem to be associated with the process other than learned emotional response in behavior coincidentally with the behavioral response. And then, it is doubtful that the complete extinction of a learned emotional response is attained coincidentally with the extinction of indices on a behavioral level.

Under the circumstances, what are the indices of the complete extinction? And then, does the complete extinction appear on what stage of these indices? Does the criterion of extinction depends on what relations with the indices under the behavioral level? And further, how the complete extinction is considered? The purpose of the present study is to reply these questions.

METHOD

Subject: Naive male Wister strain albino rats¹ (6) having mean initial body weight of 234 g (range: 226–240) were used as subjects.

Apparatus: i) Wire-netting walled individual metabolism cages (15 × 15 × 20 cm) were used. Two floors were attached to the cages, so that it was possible to assort urine, feces and diet individually. Diet containers (100 ml beakers) and 60 ml inverted drinking tubes were equipped outside the cages. ii) A Mowrer-Miller type avoidance training apparatus (80 × 20 × 10 cm) was used. It had glass walls and was painted white. Wooden barrier (5 cm high, 3 mm wide) was also covered by the grid. A buzzer was fixed outside (10 cm apart) the apparatus. Inside of the apparatus was illuminated by two 5 cp. bulbs from 13 cm above the grid floor. The apparatus was fixed in a quiet dark room throughout the experiment.

Diet: Ss were supplied basal diet throughout the experiment consisted of 64% of starch (P.H. Jap. IV), 18% of vitamin free casein, 8% of olive oil, 4% of dry yeast (vitamin B₁, 120; B₂ 40 γ/g; 45 per cent of protein; contained), 2% of cod liver oil (vitamin A, 2000; D, 400 IU/g

contained)² and 4% of McCollum's salt mixture (No. 185)³.

Chemical analysis: Micro-Kjeldahl method was used for the chemical analysis of total nitrogen. A sample, urine, was collected daily in each S separately from diet and feces using a metabolism cage which was cleaned out by the pincette and was not washed out every day. Each sample of 1.0 ml was measured, and oxydized immediately after the collection. Distillation and titration were also conducted soon after the former operations. The same technique was used for the collection of a sample, feces. The sample was weighed, then crushed and mixed using a mortar soon after the collection. Then each 1.0 g of them was oxydized immediately.

Procedure: Animals were maintained in cages individually in a quiet dark room and handled carefully throughout the experiment. Each animal was fed basal diet and distilled water ad libitum for 110 days including preliminary 10 days for adaptation. Body weight (to .5 g), volumes of diet and water ingestion (to .1 g and .1 ml), volumes of urine and feces excretion (to .1 ml and .1 g) were measured, and the urine and feces were analyzed for the nitrogen contents (to 1 mg) every day with 24 hrs intervals throughout the experiment.

The experiment was consisted of 20 periods in 5 days. Initial 3 periods were served as pre-conditioning control. During 2 periods following them, conditioning trials were conducted, and then extinction trials during 2 periods followed the conditioning. Ten periods of rest (post-extinction control period) were held thereafter. Then test trials were conducted for 2 periods following the rest. And a post-test control period followed them. Avoidance conditioning was consisted of buzzer sounds (AC 4 V) and electric shocks (AC .5 mA, 100 V), with 5 sec (CS-US) intervals. The conditioning was held 20 trials with 1 min intervals during 21 min per

¹ Purchased from Osawa Farm Inc., Shinagawa, Tokyo.

² All the nutrients used were the Japanese Pharmacopeial products.

³ All the inorganic salts used were the extra pure reagent chemicals.

day for 10 days. Extinction and test trials were the same technique as used in a conditioning except presentations of shocks.

During conditioning, extinction and test trials, avoidance response latency, number of avoidance response, inter trials response and responses to CS except avoidance such as turning a body or head toward barrier, running to the barrier and raise forelegs on the barrier etc. and other emotional signs were measured every trials. During pre-conditioning, conditioning, extinction, post-extinction (rest), test and post-test period, urinary and fecal excretion of nitrogen were determined.

RESULTS AND DISCUSSION

Owing to the avoidance conditioning, two types of response such as behavioral and metabolic were resulted parallelly in all animals. Behavioral effects of the conditioning were immediately elicited. That is, they increased (started in increase on the 2nd day of the conditioning) and decreased (started in decrease on the 4th day of the extinction) rapidly according to the conditioning and extinction trials. While metabolic effects of them were gradually increased (started in increase on the 1st day, and reached at maximum on the last day of conditioning period) and continued long after the extinction period, then they

restored gradually in all animals (completely restored during the period 12 and 16 and thereafter).

As shown in Fig. 1, under the conditioning trials, a number of avoidance responses (mean per cent) was increased rapidly among animals and reached at 90 per cent avoidance on the 2nd day. It kept 85 to 100 level of avoidance thereafter throughout the conditioning period, then decreased rapidly during the extinction period reaching at 30 per cent level on the 4th day and then 25 to 0 level thereafter.

An avoidance response latency (median sec) was decreased rapidly and reached at 1.6 level of time on the 3rd day. It tended to increase and somewhat stabilize at 2.1 to 3.2 level thereafter during the conditioning period. During the extinction, it kept 2.8 to 4.9 in the early stages. And then avoidance response was extinguished during the later stages.

A number of inter trials response (mean number) tended to increase during initial and later stage of both conditioning and extinction period. It decreased among animals and somewhat fluctuated during other stages in both periods as shown in Fig. 1.

Each value in a metabolic phase was measured daily throughout the experiment

FIG. 1. Numbers of avoidance and inter trials response under conditioning and extinction trials.

Fig. 2. Bodily intake, urinary excretion and excretion ratio of nitrogen under avoidance conditioning.

and averaged every 5 days. Urinary excretions of nitrogen per milliliter of the sample (mg/ml) remained at the level of 41.0 to 43.3 (mean 42.5) during the pre-conditioning control period. It increased rather slowly showing 44.5 to 46.0 (mean 45.3) level during the conditioning period, and further increased gradually and showed 45.6 to 48.2 (mean 46.9) level during the extinction period. Then it reached at the maximum increment immediately after the extinction period showing 49.7 level. And it was rapidly decreased (restored) thereafter, then it reached at the minimum level showing 41.1 in the middle stage of the rest period, and remained decreased thereafter with a slight increase.

Total urinary excretion of nitrogen (mg/day) maintained 338 to 369 (mean 340) level among animals during pre-conditioning period. It increased rapidly soon after the onset of conditioning reaching at 361 to 410 (mean 386) level during conditioning period, and then it was decreased gradually reaching at less than the value before conditioning (263 to 408, mean 332) during the rest period, and reached

at 197 to 244 (mean 221) level during the test period and 221 level after the period (Fig. 2).

On the other hand, bodily intake of nitrogen (mg/day), calculated by dietary intake minus fecal excretion, maintained 430 to 455 (mean 443) level during pre-conditioning period. It was decreased rapidly soon after the onset of conditioning reaching at 406 level and fluctuated markedly thereafter and reached at 296 level during the conditioning period. During the extinction period, it restored slightly showing 354 to 386 (mean 370) level. And the intake of nitrogen persistently showed decreased values without restoration keeping 306 to 388 (mean 361) level during the post-extinction period. And then it decreased further during (294 to 334, mean 314) and after (280) the test period (Fig. 2).

The excretion ratio of nitrogen (total urinary excretion/bodily intake $\times 100$ = per cent) remained constant showing 76 to 81 (mean 79) level throughout the pre-conditioning period. It rapidly increased immediately after the onset of conditioning

and reached at 101 to 122 (mean 112) level during the conditioning period. Then the ratio decreased gradually, and completely restored once in the middle stage (76), and persistently restored in the later stages (76 to 79, mean 78) during the post-extinction period. It decreased further thereafter, and reached at 67 to 73 (mean 70) level during the test period, and showed 79 level (the same as pre-conditioning level) after the period (Fig. 2).

The fluctuation ratio of the nitrogen excretion (excretion ratio/ a mean excretion ratio of the 3 periods in the pre-conditioning $\times 100 - 100 = \text{per cent}$) showed the excretion pattern as per cent fluctuation. ($\uparrow$ shows increase, and $\downarrow$ shows decrease of per cent from pre-conditioning excretion ratio). It increased to 28 $\uparrow$ to 54 $\uparrow$ (mean 41 $\uparrow$) level during the conditioning, and 30 $\uparrow$ to 43 $\uparrow$ (mean 37 $\uparrow$) level during the extinction period. It restored once during the period 12 (4 $\downarrow$) and increased again showing 8 $\uparrow$ to 14 $\uparrow$ (mean 11 $\uparrow$) level in the later stages during the rest period. Fluctuation as a whole during the rest period showed 38 $\uparrow$ to 4 $\downarrow$ (mean 14 $\uparrow$) level. It decreased further during the test period (8 $\downarrow$ to 15 $\downarrow$, mean 12 $\downarrow$) and showed 0 level after the period.

Behavioral effects of avoidance conditioning mostly disappeared in the later stages of extinction period. While nitrogen excretion ratio reached at near the maximum increment in those stages. Increased excretion ratio of nitrogen continued long after those stages and gradually restored on 28th and 46th day or so after the extinction period.

When the nitrogen excretion ratio reached at the maximum increment, depressed tendency of feeding behavior was noted, otherwise the behavior was maintained fairly constant. The depression of this sort also suggests the learned emotional property.

Following the 10 periods of the rest, when nitrogen excretion ratio was completely restored, test trials were held for 10 days.

According to the test, conditioned avoidance response was not elicited again which was extinguished already in the later stages of extinction period (Fig. 3).

In addition, the number of inter trials response (and the avoidance response latencies in the few cases also) increased as compared with the value during the extinction period (Fig. 3). The responses to CS except avoidance were elicited slightly, and disappeared rapidly. During the test period, nitrogen excretion ratio did not increase again. And the ratio did not increase significantly after the test period (Fig. 2).

Individual differences of the excretion ratio of nitrogen fluctuated variously according to the period. The difference increased at the maximum increment of the excretion ratio during the period 5 ($SD=16$), the later stage of conditioning period, as compared with that of the period 3 ($SD=7$), the latest stage of the pre-conditioning period. After the extinction period, it decreased gradually and reached at minimum during the period 9 ($SD=5$), the earlier stage of the rest period. It increased gradually and reached at maximum during the period 14 ($SD=21$), the later stage of the rest, and restored thereafter. During the test period, it increased again rapidly and reached at near the maximum during the period 19 ($SD=15$). And

FIG. 3. Numbers of avoidance and inter trials response under test trials.

gradually decreased during the period 20 ($SD=12$) after the test period.

The excretion ratio of nitrogen increased significantly (t -test) at a .005 level during the conditioning period (maximum increment in mean, 122) as compared with the control value immediately before the conditioning period ($M=79$). It decreased significantly at a .005 level in a latest stage of the rest period ($M=76$) as compared with the maximum increment. The difference between the control value during the pre-conditioning and the value during the latest stage of the rest (period 17) was not significant. Therefore, the increase in excretion ratio restored completely during the latest stage of the rest period. It increased again after the test period from 67 to 79 in mean, however the difference was not significant statistically.

Increase in the excretion ratio of nitrogen reflects increase in the catabolic processes of the protein metabolism. Therefore, metabolic deficiency of protein was resulted in the present experimental animals. Thus, an increase in the protein selection resulted from the metabolic deficiency of protein elicited by the emotional learning in the previous studies was confirmed by the present result. It is suggested that increased protein catabolism of this sort made the excretion ratio of nitrogen increase in the present study, and then the protein selection increase in the previous studies.

According to the present results, it is obvious that certain emotional and learnable stimuli elicit both behavioral (conditioned avoidance response and depressed behavior, etc.) and metabolic response (increase in protein catabolism). The behavioral response of emotional learning disappeared (extinguished) during the extinction period, while the metabolic response of the learning did not decrease (disappear) during that period, but about 50 days later after the period very slowly.

When the metabolic response disappeared, the test trials were held, and it was

found that behavioral response was not elicited again, and metabolic response also did not result following it again. Consequently it is suggested that a learned emotional response was completely extinguished only when the metabolic response restored following the disappearance of behavioral response, not when the behavioral responses disappeared.

In the present study, it was shown that emotional learning elicit behavioral response immediately after the trial which is more readily extinguishable, and metabolic response of protein (nitrogen) gradually after the trial (however, within 24 hrs it was started) which is far more persistent (long acting for many weeks). The protein selecting response observed in the previous studies was also a long acting process as well as nitrogen excretion response in the present experiment. These two processes elicited have their own way in detail each other, however, proceeded parallelly with a different time sequence. Behavioral processes of the emotional response were readily extinguishable, whereas metabolic process of nitrogen was not.

It appears that the acquired emotional response is continuously proceeding in its metabolic process of nitrogen (such a process is suggested by the increased selection of protein observed in the previous studies (Shigehisa, 1962 ; 1963), and evidenced by the increased excretion ratio of nitrogen in the present study) even after the extinction (or restoration) of behavioral processes. At the time in which learned emotional response has already extinguished behaviorally, emotional tensions (covert processes) still ever remained heightened (excitable), and the retention of emotional experience was maintained metabolically. The emotional learning process involved in the metabolic process observed in the present study indicate the fact of this sort.

According to the fact that the extinction (or retention) of a learned emotion on the behavioral level did not coincide with the

extinction (or retention) on the metabolic level in the temporal sequence, it appears that an acquired emotional response per se will not extinguish even when overt emotional behavior extinguished. That is, the learned emotional effect appears differentially between an overt behavior and a covert metabolic process. Thus, the learned emotional response continued without a behavior. And therefore, it is quite conceivable that the retention of an emotional learning could not be performed without a metabolic process. The possible mechanism of a latent learning during the acquisition process and of a spontaneous recovery of behavior (latent retention) during the extinction process are suggested by the present result. Then it seems necessary to approach the emotional learning process on both sides of metabolism and behavior. The present result suggests that metabolic response of nitrogen takes an important role in the emotional learning as well as behavior. A metabolic response of protein constitutes one of the important aspects of emotional learning. Probably, a significant relation exists between the metabolism of protein (catabolic phase) elicited by the conditioning and the learned avoidance behavior. Thus, it is readily conceivable that behavioral response alone does not fully reflect the emotional learning. On this point, it may be obvious that the dual process which proceeded parallelly with a delayed temporal sequence consisted of protein catabolism and conditioned avoidance behavior is involved in the emotional learning process.

If the increase in the protein selection was resulted by the motor activities rather than emotional learning, protein store will be resulted accompanying muscular hypertrophy, and consequently excretion ratio of nitrogen will be decreased soon after the extinction trials, according to the increase in muscular protein which results in body weight increase. In the present experiment, earlier decrement in the excretion ratio of nitrogen and the marked body

weight increment were not observed. Therefore, the increase in the excretion ratio of nitrogen that means increased catabolism of the protein was the metabolic response of the emotional learning as well as the behavioral response.

According to the present experimental result, it is suggested that the extinction of the learned emotional response, which does not accompany the spontaneous recovery, will be completed with the disappearance of metabolic response of nitrogen (gradual and long acting response), which was elicited by the emotional learning coincidentally with the learned emotional response in behavior (rapid and more transient, readily extinguishable response). Indices under the behavioral level alone does not fully indicate the completion of extinction. Indices under the metabolic level indicate the fact of this sort.

SUMMARY

Further experimental analysis of the relation between behavioral response and the response of protein metabolism under the emotional learning was attempted. Fluctuations in the behavior and the excretion per intake ratio of nitrogen were determined during pre-conditioning (15 days), conditioning (10), extinction (10), post-extinction (50), test (10) and post-test (5) period in the rat. Basal diet comprises of extra pure chemicals except dry yeast was supplied. Micro-Kjeldahl method was used for the chemical analysis of total nitrogen.

Avoidance conditioning was consisted of buzzer sounds (AC 4 V) and electric shocks (AC .5 mA, 100 V) with 5 sec intervals. The conditioning was held 20 trials with 1 min intervals during 21 min per day. Extinction and test trials were the same as conditioning except shocks.

Owing to the conditioning, two types of response such as behavioral and metabolic were resulted at the same time parallelly in all animals. Behavioral effects of the conditioning and extinction trials such as

increase and decrease in the number of avoidance response and the responses to CS except avoidance, decrease and increase in the avoidance latency and the inter trials response were elicited immediately, and proceeded and disappeared rapidly. While, metabolic effects were elicited immediately, and proceeded gradually, and after persisted for long term disappeared slowly.

Urinary excretion of nitrogen per milliliter of the sample and the total urinary excretion (mg/day) increased and dietary, intake minus fecal excretion decreased gradually during and after the conditioning and extinction period, and did not restore completely. Urinary excretion per dietary intake minus fecal excretion ratio of nitrogen significantly increased during the conditioning period. It started in increase on the 1st day and increased gradually day after day in all animals. Then it reached at the maximum on the last stage of conditioning period. The ratio continuously increased long after the extinction period, and after that they restored gradually and completely in all animals.

Behavioral effects of the avoidance conditioning disappeared in the later stages of extinction period. While metabolic effects reached at near the maximum increment in those stages.

According to the test trials, which were

held after the metabolic response disappeared completely, behavioral responses were not elicited again, and metabolic response also did not result during and after the test period. That is spontaneous recovery of the learned emotional response did not result in the present study. Consequently, it is suggested that a learned emotional response was completely extinguished only when the metabolic response disappeared following the disappearance of behavioral responses, not when the behavioral responses disappeared.

The learned emotional response appeared differentially between an overt behavior and a covert metabolic process. The learned emotional response continued without a behavior.

It is suggested that the metabolic response, which was elicited by the emotional learning coincidentally with the behavioral responses, indicated the completion of extinction (without spontaneous recovery) of the learned emotional response, whereas behavioral responses did not.

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