Behaviors of Mice Given Forced-Swimming

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Abstract: Behaviors of mice in the forced swimming test are motionlessness, climbing and the other stereotypical behaviors. We observed these behaviors in different ages and sex and in repeated forced swimming trials. The findings were 1) quantities of the climbing and the other behaviors were different with the age and sex, 2) repeated per day forced swimming remarkably increased motionlessness and motionlessness is memorized for at least 14 days, and 3) climbing is the typical opposite behavior of motionlessness and was related to adrenergic but not serotonergic neuronal activity. When these behaviors are recognized as adaptation behaviors, we conclude that mice given repeated forced swimming, but not mice given one trial of forced swimming, can be considered as a model of human depression relating to adrenaline neuronal activity.

Key words: adaptation behaviors, depression, mouse given forced swimming, repeated exposure of a stressor

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Introduction

Since the report of Porsolt *et al.* [6], mice given forced swimming have been considered as a model of depression induced by a psychological stress, and the motionlessness named “immobility” is considered as the index of the depression. Certainly, severe depression patients show motionlessness, but one attack with a psychological stressor does not necessarily induce severe depression. For consideration of mice given forced swimming as a depression model, further behavioral investigations are needed. In this paper, we observed the behavior of mice of different ages and sex given forced swimming in repeated trials.

Materials and Methods

Animals

Male and female ddY mice (Japan SLC, Inc., Shizuoka), 4, 8 and 12 weeks old, were used. They were housed in a group of five with the same age and the same sex in a plastic cage (338 × 140 × 225 mm) with free access to food (F₂: Funabashi Farm Co., Tokyo, Japan) and water. The animal room was kept at 21–25°C with 50–60% humidity and illuminated from 7:00 to 19:00. For identification, each mouse in a cage was painted on the tail with different color paints. The experiments were performed from 15:00 to 17:00. All of the experiments were carried out under the control of the Ethics Committee for Animal Experiments of Akita University School of Medicine, Japan.

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Measurements of behaviors

The apparatus used to investigate the behaviors in the forced swimming test was the same to the original apparatus (cylinders; 10 cm diameter, 20 cm height, containing water of 25°C at a depth of 10 cm) [6]. The previous report indicated that depression of a mouse is demonstrated by motionlessness (only floating, sticking the face out of water surface) in the apparatus [6]. Other behaviors were swimming, circling, diving, jumping, sniffing, and attempting to climb the apparatus wall [2]. The climbing can be easily identified and its durations are long enough to measure, but the other behaviors cannot be readily identified in the narrow space of the apparatus and the durations of the other behaviors are short. Though motionlessness has been noticed in many reports, in the present study, the behaviors measured were decided on as climbing and the other behaviors, and the duration times of both of these behaviors were considered as indices of characteristics of mouse behaviors in the test. Following the practice of the original report [6], all of the mice were given pre-treatment of forced swimming for 6 min 24 hr before the experiments.

Relations to age and sex

Ten mice of each age and each sex were individually dropped into the apparatus containing water. The climbing and other behaviors were measured in the first 3 min and the second 3 min.

Detections of the learning

Ten male 8-weeks-old mice were given forced swimming for 3 min 24, 48, 72, 96 and 120 hr after the pre-treatment, and their climbing and other behaviors were measured in the test periods. Another 10 male 8-weeks-old mice were treated in the same manner 24 and 120 hr after the pre-treatment, and their climbing and other behaviors were measured in the test period. Fourteen days after the final treatment, the climbing and other behaviors of both of these mice were measured for 3 min. In order to detect whether the climbing was induced by increase of the locomotor activity or not, voluntary running of the mouse was measured for 5 min [3] just before the measurement of the final trials, and just before the measurement on the 14th day after the final trials.

Neuronal activities relating to the behaviors

Reuptake inhibitors, maprotiline (noradrenaline) and alaproclate hydrochloride (serotonin) were purchased from Funakoshi Co. Tokyo, and were prepared with 0.5% carboxymethylcellulose sodium (CMC; Funakoshi Co.). The injection of these reuptake inhibitors temporally increases the neuronal activities in mouse brain.

Thirty-five male 8-weeks-old mice were given per day-3 min forced swimming for 4 days. Twenty-four hours after the final treatment, 5 mice each were intraperitoneally injected with 30, 3 or 0.3 mg/kg of each reuptake inhibitor, or 100 μ l of 0.5% CMC. Thirty minutes after the injection, the climbing and other behaviors were measured for 3 min.

Statistical analysis

The Kruskal-Wallis rank test was used to find significant differences among clusters. When significant differences were found (p-value of less than 0.01), the Mann-Whitney U test was used.

Results

Table 1 indicates climbing and the other behaviors in different ages and sex. In the 4-week-old mice, the females showed other behaviors more than climbing, and the males showed climbing more than other behaviors. In the 8-weeks-old mice, both females and males showed marked climbing. The females showed small quantities of other behaviors, but the males showed none at all. In the 12-weeks-old mice, both females and males showed only climbing. The climbing durations of the 8-weeks-old mice were more than those of the 4-weeks-old and the 12-weeks-old mice. In all of the mice, climbing and other behaviors were found in the first 3 min, but not in the second 3 min. Significant differences were found in climbing among all ages of females and among all ages of males, and differences in other behaviors were found among all ages of females (Kruskal-Wallis rank test).

Table 2 indicates the effects of repeated per day-trials on the behaviors. The climbing durations of the mice given per day-trial for 5 days decreased with the number of the trials. The climbing was not found in the 4th and 5th trial. The other behaviors of the mice were found at the 2nd, 3rd and 4th trial, but the durations were short. Motionlessness increased following

repeated trials. The climbing durations of the mice not given the repeated trials did not change in the observation periods, and other behaviors were found at the trial 120 hr after the pre-treatment but the durations were short (Table 2, upper). In both of these groups of mice, the climbing durations at 14 days after the final repetition trial were not different from those at the final repetition trial. The other behaviors were also found in the both of these mice but the durations were short (Table 2, lower). The voluntary runnings of these mice at the final repetition trial were not different (the mice

given 5 repetition trials : 37.4 ± 7.9 , the mice not given the repeated trials: 36.2 ± 7.9 , the revolution times in the 10 mice for 5 min), and those on the 14th day after the final repetition trial were also not different (the mice given 5 repetition trials: 38.8 ± 6.5 , the mice not given the repeated trials : 37.8 ± 7.2 , the revolution times in the 10 mice for 5 min).

Table 3 indicates the effects of the reuptake inhibitors on the behaviors. Noradrenaline reuptake inhibitor maprotiline increased the climbing durations in a dose dependent manner (Kruskal-Wallis rank test and Mann-Whitney U test), but serotonin reuptake inhibitor alaproclate did not. Either drug did not induce the other behaviors.

Table 1. Climbing and other behaviors of mice of different ages and sex in the forced swimming test

Duration (seconds)			
Age (week)	Sex	Climbing	Other behaviors
4	female	$2.4 \pm 0.8^*$	$22.0 \pm 0.8^*$
	male	66.0 ± 30.6	2.0 ± 0.6
8	female	74.4 ± 35.5	$12.0 \pm 4.0^*$
	male	79.6 ± 39.2	0
12	female	20.0 ± 6.6	0
	male	19.2 ± 6.8	0

The values in this table are the mean $\pm$ SE of the durations of the 10 mice in the first 3 min (the data in the second 3 min are not shown because these behaviors were not found in any of the mice). * $p > 0.01$. Mann-Whitney U test.

Discussion

The present studies indicate that the duration of climbing and other behaviors differs among ages and sex; repeated per day-forced swimming remarkably increases motionlessness and the motionlessness is memorized for at least 14 days; climbing is the typical opposite behavior to motionlessness, and the climbing is related to the adrenergic but not the serotonergic neuronal activity.

Table 1 indicates mouse behaviors in one trial of

Table 2. Effects of repeated per day-forced swimming trials on the behaviors

1) Effect of repeated trials

Per-day trial	A (mice with repeated trials)		B (control: mice without repeated trials)	
	Climbing	Other behaviors	Cimbing	Other behaviors
1st	78.8 ± 38.1	0	76.8 ± 39.4	0
2nd	40.2 ± 19.4	1.0 ± 1.3		
3rd	1.2 ± 1.4	1.0 ± 1.3		
4th	0	2.1 ± 1.4		
5th	0	0	77.0 ± 45.1	2.5 ± 2.5

2) 14 days after the final trials

A		B	
Climbing	Other behaviors	Climbing	Other behaviors
0	1.6 ± 2.2	78.6 ± 43.5	4.6 ± 4.5

The values in these tables are the mean $\pm$ SE of the durations (seconds) of 10 male 8-weeks-old mice in the first 3 min.

Table 3. Effects of reuptake inhibitors on the behaviors of mice given per day-forced swimming for 4 days

reuptake inhibitor	Duration (seconds)		
	Dose (mg/kg)	Climbing	Other behaviors
(control)			
0.5% CMC	—	0	0
Maprotiline	0.3	5.0 ± 3.4	0
(noradrenaline)	3	52.0 ± 11.0*	0
	30	76.4 ± 7.2*	0
Alaproclate	0.3	0	0
(serotonin)	3	0	0
	30	0	0

The values in this table are the mean ± SE of the durations of the 5 male mice of 8 weeks previously given per day-forced swimming for 4 days. * $p < 0.01$ compared to the control (Mann-Whitney U test).

forced swimming. In this experiment, mice were given a physiological stressor recognized as discomfort. The mice try to escape from the circumstance giving the stressor, or endure the discomfort showing stress-coping behaviors [4]. The climbing is a typical escape behavior and some of the other behaviors (circling and sniffing) are recognized as stereotypical stress-coping behaviors. The motionlessness shown in this experiment might indicate fatigue but not depression. It is interesting that these behaviors differ among mice of different ages and sex. Clinicians have noticed that individuals with different ages and sex also show escape and stress-coping behaviors differently.

Though mice show escape and stress-coping behaviors in uncomfortable circumstances, after repeated exposure, they recognize that these behaviors are useless. In this time, the mice become motionless, not wasting their energy, and the motionless behavior is memorized as the most adequate behavior for the circumstance. This behavior indicates that the mice are in the depressive state [4], as indicated by the results in Table 2. When the escape and stress-coping behaviors are useless in an uncomfortable circumstance, humans despair. This may be the reason that Porsolt *et al.* considered the mouse given forced swimming as a depression model induced by despair [6].

It is known that stresses induced in different situations affect the brain functions differently. Namely, the stresses under the situations in which animals can move relate to the adrenergic neuronal activity [1, 7],

and the stresses under the situations in which animals cannot move relate to the serotonergic neuronal activity [5]. These findings support the present pharmacological results presented in Table 3. Another important point is that the behavior induced by the antidepressant maprotiline is climbing but not the other behaviors. This strongly suggests that the climbing is the typical opposite behavior of motionlessness.

In the present study, the behaviors of mice were investigated in forced swimming tests. Porsolt *et al.* considered mice given one trial of forced swimming as a depression model, but the present findings clearly indicate that the mice given repeated forced swimming can be considered as a model of noradrenaline-related human depression.

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