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Evidence for a Depressive-like State Induced by Repeated Saline Injections in Fischer 344 Rats

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IZUMI, J., M. WASHIZUKA, Y. HAYASHI-KUWABARA, K. YOSHINAGA, Y. TANAKA, Y. IKEDA, Y. KIUCHI AND K. OGUCHI. *Evidence for a depressive-like state induced by repeated saline injections in Fischer 344 rats.* PHARMACOL BIOCHEM BEHAV **57**(4) 883–888, 1997.—We investigated the behavioral changes induced by mild stress in animals that may be relatively susceptible to a depressive-like state, the Fischer 344 rat strain. The mild stress of repeated handling and intraperitoneal (IP) injections with saline (2 ml/kg, twice a day for 14 days) elicited a moderate suppression of body weight gain, a decrease in open field activity, and a prolonged immobility during the tail suspension test in Fischer 344 rats compared with Sprague–Dawley rats. Chronic treatment of Fischer 344 rats with imipramine (10 mg/kg IP, twice a day for 14 days) effectively suppressed open field activity and prolonged immobility. These results suggest that repeated saline injections may be a mild stressor in these rats. In the Fischer 344 strain, which may be vulnerable to the effects of mild stressors, repeated saline injections might induce a depressive-like state and could presumably represent an experimental model for depression. © 1997 Elsevier Science Inc.

Depressive state Mild stress Repeated saline injections Emotional animals Fischer 344 strain rats Open field behavior Tail suspension test Imipramine

IN THE SEARCH for effective therapeutics for the treatment of affective disorders, increasing importance has been given to the role of a depressive-like state in those disorders. Therefore, an adequate model of the depressive-like state may be of considerable use in the development of animal experiments to test novel therapeutics. In general, stress has been thought to play an important role in the etiology of depression (2). The approach of repeatedly exposing an animal to a stressor has often been used in experimental models of depression, such as learned helplessness (6), forced running stress (9,12), repeated exposure to restraint (1,4,8,10,20,25), or unpredictable stress (3,13). These models employ severe stressors in normal rats to induce relevant changes. However, the models appear not to be suitable in their similarity to the etiology of depression in humans, because the pathogenesis of affective disorders in humans appears to be due to the stress of daily living on subjects with psychiatric or emotional vulnerability. Based on this point of view, we hypothesized that poor adaptation in vulnerable animals to a repeated mild stressor to which most animals can adapt may be a more appropriate experimental model for stress-related effects in depression.

Fischer 344 rats are reported to have aberrant characteristics that may be of importance in their adaptability to stress, such as high emotionality in open field behavior (11), lack of behavioral adaptation to forced swimming (14), and greater response to stress in the hypothalamic–pituitary–adrenal axis (22). Considering this evidence, it is postulated that Fischer 344 strain rats are suitable experimental animals for modelling affective disorders because they express a vulnerable and/or emotional condition relative to other strains such as Sprague–Dawley rats.

Several procedures have been employed as mild stressors in the study of stress. Among these procedures, repeated injection of saline in pregnant rats has been confirmed to induce some biological changes in neonates, such as alterations in behavioral responsiveness (17,18), changes in brain serotonin (5-HT) turnover (16), and differences in 5-HT_2 and adrenergic

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receptor function (15,18). Therefore, at least in vulnerable animals, repeated saline injections are expected to act as a mild stressor to elicit a depressive-like state representing a model for affective disorders.

Considering the evidence that Fischer 344 strain rats have characteristic emotionality and that repeated saline injections can be a mild stressor, we thought that the combination of these two factors might elicit some behavioral changes associated with a depressive-like state. In the present investigation, we examined the behavioral features of Fischer 344 rats exposed to repeated handling and saline injections. The results are discussed in the context of their support for the validity of our proposed approach to modelling a depressive-like state.

METHODS

Animals

Male Fischer 344 rats (9 weeks old, body weight 200–220 g) and Sprague–Dawley rats (6 weeks old, body weight 220–240 g) were purchased from Charles River Japan. Animals were housed for at least 5 days under the following standard conditions: room temperature 23 ± 2°C, humidity 55 ± 10%, 12 L:12 D cycle (lights on at 0800 h). Rats were caged in groups of five with food and water available ad lib. All experiments were performed in strict accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by our Animal Care and Use Committee.

Stress Exposure

First, all animals were weighed and their activity in the open field was measured. Based on these scores, animals of both strains were divided into three (control, stress, and stress plus imipramine) equal groups. Rats in the stress group received repeated handling and intraperitoneal (IP) injections of saline (2 ml/kg) twice a day (at 0900 and 1700 h) for 14 days. In the stress plus imipramine group, imipramine hydrochloride (imipramine; Sigma Chemical Co., St. Louis, MO, USA; 10 mg/kg IP) was administered instead of saline twice a day for 14 days. In the control group, rats were housed under the same conditions as the other two groups without the repeated handling and injections. Behavioral experiments were performed 18 h after the last injection.

Open Field Activity

A hall-type circular open field was used for testing open field activity. It was composed of a gray plastic floor (60 cm in diameter) with a 50-cm-high wall. The floor was divided by black lines into 19 segments and illuminated at about 150 lux by fluorescent ceiling lamps. The test was started by placing the rat in the central circle of the open field. The number of sections crossed (ambulation), the number of rears, and the number of defecations were observed for 5 min. The test was performed between 0900 and 1100 h.

Tail Suspension Test

The tail suspension test was done following the open field test to confirm the presence of a depressive-like state (5,23, 24). The animals were hung by the tail using a clamp covered with silicone plates, in a space surrounded by a V-shaped smooth slope on which the rats place their forepaws. The duration of immobility during the 6 min of tail suspension was then measured.

Statistical Analysis

Data are summarized as means ± SEM and were analyzed by a one-way analysis of variance (ANOVA) followed by a post hoc Tukey–Kramer test or by a two-way repeated-measures ANOVA with one within factor (session) and one be-

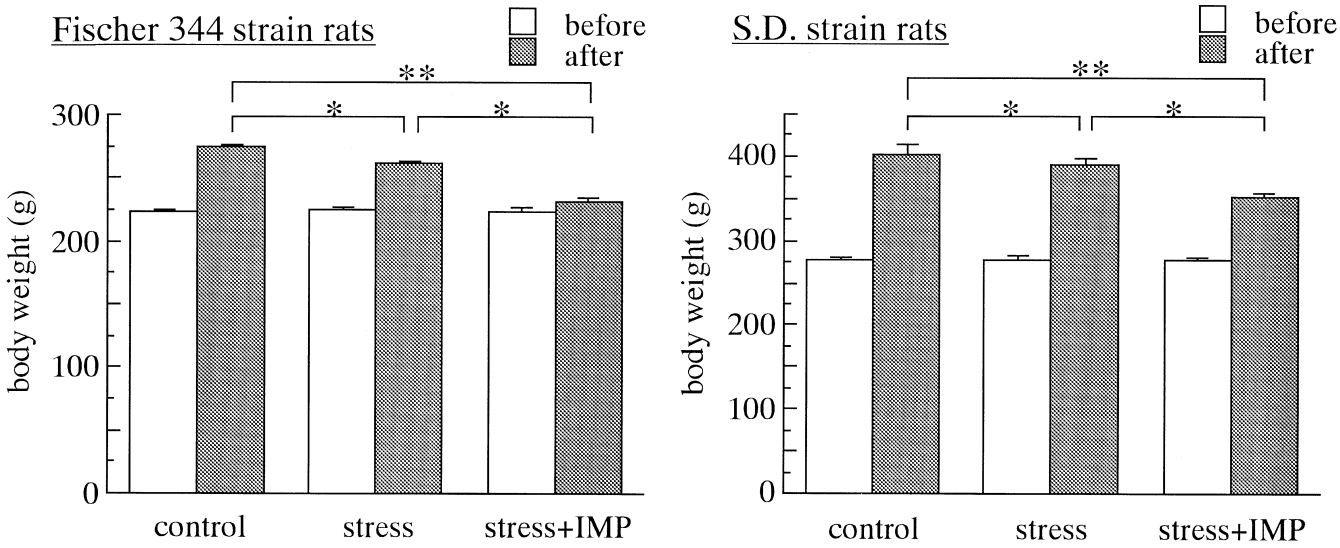

FIG. 1. Changes in body weight gain following the stress of repeated handling and saline injections in Fischer 344 and Sprague–Dawley (S.D.) rats. Animals in the stress group repeatedly received injections of saline (2 ml/kg IP, twice a day for 14 days). In the stress plus imipramine (stress + IMP) group, animals received imipramine (10 mg/kg IP) instead of saline. The control animals were housed under the same conditions without repeated handling and injections. Observation was conducted before exposure to stress and again 18 h after the last injection. Each column represents the mean ± SEM ($n = 10$). Statistical significance was evaluated by a one-way ANOVA followed by a post hoc Tukey–Kramer test; $^*p < 0.05$, $^{**}p < 0.01$, significant difference between the groups.

tween factor (stress). Differences with a p value of less than 0.05 were considered to be statistically significant.

RESULTS

Body Weight

Figure 1 shows the changes in body weight gain through the 14-day experimental protocol. In Fischer 344 rats, body weight was significantly increased through the 14-day session [main effect of session, $F(1, 18) = 1620.278$, $p < 0.001$], and the twice-daily handling and saline injections slightly, but significantly, suppressed body weight gain [main effect of stress, $F(1, 18) = 5.123$, $p < 0.001$; interaction effect of session × stress, $F(1, 18) = 38.571$, $p < 0.001$]. Fischer 344 rats in the stress plus imipramine group exhibited a considerable suppression of body weight gain ($p < 0.01$ compared with the control group, $p < 0.05$ compared with the stress group, by the Tukey–Kramer test). Further, in Sprague–Dawley rats, similar body weight results were obtained following exposure to the stress of repeated handling and injection [main effect of session, $F(1, 18) = 668.45$, $p < 0.001$; main effect of stress, $F(1, 18) = 8.718$, $p < 0.01$; interaction of session × stress, $F(1, 18) = 6.877$, $p < 0.05$; effect of imipramine, $p < 0.01$ compared with the control group, $p < 0.05$ compared with the stress group].

Open Field Activity

In Fischer 344 strain rats, decreased ambulation (the number of sections crossed) was observed in the stress group (39.1 ± 6.2, $p < 0.05$) compared with the control group (69.6 ± 8.7) [interaction of session × stress, $F(1, 18)$ ± 6.470, $p < 0.05$] (Fig. 2). Repeated administration of imipramine resulted in a

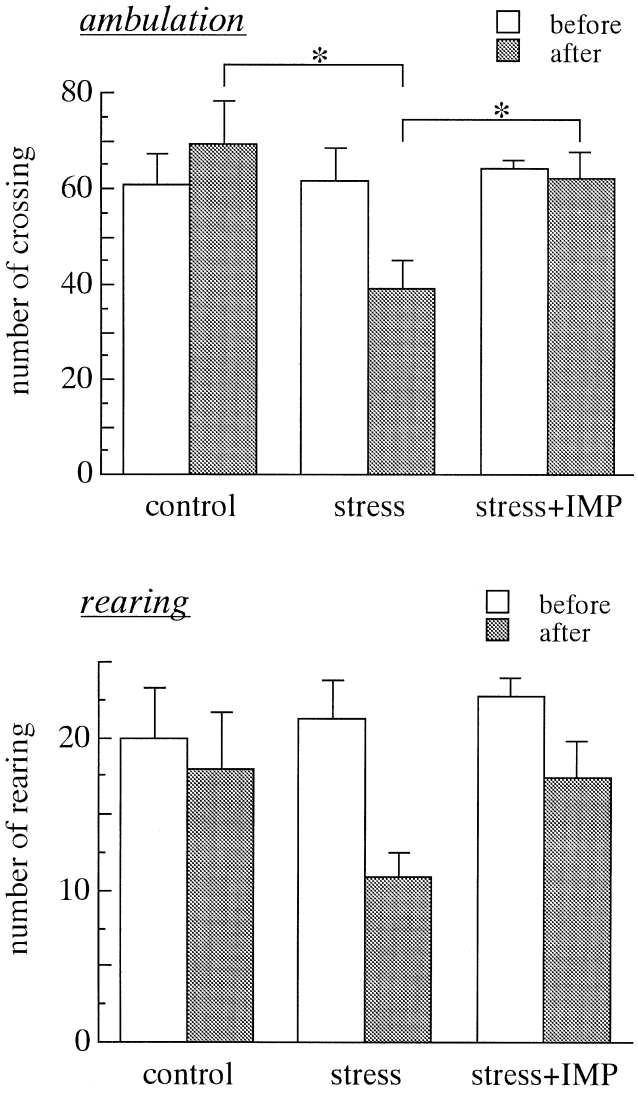

FIG. 2. Changes in open field behavior (ambulation and rearing) following the stress of repeated handling and saline injections in Fischer 344 rats. See legend of Fig. 1 for details. The number of sections crossed (ambulation) and rearing were counted for 5 min. Statistical significance was evaluated by a one-way ANOVA followed by a post hoc Tukey–Kramer test; *$p < 0.05$, significant difference between the groups.

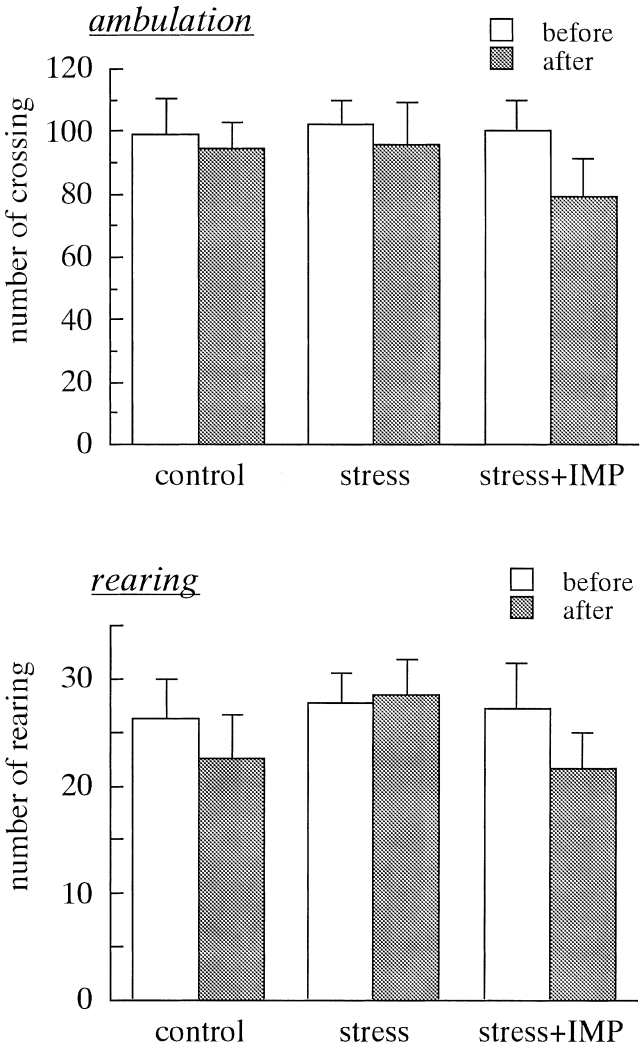

FIG. 3. Changes in open field behavior (ambulation and rearing) following the stress of repeated handling and saline injections in Sprague–Dawley rats. See legend of Fig. 1 for details. The number of sections crossed (ambulation) and rearing were counted for 5 min. No significant effects were obtained.

protective effect against the stress-induced suppression of ambulation (62.8 ± 5.8, $p < 0.05$ compared with the stress group). Both the stress and the stress plus imipramine groups yielded results similar to the ambulation data with regard to the number of rears, although statistical significance was not achieved. The number of defecations was not influenced by these manipulations.

In contrast, there was no change among the three groups in the open field behavior of Sprague–Dawley rats (Fig. 3).

Tail Suspension Test

To evaluate the depressive-like state following exposure to the stress of repeated handling and saline injections, we tested the effect of chronic imipramine on performance in a tail suspension test (Fig. 4). In Fischer 344 rats, prolonged immobility was observed in the stress group (230.6 ± 5.4 s, $p < 0.01$) compared with the control group (200.5 ± 9.4 s). The repeated administration of imipramine significantly reversed this prolonged immobility (199.8 ± 10.1 s, $p < 0.05$ compared with the stress group). In Sprague–Dawley rats, no significant difference was found in immobility among the control (162.6 ± 15.3 s), stress (172.1 ± 13.3 s), and stress plus imipramine (177.6 ± 21.3 s) groups during the 6-min tail suspension test.

DISCUSSION

In a previous study of maternal stress, it was found that repeated injections of saline could be an effective stressor for pregnant rats (15–18). In addition, it was reported that the hypothermic response to nicotine was affected by repeated handling and injection in adult Sprague–Dawley rats (7), suggesting that this manipulation could also function as a stressor in that strain. In the present study, repeated handling and saline injections were found to affect behavior as well as weight gain in "emotional" animals, rats of the Fischer 344 strain. In control animals of the Sprague–Dawley strain, a slight suppression of body weight gain was observed in the present study. As indicated by the fact that repeated exposure to stress has been widely used as an experimental model for depression, stress is thought to play an important role in the etiology of depression (2). In models of stress-induced depression, severe stress conditions have been generally used, such as inescapable shock (6), forced running stress (9,12), and repeated restraint (1,4,8,10,20,25). Considering the resemblance of milder stressors to the hypothesized etiology of some affective disorders, we preferred to use mild stressors rather than stressors of the severe type. In a study employing the chronic unpredictable stress model (5), body weight was found to be remarkably reduced after a 3-week stress session. In contrast, the repeated saline injections in the present study resulted in only slight suppression of body weight gain but no loss of body weight. It would seem that an ideal experimental model for affective disorders requires manipulations that minimize the extent of gross physical perturbations. Based on this consideration, the manipulation of repeated handling and saline injections is postulated to be a useful tool for the experimental modelling of depression.

In the study of stress-induced depression, lowered activity (3,10,21) and/or behavioral despair (4,6,19) are used as indices of the depressive-like state. In the present study, repeated saline injections suppressed open field activity in Fischer 344 rats but not in the Sprague–Dawley rats. Similarly, the manipulation induced prolonged immobility in the tail suspension test, which is considered to be an indication of behavioral de-

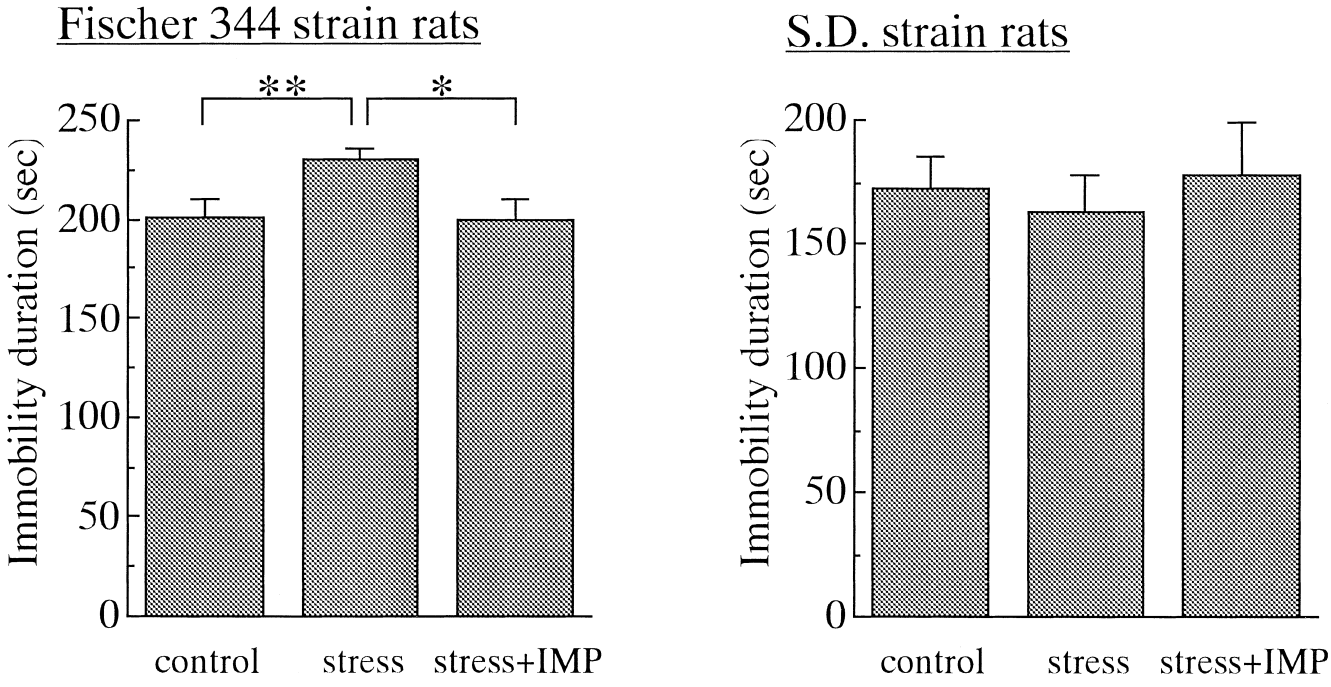

FIG. 4. Performance in the tail suspension test following exposure to the stress of repeated handling and injections in Fischer 344 and Sprague–Dawley (S.D.) rats. See legend of Fig. 1 for details. The tail suspension test was done 18 h after the last injection. Each column represents the mean ± SEM ($n = 10$), and data are expressed as the immobility duration during the 6 min of tail suspension. Statistical significance was evaluated with a one-way ANOVA followed by a post hoc Tukey–Kramer test; $*p < 0.05$, $**p < 0.01$, significant difference between the groups.

spair (5,23,24). Altered mobility was noted in Fischer 344 rats but not in Sprague–Dawley rats. These results indicate that a depressive-like state might be generated by the stress of twice-daily handling and saline injection in Fischer 344 rats. It is of considerable interest that we obtained greater stress-related effects on Fischer 344 rats than in Sprague–Dawley rats. Previous investigations have revealed behavioral and biological differences between those two strains (22) that may relate to the construct of Fischer 344 rats as "depression prone" and Sprague–Dawley rats as "normosensitive controls." Thus, the present observation that Sprague–Dawley rats were relatively resistant to the mild stress of repeated saline injections compared with Fischer 344 rats may indicate that Sprague–Dawley rats represent a useful control in models that require the demonstration of resistance to stressors to which an organism should be able to adapt. Conversely, we propose Fischer 344 rats to be suitable "vulnerable" animals for the demonstration of chronic mild stress effects. Because both the suppressed activity in the open field experiment and the prolonged immobility in the tail suspension test were significantly improved in Fischer 344 rats by chronic treatment with the tricyclic antidepressant imipramine, it might be reasonable to hypothesize that the depressive state induced by repeated saline injections possesses predictive validity as a model of clinical depression. In Sprague–Dawley rats, chronic imipramine did not engender any effects on open field activity, showing a slight decrease rather than an increase, or on the duration of immobility in the tail suspension test. The observed effect of imipramine on open field behavior in Sprague–Dawley rats seems to argue strongly against the possibility that the recovery of behavior in stressed Fischer 344 rats treated with imipramine was caused by a generalized motor stimulant action of the drug. As in previous studies (5,23), we also found an acute effect of imipramine in the tail suspension test (147.8 s of immobility, $p < 0.05$ compared with the control time of 186.0 s). However, because imipramine had been administered about 18 h before the test in the present study, the acute effect of the drug could not be expected to influence performance. Therefore, we suggest that the effect of imipramine in Fischer 344 rats might be due to protection against the development of a depressive state resulting from exposure to the repeated handling and injections. Thus, it was behaviorally and pharmacologically determined that the depressive-like state elicited by repeated handling and saline injections in Fischer 344 rats may be a suitable experimental model for depression. In addition to the behavioral changes observed in the present study, some biological factors have been identified in the stress models, such as alterations in the turnover of monoamines (8,9,12,20), changes in monoamine receptors (13,25), perturbations of the HPA axis (3) and glucocorticoid receptors (3), etc. Detailed investigations of these neurochemical parameters should be conducted for the present model as well.

In conclusion, repeated handling and saline injections elicited a depressive-like state characterized by suppressed open field activity and augmented behavioral despair in the tail suspension test in Fischer 344 rats. This depressive-like state was reversed by chronic treatment with imipramine. These results suggest that the manipulation of repeated handling and saline injections in Fischer 344 rats appears to be appropriate as an experimental model for the depressive-like state.

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