ORIGINAL ARTICLE

“Swimming-induced Head Twitching” in Rats in the Forced Swimming Test Induced by Overcrowding Stress: A New Marker in the Animal Model of Depression?

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Abstract. We have used overcrowding stress to study the pathogenesis of depression and the action of antidepressant drugs. In the present study, the influence of overcrowding on behavior was assessed by the forced swimming test. All the stressed rats revealed highly characteristic head twitching movement, which was not inhibited by repeated administration of diazepam and haloperidol, but was markedly suppressed by repeated administration of desipramine and mianserine. A significant positive correlation in the number of twitching episodes in each stressed rat between the first and second forced swimming test was seen. These findings support the use of overcrowding of rats as a stressor in the animal depression model because it fulfills the criteria of the model; face validity, construct validity and predictive validity. We propose the adoption of “swimming head twitching” as a new marker in the animal model of depression. (*Keio J Med* 41 (4): 221–224, December 1992)

Key words: crowding stress, antidepressant

Introduction

Clinical evidence suggests that affective disorders are triggered by stress or by inappropriate adaptation to psychosocially stressful situations. Biochemical and behavioral investigation of chronic stress and adaptation mechanisms in animals under such conditions may therefore prove useful in the study of the pathophysiology of these disorders.

A variety of stressors have been used in the animal depression model. Most are relatively severe, intermittent physical insults eg, restraint, electric shock, and food deprivation. However, as depression in humans is often triggered by more subtle, long term psychological stresses, milder, chronic stresses are more suitable. We used stress induced by overcrowding to study the pathogenesis of depression and the action of antidepressant drugs. In a previous paper we showed that subchronic administration of desipramine augmented norepinephrine and serotonin turnover in some regions of the rat brain under overcrowding, and suggested that this may be associated with the antidepressant action

of the drug.¹

In the present study, the influence of overcrowding on behavior was assessed by the forced swimming test which is proposed as an animal model of depression and a screening test for antidepressant drugs. During this test we observed a characteristic “head twitching movement” induced by overcrowding that was specifically inhibited by repeated administration of antidepressants. We propose the adoption of “swimming-induced head twitching” as a new marker in the animal model of depression.

Materials and Methods

Animals

Male Charles-River Wistar rats (140–150 g) were used in all experiments. Each three animals were housed to a cage with free access to food and water. Ambient temperature was maintained at 22–23°C under constant humidity. Animals were exposed to light 12 hrs per day (7:00 to 19:00). Seven days after arrival, rats were

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randomly assigned to control and chronic stress groups.

Overcrowding stress and drug treatment

Overcrowding was achieved essentially as previously described.¹ Control animals were left undisturbed in their cages (22 × 38 × 19.5 cm high). The chronic stress groups were housed under the crowded conditions of six per cage (15 × 24 × 12.5 cm, plastic cages), also with free access to food and water throughout the experiment. At 3 p.m. on each experimental day, rats were injected ip with saline or drug solutions at 1 ml/250 g for 3–14 days. Drugs were either dissolved in 0.9% saline or, if insoluble, dispersed in a suspension of propylglycol.

Drugs: Drugs used were; desipramine hydro chloride (Ciba-Geigy Japan), mianserine hydro chloride (Japan Organon), haloperidol (Dainippon Pharmaceutical), diazepam (Yamanouchi Pharmaceutical).

Forced swimming test: Each rat was subjected to the forced swimming test twice, each starting 3 hours after drug administration. The first test was on the 2nd, 6th or 13th day of overcrowding, with the second test 24 hours later. Rats were forced to swim for 15 and 5 minutes in the first and second tests respectively. The overcrowding was maintained between the first and the second tests in the chronic stress group. The test was performed as described by Porsolt *et al*.² Briefly, rats were forced to swim singly in a Plexiglas cylinder (403 × 18 cm) containing 20 cm of water (25°C). After an initial period of vigorous activity, each rat became immobile and floated in the water. The duration of immobility was measured over 5 minutes in both tests. Episodes of the characteristic “head twitching behavior” during this test, described in detail in the results section, were also counted for 5 minutes in both tests.

Statistics: Data was analyzed by one-way analysis of variance, and post hoc comparison was done using Wilcoxon’s 2-tailed rank order test. The statistically significant difference was taken as $P < 0.05$ for all comparisons.

Results

Results of the forced swimming test on 2 consecutive days are presented in Figure 1. The duration of immobility in both tests was significantly reduced by repeated injection of desipramine (20 mg/kg) for more than 6 days. Overcrowding also caused a significant reduction in the duration of immobility on most experimental days. No cumulative or synergistic effects on immobility were found between desipramine treatment and overcrowding.

Differences in test behavior between desipramine-

Fig 1 Effects of desipramine and/or overcrowding or both on immobility and swimming-induced head twitching of rats in the forced swimming test. Each group was subjected to overcrowding and/or given desipramine (DMI) for 3.7 or 14 days. The forced swimming tests were done twice for each rat. The first test was done on 2nd, 6th or 13th day, and the second test was done 24 hours after the first test. The duration of immobility (unit in second) and the number of head twitching was measured over 5 minutes in both tests. Each group consisted of at least 6 rats. * $P < 0.05$, ** $P < 0.01$ significantly different from the corresponding control. * $P < 0.05$, ** $P < 0.01$ significantly different from the corresponding stress group.

treated and stress groups after the 6th day of the study were seen. While the desipramine group often showed active escape behavior, such as climbing the wall, repeated jumping or diving, the stress group showed more “apparently aimless” behavior such head twitching, grooming or pushing off the wall. On the 2nd and 3rd experimental days, however, the stress group showed escape-directed behavior similar to the desipramine-treated groups, although some head twitching was also observed.

The most conspicuous behavioral activity in the overcrowding group was head twitching. This was never observed while rats were caged. It is described as rapid shaking of the head for periods of usually less than one second. We tentatively named it “swimming-induced head twitching”, an obvious behavioral pattern that can be easily counted. As shown in Figure 1, it was seen on the second day of overcrowding and continued until the 14th day. A significant positive correlation in the number of twitching episodes in each stressed rat between the first and second forced swimming test was seen (Fig 2).

The twitching was markedly reduced 7 days after rats were returned to the control cages after 14 days of overcrowding (Fig 3).

The twitching was significantly reduced by desipramine administration of longer than 7 days (Fig 1). Treatment for 2 or 3 days did not reduce the number of twitching episodes. Repeated administration of mianserine (10 mg/kg) also significantly inhibited twitching induced by 14 days of overcrowding (Fig 4). On the other hand,

Fig 2 Correlation of the number of swimming-induced head twitches of each rat between the first and second forced swimming test.

Fig 3 Number of swimming-induced head twitches as a function of experimental days. Seven days after termination of overcrowding for 14 days, rats were again tested by the forced swimming test. Each group consisted of 6 rats. * $P < 0.05$ as compared with the control group. ** $P < 0.01$ as compared with the rats subjected to 14 days of overcrowding.

diazepam (0.1, 0.5 mg/kg) and haloperidol (0.1 mg/kg) had no effect.

Discussion

Although many studies have examined the forced swimming test as an useful antidepressant-screening procedure, little studies concerning the effect of stress on this test have been undertaken. Platt and Stone³ reported that stress due to repeated restraint reduced immobility in this test quantitatively to the same degree as repeat administration of desipramine, and stated that this

Fig 4 Effects of repeated administration of various neuroleptic drugs on swimming-induced head twitches of stressed rats in the forced swimming test. Rats were subjected to overcrowding and given the indicated drugs for 14 days. Each group consisted of 6 rats. DMI; desipramine, MIA; mianserin, DZP; diazepam, HPD; haloperidol. * $P < 0.05$ significantly different from the control group.

confirmed their previous findings of similarities in the behavioral and neurochemical response to chronic stress and chronic antidepressant treatment. Our results showing a significant reduction in immobility by overcrowding supported these results. However, a more interesting finding in our study is the characteristic “swimming-induced head twitching” behavior in rats with subchronic or chronic overcrowding. In fact, the reduction in stress-induced immobility appeared to be caused by this abnormal behavior, rather than by escape-directed behavior as shown in the desipramine-treated rats. Platt and Stone³ also demonstrated qualitative differences in behavior between drug-treated and stressed rats; whereas the former showed vigorous escape attempts, the latter showed less vigorous movements such as grooming, sniffing, pushing off the walls of the container and elevating the head above water, although they did not observe head twitching. Thus, the behavioral effects of stress appear to vary depending on the stressor.

More importantly, the head twitching was markedly reduced by repeat administration of antidepressants and not by anxiolytics nor antipsychotics. These findings support the use of overcrowding of rats as a stressor in the animal depression model because it fulfills the criteria of the model:⁴ (1) Face validity; “swimming-induced head twitching” is a clear behavioral marker which can be easily quantified. (2) Construct validity; clinical and experimental evidence suggests the strong relationship between stress and depression. Overcrowding stress is milder and of a more psychological nature than the conventional stressors used in stress experiments. (3) Predictive validity; swimming-induced head twitching is specifically reversed by antidepressant drugs.

Furthermore, a significant correlation in the head twitching between the first and second swim test was

found. In other words, rats showing frequent episodes of the twitching in the first test also showed many episodes in the second. Such reproducibility of behavior in individual rats suggests that "swimming-induced head twitching" may also be a convenient index in the determination of individual vulnerability to stress. This may open the possibility of creating a genetic model of depression using this marker.

Regarding the physiological background of "swimming-induced head twitching", it should be noted that it became evident from the 6th experimental day and was less evident on the 2nd and 3rd days of overcrowding. At this initial stage of stress, body weight loss had not recovered and the animals were somewhat fierce, suggesting that they had not yet adapted to their environment. However, they appeared to have adapted by 6–7th day. As shown in our previous biochemical data,¹ the initial augmentation of norepinephrine and serotonin turnover also returned to normal levels at this stage of overcrowding. However, in view of the evidence that twitching appeared during this adaptive phase, one may speculate that the apparent adaptations shown in body weight, gross behavior on handling and monoamine turnover were false adaptation, easily lost in the new stressful environment. This hypothesis may relate to the pathogenesis of affective disorders.

Finally, the biochemical background of "swimming-induced head twitching" is discussed. It is well known that serotonin agonists induce head twitching in rats.⁵ As serotonin-induced twitching and the present "swimming-induced head twitching" are apparently similar, the

latter may also be induced by stimulation of the serotonergic system. However, we failed to induce "swimming-induced head twitching" by the serotonin precursor, 5-hydroxytryptamine or by serotonin agonist, 5-methoxy-N, N'-dimethyltryptamine (unpublished observation), and failed to suppress it by the serotonin antagonists, ritanserin and ketanserin. Thus, this behavior may be caused by an unknown biochemical mechanism outside the serotonergic system and may be related with the mode of action of antidepressant drugs and the biochemistry of depression.

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