Characteristics of Coping Behavior of Rats Exposed to a Long-Term Hardly Escapable Aversive Stimulus: A Possible Depression Model

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Abstract—The purpose of the present study was to induce a state of depression including both the elements of behavioral despair and chronic stress. Therefore, this study was performed under the hypothesis that a long-term exposure of rats to the experimental situation of difficult to escape from foot-shock in a Skinner box might produce animals with a state of depression containing both the elements. Male Wistar strain rats were trained to press a lever to escape from foot-shock under a fixed ratio (FR) schedule. After the training, rats were exposed daily to a schedule consisting of 20 trials (the early 10 trials, FR 5; the later 10 trials, FR 20) once a day. The exposure resulted in reduction of the number of lever presses and successful escape in FR 20. Only the animals whose number of escapes, reduced to under 20% in FR 20 were treated with psychotropic drugs once a day for 4 days. The results showed that the reduced number of escapes was most improved by anti-depressants (imipramine or mianserin), but not by haloperidol and methamphetamine. Although subchronic treatment with chlordiazepoxide partially recovered the reduced escape, the efficiency of lever pressing to escape from foot-shock was lower than that with the antidepressants. The results of the present study suggest that the behavioral suppression observed in this study might include characteristics similar to a state of depression.

Although various animal models of depression have been introduced and used as a screening model for antidepressants (1—10), controversies still exist about their use as models of human depression. Willner (11) recently reviewed the validity of 18 reported animal models as models for human depression and demonstrated the behavioral despair model and chronic stress model to be useful as human depression models. However, the depression models elicited by both the elements of behavioral despair and chronic stress have not been established. Therefore, the purpose of the present study was to induce the state of depression including both the elements of behavioral despair and chronic stress.

Seligman (12) introduced the hypothesis that the unpredictability of an aversive stimulus leads to anxiety and uncontrollability leads to depression, and proposed the theory of "learned helplessness", theoretically supporting the behavioral despair model. Therefore, we hypothesized that a long-term exposure of rats to the experimental situation of difficult to escape from foot-shock might bring rats to a state of depression related to both the elements of behavioral despair and chronic stress.

The present study consists of the following three experiments: 1) The first experiment was for establishing conditions in which rats could hardly escape from foot-shock in a Skinner box. 2) The second experiment examined whether a long term exposure of rats to such an experimental condition could...
lead them to behavioral depression. 3) The third experiment studied pharmacological characteristics of the behavioral depression using psychotropic drugs.

**Materials and Methods**

**Animals**

Male Wistar rats weighing 200–220 g at the beginning of lever press learning were used. They were housed in groups of four rats per cage in a temperature controlled room (24±1°C) with a 12 hr light-dark cycle (lights on 07:00–19:00). They were allowed free access to food and water.

**Apparatus**

The present study used the behavioral research equipment of the Ralph Gerbrands Company. The Skinner box was equipped with one lever and two cue lamps.

**Procedure**

**Experiment 1:** Following 5 min adaptation to the Skinner box, rats were trained to press a lever under a fixed ratio (FR) schedule: rats were presented a warning signal with a light and tone for 5 sec. After 5 sec of warning signal, a 2.0 mA foot-shock was supplied for 30 sec from the grid-floor by the shock generator-scrambler (Tech. Serv. Inc., U.S.A.). If a rat made an appropriate lever-pressing anytime during the warning signal and foot-shock periods, but during not the intertrial period, the sequence of warning signal and foot-shock could be interrupted for a period of 25 sec. Thus, the rats could predict foot-shock in this schedule. However, in case of higher FR, this schedule might lead rats to a situation where foot-shock was predictable but hardly controllable. Under this conditioning schedule, rats were trained to press a lever to escape from foot-shock. In order to confirm the maximum level of FR that rats could achieve to escape from the shock, FR number was gradually increased from FR 5 on the basis of 80% criteria in 10 consecutive trials of each FR schedule. Then, incidences of rats which achieved 80% criteria of escape in FR 5, FR 10, FR 20, FR 30 and FR 40 were recorded.

**Experiment 2:** In this experiment, influence of repeated exposure to FR 20 on the number of escapes was examined. Rats with reduction of the number of escapes to under 20% in FR 20 within 14 days after beginning of the conditioning were used as subjects. On the next day after rats showed this reduction, the drug experiment was started. Drugs were administered once a day for 4 days. On the first day, rats were reconfirmed to be able to accomplish escape successfully in 10 trials of FR 5, but not in those of FR 20, before the drug treatment. Then, the result in 10 trials of FR 20 was assigned as the pre-level.

Following the pre-test, drugs were administered s.c. in an injection volume of 1.0 ml/kg. At 1, 2 and 4 hr after the drug injection, rats were exposed to 10 trials of FR 20. Then, the number of lever presses and successful escapes at each time were recorded. On the 3rd and 5th day each, rats were challenged to the 20 trials of FR schedule consisting of 10 trials of FR 5 and those of FR 20 before the drug treatment in order to examine the influences of the subchronic treatments of psychotropic drugs for 2 and 4 days. Drugs used in the experiment were as follows: imipramine HCl (IMP) (10 mg/kg, n=5 and 20 mg/kg, n=5; Nippon Ciba-Geigy), mianserin HCl (MIS) (15 mg/kg, n=5 and 30 mg/kg, n=5; Organon), chlordiazepoxide HCl (CDP) (5 mg/kg, n=5 and 10 mg/kg, n=5; Roche), methamphetamine HCl (MAP) (2 mg/kg, n=5 and 4 mg/kg, n=4; Dainippon) and haloperidol (HPD) (0.1 mg/kg, n=5 and 0.25 mg/kg, n=5; Dainippon). All of these drugs were dissolved in or diluted with 0.9% NaCl solution. The 0.9% NaCl solution was used as the control.

**Statistical analyses**

Statistical evaluation was performed by the analysis of variance (ANOVA) followed by Scheffé’s test, comparing drug treatment with the saline control. The data were represented as difference from the pre-test value.
Results

Experiment 1: Figure 1 shows the incidence of rats that accomplished each FR schedule. Rats used in the present study were perfectly accomplished in FR 5 and FR 10. However, the incidence in FR 20 decreased to 78%. Only 22% of the rats used in this study completed FR 30 and no rat completed FR 40. Therefore, we examined FR 20 in the following experiments.

Experiment 2: Daily changes in the number of lever presses and successful escapes in 10 trials of FR 20 are shown in Fig. 2. In Fig. 2, the data shown are from 5 days prior to the day when the numbers of successful escapes in each rat were reduced to under 20%. The number of lever presses and escapes in FR 20, but not in FR 5, reduced gradually. The number of escapes in FR 20 reduced to under 20% within 14 days after beginning the experiment. Thus, a long-term exposure of rats to the 20 trials with FR 5 and FR 20 seems to elicit behavioral suppression on escape from foot-shock. In addition, the long-term exposure to this schedule seems to be a hardly escapable situation for rats.

Experiment 3: Figure 3 shows the effects of psychotropic drugs on lever press in FR 20 at 1, 2 and 4 hr after the drug administration on the first day. Each value represents difference from the pre-test value. The saline control showed a gradual decrease in lever presses as compared to the pre-test value. A tricyclic antidepressant IMP (10 and 20 mg/kg) increased the number of lever presses compared to the saline control [F (2, 12)=41.48, P<0.01]. In Scheffé's test, 10 mg/kg of IMP significantly increased the lever presses at 1, 2 and 4 hr after the treatment (P<0.01), while 20 mg/kg increased it at 1 hr (P<0.05). The atypical antidepressant MIS (15 and 30 mg/kg) also significantly increased the lever presses [F (2, 12)=23.57, P<0.01]. MIS at the dose of 30 mg/kg at 2 and 4 hr significantly increased the lever presses (P<0.01). An anxiolytic CDP (5 and 10 mg/kg) increased the lever presses significantly [F (2, 12)=4.00, P<0.05]. CDP at the dose of 5 mg/kg significantly increased the lever presses at 1, 2 and 4 hr after the treatment (P<0.01). The psychomotor stimulant MAP (2 and 4 mg/kg) significantly decreased the lever presses [F (2, 11)=6.64, P<0.05]. Although 2 mg/kg MAP had a tendency to increase the lever presses, 4 mg/kg MAP at 1 hr significantly decreased the lever presses (P<0.01). The neuroleptic HPD (0.1 and 0.25 mg/kg) also decreased the lever presses.
lever presses \([F (2, 12)=5.47, P<0.05]\). HPD at the dose of 0.1 mg/kg at 1 hr \((P<0.01)\) and 2 hr \((P<0.05)\) and 0.25 mg/kg at 1 hr \((P<0.01)\) significantly decreased the lever presses.

Figure 4 shows the number of successful escapes from foot-shock in FR 20 at 1, 2 and 4 hr after each drug treatment on the first day. The saline control showed no significant difference as compared to the pre-test value.

IMP increased the number of escapes compared to the saline control \([F (2, 12)=19.16, P<0.01]\). IMP at the dose of 10 mg/kg significantly increased the escape at 1 and 2 hr \((P<0.01)\) and 4 hr \((P<0.05)\), while 20 mg/kg increased it significantly at 1 hr \((P<0.05)\). MIS also significantly increased the escape \([F (2, 12)=16.01, P<0.01]\). MIS at the dose of 15 mg/kg at 1 hr \((P<0.05)\) and 30 mg/kg at 2 and 4 hr \((P<0.01)\) produced significant increases. CDP caused no significant differences compared to the saline control. However, 10 mg/kg of CDP at 4 hr after the treatment was significant \((P<0.05)\). Although MAP showed no significant difference compared to the saline control, there was a tendency for 2 mg/kg of MAP to increase and 4 mg/kg to decrease the escape. HPD elicited no significant difference compared to
the saline control. HPD at the dose of 0.1 mg/kg at 2 hr after the treatment significantly decreased the escape (P<0.05).

Figure 5 shows the effects of subchronic treatments of psychotrophic drugs for 2 and 4 days on lever press. Each value represents the difference from the pre-test value of each animal on the first day. The saline control showed a gradual decrease in lever presses as compared to the pre-test value.

IMP increased the number of lever presses compared to the saline control [F (2, 12)=11.12, P<0.01]. IMP at 10 mg/kg in the 2-day drug treatment (P<0.01) and 4-day drug treatment (P<0.05) and 20 mg/kg in the 2-day treatment (P<0.05) and the 4-day treatment (P<0.01) significantly increased the lever presses. MIS also increased the lever presses [F (2, 12)=14.61, P<0.01]. MIS at 15 mg/kg in the 2-day treatment (P<0.01) and the 4-day treatment (P<0.05) and 30 mg/kg in the 2-day and 4-day treatments (P<0.01) were significant. CDP increased the lever press significantly [F (2, 12)=7.00, P<0.01]. CDP at the doses of 5 mg/kg and 10 mg/kg in the 2-day treatment increased the lever press significantly (P<0.05). MAP also increased the lever presses significantly [F (2, 11)=4.13, P<0.05]. HPD produced no significant change com-
pared to the saline control.

Figure 6 shows the subchronic effects of psychotropic drugs on escape. In the saline control, the numbers of escapes in the 2-day and 4-day treatments showed decreased responses compared to the pre-test value.

IMP increased the number of escapes compared to the saline control \(F (2, 12) = 5.98, P<0.05\). IMP at 10 mg/kg in the 2-day treatment and 20 mg/kg in the 2-day and 4-day treatments \((P<0.05)\) showed significant increases. MIS also increased the escape \(F (2, 12) = 5.12, P<0.05\). MIS at 15 mg/kg in the 2-day treatment and 30 mg/kg in the 2-day and 4-day treatments produced significant increases \((P<0.05)\). Subchronic treatment of CDP also increased the escape significantly \(F (2, 12) = 4.51, P<0.05\). CDP at 10 mg/kg significantly increased it only in the 2-day treatment \((P<0.05)\). MAP and HPD elicited no significant difference compared to the saline control.
Fig. 6. Subchronic effects of psychotropic drugs administered for 2 and 4 days on the escapes of rats exposed to the hardly escapable situation. Each value represents the difference from the pre-test value (Mean±S.E.). *P<0.05: statistical difference from saline (Scheffé’s test).

Figure 7 represents the efficiency of lever presses for escape in rats with each drug treatment using the total number of lever presses and escapes obtained from whole data used for statistical analyses. That is, each column shows the number of lever presses needed to achieve one escape. The numbers of lever presses needed for one escape were the smallest for the antidepressants IMP and MIS as compared to those for the other drugs.

Discussion
The present study attempted to produce a new animal model of depression by means of exposing a rat to a long-term hardly escapable situation from foot-shock in a Skinner box. The long-term exposure of rats, which learned to escape from foot-shock in FR 20, to 20 trials of lever pressing consisting of FR 5 and FR 20 elicited suppression of escape behavior in FR 20, but not in FR 5. This would indicate that the decrease
of escape in FR 20 was not due to the deficit of escape learning but rather due to the exposure to a hardly escapable situation.

In the experiment of subchronic drug treatment for 2 and 4 days, saline treated rats maintained a high incidence of escape in FR 5, but had a low incidence in FR 20. This also seems to indicate that rats abandoned attempting to escape from foot-shock in FR 20, although they knew how to escape from foot-shock. Therefore, this situation is considered to expose a rat to a chronic despair condition. Willner (11) suggested that the behavioral despair and chronic stress models possessed high validity as a depression model, and stress based models were valid as models of a state of depression. Therefore, the model used in this study may be adequate as an animal model of depression.

Pharmacological analysis of this model showed that antidepressants, IMP and MIS, clearly antagonized the suppression of lever presses and escapes. Both the numbers of lever presses and escapes were increased by acute and subchronic administrations of IMP and MIS.

In contrast to the antidepressants, the neuroleptic HPD significantly potentiated the suppression in the acute stage and showed no antagonization in the subchronic stage. The psychomotor stimulant MAP at the dose of 2 mg/kg tended to increase the number of lever presses, but did not necessarily result in appropriate escape. Although psychomotor stimulants have been used as antidepressants in humans (13), they are also known to elicit behavioral excitation in animals (14). So, the increase of lever presses by MAP is considered to be due to behavioral excitation. MAP at 4 mg/kg suppressed lever presses. The suppression may be based on the stereotypy-inducing effect of MAP. Since MAP did not result in an increase of escape, the effect of psychomotor stimulants on this model is different from that of antidepressants.

On the other hand, the anxiolytic CDP partially antagonized the suppression of lever presses in acute and subchronic treatments. CDP at 5 mg/kg increased the number of lever presses, but did not increase escape. The effect in the acute administration may be related to the behavioral excitation found at
the lower dose of CDP (15). The subchronic treatment for 2 days of CDP at 10 mg/kg increased escape significantly. Since the increase by CDP was found in the recovery process by its subchronic treatment, there is a possibility that anxiolytics may be effective on this behavioral suppression. In other words, this model may be related to neurotic depression, because the method used in the present study is considered to utilize fear-motivated response. In addition, another possibility is that the depression stage obtained in this study might not be sufficient, since the drug treatment was performed just after the reduction of escape to under 20%. So, rats might require exposure to the hardly escapable situation for several days after the reduction of escape to under 20%. However, as shown in Fig. 7, the efficiency of the lever press of rats with CDP to escape from foot-shock was worse than those obtained with IMP and MIS. Therefore, the improving effect of antidepressants on the suppression of escape seems to be specific compared with the other psychotropic drugs.

On the other hand, it is known that acute exposure to unescapable foot-shock, but not chronic exposure, increases the pain threshold (16). Therefore, the decreased escape observed in this study may be related to an increased pain threshold. However, the procedure of this study means chronic exposure to foot-shock. In addition, no hyperalgesia of antidepressants, which can antagonize the suppression of escape, is known. Rather, antidepressants are reported to have an analgesic effect (17) and are clinically applied to therapy for chronic pain and neuralgias (18). Therefore, it is difficult to explain the decrease in escape response by a change of pain threshold.

Thus, the evaluation of this model by psychotrophic drugs suggests that this model may be adequate for assessing antidepressant action. However, since depression in humans is known to involve generalized behavioral suppression and several biorhythm disturbances have been observed (19), it would be necessary to investigate further whether the behavioral suppression observed in this study is generalized and whether behavioral and hormonal rhythms are changed in order to assess this behavior as a human depression model.

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References
1 McKinney, W.T.: Biobehavioral models of depression in monkeys. In Animal Models in Psychiatry and Neurology, Edited by Hanin, I. and Usdin, E., p. 117–126, Pergamon Press, Oxford (1977)
2 Suomi, S.S., Seaman, S.F., Lewis, J.K., Delizio, R.B. and McKinney, W.T.: Effects of imipramine treatment on separation-induced social disorders in rhesus monkeys. Arch. Gen. Psychiatry 34, 321–325 (1978)
3 Seligman, M.E.P.: Chronic fear produced by unpredictable electric shock. J. Comp. Physiol. Psychol. 66, 402–411 (1968)
4 Porsolt, R.D., LePichon, M. and Jalfre, M.: Depression: A new animal model sensitive to antidepressant treatment. Nature 266, 730–732 (1977)
5 Nomura, S.: A forced swimming test in mice: As a screening method for antidepressants and an animal model of depression. Clin. Psychiatry 25, 243–248 (1983) (in Japanese)
6 Hatotani, N., Nomura, J., Kitayama, I. and Oishigawa, M.: Studies on pathogenesis of depression by means of the animal model. Clin. Psychiatry 25, 249–258 (1983) (in Japanese)
7 Costa, E., Garattini, S. and Valzelli, L.: Interactions between reserpine, chlorpromazine and imipramine. Experientia 16, 461–463 (1960)
8 Everitt, B.J. and Keverne, E.B.: Models of depression based on behavioral observations of experimental animals. In Psychopharmacology of Affective Disorders, Edited by Paykel, E.S. and Coppen, A., p. 41–59, Oxford University Press, Oxford (1979)
9 Stein, L.: New methods for evaluating stimulants and antidepressants. In The First Hahnemenn Symposium on Psychosomatic Medicine, Edited by Nodine, J.H. and Moyer, S.H., p. 297–301, Lea and Fibiger, Philadelphia (1962)
10 Horowitz, Z.P., Piali, J.J., High, J.P., Burke, S.C. and Leaf, R.C.: Effects of drugs on the mouse killing (muricide) test and its relationship to amygdaloid function. Int. J. Neuropharmacol. 5, 405–411 (1966)
11 Willner, P.: The validity of animal models of depression. Psychopharmacology (Berlin) 83, 1–16 (1984)
12 Seligman, M.E.P.: Helplessness. On Depression, Development and Death. p. 75–105, W.H. Freeman and Company, San Francisco (1975)

13 Jacobson, E.: The early history of psychotherapeutic drugs. Psychopharmacology (Berlin) 89, 138–144 (1986)

14 Moore, K.E.: Amphetamines. Biochemical and behavioral actions in animals. In Handbook of Psychopharmacology, Edited by Iversen, L.L., Iversen, S.D. and Snyder S.H., Vol. 2, p. 41–98, Plenum Press, New York and London (1978)

15 Haefely, W., Schaffner, R., Polc, P. and Pieri, L.: General pharmacology and neuropharmacology of benzodiazepine derivatives. In Handbook of Experimental Pharmacology, Edited by Hoffmeister, F. and Still, G., Vol. 55/II, p. 13–262, Springer-Verlag, Berlin, Heidelberg and New York (1981)

16 Madden, J., Akil, H., Patrick, R.L. and Barchas, J.D.: Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat. Nature 265, 358–360 (1977)

17 Biegon, A. and Samuel, D.: Interaction of tricyclic antidepressants with opiate receptors. Biochem. Pharmacol. 29, 460–462 (1980)

18 Baldessarini, R.J.: Drugs and treatment of psychiatric disorders. In The Pharmacological Basis of Therapeutics. Edited by Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F., p. 387–445, MacMillan Publishing Company, New York (1985)

19 Hatotani, N.: Animal model of mental diseases. Metabolism and Disease 17, 977–987 (1980) (in Japanese)