Abstract—In SART-stressed (repeated cold-stressed) rats, shuttle-avoidance response was examined. The rats exposed to SART stress prior to training showed a high avoidance rate and no change in the intertrial response. Upon exposure to SART stress after completion of learning, the rats showed no change in the avoidance rate and an increase in the intertrial response in the retention test. These results are in contrast to the previous observations of passive avoidance response, and the abnormal behavior in such animals may be based on excessive emotionality and/or hyperreactivity rather than alterations in the process of learning and memory.

It is well known that stress plays various roles in the manifestation of behavioral abnormalities and that various stress stimuli affect behavior in experimental animals. There are some reports on the influence of stress on escape-avoidance behavior (1–7). Seligman and co-workers have reported that the prior exposure of dogs (1) or rats (3) to inescapable shock and the repeated exposure of rats to inescapable shock (4) result in interference with subsequent escape-avoidance learning. Weiss and Glazer (5) and Weiss et al. (6) have shown that a deficit in the acquisition of active avoidance response occurs in rats exposed to acute but not chronic cold swimming or inescapable footshock stress, and similar findings have also been obtained by Nomura et al. (7) using restraint plus electric shock as a stressor.

We have found that animals loaded with SART (specific alternation of rhythm in temperature) stress (8) caused by changes of environmental temperature exhibit markedly different patterns of behavior in open-field (9) and passive avoidance situations (10), in comparison with non-stressed animals. In the present study, in order to investigate in more detail the characteristics of the avoidance behavior in such stressed animals, accepted as an animal model of dysautonomia (11), active avoidance response in SART-stressed rats was examined.

Male Wistar rats (Shizuoka Laboratory Animal Center), weighing 200–250 g, were used throughout the experiments. They were housed in groups of 5 in wire-netting cages (38 x 25 x 17 cm) under normal conditions including a 12-hr light-dark cycle (lights on 07:00) and temperature of 24 ± 1 °C. Food (MF, Oriental Yeast) and water were freely available except during the experiment.

For the loading of SART stress, rats were kept alternately at 24°C and -3°C for 1-hr periods from 09:00 to 16:00 and then at -3°C from 16:00 to 09:00 the following morning. This treatment was continued for more than 5 days (12).

Active avoidance response (13) was studied by using a two-way shuttle box (Muromachi, RSY-001). The box was made of transparent plastic and consisted of two identical compartments (23 x 19.5 x 20 cm) with an electrifiable grid floor, separated by a metal wall with a gate of the arch type (7.5 cm wide and 11.8 cm high). A buzzer for presentation of the conditioning stimulus (CS) was attached to the center ceiling of the box.

The avoidance conditioning was automatically performed as follows: One trial of
avoidance conditioning consisted of the CS, which was white noise (2.8 kHz, 90 dB) for 5 sec, followed by the unconditioned stimulus (UCS) consisting of a foot shock (300 V, 3 mA) for a maximum of 20 sec delivered to the grid floor of the shuttle box through a shock generator (Muromachi, SGS-001). A constant intertrial interval of 30 sec was used. Rats received one session per day consisting of 40 consecutive trials. Numbers of avoidance responses (crossing the gate from one side of the box to the other during the CS), escape responses (crossing during the UCS after the CS had terminated), escape failures (no crossing until the UCS had terminated) and intertrial responses (spontaneous crossing during the intertrial interval) were recorded. All experiments were conducted in a soundproof chamber (Muromachi, MC-050) between 11:00 and 17:00.

In the experiment conducted to study the influence of SART stress on the acquisition of active avoidance response, rats were preexposed to SART stress for 5 days and then subjected to the training for 4 days from the 6th day of stress. For examining the influence of SART stress on the retention of active avoidance response, naive rats were given daily active avoidance training for 7 days, and only rats reaching a criterion of 70% avoidance rate at the 7th session were used. SART-stress loading was initiated on the day following the final 7th training, and the retention test was performed for 4 consecutive days from the 6th day of stress. The stressed rats were subjected to the training 1 hr after release from the cold room and then reloaded with stress again 1 hr after the end of the daily session.

Statistical significance between data was assessed by the two-tailed Mann-Whitney U-test, $x^2$-test or Fisher’s exact probability test.

Figure 1 shows the results of active avoidance training carried out in rats already exposed to stress for 5 days and non-stressed rats. As seen in panel A of Fig. 1, there was no significant difference in avoidance rate in the 1st and 2nd session between the 2 groups. In the 3rd and 4th session, however, the avoidance rate of SART-stressed rats was significantly higher than that of non-stressed rats. The percent appearance of rats showing 70% or more avoidance rates tended to be larger in the stressed group than in the non-stressed group, though there was no significant difference between the 2 groups ($x^2$-test or Fisher’s exact probability test). The appearance rate of poor learners, rats showing an avoidance rate of less than 30% in the 4th session, was 17.2% in the non-stressed group and 20.0% in the SART-stressed group (6 of 30 rats), and there was no significant difference in the appearance rates between the 2 groups (Fisher’s exact probability test). The range of avoidance rate in each rat with the exception of the above-mentioned poor learners was 40–95% in the non-stressed group and 62.5–100% in the SART-stressed group.

The rates of escape responses in the stressed group, as seen in panel B, decreased as compared with those in the non-stressed group in contrast to the increased rates of avoidance responses, and a significant differ-
ence was observed between the 2 groups in the 3rd session. The rates of escape failures of 2 groups showed no significant difference in the 1st to 4th session.

Panel C shows that the number of intertrial responses was approximately equal between the 2 groups in all sessions.

Then, the influence of SART stress on the retention of active avoidance response was investigated, and the results were shown in Fig. 2. In this case, we used 19 rats that had acquired the avoidance response by the 4th training session and shown reliable avoidance responses in the 5th to 7th training session as seen in Fig. 2. Ten of these chosen rats were loaded with SART stress.

In the retention test done 6 to 9 days after the last training, the avoidance rate and intertrial responses in non-stressed rats were almost identical to those in the 7th training session. In the case of SART-stressed rats, the avoidance rate was almost identical to that in the 7th training session, as in the non-stressed rats, but the number of intertrial responses was markedly increased, compared with that of non-stressed rats.

Weiss et al. (6) and Nomura et al. (7) assumed that in the case of chronic stress, avoidance-escape behavior did not change because of the coping mechanism or habituation. Also, they concluded that the observed changes reflected alterations in brain noradrenergic activity following acute and chronic stress. On the other hand, Rosellini and Seligman (4) have reported that animals pre-exposed to repeated inescapable shock fail to acquire active avoidance response, and they referred to this phenomenon as "learned helplessness". By the way, all of the above researchers did not carry out daily training and used response latency from the onset of CS as an index of the avoidance-escape response.

In our present study, SART-stressed rats showed no change in the avoidance rate in the 1st training session. The mean response latency, however, was observed to be short in comparison to that of non-stressed rats (data not shown). SART-stressed rats exhibited a higher rate of avoidance in the process of acquisition of the active avoidance response in the 2nd to 4th session, compared with non-stressed rats. These findings are contrary to the results obtained for acute and also chronic stresses by other researchers, and SART-stressed rats have been reported to show an increase in brain norepinephrine content (14). Thus, the active avoidance responses of SART-stressed rats observed in this study were enhanced, unlike the results obtained by other researchers (1–7). It appears that SART-stressed animals differ markedly in various aspects from stressed animals produced by monotonously repeated exposure to acute stress.

On the other hand, when well trained rats

Fig. 2. The retention of active avoidance response in non-stressed and SART-stressed rats. Open symbols, non-stressed rats; closed symbols, SART-stressed rats. Data show means±S.E. of 9 non-stressed and 10 SART-stressed rats. *P<0.05, **P<0.01 (U-test).
were loaded with SART stress, it was recognized that intertrial responses notably increased, whereas the avoidance rate did not change. These results may be attributable to the hyperreactivity observed in animals in the stable SART-stress state, which is usually established on and after the 5th day of stress. At the risk of using an anthropomorphic analogy, SART-stressed rats may thus be in such a mental state that they are unable to await the next CS.

It is noteworthy that SART-stressed mice have been observed to show a shortened test-trial latency and a decreased incidence of maximum latency in the passive avoidance response (10). From the viewpoints of learning and memory, it is difficult to interpret the difference in responses in both active and passive avoidance learning.

It has been reported that SART-stressed rats exhibit hypersensitivity to external stimuli in the GSR test (8), low-voltage fast waves in electrocorticograms (15), and hyperactivity accompanied by increased defection in the open-field test (9). These findings suggest the possibility that SART-stressed animals may be in a state of excessive emotionality, so that it is assumed that the abnormal avoidance behavior in SART-stressed animals may be associated with excessive emotionality and/or hyperreactivity rather than an abnormality in the mechanism of learning and memory.

At present, however, we consider that the appearance of some of the behavioral abnormalities including hyperreactivity and hyperemotionality in SART-stressed animals may be associated with changes in catecholaminergic and/or cholinergic neuron activities in the hypothalamus and other areas of the brain (14, 16), although further studies will be needed in order to investigate the behavioral characteristics and neural, hormonal, and other mechanisms of abnormal behavior in this animal model.

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