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Sex differences in the long-lasting effects of a single exposure to immobilization stress in rats

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ABSTRACT

In male rats, a single exposure to a severe stressor such as immobilization (IMO) results in marked activation of the HPA axis and reduction of body weight gain. In addition, the HPA response to the same (homotypic) stressor is reduced, whereas the response to a different (heterotypic) stressor is enhanced for days. Although sex differences in the responsiveness of the HPA axis have been described, there are few studies about the influence of sex on long-lasting effects of stress. Thus, we have compared the consequences of a single exposure to IMO in male and female rats. Females showed a similar ACTH response to the first IMO associated with higher corticosterone, but they were more resistant than males to stress-induced loss of body weight. Unstressed females showed higher resting levels of ACTH and corticosterone, but they did not show the increase in the resting levels of HPA hormones observed in males on the day after IMO. During exposure to a different stressor (open-field) two days after IMO, enhanced corticosterone response and hypoactivity was observed in males, but not in females. Finally, a second exposure to IMO 8 days after the first one resulted in a reduction of the HPA response and of the negative impact on body weight as compared to the first exposure, and this protective effect was greater in females. In sum, IMO-exposed females showed a greater reduction of the response to a second IMO and appear to be more resistant than males to some of the negative impacts of IMO.

Introduction

In the last decades numerous reports have demonstrated that a single exposure to certain stressors can induce long-lasting changes in anxiety-like behavior as well as in the physiological and behavioral responses to further stressors (Armario et al., 2008). Focusing on the hypothalamus–pituitary–adrenal (HPA) axis, the best characterized biological marker of stress, it has been demonstrated that a single exposure to a severe stressor such as immobilization on boards (IMO) is able to induce a long-lasting reduction of the response of the HPA axis to the same (homotypic) stressor that can be noted both peripherally and in the paraventricular nucleus of the hypothalamus (PVN), the key brain area for the control of the HPA axis (Martí et al., 2001; Vallès et al., 2003). This phenomenon has been observed not only with predominantly emotional stressors such as IMO, but also with a systemic stressor such as endotoxin at a high dose (Vallès et al., 2002). Interestingly, when stressors differing in intensity, as evaluated by the area under the curve of the response (during the stressor and the post-stress period) of classical stress markers, including plasma levels of ACTH, corticosterone, prolactin and glucose (Armario et al., 2012), have been compared, the greater the intensity the greater the reduction of the HPA response to the homotypic stressor (Armario et al., 2004; Martí et al., 2001). This property differs from the characteristics of habituation (Armario et al., 2004), the process assumed to underlie adaptation of the HPA axis to daily repeated stressors (Grissom and Bhatnagar, 2009).

Regardless of whether or not the reduction of the HPA response to the homotypic stressor fits with habituation, it is clear that this phenomenon involves some kind of learning process as the HPA response to different (heterotypic) stressors is increased rather than reduced (Belda et al., 2008, 2012; Gagliano et al., 2008; Martí et al., 2001; Muñoz-Abellán et al., 2008), suggesting a non-specific sensitization of the HPA axis. In fact, this HPA sensitization has also been consistently found after exposure to a session of tail-shocks typical of the learned helplessness paradigm developed by Maier and colleagues (Johnson et al., 2002; O'Connor et al., 2003). The latter authors demonstrated that this peripheral sensitization was associated with enhanced activation of the PVN (O'Connor et al., 2004) and it appears to involve a certain resistance to negative glucocorticoid feedback (O'Connor et al., 2003).

The above described effects of a single exposure to stress appear to be quite consistent, but surprisingly, all data have been obtained in

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males. Therefore, it is unclear whether females behave differently regarding long-lasting effects of a single exposure to stress on both homotypic reduction of the HPA response and heterotypic sensitization of the HPA axis. This is particularly important because some of the effects of a single exposure to severe stressors may be relevant for post-traumatic stress disorder (PTSD) and women appear to be more sensitive than men to this pathology (see Cohen and Yehuda, 2011 for a review). Our available results in male rats indicate that a single IMO exposure induces a long-lasting impairment of spatial memory in the Morris water maze (Andero et al., 2012), fear extinction (Andero et al., 2011) and an increase in the acoustic startle response (Fuentes et al., 2014), changes that mimics those reported in PTSD patients (Yehuda and LeDoux, 2007). Thus, the purpose of the present study was to compare the response of male and female rats to an acute severe stressor (IMO) in terms of: (i) HPA response to the first and a second exposure to IMO; (ii) the impact of IMO on resting HPA activity and the HPA and behavioral response to a heterotypic stressor (a novel environment); and (iii) IMO-induced changes in body weight gain, which is related to food intake and both parameters are sensitive to both the intensity of stressors (Márquez et al., 2002; Vallès et al., 2000) and previous experience with a particular stressor (Dal-Zotto et al., 2004).

Materials and methods

Animals

Sprague–Dawley rats (22 males and 22 females) obtained from the breeding center of the Universitat Autònoma de Barcelona, of about 75 days old (body weight of about 439 ± 3 and 266 ± 3 g, respectively) at the beginning of the experiments, were used. They were individually housed, in polypropylene opaque wire-topped cages with solid bottom (21.5 × 46.5 × 14.5 cm; Type '1000 cm2', Panlab S.L.U., Barcelona, Spain) containing sawdust bedding (Ultrasorb, Panlab, S.L.U.). Rats were maintained in standard temperature conditions (21 ± 1 °C) on a 12-h light/12-h dark schedule (lights on at 08:00 h). They were fed with Diet 'A04' (Safe-Panlab S.L.U) and food and filtered water were available ad libitum. Males and females were in the same room with no other experiments going on. The experimental protocol was approved by the Committee of Ethics of the Universitat Autònoma de Barcelona, following the "Principles of laboratory animal care" and was carried out in accordance with the European Communities Council Directive (86/609/EEC).

General procedure

The experimental procedures were always done in the morning and can be seen in Fig. 1. All animals were handled three times (every other day) for approximately 2 min a day. On the last day of handling, blood samples were taken under basal conditions by tail-nick to habituate the animals to the procedure. Two rats were sampled simultaneously (two experimenters were sampling at the same time and a third was gently holding the two rats). The tail-nick consisted of gently wrapping the animals with a cloth, making a 2 mm incision at the end of one of the tail veins and then massaging the tail to collect 300 μl of blood, within 2 min, into ice-cold EDTA capillary tubes (Sarsted, Granollers, Spain). This procedure is extensively used in our lab and by others because very low resting levels of hormones are obtained under appropriate conditions (Belda et al., 2004; Vahl et al., 2005). From the blood samples, adrenocorticotropic hormone (ACTH) and corticosterone were measured. Blood samples were always obtained between 09:00 and 13:00 h.

Before the beginning of the experiment, the animals were divided in four groups: 1) M-C (male-control, n = 10), 2) M-IMO (male-IMO, n = 12), 3) F-C (female-control, n = 10) and 4) F-IMO (female-IMO, n = 12). IMO animals were immobilized during 1 h by taping their four limbs to metal mounts attached to a board (Gagliano et al., 2008). Head movements were restricted with two plastic pieces (7 cm × 6 cm) and the body was subjected to the board by means of a piece of plastic cloth (10 cm wide) attached with velcro which surrounded all the trunk. On day 1, M-IMO and F-IMO groups were immobilized and blood samples

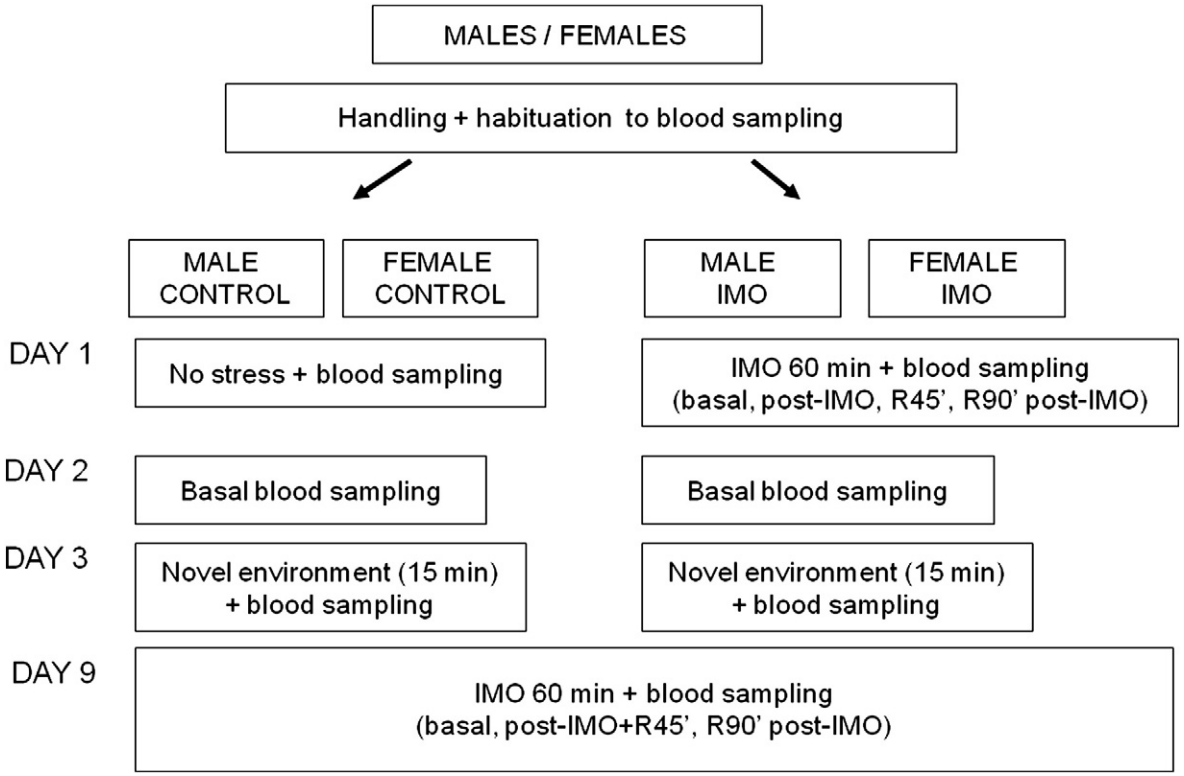

Fig. 1. Summary of the experimental procedures. IMO: immobilization; R45′ and R90′: recovery times 45 and 90 min after the end of the IMO.