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Stress Exposure using Small Mobile Robot both in Immature and Mature Period Induces Mental Disorder in Rat

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***Abstract*- The number of patients with mental disorders is increasing in advanced countries, hence more effective psychotropic drugs are recently desired. In process of development of psychotropic drugs, animal experiments have been playing a very important role. Mental disorder model animals which exhibit behavior disorder like patients with mental disorders are used in these experiments. These animals are normally developed by genetic manipulation, surgical operation in their brain or drug administration. A candidate for a new drug is administrated in these animals to evaluate its effect. However, we have some doubts about conventional mental disorder model animals because they are induced these disorders by using methods which are quite different from causes of mental disorder of human beings. Therefore, the purpose of this study is to develop an novel methodology to create mental disorder model animals. We then developed a small mobile robot and a control system for it. Using them, we have performed some experiments to create a mental disorder model rat. We had then succeeded in developing a mental disorder model rat by exposing stress using the robot during immature period. This rat exhibits low activity in some behavior tests during mature period. For better understanding of how stress exposure induces mental disorder in a rat, we conducted another experiment based on stress vulnerability hypothesis. In this experiment, stress was exposed during both immature and mature period while that had been exposed only during immature period. We prepared several conditions of stress exposure by changing robot behavior pattern to find the one to induce much stress in a rat. From a result of experiment, we found that a rat which received gentle chase by the robot during immature period was induced much stress when it received robot attack during mature period. Thus, we consider that this rat is more appropriate to the mental disorder model than that was developed in our past experiment.**

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I. Introduction

RECENTLY, the number of patients with mental disorders is increasing in advanced countries such as Japan, USA or European countries. For instance, advanced counties spend large amount of money for this issue [1]. Therefore, more effective psychotropic drugs are desired. In process of development of psychotropic drugs, animal experiments have been playing a very important role. Mental disorder model animals are living animals that represent phenotypes of patients with mental disorders, such as depression or anxiety disorder. They are produced by genetic manipulation, surgical operation on the brain, psychotropic drugs or stressful environment [2]. In the process of drug development, they are used to screen candidates for psychotropic drugs [3-5].

We recognize that mental disorder model animals have much contributed to developments of psychotropic drugs. However, there are few studies that are focused on the effects of stress from environment while it plays a key role in mental disorders. The stress induced from the environment should be more considered to make more suitable mental disorder model animals. There are some experiments where a rat is induced stress by exposing electric shock or cold window. However, we consider that these kinds of stress are quite different from stress in human society. The reason why there are few studies focused on the social stress is that there are few useful experimental setups to expose and control it.

On the other hand, we had been developed several small

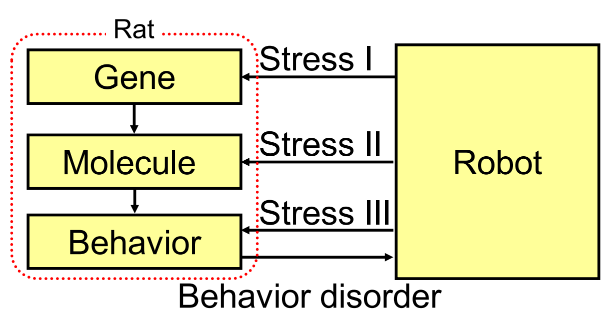


Fig. 1 Basic idea to develop novel mental disorder model.

mobile robots which interact with a rat, and performed several experiments on interactions between a rat and the robot [6-8]. We then considered that the small mobile robot can be used as a stressor for a rat. The basic idea is shown in Fig. 1. It is developed based on the "stress-vulnerability hypothesis" that is well recognized as one of the most suitable ideas to explain how mental disorders occur in humans [9-10]. There are few doubts that stress from the environment plays a very important role when a mental disorder occurs in a patient. In this hypothesis, each person has his/her own stress-vulnerability, the threshold for stress, and he/she is suffered mental disorder when intensity of stress is over the threshold. Some researchers said there are several kinds of stress-vulnerability, hence several cases can be explained using this hypothesis [11-13].

In the idea shown in Fig. 1, a mental disorder model animal can be created by stress induced by the robot during immature period (stress I and II). Some changes might be then occurred in the neural circuit or biochemical system of the animal. The animal becomes to behave like a patient with mental disorders when it would be induced stress again during mature period. Based on this concept, we succeeded in developing a mental disorder model rat which exhibited low activities during mature period by exposing stress using the robot during immature period [14]. For deeper understanding of this mental model rat , we performed another experiment. Referring to the stress-vulnerability hypothesis, vulnerability has been developed in a rat by the stress exposure during its immature period, and mental disorder would be developed when it would receive additional stress on its vulnerability in mature period. Therefore, we design a experiment where a rat receives stress exposure during its immature period to develop vulnerability for the stress and receives the stress again when it becomes mature to develop mental disorder. We performed an experiment based on this idea and describe it in this paper.

II. Robot and Experimental Setup

A. Small Mobile Robot: WM-8

The small mobile robot, WM-8 (Waseda Mouse No. 8) was developed. A photo of the robot is shown in Fig. 2 and its specifications are shown in Table 1. Dimensions and performance are equal to a mature rat. A Li-ion battery is mounted on WM-8 and it keeps operation for 120 [min].

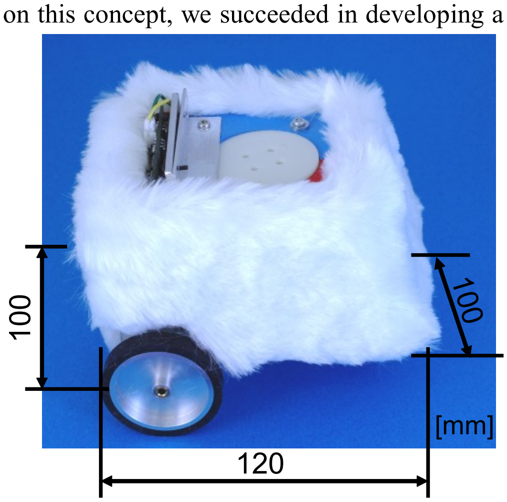


Fig. 2 Small mobile robot WM-8.

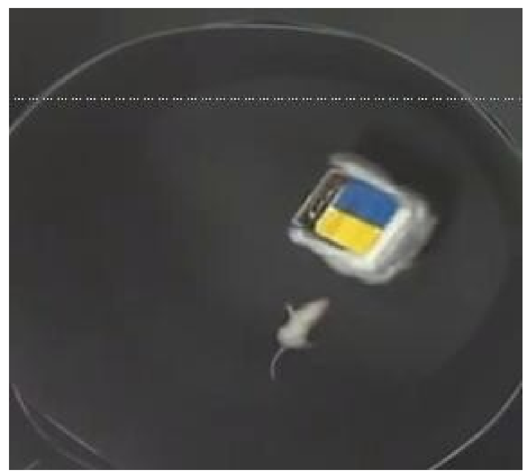


Fig. 4 Open-field, a robot and a rat are there.

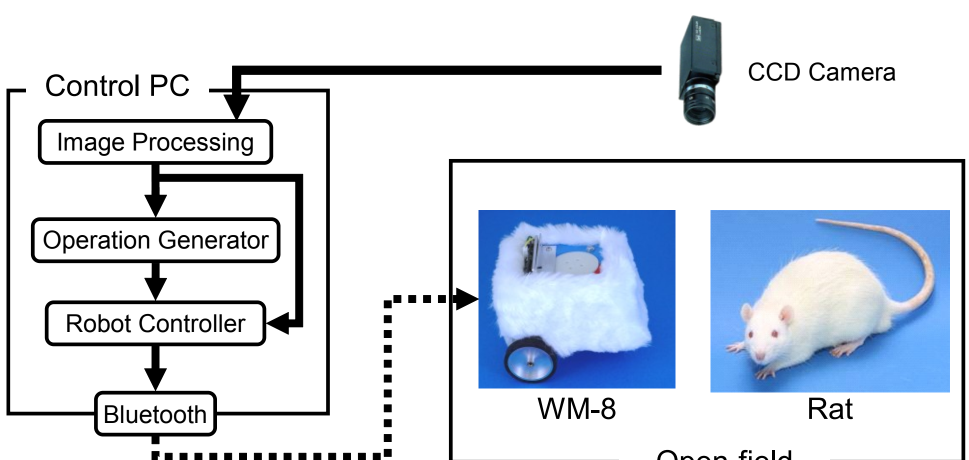


Fig. 3 Overview of experimental setup [7][8].

During the experiment, WM-8 is covered with white fake fur with a color sheet (red and blue) for image processing.

WM-8 has two drive wheels and a passive omni-directional caster. The drive wheels are mounted at right and left side of rear end and a passive omni-directional caster is placed at the front end. Therefore, motion of WM-8 receives non-holonomic constraint.

A microcontroller, a Bluetooth wireless communication module and motor control drivers are implemented in WM-8. The motion of WM-8 is controlled by the PC via Bluetooth wireless communication. The PC sends the instruction of the velocity of each motor. When it receives an instruction from the PC, the microcontroller sends a target value of the velocity of each motor to each driver. Each driver then controls the velocity of each DC motor via virtual velocity feedback control. This driver controls the velocity of the motor with a circuit to calculate velocity of the motor from the back electromotive force.

Table 1 Specification of WM-8.

Dimension	mm	100x100x120
Weight	g	585
Max translational vel.	m/s	1.0
Max rotational vel.	rad/s	10.0

B. Experimental Setup

This setup consists of an open-field, a CCD camera and a control PC as shown in Fig. 3. The CCD camera is placed above the open-field. The experiments with a rat and WM-8 are performed in the open-field. A picture of the open-field taken by the CCD camera is shown in Fig. 4.

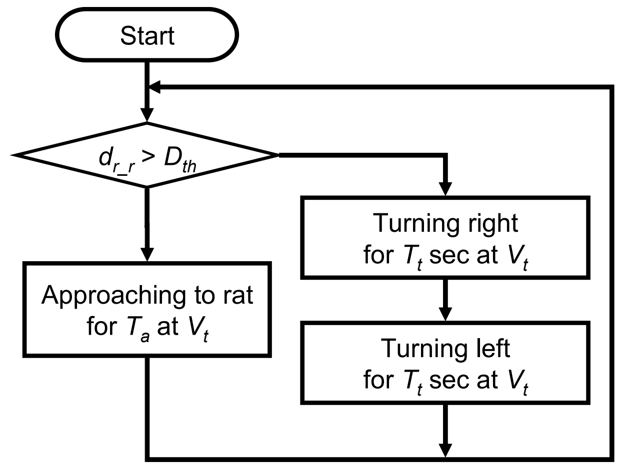
The robot is controlled by a control PC with software that consists of an image processing module, an operation generator module and robot controller module. In this software, the positions of the rat and the robot are calculated every 50 milliseconds. Motion of the robot is generated based on the algorithm in operation generator module. It is implemented using a format as shown in Fig. 5.

III. EXPERIMENT

A. Objective

The objective of this experiment is to confirm the effect of the stress exposure by the robot during mature period on a rat which has received it during immature period. In our past experiment, stress exposure by the robot during immature period had an effect to develop at least stress-vulnerability in a rat. referring to stress-vulnerability hypothesis, additional stress exposure may induce mental disorder in the rat.

In this experiment, stress is exposed by WM-8 during immature period in the same way of our past experiment. Additional stress then is exposed on the rat when it becomes mature under different experimental condition.



d_{r_r}: Distance between the rat and the robot
D_{th}: Threshold of d_{r_r}
V_t: Velocity of the robot
T_a: Time the robot approaches to the rat
T_t: Time the robot rotates

Fig. 5 Algorithm of the robot motion.

B. Method

1) Subject: In this experiment, we choose F344 rats as subjects. These rats arrived at the laboratory when they were two weeks old with their mother. Since then, each litter was bread in a cage with their mother. They were then divided into 4 groups and experienced different situation.

2) Procedure in Immature period: The rats in each group were experienced the experimental situation as shown in Table 2 for 5 days from when they were 3 weeks old. During these 5 days, each rat was put in the open-field and experienced experimental situation. The experiment with each rat is

Table 2 Experimental condition of 4 groups.

		Group A	Group B	Group C	Group D
Number of rats		6	6	6	6
Experimental condition in immature period	Shape of open-field	Circular (d=500mm)			Rectangular (350mm×200mm)
	Robot	Attacking	Gently chasing	No	No
	Other stressor	No	No	No	Electric shock (2000 V)

performed for 10 minutes. After these 10 minutes, the rat put back in the breeding cage.

Group A is an experimental group. A rat in this group was induced severe stress by WM-8. It was put into the open-field with WM-8. In the open-field, WM-8 was controlled to keep the distance between its center and center of the rat under 50 [mm] using the algorithm as shown in Fig. 5. When the distance is under 50 [mm], the robot also kept turning right and left by turns. With this algorithm, the robot keeps hitting the rat.

Group B is also an experimental group. A rat in this group was chased gently by WM-8. It was put into the open-field with WM-8. In the open-field, WM-8 was controlled to keep the distance between it and the rat around 300 [mm]. When the distance is under 300 [mm], the robot kept turning right and left by turns.

Group C is a control group. A rat in this group was put into the open-field and released there alone. It wasn't induced any specific stress in the open-field.

Group D is a kind of control group. A rat in this group was induced stress by electrical shock generator. It was put into the box set in the open-field and provided the electric shock from the floor of the box. Short (20 us) but strong (around 2000 V) electric shock was provided every 2 seconds.

3) Procedure in Mature period: Behavior tests were performed to evaluate their characteristics of the rats in each group, activity in each condition. Therefore, we performed open-field test and robot attack test with all the rats in group A to D.

Open-field test is a test to evaluate activity of a rat. In this test the rat is released into the open-field and left there for 10 minutes. The activity of the rat, total travel distance of the rat, is measured by image processing as described in Chapter II.

Robot attack test is a test to evaluate the response of a rat to the robot attack. In this test the rat is released into the open-field with WM-8 for 10 minutes. WM-8 is controlled using the algorithm as shown in Fig. 5. Each rat experiences this experiment 5 days. The distance traveled by the rat is measured by image processing.

C. Result

Experimental result of the open-field test, activity of the rats in each group, is shown in Fig. 6. Those of 1st day and 5th day of the robot attack test are also shown in Fig. 7 and 8. Changes of activities of rats in group A, C and D are shown in Figure 9. Approximation line obtained by linear approximation is also shown in figure 9.

Significant differences between each group are confirmed by student's t-test using excel 2003 and shown with * marks in each figure.

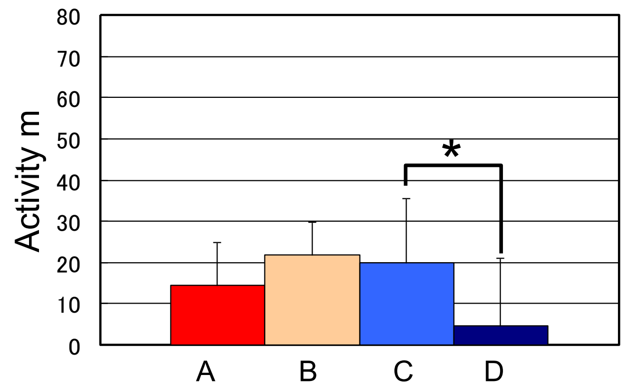


Figure 6 activities in open field test (mean and SD of 6 subjects in each group)

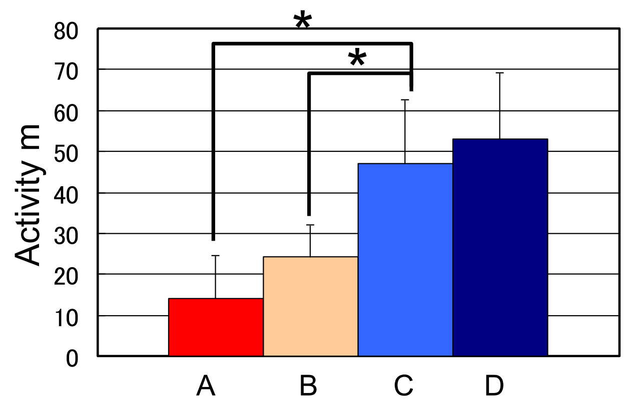


Figure 7 activities in 1st day of robot attack test (mean and SD of 6 subjects in each group)

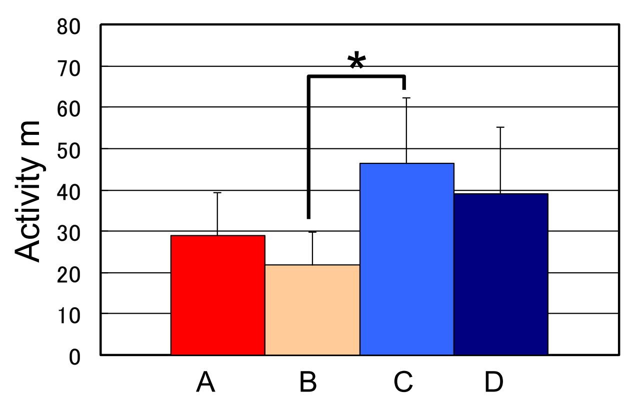


Figure 8 activities in 5th day of robot attack test (mean and SD of 6 subjects in each group)

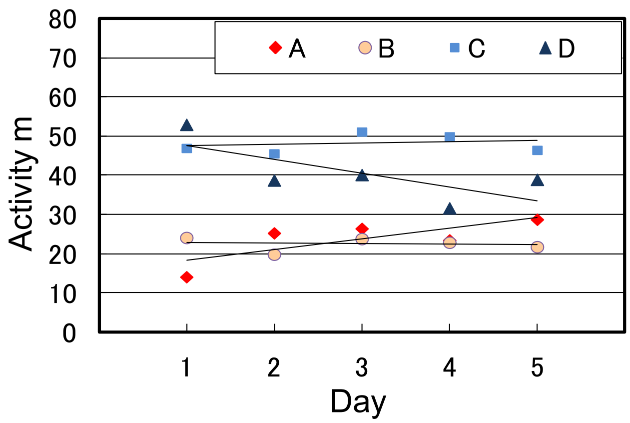


Figure 9 changes of activities in robot attack test (mean of 6 subjects in each group)

IV. Discussion

In this experiment, group C is the control group, hence comparisons between group C and other groups induce some considerations. As you can see in Fig. 6, activities of the rats in group A, B and C are almost same while that in group D is much lower than that in C. "Fear conditioning" is a well known effect in psychology [15]. A rat that was exposed severe fear, normally induced by electric shock, shows "freezing (stopping and shivering)" when it is put under the same or similar environmental stimulus as it experienced the fear once. In conventional experiments, freezing time is measured by human observer to evaluate how much a rat freezes. In our experiment, we measure the activity of a rat which can be computed by image processing to avoid noises caused by human observation.

We can describe the reason why activity of the rats in group D is lower than those in other groups by referring to fear conditioning effect. Activity of the rats in group A is much lower than those of group C while that of group D is almost same as that of group C in the 1st day of the robot attack test. Therefore, we consider that fear is exclusively conditioned with the robot, not other stimulus in the experimental setup, when a rat is attacked by the robot in immature period, while it is conditioned with the environmental stimulus when a rat is exposed electric shock.

In the 5th day of the robot attack test, a significant difference is not confirmed between activity of the rats in group A and that of C. It means effect of fear conditioning is decreased. As you can see in Fig. 9, activity of the rats in group A increased with trials. "Extinction" is a well known effect in psychology also. The basic concept of extinction is that it leads to a decline in that behavior when a conditioned behavior is no longer reinforced. It is applicable to feared conditioning. It lead to a decline in the freezing when a fear is no longer provided. We consider increase of activity of the rat in group A during robot attack test is lead by the extinction. Therefore, robot attack induces less stress on mature rats, and the stress level of a rat is less than the threshold of the stress vulnerability.

On the other hand, activity of the rats in group B is lower than that of group C both in the 1st and 5th day of robot attack test. In Fig. 9, the activities of the rats in groups C keeps low level. Therefore, we consider that the rat experienced gentle chase by the robot during immature period is induced much stress from the attack by the robot comparing to the one experienced the attack by the robot during immature period.

V. Conclusions

We confirmed that a rat which had been exposed stress by the robot during immature period exhibited lower activity when it became mature. Based on the stress-vulnerability hypothesis, an additional stress, attack by the robot was exposed to the rat when it became mature. However, the attack by the robot during mature period did not have similar effect of that during immature period and induced less stress in the rat. Therefore, attack by the robot during mature period is not appropriate as an additional stress. On the other hand, attack by the robot during mature period induce much stress in a rat which experienced gentle chase by the robot during immature period. The rat exhibit the lowest activities in all the behavior test. Thus, a rat which has been exposed gentle chase by the robot during immature period and exposed attack by the robot during mature period is more appropriate to a mental disorder model animal than the one developed in our previous experiment. The next step is to confirm which kind of mental disorder this model represents [16][17]. We are going to administrate some psychotropic drugs in the rat and check the modification of its behavior to conclude the type of mental disorder.

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