ORIGINAL ARTICLE

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

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Summary: Our previous findings suggested the existence of stressor-specific behavioural and cognitive responses in rats. In the present study, restraint stressor (immobilization, IMO) and restraint stressor combined with partial immersion of rats into water (IMO+C) were applied for 1 hour to Wistar male rats and their spontaneous behaviour was examined in the open field test. The classic behavioural parameters were recorded: crossing, rearing, and resting. When tested 1 and 4 hours after IMO+C, animals exhibited strong suppression of locomotor and exploratory activity (crossing and rearing); partial inhibition of both behavioural variables was found after IMO. Thus, substantial differences were observed in dependence on the length of period between the end of stressor application and the start of testing. In testing performed one week later, the locomotor and exploratory activity levels of both IMO and IMO+C animals corresponded to the control ones. These data suggest a differential behavioural response to both used stressors that may result from their different proportion of psychical and physical components. In conclusion, our results provide other data for the support of differential effects of two types of restraint stressors on spontaneous behaviour of animals exposed to a novel environment.

Key words: *Open field test; Restraint stressor; Restraint/cold stressor; Spontaneous behaviour; Rat; Stress*

Introduction

Marked advances in stress research have been reached in the last two decades. At present, stress is defined as state of threatened homeostasis leading to adaptive responses (1,2,9), and stress response can either be generalized and non-specific or specific to various stressors (13,14). The existence of the latter one changes the original Selye's doctrine of non-specificity and offers many new avenues of stress research.

In our previous studies, stress-induced changes in cognitive functions of rats were examined using the passive avoidance task (4,5,6) and the active learning task (18). The findings suggest the existence of stressor-specific behavioural and cognitive responses; restraint (immobilization, IMO) and restraint combined with partial immersion of rats into water (IMO+C) suppressed differentially the learning and memory response. Besides, when the rats were exposed to IMO and IMO+C, stress hormones, namely ACTH and corticosterone, responded differentially (6).

Differential responses to various stressors, such as IMO and IMO+C, may be explained by prevailing psychological or physical component of their action (12,14). The stressors could impair the sensorimotor abilities and decrease the attention or the associability of the perceptual stimuli of the testing device; the occurrence of fear related behaviour has to be taken into account too. Therefore, we examined the effect of two types of restraint stressors, IMO and IMO+C, on the spontaneous behavioural response of Wistar rats introduced into a novel and unfamiliar environment of an open field arena. In order to determine whether the stressors induce longer lasting effects, we performed the behavioural testing twice: (a) shortly after the restraint exposure, and (b) one week later without exposure to the stressor.

Methods

Animals

Wistar male rats (Velaz, Czech Republic) with the average body weight of 260±19 g were used. Animals were housed four per cage and maintained on a 12 h light/12 h dark phase (light on at 6 a.m.), at a constant temperature (21±1 °C) and relative humidity (50–70%). Experiments were performed in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals (DHEW Publication, NHI 80–23).

Stress procedure

Wistar rats were exposed to two types of acute restraint stressors (6,7,8). IMO stressor was applied by fixing front and hind legs of the rat with adhesive plaster; then the ani-

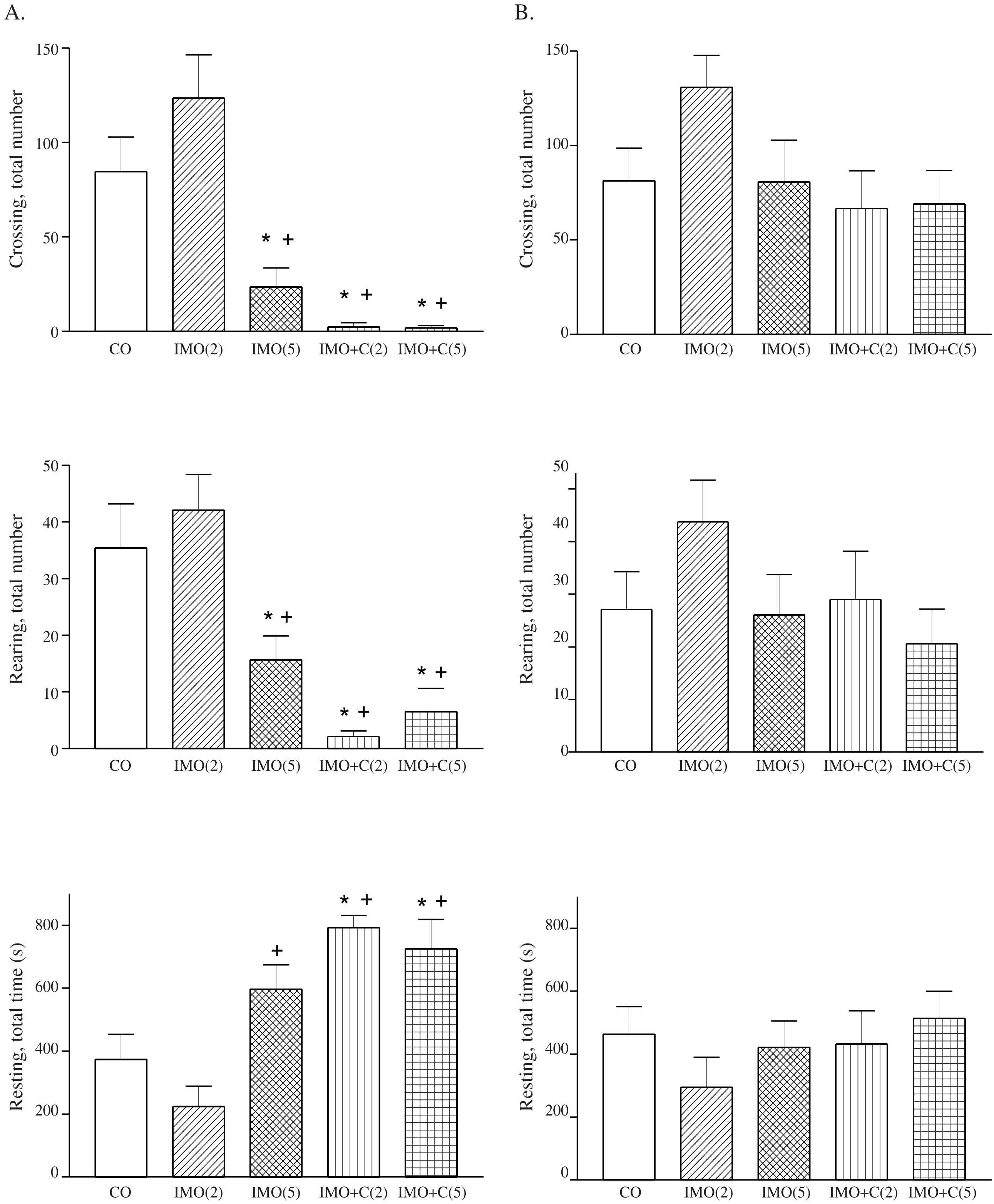

Fig. 1: The effect of IMO and IMO+C on the behavioural performance of male rats in the open field test. A. First testing: shortly after exposure to stressors; B. Second testing: one week later. Given mean values ± S.E.M. Statistical significance obtained from Bonferroni's post test for $p<0.05$: *versus control group, $^{+}$versus IMO(2).

mal was restrained in a snug-fitting 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 a combination of restraint with water immersion, the restrained rats were further immersed in the water bath (22 °C) in such a way that the upper 1/4 of the animal was outside of water; the physical component is probably more expressed in this stress. After the exposure to either of the stressors for one hour, the animals spent another one or four hours in the home cage. Then, the first part of behavioural testing started; the interval of the start of stressor application, related to the beginning of behavioural testing, is given in the parentheses of experimental groups, like IMO(2) or IMO+C(5) – i.e. IMO+C started 5 and finished 4 hours before the start of testing. The used animals were divided into five groups: (a) control rats (n = 8) received no treatment; (b) IMO(2) (n = 7), (c) IMO(5) (n = 7), (d) IMO+C(2) (n = 7), and (e) IMO+C(5) (n = 6). After a week during which the rats were left undisturbed in their home cages, the second part of the experiment was performed with the same animals, however, without application of stressors prior testing.

Open field test

Behavioural testing was performed between 8 a.m. and 1 p.m. Open field arena (60 x 60 cm) surrounded by transparent Plexiglas walls 40–cm high was located in a dimly illuminated room. The floor of the arena was divided into 16 equal squares (15 x 15 cm) by black lines. Each rat was gently placed on the right rear corner of the arena and allowed to explore the arena for 15 min. The following behavioural parameters were recorded: (a) crossing – horizontal locomotor activity expressed by the number of sectors crossed, (b) rearing – vertical exploratory activity expressed by the number of rearing on hind limbs, very often against the wall of the arena, and (c) resting – the total time (s) spent in sitting or lying (e.g. 3,11).

Data analysis

A one-way analysis of variance (ANOVA) followed by the Bonferroni's method was used to compare the data on the spontaneous behavioural variables. Always, statistical significance was accepted when $P \leq 0.05$.

Results

Fig. 1 depicts the effect of both IMO and IMO+C on behavioural parameters measured. In the first testing (Fig. 1A), the overall analysis revealed significant differences: for crossing, $P<0.001$; for rearing, $P<0.001$; for resting, $P<0.001$. When compared with the control group, there was a significant reduction of the total number of crossings in animals subjected to IMO(5), IMO+C(2) and IMO+C(5); these groups were also significantly different from IMO(2) group. Further, the rats exposed to IMO+C exhibited the lowest level of crossings. As to the total number of rearing, similar differences among groups were observed. Concerning the total time spent in resting, the animals exposed to both IMO+C stress conditions exhibited the highest values. Also value of IMO(5) was significantly higher than in IMO(2). No difference between both IMO groups and the controls was found.

In the second testing (Fig. 1B), the overall analysis revealed no significant difference among groups: for crossing, $P=0.14$; for rearing, $P=0.34$; for resting, $P=0.51$.

Discussion

In contrast to minor differences between the effects of IMO and IMO+C on the performance of rats in the Y-maze task (18), the open field experiments showed a strong suppression of the horizontal locomotor and vertical exploratory behaviour after IMO+C and a weaker effect of the immobilization per se. The exposure to IMO+C terminating both 4 and 1 h before the open field test practically abolished all locomotor and rearing activities. While IMO did not produce any decrease in the crossing and rearing 1 h after the cessation of stressor exposure, a deficit in horizontal but not vertical activity emerged 4 h after stress termination. The marked difference between the two stressors terminating 1 h before the start of open field test may be caused by the physical component of the stressor imposed by exposure of rats to water (22° C cold). On the other hand, the difference between the behavioural responses of both IMO treated groups may be related to the long-term consequences of restraint stressors. For example, rats exposed to cold and restraint stress ceased traversing the radial maze after visiting two or three arms and the performance deficit persisted for further 24 h (17). In contrast, in the open field test stressed rats (restraint, forced swim stress, and inescapable foot shock) did not exhibit differences in spontaneous behaviour (12).

In the second behavioural testing performed without application of stressor after 7 days after the first one, no differences among the groups were found: animals of all groups displayed a comparable locomotor and exploratory activity. This finding indicates that the decreased locomotor and exploratory behaviour one hour or several hours following application of the stressor was an immediate and short-term effect and no contextual conditioning occurred. In searching for factors underlying the low locomotor and exploratory response to novelty induced by IMO+C and partly by IMO(5), we must consider the already mentioned physical insult. The reduced exploratory activity may be related to freezing as a passive defensive reaction. Rats show a general tendency to respond to stressful conditions in an inactive way that is with freezing and immobility (10,11,15). Novel environment represents a stressful event; open field exposure evoked an increase in plasma levels of ACTH and corticosterone in rats (16). Therefore, it is conceivable that the open field exposure, a stressful event per se, too mild to evoke a prolonged freezing reaction in the non-stressed animals, could act as a strong stressful stimulus in animals sensitised by the previous stressor exposure.

In conclusion, the open field experiments showed the strong suppression of spontaneous horizontal locomotor and vertical exploratory behaviour after IMO+C and the weaker or no effect after IMO itself. The exposure to IMO+C, terminating 1 or 4 hours before the open field test, practically abolished all motor activities. Our results provide other data for the support of differential effects of two types of restraint stressors on spontaneous behaviour of rats exposed to novel environment.

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