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Evaluation of Depression in Rats Exposed to Chronic (Unpredictable) Electric Shock

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NARUO, T., C. HARA, S.-I. NOZOE, H. TANAKA AND N. OGAWA. *Evaluation of depression in rats exposed to chronic (unpredictable) electric shock.* PHARMACOL BIOCHEM BEHAV 46(3) 667–671, 1993.—The present study was undertaken to evaluate the applicability of a proposed behavioral stress paradigm as an animal model for depression. Rats were trained to press a lever under a fixed ratio (FR) 5 schedule in a Skinner box for 10 days and were subsequently exposed to a daily regimen of 20 cycles of FR 5 and 10 cycles of variable ratio (VR) 10 for about a week. This exposure resulted in a reduction of the number of lever presses and successful escapes compared to the level achieved after training. In addition, weight gain was significantly suppressed compared with other treatments. Acute and chronic administration of psychotropic drugs (imipramine and chlordiazepoxide) showed that treatment with imipramine increased both the number of lever presses and successful escapes while chlordiazepoxide increased only the number of lever presses. The results suggest that this simplified animal model utilizing chronic unpredictable electric shock may be useful in the study of human depression.

Skinner box Fixed ratio (FR) Variable ratio (VR) Unpredictable electric shock Depression

ALTHOUGH the relevance of animal models in the study of human depression is controversial, behavioral stress models remain important for indicating the relationship between stress and depression (6,7). It has been reported that models of chronic intermittent stress (CIS), learned helplessness, and behavioral despair were promising for the study of depression (15).

The CIS model involved exposing rats to a variety of stresses for a period of 3 weeks: the animals were subjected to a variety of different stressors, including, among others, electric shocks, immersion in cold water, and reversal of the light/dark cycle. The resulting decrease in open field activity was considered to be analogous to endogenous depression in humans (8,9). The learned helplessness model was originally described in dogs that, when unable to avoid the repeated aversive stimuli to which they were exposed, gave up trying to escape (13). It was suggested that this acceptance of an uncontrollable situation was analogous to the apathetic despair seen in human depression. In the behavioral despair model, rats that were forced to swim until exhausted displayed apparent surrender by floating. Similar to learned helplessness, this model also involved exposure to stress factors from which there was no escape. It was claimed to be sensitive to a variety of antidepressants (12).

The main criticisms of these studies are that a) the antidepressants were given acutely either before or during the procedure that produced the stress, and b) the criteria showed a lack of consistency, objectivity, or reproducibility (7).

Recently, a depression model that involved long-term exposure of rats trained under difficult escape conditions using the Sidman escape schedule was proposed (14). The authors claimed that this model included elements of the behavioral despair and chronic stress models. However, high levels of chronic stress cannot substitute for an inescapable situation as required by the behavioral despair model. Second, the selection criteria (80% escape at FR 20) was so stringent that only a few rats (less than 40%) could qualify for subsequent studies (Takaoka, 1989 personal communication).

The current experiment was therefore designed to improve the previous model through a) the utilization of a clear procedure to induce depression, and b) a reduction of the FR number requirement to prevent undue stress on the test rats. In our first experiment, we evaluated the effects of chronic and unpredictable electric shock on conditioned behavior and weight gain. In the second experiment, rats with suppressed behavior and weight gain induced by the previous procedure were then administered psychotropic drugs and their behavior and weights were monitored.

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EXPERIMENT 1

METHOD

Subjects

Male Wistar rats at 63 days of age, weighing 240–260 g, were housed in groups of three in plastic home cages (22 × 38 × 15 cm) and maintained under controlled temperature (24 ± 1°C) with a 12L : 12D cycle (lights on 0700–1900). They were allowed free access to food and water. All treatments and measurements took place in the light phase of the light/dark cycle. After 7 days of rest, they were moved to stainless steel cages (15 × 20 × 18 cm) to receive the experimental treatments.

Apparatus

The behavioral research equipment of Muromachi Kikai Company was used. The Skinner box (20 × 25 × 25 cm) was equipped with one lever, one buzzer, and two cue lamps. Electric shock was delivered through stainless steel grids on the floor by the built-in shock generator-scrambler. In addition, the lever was also made to supply gradually increased levels (0.8 to 1.6 mA) of electric shock to prevent prolonged pressing.

Procedure

The effect of treatment A, chronic unpredictable electric shock on conditioned behavior and weight gain, was examined and compared with three other treatments.

Treatment A. After a 5-min period of adaptation to the Skinner box, a conditioned stimulus (CS) consisting of a warning signal, light, and buzz was provided at first and then, 5 s later, a 2.0-mA foot shock was supplied as the unconditioned stimulus (UCS) for 30 s, followed by a 15-s rest. If the rats pressed the lever for the required number of times according to the FR level, at any time during the CS or UCS, the signal and/or shock were immediately terminated and the rats were allowed additional resting time corresponding to the remaining UCS period. Pressing the lever for the required number of times during the CS or the UCS was considered successful escape. In this study, FR levels ranged from 1 to 5; FR 1 required the rats to press the lever once to terminate the UCS while FR 5 required five lever presses to terminate the shock treatment.

Twenty-one rats were trained with 30 consecutive cycles of FR 1 during the first day, and the FR level was increased once a rat was able to exhibit 80% escape at a given FR level. Training was terminated once the rats exhibited 80% successful escape at FR 5.

To induce helplessness or a desperate situation in the animals, each of the 15 trained rats was exposed daily to 20 consecutive CS + UCS cycles of FR 5 followed by 10 cycles of VR 10 (a combination of 10 random FR levels from 1 to 20 with mean FR level = 10). This VR procedure was used to introduce the element of unpredictability. The number of lever presses and successful escapes in 20 cycles of FR 5 were recorded.

Treatment B. Five rats without previous training were exposed daily to 30 consecutive CS + UCS cycles of FR 5.

Treatment C. Every day, after being weighed, five rats were placed in the Skinner box and exposed to 30 cycles consisting of a 5-s CS and a 45-s rest.

Treatment D. As a control, seven rats were weighed daily and returned to their housing cages after the weighing.

Statistics

Data were evaluated using analysis of variance (ANOVA) followed by Scheffe's multiple comparison test. Data represent differences from pretest values.

RESULTS

Behavioral Changes

Figure 1 shows the daily changes in the number of lever presses and successful escapes in 20 cycles of FR 5 in treatment A (n = 15). Both the number of lever presses and the number of successful escapes were reduced gradually, falling below 20% within 6 days (mean, 5.7 days) after exposure to 10 cycles of VR 10. Only 15 rats showed 80% successful escape behavior. In treatment B, initially some rats were able to escape but they soon gave up all attempts even to press the lever. Thus, basically, the untrained rats just froze and received the shock treatment without attempting to press the lever.

Changes in Body Weight

Figure 2 illustrates mean weight gain in the four groups in Experiment 1. During the first 10 days, the rats in treatment A were exposed to 30 cycles of CS + UCS. During the next 6 days, they were exposed to 30 cycles (20 cycles of FR 5 and 10 cycles of VR 10). During these 16 days, rats in treatment B, C, and D were treated as described above under. A mixed-design ANOVA (group × day) revealed a significant group effect, $F(3, 28) = 20.73$, $p < 0.01$, a day effect, $F(1, 28) = 98.64$, $p < 0.01$, and a group × day interaction, $F(3, 28) = 8.49$, $p < 0.01$. Scheffe's test yielded a highly significant difference from treatment D for rats in both treatments A and B on days 10 and 16 ($p < 0.01$). This test also detected highly significant differences between treatments A and C on days 10 and 16 ($p < 0.01$); however, the difference between treatments B and C was significant only on day 16 ($p < 0.05$). There was no significant difference between treatments A and B. Treatment C also showed lower weight gain, and Scheffe's test revealed

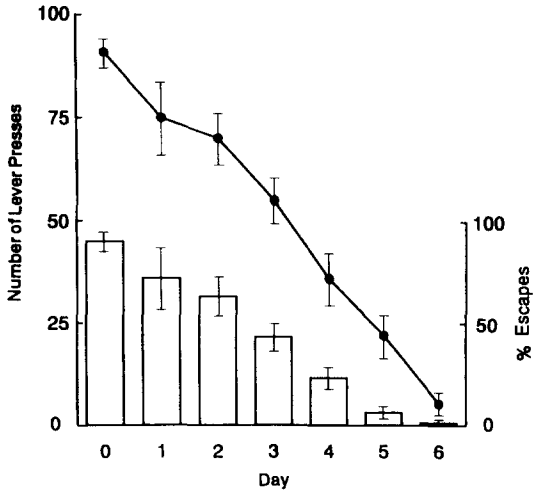

FIG. 1. Daily changes in the number of lever presses and incidence of successful escapes in 20 cycles of FR 5 in treatment A (n = 15). Each column represents the percent of escape response (% escapes) in 20 trials of FR 5 (mean ± SE). The solid line represents the number of lever presses in 20 trials of FR 5 (mean ± SE).

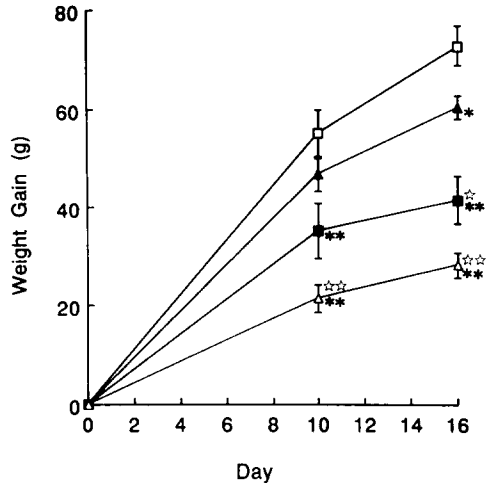

FIG. 2. Solid lines represent the weight gain of the rats receiving four different treatments in Experiment 1: treatment A (△, n = 15), treatment B (■, n = 5), treatment C (▲, n = 5), and treatment D (□, n = 7). ☆Significantly different from treatment C ($p < 0.05$). ☆☆Significantly different from treatment C ($p < 0.01$). *Significantly different from treatment D ($p < 0.05$). **Significantly different from treatment D ($p < 0.01$) in Scheffe's test.

a significant difference from treatment D on day 16 ($p < 0.05$).

EXPERIMENT 2

METHOD

In this experiment, the effects of psychotropic drugs on the 15 rats that showed less than 20% escape in 20 cycles of FR 5 from treatment A were examined. During the experiment, the FR level was reduced to 3 to prevent undue stress.

Acute Administration

The rats were assigned to three groups and drugs were administered. Two hours after injection, the rats were exposed to 20 cycles of FR 3 and the number of lever presses and successful escapes were recorded.

Chronic Administration

Following the determination of the acute effects (day 1), the animals were tested for 10 days. At 48-h intervals, each rat was exposed to 20 cycles of FR 3, and the number of lever presses and successful escapes were recorded. Weight gain was recorded every 2 days.

Drugs

Drugs used in this experiment were imipramine HCl (10 mg/kg, n = 5; Nippon Ciba-Geigy) and chlordiazepoxide HCl (5 mg/kg, n = 5; Roche). Both drugs were dissolved in 0.9% NaCl solution. Controls were given 0.9% NaCl (n = 5). All doses were administered IP in an injection volume of 1.0 ml/kg.

Statistics

Data were evaluated using the same methods as in Experiment 1.

RESULTS

Acute Drug Administration

The upper panel of Fig. 3 illustrates the effects of psychotropic drugs on lever presses. The lower panel illustrates successful escapes in FR 3 at 2 h after the drug administration. Each value represents differences from the pretest value in FR 3. Acute administration of imipramine and chlordiazepoxide did not increase lever presses nor successful escapes. A between-groups ANOVA showed no significant differences among groups for lever presses, $F(2, 12) = 1.98$, NS, or for successful escapes, $F(2, 12) = 0.29$, NS.

Chronic Administration

The upper panel of Fig. 4 illustrates the effects of 10 days of daily drug administration on lever presses. Rats treated with imipramine and chlordiazepoxide showed a gradual increase in the number of lever presses compared to the pretest values, while the rats injected with saline solution showed a decreased number of lever presses. A mixed-design ANOVA (group × day) revealed a significant group effect, $F(2, 12) = 8.52$, $p < 0.01$, and day effect, $F(5, 60) = 3.51$, $p < 0.01$, but not a group × day interaction, $F(10, 60) = 1.69$, $p > 0.05$. Scheffe's test showed a highly significant difference between imipramine and saline treatment on days 7 and 11 ($p < 0.01$). Relative to the saline-treated group, chlordiazepoxide also significantly increased the lever presses on days 7 and 11 ($p < 0.05$), but Scheffe's test failed to detect significant differences between the two drugs.

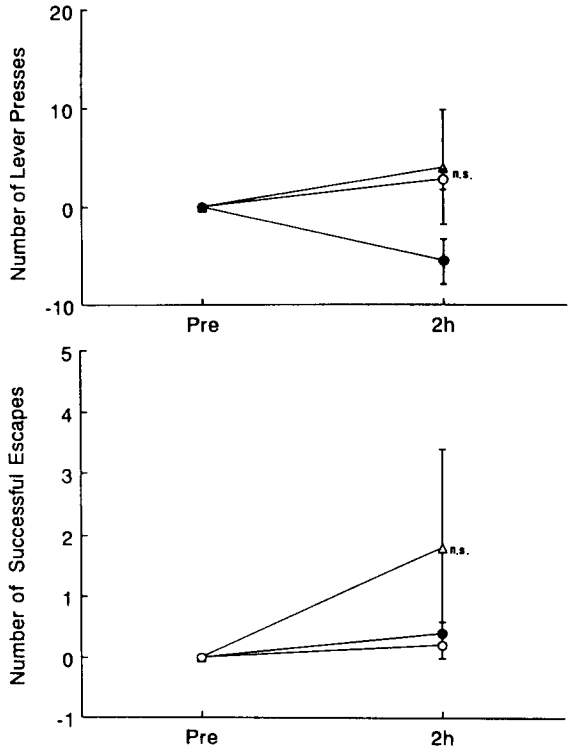

FIG. 3. The effects of acute administration of imipramine (○, n = 5), chlordiazepoxide (△, n = 5), and saline (●, n = 5) on suppressed behavior. The upper panel illustrates the effects of drugs on the number of lever presses and the lower one illustrates the number of successful escapes. n.s.: $p > 0.05$ in Scheffe's test.

The lower panel illustrates the effects of chronic treatment on successful escapes. Imipramine increased gradually on day 7 and day 11. On the other hand, chlordiazepoxide showed no significant differences from the saline group. A mixed-design ANOVA revealed a significant group effect, $F(2, 12) = 4.70$, $p < 0.05$, and day effect, $F(5, 60) = 4.87$, $p < 0.01$, but not a group × day interaction, $F(10, 60) = 1.83$, $p > 0.05$. Scheffe's test showed that only imipramine significantly increased the number of successful escapes on day 7 ($p < 0.05$) and day 11 ($p < 0.01$), and there was no significant difference between imipramine and chlordiazepoxide on any day.

Effects of Chronic Administration on Weight Gain

Figure 5 illustrates the changes in weight gain. A mixed-design ANOVA revealed a significant group effect, $F(2, 12) = 4.12$, $p < 0.05$, day effect, $F(4, 48) = 60.29$, $p < 0.01$, and a group × day interaction, $F(8, 48) = 2.26$, $p < 0.05$. In particular, the imipramine-treated group showed lower weight gain compared to the groups treated with chlordiazepoxide or saline solution. By Scheffe's test, while the imipramine-treated group showed significant differences from the saline group on days 7, 9, and 11 ($p < 0.05$), the chlordiazepoxide-treated group showed no significant difference from the saline group.

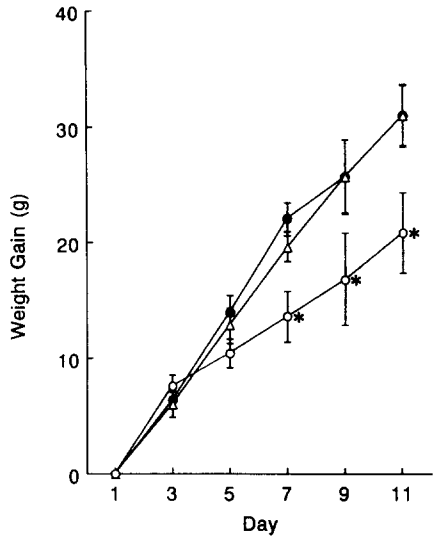

FIG. 5. Effects of the drugs on weight gain in rats with suppressed behavior. Each value represents body weight gain during chronic treatment with imipramine (○, $n = 5$), chlordiazepoxide (△, $n = 5$), and saline (●, $n = 5$). *$p < 0.05$ significantly different from the saline group (Scheffe's test).

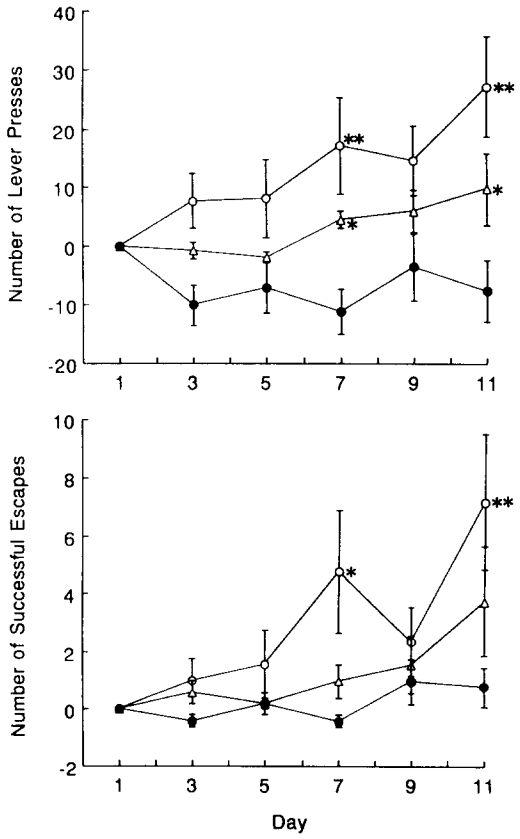

FIG. 4. The effects of 10 days of daily drug administration. The upper panel shows the number of lever presses and the lower panel shows the number of successful escapes during the course of chronic imipramine (○, $n = 5$), chlordiazepoxide (△, $n = 5$), and saline (●, $n = 5$) administration. *$p < 0.05$, **$p < 0.01$ significantly different from the saline group (Scheffe's test).

DISCUSSION

Experiment 1 basically evaluated the effects of training on the number of lever presses, successful escapes, and body weight of rats subjected to conditions under chronic and unpredictable electric shock. Figure 1 illustrates that 10 cycles of VR 10 effectively suppressed both the number of lever presses and escapes of well-trained rats. Figure 2 shows that gain in body weight of treatments A, B, and C were suppressed relative to treatment D. The data from treatment A on day 10 indicates that, even with training, exposure to CS and UCS at FR 5 led to a suppression of weight gain. Even for treatment C, the statistical analysis revealed significantly lower weight gain, indicating that 30 cycles of warning signal had a pronounced stressful effect on weight gain.

The rats in treatment B were totally unable to exhibit escape behavior and did nothing but freeze and receive the foot shocks. Despite this, these rats gained more weight during the experimental period as compared to treatment A, though the statistical analysis failed to show any significant difference between these two groups. This indicates that treatment A was more stressful than treatment B, most probably because the unpredictable nature of the VR 10 procedure in the former induced a psychologically depressive situation that was more stressful than the simple physiological stress suffered by the rats in treatment B. In this context, treatment A seemed to include psychological and physical stressful factors of CIS (8). Since, aside from training, the only difference between the two groups was the VR 10 procedure, it is possible that the uncontrollability of the VR 10 procedure induced a hopeless or desperate situation resulting in the depressed weight gain data of treatment A. Hopelessness and despair are key factors in the etiology of human depression (12,13).

Using treatment A, 15 rats (71%) were able to demonstrate 80% escape as compared to Takaoka's 40%. This confirms our idea that our procedure is less stringent. This advantage is especially important in laboratories with limited resources.

When studying the standard paradigm of learned helplessness, some investigators have reported the effectiveness of chronic treatment with antidepressants on the escape deficit (2,4,9,11). In the present study, chronic treatment with imipramine and chlordiazepoxide enabled the rats to gradually increase the number of presses and the number of successful escapes (Fig. 4). The response to imipramine was earlier than that to chlordiazepoxide, indicating that imipramine seems to be better than chlordiazepoxide in eliciting the appropriate escape behavior. The strong therapeutic potency of imipramine in elevating mood, as reported in clinical studies, may be responsible for the increased number of lever presses and successful escapes in the imipramine-treated group (10).

In the present study, an electric shock (from 0.8 to 1.6 mA) was supplied from the lever to prevent prolonged pressing. Thus, the trained rats also seemed to suffer from conflict during lever pressing. Chlordiazepoxide is reported to have potent effects in "conflict" situations, and this may be the reason for the increased number of lever presses in the chlordiazepoxide-treated group (5). As expected from an anxiolytic agent, chlordiazepoxide treatment enabled the rats to increase the number of lever presses but it did not dramatically increase the number of successful escapes. The same effect of chlordiazepoxide on lever presses and successful escape has also been reported (14). We assumed here that the fear-motivated response used in the conditioning procedure and the different pharmacological effects of chlordiazepoxide and imipramine on behavior were possible causes of the different effects of these two drugs on lever presses and successful escapes.

A major objective of animal models is to discriminate between different types of drugs. In the current model, imipramine, an antidepressant, was statistically differentiated from chlordiazepoxide, an anxiolytic agent, through the increase in successful escapes. In addition, there were noticeable differences in the effects of these two drugs in terms of lever presses (upper panel in Fig. 4) and weight gain (Fig. 5).

The data in Fig. 5 contain contradictory but important results: treatment with imipramine resulted in lower weight gain than treatment with chlordiazepoxide. The lower weight gain induced by imipramine in the present study is corroborated by a previous study in which it was found that this antidepressant, administered at 10 mg/kg, reduced food intake and decreased weight gain in rats (1).

In conclusion, the following criteria were used to evaluate depression in rats exposed to chronic unpredictable electric shock: a) effects on body weight, b) effects on escape behavior, c) effect of acute or chronic treatment of psychotropic drugs on b), and d) identification of the specific effects of these drugs. The results indicate that this procedure may be useful in the study of human depression.

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