ORIGINAL ARTICLE

EFFECTS OF TWO TYPES OF RESTRAINT STRESS ON THE LEARNED BEHAVIOUR IN RATS

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Summary: To study the effects of stress on cognitive functions, Wistar and Lewis rats were exposed to restraint (immobilization stressor) (IMO) or restraint combined with partial immersion into water (IMO+C). Learned discriminatory avoidance response in Y-maze, with foot-shock as an unconditioned stimulus, was used as a memory test. The latency to enter the correct arm and number of wrong entries were daily recorded during the training period (20 days) until the criterion was reached, which was set at 90 % avoidances (choosing the correct arm). After exposure of rats to one of the stressors for 60 min, the rats were returned to the home cage; the latency to enter the safe arm was recorded in 6 daily trials that started 1 h after application of stressor. Both stressors significantly prolonged the avoidance latencies for 2 or 3 days in Wistar and Lewis rats, respectively; then the latencies returned to the values obtained before the stress exposure. In Lewis rats, the latencies more increased after IMO+C than after IMO stressor, and the maximal increase in latencies was higher in Lewis rats than in Wistar rats. The latency did not reach the time limit for foot-shock delivery, and the number of correct choices remained unchanged in both strains. The results indicate that the used restraint stressors did not affect the long-term memory; rather a transient impairment of retrieval can be considered. Further, differences in response of Lewis and Wistar rats may be interpreted by different activity of hypothalamic-pituitary-adrenal axis activity in used strains.

Key words: *Y-maze; Restraint and restraint/cold stressors; Lewis and Wistar rats; HPA axis*

Introduction

Various stressors have been shown to exert modulatory effects on animal cognition. The corticosteroid hormones secreted by the adrenal cortex in response to stress promote the acquisition and storage of novel information and also facilitate extinction of behaviour that is no longer relevant (15). On the other hand, the exposure of laboratory rodents to inescapable stress has been demonstrated to disrupt cognitive processes in several models of dissociative or spatial learning tasks (18,19,20). In our previous experiments, prior exposure of rats to restraint combined with cold water immersion resulted in the impairment of passive avoidance acquisition (14). In that study, in an attempt to assess the participation of stress hormones in learning of this task, we compared the performance of Sprague-Dawley and Lewis rats, the latter strain being known to have deficits in HPA axis responses to stress exposure (4, 6, 8,16, 23, 28). We observed differences in several parameters of the studied behaviour such as an occurrence of habituation or differences in the rate of acquisition and extinction of the inhibitory response between both strains (14). In experiments exploring the effect of restraint on stress hormones, we also found lower response of circulating corticosterone in Lewis compared with Sprague-Dawley rats; no difference occurred in plasma levels of prolactin (13).

Generally, the intensity and duration of stress appear to be decisive for the resulting impact on animal cognition. For example, combined restraint and cold stressors induced more severe impairment of spatial memory in the radial maze task than restraint alone (24). A deficit in spatial memory water maze task or conditioned reflex activity was increased with a more pronounced hypothermia too (3,21). In the active avoidance learning task, the exposure to a strong stressor, consisting of repeatedly applied inescapable electric foot-shock or also swimming in cold water, impaired the acquisition of the escape response in a shuttle box (1,5,26,27). Therefore, in the present study, we used two types of immobilization stressors, restraint (IMO) or restraint combined with partial immersion of rats in (cold) water (IMO+C), during application of stress and examined their effects on the associative memory, formed during conditioning of active avoidance response in the Y-maze task. We also included two rat strains, Wistar and Lewis, to see whether lower responsiveness of hypothalamic-pituitary-adrenal (HPA) axis activity to acute stressor in the

latter strain could be revealed in the Y-maze task under the influence of both studied stressors.

Methods

Animals

The experiments were carried out using male Wistar (WI) rats (Velaz, Czech Republic) and male Lewis (LE) rats (Charles River Laboratories, Sulzfeld, Germany). At the beginning of experiment, the average body weight of rats was 260 (Lewis rats) or 290 g (Wistar rats) (age 90 days). Animals had free access to standard pellet food and water. Rats were housed 4 per cage (42 x 26 cm) and maintained on a 12 h light/12 h dark phase (change performed at 6.00 h and 18.00 h), at a constant temperature (21±1 °C) and relative humidity (50–70 %). Training and testing were performed between 8.00 h and 13.00 h. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals (DHEW Publication, NHI 80–23).

Stress procedure

The experimental rats were exposed to two types of restraint stressors (11,12). Restraint (immobilization) stress (IMO) was induced by fixing front and hind legs of the rat with adhesive plaster; then the animal was restrained in a snug-fitting vertical plastic-mesh. This mesh was bent to conform to the size of individual animal and a bandage fixed this shape of mesh. In the case of combination of restraint with water immersion (IMO+C), the restrained rats were immersed in the water bath (22° C) in such a way that the upper 1/4 of the rat was outside of water. After the exposure of rats to either of the stressors for one hour, the animals were returned into the home cage for another hour and then the behavioural testing started. Control animals remained untreated. The rats were exposed to the stressors only once. In previous studies, we found that the colonic temperature in the 22° C cold-water exposed rats returned to normal values before the one-hour limit (unpublished).

Y-maze test

The employed Y-maze was a fully automated apparatus with electric foot-shock serving as aversive motivation. It consisted of a square start area (285 x 480 mm) separated by plexiglass sliding doors from two trapezoid, black and white arms – choice area (140 x 324 mm). The walls were 240 mm high. The grid-floor in the start and choice areas was electrifiable. The trial started by placing the animal in the start area. After 48 s, electric foot-shock (60 V, 50 Hz, of 5 s in duration) was applied through the grid floor. The animals were trained to avoid punishment by escaping to the safe – white arm of the maze. The latency (s) to enter the correct arm and number of wrong entries were recorded. Two trials were run daily during the training period until the criterion was reached, which was set at 90 % avoidances (choosing the correct arm), with average avoidance latencies occurring within <1.5 s (9,10). Experiments on the two strains were run simultaneously. After having reached the criterion (20 days), the experimental rats were exposed to one of the stressor and the testing trial started 1 h later. Testing without stressor application continued once daily every 24 h for total 6 days.

Data analysis

The data on the escape latency into the safe compartment were analysed by SYSTAT 10 program. To analyse a difference between the last pre-stress and the first post-stress trial within a given group, the Wilcoxon matched-pairs signed ranks test was used. Then, a three-way analysis of variance (ANOVA) on individual post-stress entrance latencies, involving the factors: strain, stress treatment and repeated trials, was performed. Further, to compare the differences among groups during repeated trials within a particular strain a two-way ANOVA was used. A one-way ANOVA followed by the Bonferroni method for post test evaluation was used with the aim to compare the difference in latencies: (a) among groups within a particular day separately in WI and LE strain, (b) between strains within both a particular day and a given experimental condition. The statistical significance was accepted when $P \leq 0.05$.

Results

During the last few training sessions, all rats met the criterion: they entered the white-safe arm of the maze within 1.5 s without making any wrong choice. Irrespective of the strain, neither IMO nor IMO+C impaired the discrimination between the safe-white and the shock-black arm of the maze.

Fig. 1 presents the mean and SEM values of the escape latency (in seconds) on the last pre-stress trial and during the post-stress trials for the two strains (WI, LE).

The Wilcoxon test (always, df 1,6) proved a significant increase of the escape latency in animals subjected both to IMO and to IMO+C but not in the controls. A three-way ANOVA revealed a significant main effect of all three factors: strain, treatment and trials. All the two-way interactions were significant: strain x treatment, strain x trials, treatment x trials, and three-way interaction: strain x treatment x trials. Separate two-way ANOVA of latencies revealed main effects attributable to the factors treatment and trials for WI and LE strains: treatment, trials, and treatment x trials.

Further, a one-way ANOVA of latencies in WI strain within a particular day (always, df 1,8) revealed a significant difference between controls and both stressors for day 1 and 2 but not for day 3. On the day 1 and 2, the Bonferroni method showed significantly longer latencies in both IMO and IMO+C animals as compared to the controls; no difference was found between IMO and IMO+C exposure. Thus, WI rats responded equally to both stressors.

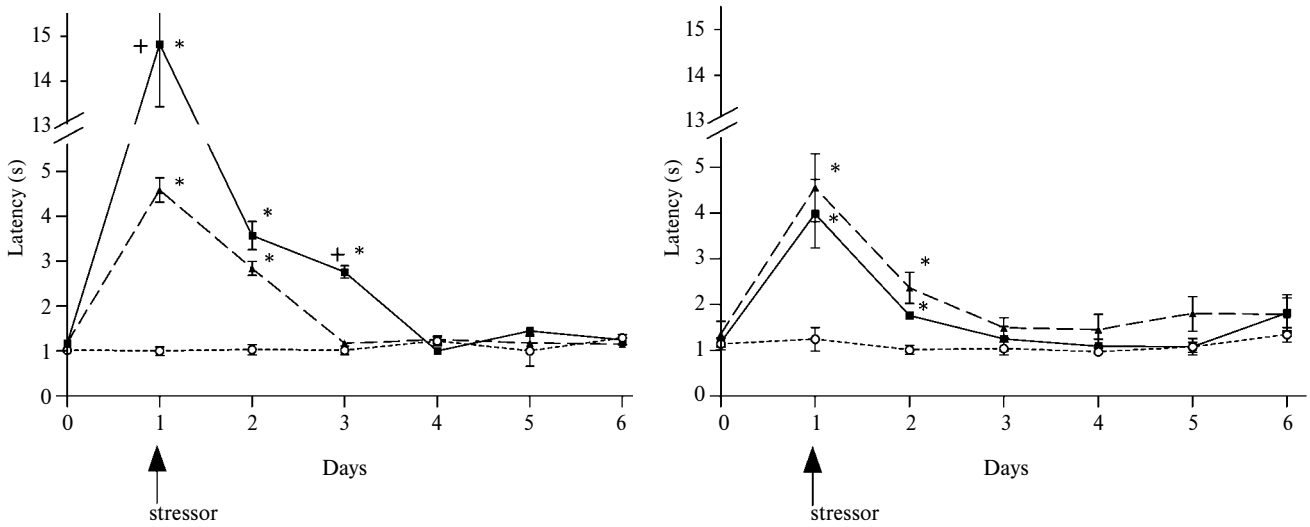

Fig. 1: Active avoidance performance of Lewis (left panel) and Wistar rats (right panel) in the Y-maze. Data in escape latencies in seconds (s) are expressed as the mean ± S.E.M. values. Arrow indicates the day of application of stressor. - - - o - - - = control group, – – – ▲ – – – = IMO, —— ■ —— = IMO+C. Significant differences for $P<0.05$: * vs control group, + vs IMO group.

The same analysis in LE strain (always, df 2,18) revealed a significant difference between control and both stressors for days 1, 2 and 3. The Bonferroni method showed: on the day 1, IMO+C animals had significantly longer latencies than IMO ones; on the day 2, statistical difference between IMO and IMO+C animals was not reached; on the day 3, the latencies in IMO+C animals were again significantly longer than those in IMO ones. Thus, LE rats responded differently to the two stressors. We add that on the day 4, 5 and 6 no significant differences among all groups were found.

In addition, a comparison between WI and LE strain in particular groups separately for day 1, 2 and 3 was performed. A one-way ANOVA revealed no significant difference for the controls; the animals exhibited very short latencies. There was no significant difference between WI and LE animals subjected to IMO for days 1,2 and 3. On the contrary we found significantly longer latencies in LE than in WI rat strains subjected to IMO+C for days 1 to 3.

Discussion

In the Y-maze, both stressors significantly influenced the acquired active avoidance performance of both WI and LE rats. The escape latency increased after IMO and IMO+C and this increase persisted for another 1 or 2 days in WI and LE rats, respectively. However, the latency never reached the time of the foot-shock delivery, and it spontaneously returned to the pre-shock values. Neither stressor impaired the acquired ability to solve the task by discriminatory response strategies. Altogether, the transient increase in the latency suggests that the long-term discriminatory memory was not substantially impaired. There was a significant difference between the strains in response to the stressors: LE compared to WI rats exhibited longer latencies induced by IMO+C but not by IMO. In addition, only LE rats displayed longer latencies due to IMO+C than IMO exposure. The lack of significant difference in LE rats for day 2 was caused by higher variations of latencies in IMO+C group.

Our results as well as data previously published (8) may be interpreted by different activity of hypothalamic-pituitary-adrenal (HPA) axis activity in the used strains. Due to the lower responsiveness of HPA axis to stressors in LE rats (4,6,16,23), we may assume differential levels of CRH, ACTH and corticosterone in comparison with WI rats; however, no direct comparison of stress hormones levels between these two strains was performed.

The study of stressor-specific response of various stressors on cognitive functions by different behavioural tests seems to be important. In our previous work (14) performed in the passive avoidance device, we found the strong amnesic effect of IMO+C, while the relatively weak impairment of the performance induced by both stressors was only observed in the Y-maze. Among possible explanations for the observed effects of both IMO and IMO+C stressors, we may consider the robustness of the well learned, by the foot-shock motivated and by motor memory supported, escape response in the Y-maze. The finding that both stressors produced only short and transient delay in the escape responses poses a question, to what extent the memory dependent processes were affected. The animal, after being placed in the device, has to detect the known environment by comparing it with the stored information that would enable him to select behaviours relevant for the situation. In

the stressed animals, we may consider changes in arousal to be induced with ensuing disturbances of attention and perception, which can be reflected in a short hesitation before escaping to the safe compartment. Another source of the delayed escape response may be caused by physical impairment, e.g. impaired joint and muscle function as well as general motor coordination (24).

The stressor consisting of 1 h IMO or IMO+C can influence the performance of animals in the Y-maze not only by direct effects on learning and memory processes. For example, the stressors could impair the sensorimotor abilities and decrease the attention or the associability of the perceptual stimuli of the device. In this test, we must also consider the emergence of fear related behaviour, like freezing (2,7,17,22).

The observed behavioural changes due to action of IMO or IMO+C are most probably related to stress hormones of HPA axis; their levels depend on the type of the used stressor and on the duration of its exposure (4,23, 25,29). In our previous studies with Wistar rats, we found differential response of plasma ACTH to the action of IMO and IMO+C stressors (12). As oxytocin has been shown to participate in the response of organism to stress stimuli and to influence learning and memory processes (27), we determined also this hormone after IMO and IMO+C in order to disclose a possible differential response to these stressors. In the unpublished results we found that IMO+C induced significantly higher levels of oxytocin that IMO alone. Also all these findings indicate a differential response of rats to the two stressors, however, the elucidation of the causal relationship between hormone levels and behaviour of rats requires more experimental data.

In summary, our results investigating the effects of two types of stressors, IMO and IMO+C, on rat behaviour in the Y-maze indicate that the used restraint stressors did not affect the long-term memory; rather a transient impairment of the retrieval phase of memory processing can be considered. These results are in large contrast to more pronounced effects in the passive avoidance task (11,14). Further, the differences in response of Lewis and Wistar rats may be interpreted by different activity of hypothalamic-pituitary-adrenal axis activity in the used strains.

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