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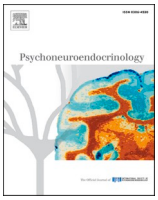

Individual differences in the neuroendocrine response of male rats to emotional stressors are not trait-like and strongly depend on the intensity of the stressors

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ABSTRACT

Biological response to stressors is critical to understand stress-related pathologies and vulnerability to psychiatric diseases. It is assumed that we can identify trait-like characteristics in biological responsiveness by testing subjects in a particular stressful situation, but there is scarce information on this issue. We then studied, in a normal outbred population of adult male rats (n = 32), the response of well-characterized stress markers (ACTH, corticosterone and prolactin) to different types of stressors: two novel environments (open-field, OF1 and OF2), an elevated platform (EP), forced swim (SWIM) and immobilization (IMO). Based on both plasma ACTH and prolactin levels, the OF1 was the lowest intensity situation, followed by the OF2 and the EP, then SWIM and finally IMO. When correlations between the individual responses to the different stressors were studied, the magnitude of the correlations was most dependent on the similarities in intensity rather than on other characteristics of stressors, with good correlations between similar intensity stressors and no correlations at all were found between stressors markedly differing in intensity. In two additional confirmatory experiments (n = 37 and n = 20) with HPA hormones, we observed good correlation between the response to restraint and IMO, which were close in intensity, and no correlation between OF1 and SWIM. The present results suggest that individual neuroendocrine response to a particular stressor does not predict the response to another stressor greatly differing in intensity, thus precluding characterization of low or high responsive individuals to any stressor in a normal population. The present data have important implications for human studies.

1. Introduction

Exposure to stress has been associated to a wide range of pathologies, including immune suppression, anxiety, depression and susceptibility to drug addiction. Nevertheless, it is acknowledged that the particular consequences of exposure to stress are markedly dependent on genetic or environmentally acquired individual differences in susceptibility. It is thus critical to characterize such individual differences in vulnerability as a means to predict the detrimental impact of stress and prevent, if possible, exposure to severe stressors or its consequences.

Although stress alters numerous physiological systems, the most extensively studied biological variables are those associated with the activation of the hypothalamic-pituitary-adrenal (HPA) axis (e.g. plasma levels of ACTH and glucocorticoids) and the autonomic nervous system (ANS), mainly the sympathetic branch (Armario et al., 2020). ANS activation is mainly reflected in the release of adrenaline from the adrenal medulla and that of noradrenaline from the adrenal medulla and sympathetic terminals, together with important cardiovascular (CV) changes. Other well-studied physiological functions under stress are the endocrine axis of the anterior pituitary (e.g. prolactin) and the immune system. It is reasonable to assume that individual differences in susceptibility to stress-related pathologies are linked to differences in the

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biological response to stressors, particularly in those systems whose response is related to the intensity and duration of stressors (Charmandari et al. 2005; Armario et al., 2020).

However, before establishing a putative relationship between the HPA or other stress responsive systems and particular behavioral traits or individual differences in susceptibility to stress, it is important to know whether individual differences in biological responsiveness to stress are reliable, particularly regarding stressors having a major emotional component. The study of the consistency of individual differences in response to stress has two main steps. This first one is to know whether the response to a particular type of stressors is reliable when measured in more than two occasions, days, months or years later. The second is whether low or high responsiveness is maintained when exposed to stressors differing in nature or intensity. More precisely, whether or not we can actually define universal stress hypo- or hyper-responsive phenotypes based on the data obtained from specific stress situations.

In humans, reliability of the neuroendocrine, cardiovascular and immune response to stressors has attracted great interest, although results are not conclusive particularly when comparing different situations on different days (Parati et al., 1988; Cohen et al., 2000; Halpern et al., 2002; Hankin et al., 2015). A more recent study showed a good correlation in salivary cortisol response to real-life academic examination and the laboratory trier social stress test (Henze et al., 2017), although the two stressors were likely to be of similar intensity. Surprisingly, there are to our knowledge very scarce studies on this topic in rodents despite the vast literature dealing with the relationship between behavioral traits and endocrine responsiveness. In a normal population of adult outbred male rats, individual differences in the catecholamine response to immobilization (Taylor et al., 1989) or the corticosterone response to a same novel environment (Cavigelli et al., 2009) appeared to be quite stable when assessed several months apart. Similarly, we have previously reported good correlations in ACTH, corticosterone and prolactin responsiveness to different novel environments (circular corridor, elevated plus maze and hole-board), which represent stressors of similar nature and relatively low intensity (Márquez et al., 2005; 2006). However, there is no evidence that such correlation might be maintained after exposure to stressors differing in nature and intensity.

If there is no consistent individual HPA response across different types of emotional stressors and that response is critically dependent on the particular stressful situation, this inconsistency could at least in part explain the controversial results regarding HPA activity and particular behavioral traits (Armario and Nadal, 2013). Therefore, the aim of the present work was to characterize the correlation of individual differences in the response of adult male rats to a set of predominantly stressors chosen because there is evidence that they greatly differ in nature and intensity on the basis of well-known biological indexes, including HPA hormones, prolactin and food intake (Belda et al., 2016; Márquez et al., 2002; Pace et al., 2005; Rabasa et al., 2015; Rotllant et al.,2007), all of them good markers of stressor intensity in animals and humans (Armario et al., 2020).

2. Materials and methods

2.1. Animals and general procedure

Male Sprague–Dawley rats obtained from the breeding centre of the Universitat Autònoma de Barcelona were used. They were 2 months-old at the beginning of the experiments. The animals were housed in pairs under standard conditions of temperature (21 ± 1 °C) in a 12:12 h light/dark schedule (lights on at 07:00 h), with food and water ad libitum. The experimental protocol was approved by the Committee of Ethics of the Universitat Autònoma de Barcelona and by the Generalitat de Catalunya and was carried out in accordance to the European Communities Council Directive (2010/63/EU) and Spanish legislation (BOE53-2013).

Starting at least two days after their arrival to the facility, all animals were handled at least three times on different days for approximately 2 min a day. In addition, one blood sample was taken by tail-nick as described previously (Belda et al., 2004), in order to habituate the animals to the procedure. Tail-nick is extensively used in our lab and others because very low resting levels of hormones are obtained under appropriate conditions (Belda et al., 2004; Vahl et al., 2005). All experimental procedures were done in the morning. Cage-mates were sampled simultaneously (two experimenters were sampling at the same time and a third was gently holding the two rats). Blood was centrifuged at 4930× *g* (15 min, 4 °C), and plasma was frozen (−20 °C) until assay. Animals were assigned at random to the different experimental groups in function of their date of birth and body weight.

2.2. Experimental designs (Fig. 1)

2.2.1. Main experiment (Exp. 1)

Rats were assigned to control (n = 10) and stress (n = 32) groups. The stress group rats were sequentially exposed to various stressors for 20 min: open-field 1 (OF1) on day 1, elevated platform (EP) on day 4, forced swim (SWIM) on day 8, OF2 on day 11 and immobilization on boards (IMO) on day 15. Controls rats were only exposed to OF2 on day 11. Both control and stress rats were blood sampled immediately after the stressors or after being taken from their home-cages if not stressed. In the particular case of IMO, rats were again sampled 30 and 60 min after the termination of the stressor (R30 and R60), because this is a severe stressor and the overall HPA response is better evaluated following the post-stress period (García et al., 2000; Márquez et al., 2002).

The OF1 consisted of a grey rectangular box (56 × 36 × 31 cm) placed in a room with dim light. The EP consisted of a non-protected white small platform (15 × 15 cm) 100 cm above the floor. The OF2 consisted of a rectangular box (68 × 56 × 42 cm) with a white floor and black walls, placed in a room with high intensity light. OFs and EP were cleaned carefully between animals with a tap water solution containing ethanol (5% v/v).

SWIM was done in a transparent cylindrical tanks (height: 40 cm, internal diameter: 19 cm) with 24 cm of water (36–37 °C) and water was changed between animals (Rabasa et al., 2015).

IMO rats were immobilized on boards as previously described (Rabasa et al., 2015). Rats were restrained in a prone position by attaching their four limbs to metal mounts with adhesive tape. Head movements were restricted by means of two metal loops around the neck, 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.

2.2.2. Complementary experiments

In Exp. 2, thirty-seven rats were exposed for 15 min to the OF1 and two days later to SWIM. In Exp. 3 twenty rats were firstly exposed to 30 min restraint in tubes and 12 days later to 30 min IMO. Plexiglas cylindrical restrainers (WPI, UK, Ref. STR554) were used, measuring 6 cm in diameter and 21.5 cm in length, with several holes in the walls of the cylinder to provide fresh air (Rabasa et al. 2015).

2.3. Hormone analysis

Plasma ACTH and corticosterone levels were determined by double-antibody radioimmunoassay (RIA) following our general procedures (Muñoz-Abellán et al., 2011). In brief, ACTH RIA used ^{125}I-ACTH (PerkinElmer Life Science, Boston, USA) as the tracer, rat synthetic $ACTH_{1–39}$ (Sigma, Barcelona, Spain) as the standard and an antibody raised against rat ACTH (rb7) kindly provided by Dr. W.C. Engeland (Department of Surgery, University of Minnesota, Minneapolis, USA). The characteristics of the antibody have been described previously (Engeland et al., 1989) and we followed a non-equilibrium procedure. Corticosterone RIA used ^{125}I-corticosterone-carboximethyloxime-tyrosine-methylester (ICN-Biolink

2000, Barcelona, Spain), synthetic corticosterone (Sigma, Barcelona, Spain) as the standard and an antibody raised in rabbits against corticosterone–carboximethyloxime-BSA kindly provided by Dr. G. Makara (Institute of Experimental Medicine, Budapest, Hungary). The characteristics of the antibody and the basic RIA procedure have been described previously (Zelena et al., 2003) and we followed an equilibrium procedure. Prolactin was determined by RIA using ^{125}I-prolactin (NEN, Boston, MA, USA) as the tracer, rat prolactin (rat PRL-RP-3) as the standard and an antibody raised against rat prolactin (anti-rPRL-S-9), kindly provided by Dr. A. F. Parlow (NIDDK National Hormone and Peptide Program, CA, USA). All samples were run in the same assay to avoid inter-assay variability. The intra-assay coefficient of variation was 3.3% for ACTH, 7.8% for corticosterone and 4% for prolactin. The sensitivity of the assay was 25 pg/ml for ACTH, 2 ng/ml for corticosterone and 0.5 ng/ml for prolactin. Samples were run at least in duplicates. No data lower than the minimum detection level of the assay was found.

2.4. Statistical analysis

Data were analyzed by means of the Statistical Program for Social Sciences (SPSS-IBM for Windows, version 24, Armonk, NY, IBM Corporation). Hormonal data were log-transformed to achieve normality (Shapiro-Wilk). A repeated-measures analysis of variance was used (within-subjects factor: time or type of stressor), followed by additional pair-wise comparisons. In other cases, when only two observations were compared, *t*-tests for independent o dependent means were performed. Pearson coefficient (two-tailed) was used to assess correlations between the different hormones. The area under the curve (AUC) for each animal was calculated with Graph Pad Prism 5 (GraphPad Software, La Jolla, CA, USA). ACTH and corticosterone concentrations (pg/ml and ng/ml, respectively) were plotted in the y-axis versus time (minutes) in the x-axis. The area is computed connecting a straight line between every set of adjacent points defining the curve, and calculating the area beneath these lines. The criterion for significance was set at $p < 0.05$. Data are available upon request.

3. Results

In Exp. 1, the repeated-measures ANOVAs revealed significant effect of the type of stressor for ACTH [$F(4, 124) = 287.6$, $p < 0.001$], corticosterone [$F(4, 124) = 38.2$, $p < 0.001$] and prolactin [$F(4, 124) = 158.8$, $p < 0.001$]. Further pair-wise comparisons (detailed statistical differences can be seen in Fig. 2) showed that the order of intensity of the stressors in terms of ACTH was OF1 < OF2 = EP < SWIM < IMO. The same pattern was observed with corticosterone, except that corticosterone levels after IMO were lower than after SWIM. Finally, prolactin follows the same pattern as ACTH, although the response to the EP was a bit lower than that to OF2. Accordingly with the overall response the stressors were ordered in Figures as follows: OF1, OF2, EP, SWIM and IMO.

In response to the OF2, no differences were observed between the control group (only exposed to blood-sampling) and the stress group (previously exposed to OF1, EP and SWIM), suggesting that prior stress experience did not alter the response to the new novel environment (Fig. 3). As expected, exposure to IMO resulted in a HPA response that still persisted 60 min after the termination of IMO (Fig. 4).

Correlations of the hormonal responses between the various stressors can be seen in Table 1. A heat-map representing correlations are presented as Supplementary Fig. S1. The AUCs of ACTH and corticosterone responses to IMO are also included to rule out that a ceiling effect could have determined the lack of correlations. The pattern of correlations was similar, although not identical, for the three hormones: moderate to good between stressors of similar intensity and poor between stressors greatly differing in intensity. When correlations between ACTH and corticosterone were calculated for each particular stressor, they were as follows: OF1 ($r = 0.80$, $p < 0.001$), EP ($r = 0.62$, $p < 0.001$), OF2 ($r = 0.62$, $p < 0.001$), SWIM ($r = -0.25$, NS), IMO-post ($r = 0.36$, $p = 0.02$) and IMO-AUCs ($r = 0.51$, $p = 0.003$). Correlations between HPA hormones and prolactin for each particular stressors only yielded significance regarding ACTH and prolactin after IMO ($r = 0.38$, $p = 0.03$).

Classical studies on individual differences classified animals in two or more groups in function of a given variable and studied the consequences on other variables. Although we expected that information given by this approach will not essentially differ that derived from the correlations, we tested this classifying rats in low ($n = 10$), intermediate ($n = 12$) and high responders ($n = 10$) for each variable and the two stressors most differing in intensity (OF1 and IMO). The results were in accordance with the correlational data and can be seen in the Supplementary Table S1.

In Exp. 2, ACTH and corticosterone responses to the OF1 (Fig. 5A) were much lower than to SWIM [ACTH: $t(36) = 17.9$, $p < 0.001$; corticosterone: $t(36) = 10.1$, $p < 0.001$], and no correlation between the response to the two stressors was found for ACTH [$r(35) = +0.16$], although for corticosterone it approached significance [$r(35) = +0.32$, $p = 0.056$].

In Exp. 3, ACTH response to restraint was lower than that to IMO [$t(19) = 4.8$, $p < 0.001$] and a good correlation was found [$r(18) = +$

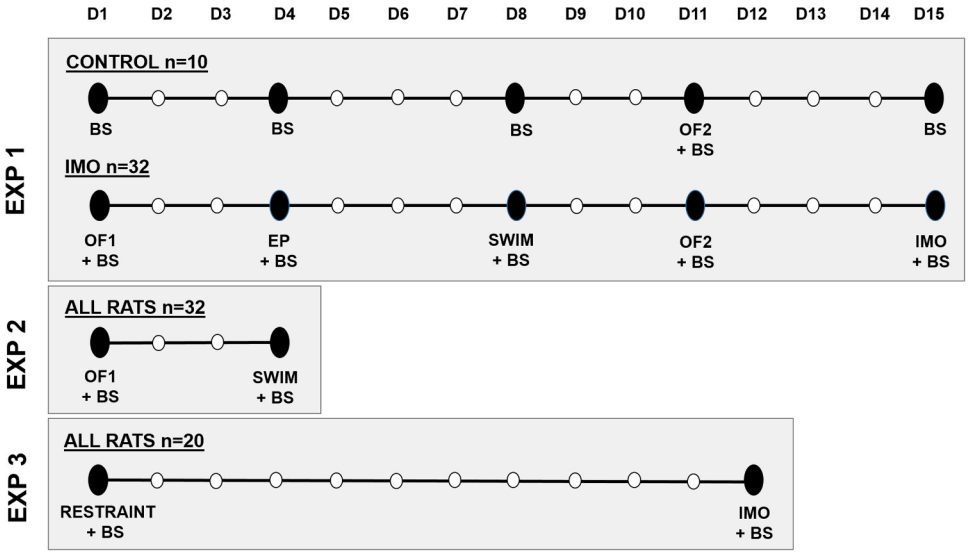

Fig. 1. Study design in Experiments (EXP) 1, 2 and 3. Male Sprague-Dawley adult rats were exposed to each stressor on the indicated days (D) and blood sampled (BS) to analyze hormone response. The duration of the stressors was 20 min in EXP1, 15 min in EXP2 and 30 min in EXP3. The stressors were two different open-fields (OF1, OF2), elevated platform (EP), forced swim (SWIM), restraint in tubes (RESTRAINT) and immobilization on boards (IMO). Additional details in the Materials and methods Section.

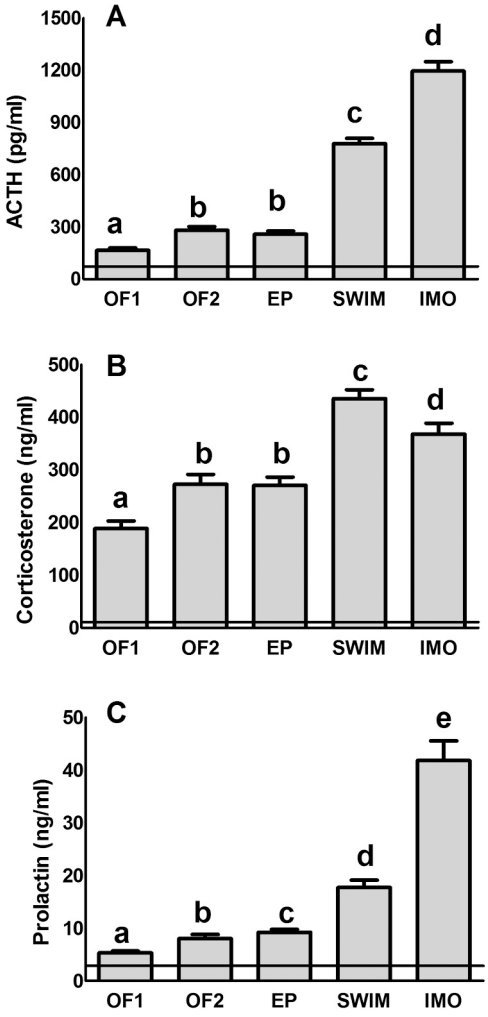

Fig. 2. Neuroendocrine response to different stressors (20 min exposure). Means and SEM (n = 32) of plasma levels of ACTH (A), corticosterone (B) and prolactin (C) are represented. Bars with different letters are statistically different. Horizontal lines indicates average basal values obtained in controls rats sampling in parallel. The stressors used were open-field-1 (OF1), open-field 2 (OF-2), elevated platform (EP), forced swim (SWIM) and immobilization (IMO).

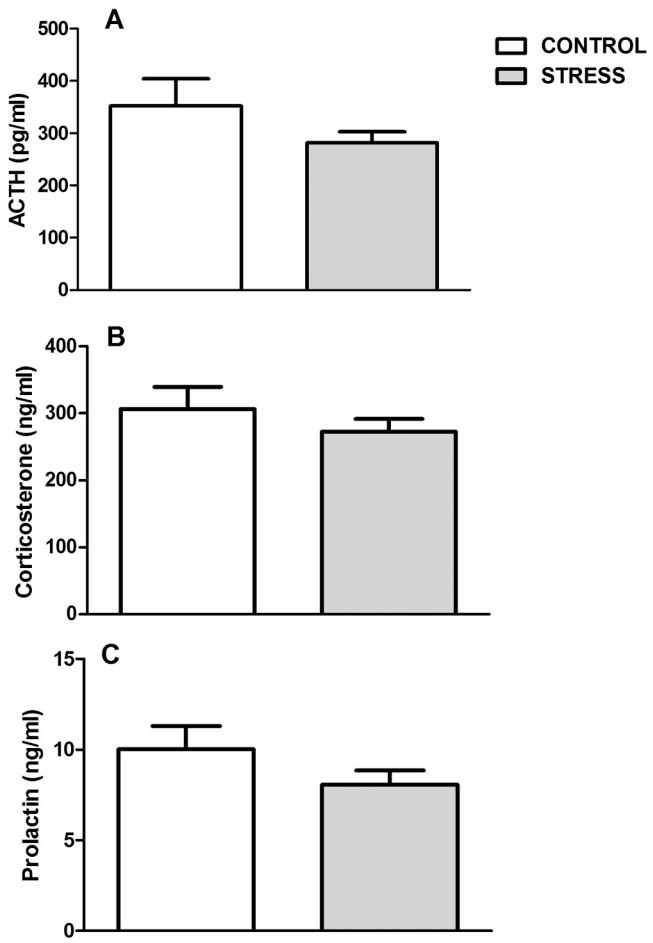

Fig. 3. Comparison of the neuroendocrine response to the second open-field (OF2) in stress-naïve (control, n = 10) and previously stressed (n = 32) rats. Means and SEM of plasma levels of hormones are represented (ACTH in (A), corticosterone in (B) and prolactin in (C)). No significant group differences were found.

0.64, p = 0.002] between the two stressors (Fig. 5B). Corticosterone response to the two stressors was similar, with a good correlation between the stressors [r(18) = +0.54, p = 0.013].

4. Discussion

The present data indicate that we cannot characterize trait-like stress hypo- or hyper-responsive subjects in a normal population of adult male rats in terms of HPA and prolactin response. Instead, individual differences in responsiveness appear to be markedly dependent on the intensity of the stressor chosen to evaluate them.

When the same animals were exposed to different types of stressors, all of them having a strong emotional component, we observed (as expected), that the lowest ACTH and prolactin response corresponded to the small OF1 and the highest one to IMO. The hormonal response to the EP was similar to that of the OF2 for ACTH and corticosterone, but a bit higher for prolactin, although the hormonal responses to the EP and OF2 were clearly lower than SWIM. ACTH and prolactin give rise to a quite similar order of classification of the stressors, thus supporting previous studies demonstrating that the two hormones have been found to be good markers of such an intensity (Armario et al. 2012, 2020). Corticosterone followed a similar pattern, but, surprisingly, corticosterone levels just after the stressor were higher after SWIM than after IMO despite lower ACTH response to the former stressor.

We expected similar corticosterone levels after SWIM and IMO, as the ACTH levels achieved with both were enough to saturate the adrenal cortex (Keller-Wood et al., 1983). We have no clear explanation for this result that has nevertheless been replicated in another study from our lab (unpublished data). Interestingly, whereas significant positive correlations between ACTH and corticosterone were found with all stressors, including IMO (particularly AUCs), a non-significant (negative) correlation was found after SWIM, suggesting that some factor specifically associated with this stressor modulates the adrenal response to ACTH. SWIM at 36 °C did not change body temperature so that a putative factor could be muscular activity associated to swim, which might modulate the adrenal responsiveness to ACTH. We are not aware of any experimental data directly supporting this possibility, but chronic voluntary running wheel exercise in male rats has been found to alter corticosterone response to acute stressors independently of ACTH levels (Droste et al., 2007). Interestingly, the direction of the changes in stress-induced corticosterone in exercise rats with respect to controls were opposite in response to novel environment and to forced swim, suggesting that the modulatory role of extra-ACTH factors were dependent of the type of stressor. Evidence for an extra-ACTH regulation of the adrenal cortex has been accumulating over the years since the pioneering research of Dallman`s laboratory with the study of the circadian rhythm of HPA hormones (Dallman et al., 1978; Engeland

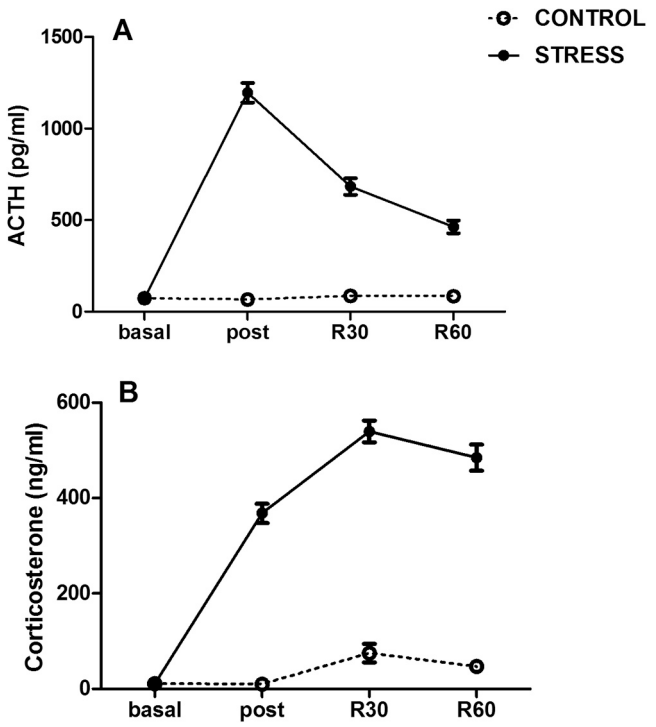

Fig. 4. The dynamics of the response to IMO as compared to that of unstressed animals. Means and SEM of plasma levels of hormones are represented (control n = 10; IMO n = 32). Samples were taken just after 20 min IMO (post) and at 30 and 60 min after the termination of IMO (R30 and R60, respectively). Basal values are the average of various previous sampling days (control group). Differences between control and IMO rats were always $p < 0.001$ and are not indicated.

et al., 1977) and has also been found comparing inbred rat strains (Gómez and Lahmame, 1996). It is unlikely that the lower ACTH levels after SWIM than IMO reflects a greater negative glucocorticoid feedback rather than a lower intensity of the former stressor. When comparing both, the impact of IMO in terms of the post-stress recovery time, impact on food intake in the next 24 h and heterotypic HPA sensitization is stronger (Belda et al., 2016; Rabasa et al., 2015).

To avoid carry over effects of prior exposure to other stressors, we exposed the animals first to presumably low intensity stressors (OF1, EP), then to SWIM and finally to the most severe stressor (IMO). We also included in the study two different novel environments, OF1 and OF2, which represent qualitatively similar situations. As exposure to the OF2 was done after exposure to SWIM, we wanted to rule out that such response was altered by prior experience of the rats with the other stressors by comparing stress-experienced rats with a group of control (stress-naive) rats. No differences between the groups were observed, indicating that the response to the OF2 was not altered by the prior history of stress. Behavioral response to the OF2 was also not affected by prior stress experience (not shown).

On the basis of the overall endocrine data, we can assume that the ACTH and prolactin responses, and corticosterone with some limitations, appear to similarly classify the predominantly emotional stressors used in the present study in terms of intensity. We are aware of the difficulty of objectively determining the intensity of an emotional stressor and consequently a high degree of circularity is involved when using the biological response to assign a level of intensity to a stressor. However, we consider that this is a reasonable approach considering that all parameters that has been found to be sensitive to intensity change show always the same pattern in an important number of experimental studies (see Armario et al., 2020).

Regarding individual consistency across stressors, with plasma ACTH levels, the highest correlations were between the OF1 and the OF2, and between OF2 and EP, whereas that between the OF1 and the EP was statistically significant but lower. The OF1 and the OF2 represent qualitatively similar situations involving novelty and potential danger as well as free active and exploratory behavior. Nevertheless, the higher response to the OF2 suggests that the latter was more aversive than the OF1, probably because the latter was smaller and done under dim light conditions. The EP represents a potential risk for predation and falling out, and there is no possibility for free activity of the animals. Therefore, the EP has important differences with the OF2, but both were of similar intensity. These data tentatively suggest that being of similar magnitude and sharing certain characteristics are important to elicit a similar ACTH response. This hypothesis is supported by the correlations that included the two most severe stressors: (i) correlations of the low intensity stressors with SWIM and IMO were low and non-significant; (ii) correlations between the latter two stressors was moderate. Correlations of corticosterone did not parallel ACTH and were better between stressors of intermediate intensity (EP, OF2, SWIM), which poorly correlated with those of low intensity (OF1) or high intensity (IMO). When the correlation of the AUCs of the ACTH and corticosterone response to IMO with

Table 1
Correlations of the response of ACTH, corticosterone and prolactin to the different stressors.

Stressor	Hormone	OF1	OF2	EP	SWIM	IMOpost	IMO-AUC
OF1	ACTH	–	+0.65***	+0.42*	+0.22	+0.25	−0.02
	Corticosterone	–	+0.32#	+0.14	−0.02	−0.10	−0.07
	Prolactin	–	+0.52**	+0.38*	+0.13	+0.01	NA
OF2	ACTH		–	+0.64***	+0.27	+0.15	+0.02
	Corticosterone		–	+0.65***	+0.45***	+0.28	+0.34#
	Prolactin		–	+0.53**	+0.62**	+0.29	NA
EP	ACTH			–	+0.18	+0.14	+0.18
	Corticosterone			–	+0.56**	+0.21	+0.16
	Prolactin			–	+0.51***	+0.25	NA
SWIM	ACTH				–	+0.33#	−0.02
	Corticosterone				–	+0.69***	+0.68***
	Prolactin				–	+0.58***	NA
IMOpost	ACTH					–	+0.56***
	Corticosterone					–	+0.85***
	Prolactin					–	NA

Pearson coefficient correlations (two-tailed) are indicated (n = 32). ACTH: adrenocorticotropic hormone; AUC: area under the curve; EP: elevated platform; IMO: immobilization; OF1: open field 1; OF2: open-field 2.

* At least $p < 0.05$.

** At least $p < 0.01$.

*** At least $p < 0.001$.

Marginal; NA: non-applicable.

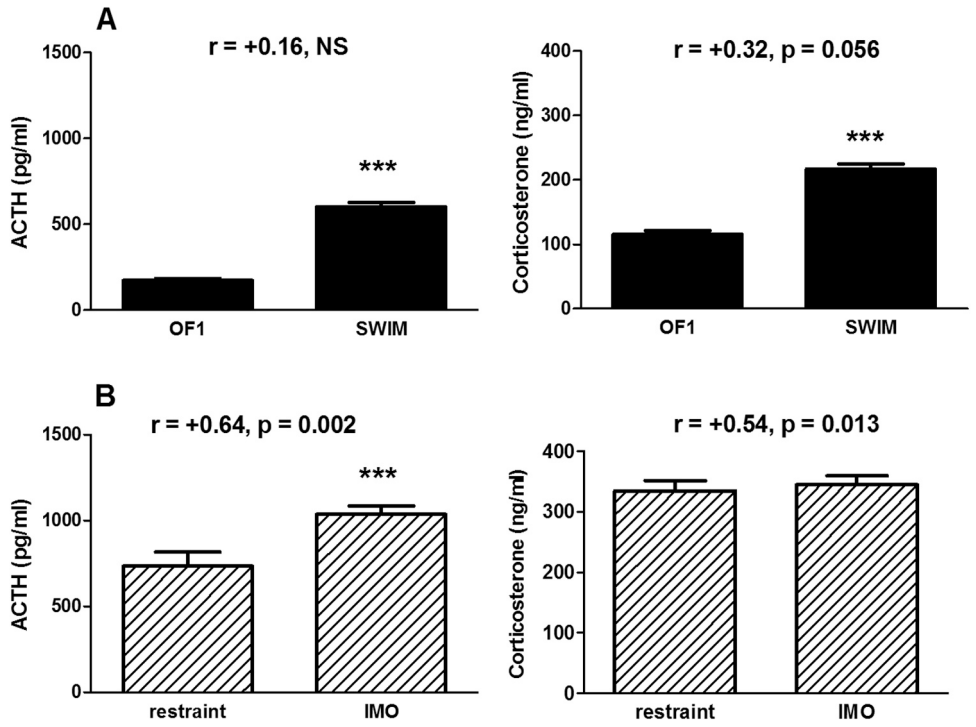

Fig. 5. Comparison of the neuroendocrine response of the same rats to two different stressors. Means and SEM of plasma levels of hormones are represented: in (A) (n = 37) the ACTH and corticosterone responses to an open-field (OF1) and SWIM; in (B) (n = 20) the ACTH and corticosterone responses to restraint and immobilization (IMO). Correlations between the two stressors are also indicated. ***$p < 0.001$ versus the other stressor.

the other stressors was introduced, no improvement was found, suggesting that is a characteristic of the stressor rather than a problem of catching the integrated response to the situation. Prolactin followed the same pattern as ACTH, although correlations were in general higher than those of ACTH. Therefore, the overall results suggest that the endocrine response to stressor markedly differing in intensity poorly correlates and this is not restricted to a particular endocrine system as were obtained with ACTH and prolactin, two independent neuroendocrine system.

To demonstrate that the results can be consistent across studies we calculated, in two independent experiments, the correlation of the response of HPA hormones after exposure to OF1 vs SWIM (two days apart), and after exposure to restraint vs IMO (12 days apart). The conclusions were similar to the main experiment: no correlation between stressors greatly differing in intensity (OF1 vs SWIM) and good correlation between those close in intensity (restraint vs IMO). This consistency was found despite certain differences in the duration of stressors between the experiments: 20 min (Exp. 1), 15 min (Exp. 2) and 30 min (Exp. 3). Therefore, minor differences in the procedure cannot affect the conclusions.

We have previously reported good correlations between the response of HPA hormones and prolactin across different novel environments (e.g. elevated plus-maze, hole-board), but in those studies the endocrine response was very similar in all environments (Márquez et al., 2005; 2006). Therefore, the latter results are compatible with the suggestion that good correlations are observed when the situations are similar in magnitude and also qualitatively.

To our knowledge, there is no previous similar study in normal populations of rats, but some results obtained in rats genetically selected for anxiety give support to the present conclusions. For instance, in rats genetically selected for low or high avoidance in a shuttle-box, presumably related to high vs low anxiety, respectively, differences in HPA and prolactin response has been observed but only in response to low intensity but not high intensity stressors (Gentsch et al., 1982). Also, rat lines selected for anxiety behavior in the elevated plus maze (LAB and HAB rats) did not show consistent differences in the HPA response to stressors as they are markedly dependent on the type of stressor (Liebsch et al. 1998; Landgraf et al. 1999; Frank et al. 2006). More consistent response to different types of stressors are expected if animals have been genetically selected on the basis of their neuroendocrine response to stressors. This has been done regarding corticosterone response to restraint stress in mice that resulted in low, intermediate and high responsive lines (Touma et al., 2008). However, only two stressors have been tested (restraint vs open field plus forced swim), which showed a similar pattern (Mattos et al., 2013). Interestingly, in the mouse lines discussed above (Knapman et al., 2010; Touma et al., 2008) as well as in rat lines also genetically selected for the corticosterone response to stressors (Walker et al., 2017; Walker and Sandi, 2018), differences in coping as well as aggressiveness were also observed, although the direction of the changes were not concordant.

The present data bear important implications regarding characterization of putative phenotypes differing in responsiveness to stress in normal populations of animals and humans in that they indicate that we cannot identify individuals characterized by a generalized hypo or hyper-responsiveness to stressors. The results clearly illustrate the importance of paying attention to the intensity of stressors, but they do not rule out that behavioral traits such as coping style could interact with qualitative aspects of stressors (e.g. controllability and unpredictability) to determine the biological response (Koolhaas et al., 2010). In this regard, availability of coping (presence of bedding material allowing burying) has been found to alter the plasma corticosterone and catecholamine response to an electrified prod in the cage (De Boer et al., 1990). It would be extremely interesting to study how coping style interacts with certain characteristics of stressors.

Although considerable attention has been paid in human research to study reliability of the biological response to stressors, most of it has focused on repetition of the same situation/task or tasks of similar intensity (see for instance Carroll et al., 1984; Parati et al., 1988; Kirschbaum et al., 1995; Cohen et al., 2000; Hawkley et al., 2001; Burleson et al., 2003; Henze et al., 2017; Bachmann et al., 2019), and studies

specifically comparing stressor markedly differing in intensity are lacking. This is of major relevance when we want to know whether the endocrine response to laboratory stressors, usually of low intensity, are related to the vulnerability to severe stressors (i.e. development of post-traumatic stress disorder). Although we are aware of ethical concerns, information regarding the response to real-life (non-provoked), relatively severe stressors can contribute to shed lights to this issue in humans.

In conclusion, the present data indicate that testing individual differences in neuroendocrine responsiveness to a particular, predominantly emotional, stressor does not predict individual differences in the response to other emotional stressors markedly differing in intensity. Therefore, we cannot identify individuals characterized by a consistent and generalized hypo- or hyper-responsiveness to any type of emotional stress on the basis of results obtained with a particular situation. Intensity, rather than qualitative aspects of stressors, appear to be critical to detect consistent individual differences. Clearly studies in humans dealing with this problem are needed.

Ethical statement

The experimental protocol was approved by the Committee of Ethics of the Universitat Autònoma de Barcelona and by the Generalitat de Catalunya and was carried out in accordance to the European Communities Council Directive (2010/63/EU) and Spanish legislation (BOE53-2013).

CRediT authorship contribution statement

AA obtained funding, designed the experiments, analyzed and interpreted the data and wrote the draft of the manuscript; RN obtained funding, contributed to the design, performed statistical analysis and participated in the elaboration of the manuscript; MG-S did the great part of the experimental work of the main experiment in collaboration with MS-O; HG and XB did the complementary experiments; MG-S, HG and XB did the hormone analyses.

Declaration of Competing Interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105127.

References

Armario, A., Nadal, R., 2013. Individual differences and the characterization of animal models of psychopathology: a strong challenge and a good opportunity. Front. Pharm. 4, 137.

Armario, A., Daviu, N., Muñoz-Abellán, C., Rabasa, C., Fuentes, S., Belda, X., Gagliano, H., Nadal, R., 2012. What can we know from pituitary-adrenal hormones about the nature and consequences of exposure to emotional stressors? Cell Mol. Neurobiol. 32 (5), 749–758.

Armario, A., Labad, J., Nadal, R., 2020. Focusing attention on biological markers of acute stressor intensity: empirical evidence and limitations. Neurosci. Biobehav. Rev. 111, 95–103.

Bachmann, P., Finke, J.B., Rebeck, D., Zhang, X., Larra, M.F., Koch, K.P., Turner, J.D., Schäschinger, H., 2019. Test-retest reproducibility of a combined physical and cognitive stressor. Biol. Psychol. 148, 107729.

Belda, X., Márquez, C., Armario, A., 2004. Long-term effects of a single exposure to stress in adult rats on behavior and hypothalamic-pituitary-adrenal responsiveness: comparison of two outbred rat strains. Behav. Brain Res. 154 (2), 399–408.

Belda, X., Nadal, R., Armario, A., 2016. Critical features of acute stress-induced cross-sensitization identified through the hypothalamic-pituitary-adrenal axis output. Sci. Rep. 6, 31244.

Burleson, M.H., Poehlmann, K.M., Hawkley, L.C., Ernst, J.M., Berntson, G.G., Malarkey, W.B., Kiecolt-Glaser, J.K., Glaser, R., Cacioppo, J.T., 2003. Neuroendocrine and cardiovascular reactivity to stress in mid-aged and older women: long-term temporal consistency of individual differences. Psychophysiology 40 (3), 358–369.

Carroll, D., Turner, J.R., Lee, H.J., Stephenson, J., 1984. Temporal consistency of individual differences in cardiac response to a video game. Biol. Psychol. 19 (2), 81–93.

Cavigelli, S.A., Ragan, C.M., Michael, K.C., Kovacsics, C.E., Bruscke, A.P., 2009. Stable behavioral inhibition and glucocorticoid production as predictors of longevity. Physiol. Behav. 98 (1–2), 205–214.

Charmandari, E., Tsigos, C., Chrousos, G., 2005. Endocrinology of the stress response. Annu Rev. Physiol. 67, 259–284.

Cohen, S., Hamrick, N., Rodriguez, M.S., Feldman, P.J., Rabin, B.S., Manuck, S.B., 2000. The stability of and intercorrelations among cardiovascular, immune, endocrine, and psychological reactivity. Ann. Behav. Med. 22 (3), 171–179.

Dallman, M.F., Engeland, W.C., Rose, J.C., Wilkinson, C.W., Shinsako, J., Siedenburg, F., 1978. Nycthemeral rhythm in adrenal responsiveness to ACTH. Am. J. Physiol. 235 (5), R210–R218.

De Boer, S.F., Slangen, J.L., Van der Gugten, J., 1990. Plasma catecholamine and corticosterone levels during active and passive shock-prod avoidance behavior in rats: effects of chlordiazepoxide. Physiol. Behav. 47 (6), 1089–1098.

Droste, S.K., Chandramohan, Y., Hill, L.E., Linhorst, A.C.E., Reul, J.M.H., 2007. Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. Neuroendocrinology 86 (1), 26–32.

Engeland, W.C., Shinsako, J., Winget, C.M., Vernikos-Danellis, J., Dallman, M.F., 1977. Circadian patterns of stress-induced ACTH secretion are modified by corticosterone responses. Endocrinology 100 (1), 138–147.

Engeland, W.C., Miller, P., Gann, D.S., 1989. Dissociation between changes in plasma bioactive and immunoreactive adrenocorticotropin after hemorrhage in awake dogs. Endocrinology 124 (6), 2978–2985.

Frank, E., Salchner, P., Aldag, J.M., Salomé, N., Singewald, N., Landgraf, R., Wigger, A., 2006. Genetic predisposition to anxiety-related behavior determines coping style, neuroendocrine responses, and neuronal activation during social defeat. Behav. Neurosci. 120 (1), 60–71.

García, A., Martí, O., Vallès, A., Dal-Zotto, S., Armario, A., 2000. Recovery of the hypothalamic-pituitary-adrenal response to stress. Effect of stress intensity, stress duration and previous stress exposure. Neuroendocrinology 72 (2), 114–125.

Gentsch, C., Lichtsteiner, M., Driscoll, P., Feer, H., 1982. Differential hormonal and physiological responses to stress in Roman high- and low-avoidance rats. Physiol. Behav. 28 (2), 259–263.

Gómez, F., Lahmame, A., De Kloet, E.R., Armario, A., 1996. Hypothalamic-pituitary-adrenal response to chronic stress in five inbred rat strains: differential responses are mainly located at the adrenocortical level. Neuroendocrinology 63 (4), 327–337.

Halpern, C.T., Campbell, B., Agnew, C.R., Thompson, V., Udry, J.R., 2002. Associations between stress reactivity and sexual and nonsexual risk taking in young adult human males. Horm. Behav. 42 (4), 387–398.

Hankin, B.L., Badanes, L.S., Smolen, A., Young, J.F., 2015. Cortisol reactivity to stress among youth: stability over time and genetic variants for stress sensitivity. J. Abnorm. Psychol. 124 (1), 54–67.

Hawkley, L.C., Burleson, M.H., Poehlmann, K.M., Berntson, G.G., Malarkey, W.B., Cacioppo, J.T., 2001. Cardiovascular and endocrine reactivity in older females: intertask consistency. Psychophysiology 38 (6), 863–872.

Henze, G.I., Zänkert, S., Urschler, D.F., Hiltl, T.J., Kudielka, B.M., Pruessner, J.C., Wüst, S., 2017. Testing the ecological validity of the Trier Social Stress Test: association with real-life exam stress. Psychoneuroendocrinology 75, 52–55.

Keller-Wood, M.E., Shinsako, J., Dallman, M.F., 1983. Integral as well as proportional adrenal responses to ACTH. Am. J. Physiol. 245 (1), R53–R59.

Kirschbaum, C., Prüssner, J.C., Stone, A.A., Federenko, I., Gaab, J., Lintz, D., Schommer, N., Hellhammer, D.H., 1995. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. Psychosom. Med. 57 (5), 468–474.

Knapman, A., Heinzmann, J.M., Hellweg, R., Holsboer, F., Landgraf, R., Touma, C., 2010. Increased stress reactivity is associated with cognitive deficits and decreased hippocampal brain-derived neurotrophic factor in a mouse model of affective disorders. J. Psychiatr. Res. 44 (9), 566–575.

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., Buwalda, B., 2010. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Front. Neuroendocrinol. 31 (3), 307–321.

Landgraf, R., Wigger, A., Holsboer, F., Neumann, I.D., 1999. Hyper-reactive hypothalamo-pituitary-adrenocortical axis in rats bred for high anxiety-related behaviour. J. Neuroendocrinol. 11 (6), 405–407.

Liebsch, G., Linthorst, A.C., Neumann, I.D., Reul, J.M., Holsboer, F., Landgraf, R., 1998. Behavioral, physiological, and neuroendocrine stress responses and differential

sensitivity to diazepam in two Wistar rat lines selectively bred for high- and low-anxiety-related behavior. Neuropsychopharmacology 19 (5), 381–396.
Márquez, C., Belda, X., Armario, A., 2002. Post-stress recovery of pituitary-adrenal hormones and glucose, but not the response during exposure to the stressor, is a marker of stress intensity in highly stressful situations. Brain Res. 926 (1–2), 181–185.
Márquez, C., Nadal, R., Armario, A., 2005. Responsiveness of the hypothalamic-pituitary-adrenal axis to different novel environments is a consistent individual trait in adult male outbred rats. Psychoneuroendocrinology 30 (2), 179–187.
Márquez, C., Nadal, R., Armario, A., 2006. Influence of reactivity to novelty and anxiety on hypothalamic-pituitary-adrenal and prolactin responses to two different novel environments in adult male rats. Behav. Brain Res. 168 (1), 13–22.
Mattos, G.E., Heinzmann, J.M., Norkowski, S., Helbling, J.C., Minni, A.M., Moisan, M.P., Touma, C., 2013. Corticosteroid-binding globulin contributes to the neuroendocrine phenotype of mice selected for extremes in stress reactivity. J. Endocrinol. 219 (3), 217–229.
Muñoz-Abellán, C., Rabasa, C., Daviu, N., Nadal, R., Armario, A., 2011. Behavioral and endocrine consequences of simultaneous exposure to two different stressors in rats: interaction or independence? PLoS One 6 (6), e21426.
Pace, T.W.W., Gaylord, R., Topczewski, F., Girotti, M., Rubin, B., Spencer, R.L., 2005. Immediate-early gene induction in hippocampus and cortex as a result of novel experience is not directly related to the stressfulness of that experience. Eur. J. Neurosci. 22 (7), 1679–1690.
Parati, G., Pomidossi, G., Casadei, R., Ravogli, A., Groppelli, A., Cesana, B., Mancia, G., 1988. Comparison of the cardiovascular effects of different laboratory stressors and their relationship with blood pressure variability. J. Hypertens. 6 (6), 481–488.
Rabasa, C., Gagliano, H., Pastor-Ciurana, J., Fuentes, S., Belda, X., Nadal, R., Armario, A., 2015. Adaptation of the hypothalamus-pituitary-adrenal axis to daily repeated stress does not follow the rules of habituation: a new perspective. Neurosci. Biobehav. Rev. 56, 35–49.
Rotllant, D., Nadal, R., Armario, A., 2007. Differential effects of stress and amphetamine administration on Fos-like protein expression in corticotropin releasing factor-neurons of the rat brain. Dev. Neurobiol. 67 (6), 702–714, 702-214.
Taylor, J., Weyers, P., Harris, N., Vogel, W.H., 1989. The plasma catecholamine stress response is characteristic for a given animal over a one-year period. Physiol. Behav. 46 (5), 853–856.
Touma, C., Bunck, M., Glasl, L., Nussbaumer, M., Palme, R., Stein, H., Wolferstätter, M., Zeh, R., Zimbelmann, M., Holsboer, F., Landgraf, R., 2008. Mice selected for high versus low stress reactivity: a new animal model for affective disorders. Psychoneuroendocrinology 33 (6), 839–862.
Vahl, T.P., Ulrich-Lai, Y.M., Ostrander, M.M., Dolgas, C.M., Elfers, E.E., Seeley, R.J., D'Alessio, D.A., Herman, J.P., 2005. Comparative analysis of ACTH and corticosterone sampling methods in rats. Am. J. Physiol. Endocrinol. Metab. 289 (5), E823–E828.
Walker, S.E., Sandi, C., 2018. Long-term programing of psychopathology-like behaviors in male rats by peripubertal stress depends on individual's glucocorticoid responsiveness to stress. Stress 21 (5), 433–442.
Walker, S.E., Zanoletti, O., Guillot de Suduiraut, I., Sandi, C., 2017. Constitutive differences in glucocorticoid responsiveness to stress are related to variation in aggression and anxiety-related behaviors. Psychoneuroendocrinology 81, 1–10.
Zelena, D., Mergl, Z., Foldes, A., Kovács, K.J., Tóth, Z., Makara, G.B., 2003. Role of hypothalamic inputs in maintaining pituitary-adrenal responsiveness in repeated restraint. Am. J. Physiol. Endocrinol. Metab. 285 (5), E1110–E1117.