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Responsiveness of the hypothalamic–pituitary–adrenal axis to different novel environments is a consistent individual trait in adult male outbred rats

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Summary Susceptibility to some stress-induced pathologies may be strongly related to individual differences in the responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis to stressors. However, there have been few attempts in rodents to study the reliability of the individual differences in the responsiveness of the HPA to stressors and the relationship to resting corticosterone levels. In the present work, we used a normal population of Sprague–Dawley rats, with a within-subject design. Our objectives were to study: (a) the reliability of the ACTH and corticosterone response to three different novel environments widely used in psychopharmacology and (b) the relationship between stress levels of HPA hormones and the daily pattern of corticosterone secretion (six samples over a 24-h-period). Animals were repeatedly sampled using tail-nick procedure. The novel environments were the elevated plus-maze, the hole-board and the circular corridor. Animals were sampled just after 15 min exposure to the tests and again at 15 and 30 min after the termination of exposure to them (post-tests). The hormonal levels just after the tests indicate that the hole-board seems to be more stressful than the circular corridor and the elevated plus-maze, the latter being characterized by the lowest defecation rate. Correlational analysis revealed that daily pattern of resting plasma corticosterone levels did not correlate to HPA responsiveness to the tests, suggesting no relationship between resting and stress levels of HPA hormones. In contrast, the present study demonstrates, for the first time, a good within-subject reliability of the ACTH and corticosterone responses to the three environments, suggesting that HPA responsiveness to these kind of stressors is a consistent individual trait in adult rats, despite differences in the physical characteristics of the novel environments.

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1. Introduction

It is well-accepted in humans that there are major individual differences in susceptibility to psychiatric pathologies and stress-related pathological processes. Unfortunately, our knowledge of the biological bases of such susceptibility is hampered by obvious limitations of conducting research in humans. Thus, characterization of individual differences in animals (i.e. rodents) may help to find the origin of susceptibility in humans. To date, neurobiological bases of individual differences in rodents have been mainly focused on the hypothalamic–pituitary–adrenal (HPA) axis, one of the prototypical stress systems, and its relationship to behavioral traits such as fear/anxiety and novelty-seeking (attraction for novelty) (Dellu et al., 1996; Kabbaj et al., 2000; Landgraf and Wigger, 2003; Steimer and Driscoll, 2003). This interest relies on the major role of the final hormones of the HPA axis, glucocorticoids, in stress-induced pathologies.

However, before establishing a putative relationship between HPA responsiveness to stress and particular behavioral traits, it is important to know whether or not we can reliably evaluate individual differences in responsiveness to stress so that we can define hypo or hyperresponsive phenotypes. In humans, a low to good within subject reliability of the response of the HPA axis to stress has been found (for a review, see Cohen and Hamrick, 2003). However, it is surprising that, to our knowledge, there is no study on this topic in rodents despite the vast literature dealing with the relationship between behavioral traits (i.e. anxiety) and HPA responsiveness. The lack of a consistent individual HPA response to stressors could, at least in part, explain the controversial results regarding HPA activity and behavioral traits (Abel, 1991; Brush, 1991; Dellu et al., 1996; Kabbaj et al., 2000; Landgraf and Wigger, 2003; Steimer and Driscoll, 2003).

Exposure to novel environments is a common procedure used to evaluate some behavioral traits in rodents. The behavior of animals in such environments is the result of the interaction of several factors, including activity, motivation to explore and fear/anxiety. Since exposure to novel environments generates fear/anxiety in animals and is stressful as evaluated by the activation of the HPA axis (Pellow et al., 1985; Misslin and Cigrang, 1986; Appenrodt et al., 1999), to appropriately define the influence of fear/anxiety in the behavior of animals in these environments it is important to characterize the degree of stress experienced by them. Circulating levels of ACTH and corticosterone, the two peripheral hormones of the HPA axis, are, under appropriate conditions, positively related to the intensity of the stressors (Hennessy and Levine, 1978; Armario et al., 1986; De Boer et al., 1990) and therefore can give us objective information about the stressful properties of the different tests. However, because of the approximately 15 min delay between ACTH release and the subsequent optimum activation of the adrenal cortex, and because of the saturation of adrenal capability to synthesize corticosteroids with moderate levels of ACTH (Keller-wood et al., 1981), it is important to follow circulating levels of the two hormones for a time after the termination of stress to avoid apparent discrepancies between the two hormones.

On the basis of the above, the aim of the present work was to study resting levels of corticosterone over a 24-h-period and the HPA response of adult male Sprague–Dawley rats to 15 min of exposure to various novel environments, with the purposes of characterizing: (a) the degree of stress elicited by some novel environments commonly used to characterize behavioral traits in rodents; (b) the reliability of individual differences in the stress response of the HPA axis elicited by such novel environments; and (c) the relationship between resting and stress levels of HPA hormones.

2. Methods

2.1. Animals and general procedure

Eighteen male Sprague–Dawley rats from the breeding centre of the Universitat Autònoma de Barcelona, 55 days old at the beginning of the experiment, were used. They were housed two per cage in standard conditions of temperature (22 °C ± 1) and on a 12–12 h light–dark schedule (lights on at 7 am). Food and water were provided *ad libitum*. Cages were cleaned twice a week. The experimental protocol was approved by the committee of Ethics of the Universitat Autònoma de Barcelona and was carried out in accordance the European Communities Council Directive (86/609/EEC).

During the second week after their arrival, all animals were handled three times (every other day) and then, blood samples were taken under basal conditions by tail-nick to obtain resting hormone levels and habituate the animals to the sampling procedure. Eight days later, exposure to three different novel environments started. Over 6 days, all rats were exposed to hole-board, elevated plus-maze and circular corridor for 15 min. One rat from each cage was tested on one day and the other one