Behavioral sexual dimorphism in models of anxiety and depression due to changes in HPA axis activity

Nikolaos Kokras\textsuperscript{a,b}, Christina Dalla\textsuperscript{a}, Antonios C. Sideris\textsuperscript{a}, Artemis Dendi\textsuperscript{a}, Hudu G. Mikail\textsuperscript{a}, Katerina Antoniou\textsuperscript{c}, Zeta Papadopoulou-Daifoti\textsuperscript{a,d}\textsuperscript{*}

\textsuperscript{a}Department of Pharmacology, Medical School, University of Athens, 75 Mikras Asias Street, 11527 Goudi, Athens, Greece
\textsuperscript{b}First Department of Psychiatry, Eginition Hospital, Medical School, University of Athens, Greece
\textsuperscript{c}Department of Pharmacology, Medical School, University of Ioannina, Greece

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\textbf{A B S T R A C T}

Anxiety and depression are considered as stress-related disorders, which present considerable sex differentiation. In animal models of anxiety and depression sex differences have been described and linked to the sexually dimorphic hypothalamus–pituitary–adrenals (HPA) axis. The present study aimed to adjust corticosterone, the main HPA axis stress hormone, in male and female adrenalectomized rats with oral (25 μg/ml) corticosterone replacement (ADXR). Subsequently we investigated the behavioral performance of ADXR rats in the open field, light/dark and forced swim test (FST). Male ADXR rats showed less anxiety-like behavior when compared to sham-operated controls, despite adequate corticosterone replacement. They further showed increased swimming and reduced climbing behavior in the FST, while immobility duration did not differ from sham-operated males. On the contrary, adrenalectomy and corticosterone replacement did not have significant effects on the female behavioral response. Females were generally more active and presented less anxiety-like behavior than males; while they exhibited higher depressive-like symptomatology in the FST. ADXR affected behavioral responses predominantly in males, which in turn modified sex differences in the behavioral profile. Females in proestrous and estrous did not differ from females in diestrous and metestrus in any measured behavioral response. Present results suggest that the male and not the female behavioral responses in models of anxiety and depression were mainly affected by ADXR. These findings may play a significant role in explaining the differential coping strategy of the two sexes in response to stressful experiences.

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1. Introduction

It is now well-established that stress is crucially related to the pathophysiology of affective disorders (Goel and Bale, 2009; Oitzl et al., 2010; Young and Korszun, 2010), while the sex differentiation in stress response attracts particular attention since women are more vulnerable to anxiety and depression (Alonso et al., 2004; Kessler et al., 1994; Young and Korszun, 2010). The physiological stress response in both sexes involves a complex neurocircuitry which ultimately results into the hypothalamus–pituitary–adrenals (HPA) axis releasing glucocorticoids to the systemic blood stream (Riedemann et al., 2010). Numerous studies have established that in females the HPA axis shows a higher baseline tone and during the stress response, release of glucocorticoids is more rapid and intense, while de-escalation of the HPA axis drive is slower (Galea et al., 1997; Kitay, 1961). Earlier observations in humans discovered the link between glucocorticoids and affective states because patients with elevated glucocorticoids develop depressive-like symptomatology and inversely, depressed patients show impairments in HPA axis function (Holsober and Ising, 2010; Ising et al., 2005). Furthermore, previous human studies have shown that glucocorticoids levels also correlate well with anxiety disorders (Vreeburg et al., 2010), that depression correlates more with glucocorticoids when co-morbid anxiety is present (Vreeburg et al., 2009) and that glucocorticoid levels are influenced by gonadal hormones (Lederbogen et al., 2010). In addition, it was previously shown that in depression the HPA axis dysregulation differs in men and women (Young and Ribeiro, 2006) and antidepressant treatment affects the HPA axis activity and stress.

Abbreviations: ADXR, Adrenalectomy with Corticosterone Replacement; ANOVA, Analysis of Variance; CRH, Corticotropin releasing hormone; D, Diestrous; E, Estrous; FST, Forced Swim Test; HPA, Hypothalamus–pituitary–adrenals; M, Metestrus; P, Proestrous.

* Corresponding author. Tel.: +30 210 7462702; fax: +30 210 7462554.
E-mail address: zdaifoti@med.uoa.gr (Z. Papadopoulou-Daifoti).

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response in a sex-dependent manner (Binder et al., 2009; Stewart and Roper, 2008).

Animal models are widely used to study the neurobiology of depression and anxiety as well as the mechanism of action of candidate and established treatments (Cryan et al., 2002; Dalla et al., 2011; Palanza, 2001). The open field and the light/dark box have been extensively used to assess anxiety, based on the aversion of rodents for open and illuminated spaces (Belzung and Griebel, 2001; Merlo Pich and Samanin, 1989). On the other hand, the Forced Swim Test (FST) has been extensively used to assess antidepressant potential and it can successfully differentiate serotonergic and noradrenergic behavioral responses (Cryan et al., 2002, 2005). However most research is performed on male animals and relatively less effort has been devoted into validating and investigating models of anxiety and depression for females (Becker et al., 2007; Dalla et al., 2011; Palanza, 2001). Indeed, our recent studies showed an increased female vulnerability to the detrimental effect of stress on mood- and anxiety-related behaviors along with serotonergic, dopaminergic and glutamatergic differentiation at baseline and following antidepressant treatment (Dalla et al., 2005, 2008; Drossopoulou et al., 2004; Kokras et al., 2008b; Pitychoulis et al., 2009).

Moreover, we recently showed that antidepressant treatment can result in convergent behavioral responses from both sexes; however the magnitude of such behavioral response depends on the sex of the animal (Dalla et al., 2010; Kokras et al., 2009a, 2011, in press). The human analogy in anxiety and depression would be of women presenting a differential loading of symptoms than men (Marcus et al., 2008), and although treatment generally alleviates the disease in both sexes, the therapeutic response often appears of different efficacy between the two sexes (Kornstein et al., 2000). Besides other contributing factors, involving pharmacodynamics and pharmacokinetics (Kokras et al., 2011), it is also possible that the apparent difference in antidepressant response is due to the reported differences in baseline anxiety and depressive symptoms between men and women (Frank et al., 1988; Marcus et al., 2008, 2005). Finally, it should be noted that sex hormones are essentially involved in the female stress and antidepressant response (Estrada-Camarena et al., 2009; Young et al., 2007).

Therefore in order to better estimate the magnitude of a sex-differentiated response, the baseline sex differences should be taken into account. Based on the above mentioned rationale, the present study aimed to investigate the sex differences in tests of anxiety and depression in the presence of absence of manipulated peripheral corticosterone levels. In particular, we artificially adjusted in males and females the peripheral corticosterone levels using adrenalectomy and corticosterone replacement, and compared the resulting sex-differentiated behavioral profile in the spontaneous open field activity, the light/dark paradigm and the forced swim test. Despite the well-established sex differences in peripheral glucocorticoids at baseline and following stress, we hypothesized that an abolishment of such peripheral differentiation would still permit the appearance of behavioral sex differences, although affected by our manipulation.

2. Methods

2.1. Animals

Forty-eight adult male and female Wistar rats, aged 12 weeks at the beginning of the experiment, were used. Sham-operated male and female rats weighed 304 ± 46 and 212 ± 19 g at the beginning of the experiment and 352 ± 39 and 228 ± 18 g at the end of the experiment. Adrenalectomized male and female rats with corticosterone replacement (ADXR) weighed 302 ± 32 and 201 ± 20 at the beginning of the experiment and 338 ± 38 and 221 ± 17 at the end of the experiment. Animals were group-housed under controlled 12:12 light/dark cycles (lights on at 07:00 a.m.) and temperature (22 ± 2 °C), with free access to food and either tap water pre-operatively or an aqueous solution post-operatively, as described in Section 2.3.

Behavioral testing took place during the morning, between 0900 and 1200 h. A timeline of the experiment is depicted in Fig. 1. All efforts were made to minimize animal suffering and to reduce the number of animals used. All animal experiments were carried out in accordance with the EEC directive 86/609.

2.2. Estrous cycle

In the case of females, a semi-random process controlled for disparities regarding the phases of the estrous cycle. Specifically, female rats were selected from a larger pool of experimental animals on the basis of a regular 4 day cycle and assigned to surgery groups (Sham-operated or ADXR). The equal distribution of estrous cycle phases was then daily monitored by vaginal smears for one week before commencement and during behavioral testing, as described elsewhere (Becker et al., 2005). Although previous studies have shown that stress (Paredes et al., 1998) and adrenalectomy alone without corticosterone replacement (Galvez et al., 1999) may affect ovarian function, in our ADXR rats we did not observe any disruption in the estrous cycle. For the purpose of increasing statistical power, female rats in proestrus and estrous phase of the cycle were grouped together (females P + E) and similarly females in metestrus and diestrus phase of the cycle were also grouped together (females M + D), under the understanding that P + E females are generally under the influence of higher estrogens levels than M + D female rats (Consoli et al., 2005; Salmon et al., 2011).

2.3. Adrenalectomy for corticosterone replacement

Assignment to sham-operated or adrenalectomy with corticosterone replacement (ADXR) groups was random for male rats and semi-random for females, as described in Section 2.2. The bilateral adrenalectomy was aseptically performed via the dorsal approach with animals under anesthesia with 100 mg/kg ketamine, 10 mg/kg xylazine and 0.5 mg/kg atropine intraperitoneally. Tissue materials removed from the adrenal glands. In sham-operated animals the adrenal glands were reached and gently manipulated but not removed. Immediately after surgery and continuously regarding the phases of the estrous cycle. Specifically, female rats were selected from a larger pool of experimental animals on the basis of a regular 4 day cycle and assigned to surgery groups (Sham-operated or ADXR). The equal distribution of estrous cycle phases was then daily monitored by vaginal smears for one week before commencement and during behavioral testing, as described elsewhere (Becker et al., 2005). Although previous studies have shown that stress (Paredes et al., 1998) and adrenalectomy alone without corticosterone replacement (Galvez et al., 1999) may affect ovarian function, in our ADXR rats we did not observe any disruption in the estrous cycle. For the purpose of increasing statistical power, female rats in proestrus and estrous phase of the cycle were grouped together (females P + E) and similarly females in metestrus and diestrus phase of the cycle were also grouped together (females M + D), under the understanding that P + E females are generally under the influence of higher estrogens levels than M + D female rats (Consoli et al., 2005; Salmon et al., 2011).

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![Timeline of the experiment.](image-url)
2.4. Open field test

Spontaneous motor activity following exposure to a novel open field environment (Hall, 1934; Valle, 1970) was measured for a 5 min period. As previously described (Polissidis et al., 2009, 2010), all rats were acclimatized to the test room for 1 h and thereafter placed in a clear Plexiglas chamber (Med Associates Inc., St Albans, VT). Ambulatory counts as an index of horizontal activity and vertical counts as an index of rearing behavior (vertical activity) were assessed for the entire registration period. Additionally, the latency to escape from the center of the open field and the time spent in the center served as indices of anxiety (Belzung and Griebel, 2001; Prut and Belzung, 2003; Rentenius et al., 2010).

2.5. Light dark test

Anxiety-like behavior was assessed using the light/dark box (Crawley, 1985; Merlo Pich and Samanin, 1989). A Plexiglas chamber (Med Associates Inc.) measuring 430 × 430 × 300 mm was equally divided in two compartments, one transparent, brightly illuminated and a second opaque and dark using the appropriate feeder. The two compartments were communicating through an opening measuring approximately 105 mm height and width. All rats were placed in the middle of the illuminated compartment facing away from the opening towards the dark compartment and allowed to explore the maze for 5 min. All sessions were videotaped and the scored behaviors were: total transitions from one compartment to the other, latency to escape from the illuminated compartment and total time spent in the illuminated compartment. A transition from one compartment to the other was recorded when all four paws were in one compartment.

2.6. Forced Swim Test

For the FST (Porsolt et al., 1977) as modified by Detke at al (Detke et al., 1995), rats were individually placed in a cylindrical tank measuring 50 cm in height and 19 cm in diameter. The tank was filled up with tap water which was set to 24 °C and was renewed after each FST session. The water depth was adjusted to a height that did not allow the animals to touch the tank’s bottom with their hind paws or their tail (Detke and Lucidi, 1996). The animals were forced to swim for a 15-min pre-test period (data not shown) and 24 h later were subjected to a 5-min swimming test session. Following FST sessions, the rats were removed from the tank, carefully dried in heated cages and then returned to their home cages until decapitation. All sessions were videotaped and blindly scored offline by trained observers (NK, AS) using a computer-assisted method which allowed the calculation of the total duration of immobility, swimming and climbing, using a modified computer program (Antoniou and Kafetzopoulos, 1996) as previously described (Dalla et al., 2008). Rats were considered to be immobile when they were making only those movements necessary to keep their heads above the water. Swimming was recorded when they were actively swimming around in circles (horizontal movement). Climbing was scored when rats were climbing at the walls of the cylinder (vertical movement). As previously shown, increased passive behavioral responses in FST such as immobility and decreased active behaviors like swimming and climbing, are thought to be a clear indication of depressive-like symptomatology and those behavioral parameters are sensitive to antidepressant treatments (Cryan et al., 2002; Detke et al., 1995; Reneric and Lucidi, 1998).

2.7. Corticosterone assay

Baseline blood samples were collected during the morning of the day that the FST test session took place, from the rats’ lateral tail veins. Post-stress blood samples were collected from trunk blood after sacrifice, 20 min after the FST test session as previously described (Dalla et al., 2005; Drossopoulou et al., 2004; Pityuchotis et al., 2011). All samples were processed to recover serum (centrifugation at 4000 g, 30 min, 4 °C); and the serum samples were stored at −20 °C before being assayed for corticosterone by a standard radioimmunoassay (MP Biomedicals, Costa Mesa, CA). The inter- and intra-assay coefficients of variation were both 8%.

2.8. Statistical analysis

All results presented herein were analyzed with a factorial two-way ANOVA design using the General Linear Model of SPSS version 19 (IBM Corp, Somers, NY, USA). The independent variables were “ADXR” (two levels: Sham-operated, Adrenalectomized with corticosterone replacement) and “sex” (three levels: Males, Females M + D, Females P + E). Subsequent one-way ANOVAs were performed to elucidate significant interactions when indicated by the factorial model. Dunnett’s post-hoc tests were used in order to elucidate differences between females M + D and P + E groups vs. their corresponding sham-operated or ADXR males. All results were compared against ANOVA assumptions regarding normality and equality of variance in analyzed data. When those criteria were not met and no outliers could be detected by computer-aided statistical evaluation, then the non-parametric Kruskal–Wallis chi-square and the Mann–Whitney U tests were used to investigate significant differences. Additionally, for corticosterone levels, a two-way repeated measures ANOVA was used, in order to compare corticosterone levels before and after stress in male and female sham-operated and ADXR rats. A probability value of p ≤ 0.05 was considered as significant whereas non-significant trends were considered only if dp ≤ 0.10.

3. Results

3.1. Open field test

3.1.1. Horizontal activity: ADXR decreases activity only in males

Male ADXR rats exhibited lower horizontal activity following adrenalectomy and corticosterone replacement, in comparison to sham-operated males (F = 6.794 p < 0.016). On the contrary, ADXR had no effect in the horizontal activity of females (p = n.s.). Thus, a significant interaction between ADXR and sex was observed (F = 4.005 p < 0.026).

Although generally males were less active than females, this effect reached statistical significance only in ADXR groups (F = 18.742 p < 0.001). In fact, male ADXR rats were less active than female ADXR groups M + D and P + E (p < 0.001; p < 0.001 respectively) (Fig. 2A).

3.1.2. Vertical activity: females display more exploration than males, while ADXR has no effect

Female rats of both M + D and P + E groups had higher vertical counts in the open field test than males [main effect F (4,24) = 12.149 p < 0.001, post-hoc tests p < 0.001 and p < 0.002, respectively for M + D and P + E groups vs. males]. ADXR had no effect on vertical activity (Fig. 2B).

3.1.3. Escape latency: ADXR decreases anxiety index only in males

Adrenalectomy with corticosterone replacement (ADXR) increased the latency to escape from the center of the arena only in male ADXR rats (F = 10.570 p < 0.004) and as a consequence this measurement was higher in ADXR males, in comparison to M + D and P + E ADXR females (p < 0.033 and p < 0.010, respectively). Consequently, there was a significant interaction between ADXR and sex (F = 3.526 p < 0.038), due to sex differences in ADXR rats (F = 6.129 p < 0.007). No sex differences were detected in sham-operated rats (Fig. 2C).

The factorial model did not indicate any significant differences in the time spent at the center of the open field, although ADXR male rats tended to spent more time in the center of the open field than their control sham-operated males (Fig. 2D).

3.2. Light–dark test: males exhibit more anxiety-like behavior than females, and ADXR decreases anxiety measures only in males

Analysis of data regarding the latency to escape the illuminated compartment of the apparatus showed that the variances were not equal between groups [Levene’s Test: F(4,24) = 3.268 p < 0.014]. A Kruskal–Wallis non-parametric test showed significant differences across all groups [x² = 13.425 df = 5 p < 0.020] and further analysis showed that there were significant differences within sham-operated but not ADXR rats [x² = 9.030 df = 2 p < 0.011]. Mann–Whitney U tests showed that sham-operated male rats presented more anxiety-like behavior because they had a lower escape latency than female M + D and P + E rats [U = 2, z = -2.817 p < 0.005; U = 14.5, z = -2.002 p < 0.043 respectively]. Notably,
this sex difference was abolished by adrenalectomy and corticosterone replacement, because ADXR M + D and P + E females did not differ from ADXR males. This was due to the significant increase in latency to escape of ADXR males in comparison to sham-operated males (p < 0.05). Furthermore, the latency to escape from the center of the arena was higher in ADXR males, in comparison to M + D and P + E ADXR females (p < 0.05). D. There were no significant differences in the time spent at the center of the open field between groups. Means ± standard errors are presented in all graphs. An asterisk (*) denotes ADXR effect (ADXR vs. Sham-operated) whereas a plus sign (+) denotes a sex difference (males vs. corresponding females).

3.3. Forced swim test

3.3.1. Immobility: females exhibit higher “depressive-like” symptomatology than males

Female rats in both P + E and M + D phases of the cycle had higher immobility time during the 5 min FST test session than males (sex effect: F(2,42) = 5.750 p < 0.0066 and post-hoc tests: p < 0.011 and p < 0.015, respectively), while adrenalectomy with corticosterone replacement (ADXR) had no effect (Fig. 4A).

3.3.2. Swimming and climbing: ADXR decreases climbing and enhances swimming only in males

Male ADXR rats swam more than sham-operated males [F(1,21) = 13.946 p < 0.001]. On the contrary, no effect of ADXR was detected in females, regardless the phases of the estrous cycle. Furthermore, sham-operated males did not differ in swimming duration from sham-operated females. On the contrary, male ADXR rats swam for more time than ADXR females in P + E phases of the estrous cycle, where estrogens are higher (p < 0.044), but only marginally (p < 0.070) in M + D phases of the cycle [sex effect in ADXR rats: F(2,22) = 4.079 p < 0.030]. Thus, a significant interaction between ADXR and sex was observed for the duration of swimming during the test session of the FST [F(2,42) = 3.622 p < 0.035] (Fig. 4B).

Analysis of data regarding climbing duration showed that the variances were not equal between groups [Levene’s Test: F(5,42) = 2.450 p < 0.049]. The Kruskal–Wallis non-parametric test showed marginally significant differences across groups (p = 0.054). Further analysis indicated that male ADXR rats climbed significantly less than sham-operated males [Mann–Whitney U = 32.5, z = -2.016, p < 0.044], while no differences were detected in female rats (Fig. 4C).

3.4. Corticosterone levels

3.4.1. Baseline: females have higher corticosterone levels than males

Sham-operated males had lower baseline corticosterone levels than sham-operated M + D and P + E females [F(2,19) = 7.917 p < 0.003; post-hoc p < 0.003 and p < 0.028, respectively]. As expected, no sex differences were detected in ADXR rats. Thus, while no differences existed between ADXR vs. sham-operated males,
female ADXR M + D and P + E rats had lower baseline corticosterone levels than control sham-operated females \( F(1,13) = 10.406, p < 0.007; F(1,18) = 8.714, p < 0.018 \), respectively. As a result, a significant interaction between ADXR and sex was observed \( F(2,42) = 5.384, p < 0.008 \) (Fig. 5A).

4.2. Stress: sex-differentiated corticosterone increase in sham-operated rats

Twenty minutes post-FST, sham-operated males had lower stress-induced levels of corticosterone than stressed females in M + D and P + E phases of the cycle \( F(2,19) = 13.217, p < 0.001 \); post-hoc \( p < 0.001 \) and \( p < 0.001 \), respectively. Furthermore, all sham-operated rats had higher corticosterone levels than ADXR rats [males \( F(1,13) = 44.350, p < 0.001 \); females M + D \( F(1,13) = 77.442, p < 0.001 \); females P + E \( F(1,18) = 151.142, p < 0.001 \)]. Thus, a significant ADXR \( \times \) sex interaction was found on stress-induced corticosterone levels \( F(2,42) = 10.196, p < 0.001 \) (Fig. 5B).

4.3. Comparison of pre- and post-stress corticosterone levels

A repeated measure ANOVA before and after stress, showed that stress failed to induce corticosterone levels in ADXR rats. On the contrary, comparison of pre- and post-stress corticosterone in sham-operated rats showed that stress clearly increased corticosterone in all groups of sham-operated rats [males \( F(1,13) = 52.657, p < 0.001 \); females M + D \( F(1,13) = 32.702, p < 0.001 \); females P + E \( F(1,18) = 39.409, p < 0.001 \)]. Thus, significant interactions between ADXR \( \times \) stress, and stress \( \times \) sex were revealed \( F(1,42) = 119.590, p < 0.001; F(1,42) = 4.018, p < 0.025 \) respectively.

4. Discussion

The present study aimed to investigate sex differences in the presence or absence of adjusted peripheral corticosterone levels. Our findings show that the abolishment of such peripheral differentiation still permits the appearance of sex differences in the behavioral profile. In particular, ADXR males showed signs of reduced anxiety levels when compared to sham-operated males and exhibited a different organization of their active response during the FST test session. On the contrary, ADXR females were not essentially affected by adrenalectomy and corticosterone replacement in comparison to their sham-operated counterparts.

In our study, the corticosterone replacement led to comparable levels of baseline corticosterone between sham-operated and ADXR male rats, but the latter were not able to experience elevated stress-induced corticosterone levels. Interestingly, male ADXR rats were less active in the novelty stress and less prone to abandon the center of the arena. Similarly, they were less prone to leave the illuminated area, without significant differences in total entries when compared to sham-operated rats. Together with their tendency to remain more time in the center of the open field and in the illuminated compartment of the light/dark box, these results suggest that adjusted peripheral corticosterone levels lead to...
reduced anxiety levels in male rodents. The lower horizontal activity of ADXR male rats in the novel open field arena, along with the increased latency to escape from the center of the arena, might also reflect a reduction in motor activity in comparison to sham-operated males. This interpretation however, needs further investigation given that total transitions in the light/dark test (a safer index of motor activity) were not differentiated between ADXR and Sham-operated rats. Previous studies have established that

Fig. 4. Sex differences in forced swim test and effect of ADXR. A. Females exhibit higher "depressive-like" symptomatology than males, because all females in both methestrous and diestrous (M + D) and proestrous and estrous (P + E) phases of the cycle had higher immobility time during the 5 min Forced Swim Test (FST) session than males (*p < 0.05). Adrenalectomy with corticosterone replacement (ADXR) had no effect. B. Male ADXR rats swam more than sham-operated males during the 5 min FST session (*p < 0.05), while ADXR had no effect in females. Furthermore, male ADXR rats swam for more time than ADXR females in P + E phases of the estrous cycle (*p < 0.05). C. Male ADXR rats climbed for less time than sham-operated males during the 5 min FST session (*p < 0.05), while no differences were detected in female rats. Means ± standard errors are presented in all graphs. An asterisk (*) denotes ADXR effect (ADXR vs. Sham-operated) whereas a plus sign (+) denotes a sex difference (male vs. corresponding female).

Fig. 5. Sex differences and effect of ADXR in corticosterone levels before and after swim stress exposure. A. Sham-operated females in both methestrous and diestrous (M + D) and proestrous and estrous (P + E) phases of the cycle had higher corticosterone levels than sham-operated males (*p < 0.05), while no sex differences were detected in adrenalectomized rats with corticosterone replacement (ADXR). Also, female ADXR M + D and P + E rats had lower baseline corticosterone levels than control sham-operated females (*p < 0.05), while no differences were evident in male rats. ADXR prevented the stress-induced increase in corticosterone levels in both sexes, because all sham-operated rats had higher corticosterone levels than all ADXR rats 20 min post-FST exposure (*p < 0.05). Furthermore, sham-operated males had lower stress-induced levels of corticosterone than stressed M + D and P + E females (*p < 0.05). Finally, corticosterone levels were increased following stress in all groups of sham-operated rats (#p < 0.05), while no differences were seen in ADXR rats. Means ± standard errors are presented in all graphs. An asterisk (*) denotes ADXR effect (ADXR vs. Sham-operated) whereas a plus sign (+) denotes a sex difference (male vs. corresponding female). A hash sign (#) denotes a significant stress difference (before vs. after FST).
adequate corticosterone replacement, which mimics baseline levels, prevents the behavioral and neurobiological effects of adrenalectomy (Edwards et al., 1990; Tejani-Butt and Labow, 1994). Interestingly, a behavioral study employing intact animals in rodent tests of anxiety and depression indicated that male behavior is driven by anxiety whereas female by activity (Fernandes et al., 1999). It was previously suggested that adjusted glucocorticoid levels may facilitate a stress inoculation effect in ADXR rats, and thus leading to emotional stability and risk-taking behavior (Chorpita and Barlow, 1998). Present findings support these conclusions and provide further evidence that male behavior in rodent tests is linked to HPA axis activity and corticosterone levels.

Our findings further show that immobility during FST did not differ between ADXR and sham-operated rats, despite the application of a two-day swim stressor and the documented inability of ADXR rats to exhibit increased corticosterone levels, in response to stress. Interestingly though, the organization of the active response to the FST was substantially altered only in ADXR males. Elevated glucocorticoid levels after repeated stress or high doses of exogenous glucocorticoids induce a depressive-like state during the FST in male rats (Marks et al., 2009; Molina et al., 1994). However, previous studies on adrenalectomized male rats without corticosterone supplementation offered conflicting results. Adrenalectomy either reduced (De Kloet et al., 1988; Jefferies et al., 1985) or did not influence immobility in the FST (Abel and Bitziuk, 1992; Marti and Armarino, 1996). However, it was earlier shown that an adequate corticosterone replacement results in no differences in immobility (Edwards et al., 1990). It was then suggested that the presence of corticosterone is crucial for the incorporation of the learned response after swim stress. Therefore corticosterone was implicated in helpless behavior during FST. In line with our findings, previous studies confirmed that the passive behavior in the FST is not related to pharmacological adrenalectomy (Gomez et al., 1998) and a recent study using male mice equally showed no differences in passive behaviors after adrenalectomy and corticosterone replacement (Xu et al., 2009). As already mentioned, active behavioral FST responding was altered in ADXR males. SSRIs are known to increase swimming whereas SNRIs increase climbing in FST, because of enhanced serotonergic and noradrenergic neurotransmission respectively (Detke et al., 1995). Therefore, the observed reduction in climbing and increase of swimming behavior in ADXR males during this study may either reflect a decrease in noradrenergic activity or more probably, a shift in balance between noradrenergic and serotonergic activity, in favor of the latter. Previous studies have demonstrated that following stressors such as the novelty stress and the FST, elevated glucocorticoid levels mobilize via feedback the CRH system and activate the noradrenergic system (Curtis et al., 1999; Santibanez et al., 2005). It is reasonable to speculate that in ADXR male rats, manipulated corticosterone levels do not facilitate the noradrenergic activation and thus allow the serotonergic neurotransmission to dominate the FST behavioral response.

Concerning female rats, they were generally more active than males in the open field and the light/dark box, in agreement with previous reports (Brotto et al., 2000; Dalla et al., 2005; Valle and Gonzálka, 1980). Furthermore, females exhibited less anxiety than males when examining thigmotaxis and avoidance of illuminated space, as previously observed (Ramos et al., 1998; Roman and Arborelius, 2009; Zimmerberg and Farley, 1993). Interestingly, the female behavioral profile in the open field and light/dark box was not affected by adrenalectomy and corticosterone replacement. In addition, whereas in ADXR males corticosterone replacement successfully mimicked baseline levels, in female ADXR rats it resulted in somewhat lower levels in comparison to baseline corticosterone levels of sham-operated females. This effect was probably observed because sham-operated females exhibit higher baseline corticosterone levels than the corresponding males, as previously shown (Drossopoulou et al., 2004). Yet, despite the corticosterone difference between female ADXR vs. sham-operated rats, the female behavioral response was not substantially altered. In line with recent reports (Aoki et al., 2010; Kokras et al., 2011), present findings suggest that female behavior was not essentially affected by our manipulation in the peripheral corticosterone levels.

Present findings further reveal that females generally presented more immobility and less active behaviors than males during the second FST session, irrespectively of the corticosterone manipulation. Similar sex differences in FST performance have been reported from our group and others (Brotto et al., 2001; Dalla et al., 2005; Hill et al., 2003; Pitychoutis et al., 2009, 2011; Tonelli et al., 2008). It was previously suggested that the higher immobility of females during the FST is a result of females having higher corticosterone levels, in comparison to males (Hill et al., 2003). This suggestion was based firstly on the observation that females naturally have higher corticosterone levels than males and secondly, on studies conducted in males which linked corticosterone levels to the occurrence of immobility (Johnson et al., 2006; Marks et al., 2009). However, in line with our results, Walf and Frye (Walf and Frye, 2005) showed that in the FST, physiological corticosterone replacement did not differentiate ADXR females from the control sham-operated females. Present findings clearly show that the female FST behavioral response was not altered by adrenalectomy with low and adjusted corticosterone replacement (ADXR). In fact, in the animal tests used in the present study, when behavioral sex differences were affected, this was because of the ADXR male behavior converging or diverging from the female and not the opposite.

On the other hand, the estrous cycle did not significantly modulate behavioral outcomes in the present study in either sham-operated or ADXR female rats. This finding is in agreement with previous observations (Andrade et al., 2010; Salomon et al., 2011; Tonelli et al., 2008) although others have reported a role of female gonadal hormones in the expression of anxiety and depressive behavior (Brotto et al., 2001; Consoli et al., 2005; Papalexi et al., 2005; Walf et al., 2009). Interestingly, the contribution of the female hormonal milieu to behavioral responses may fluctuate according to light conditions (Mora et al., 1996). Aside differences in light intensity, rat strain and other methodological differences, including the period of data collection, may also explain the divergent results of such studies (Marcondes et al., 2001). Taking into consideration the limitation that herein females were grouped in two phases of the cycle in order to increase statistical power, we conclude that under normal illumination, physiological gonadal–hormone variations, as in the case of normal cycling females, seem not to affect the behavioral performance in the behavioral tests used in this study.

It should be noticed that in our ADXR rats, the adrenal medulla was removed together with the adrenal cortex. Therefore, in the observed anxiety- and depression-like behaviors, the possible involvement of other cortical and medullar adrenal hormones should be taken into account. Interestingly, Oitzl and de Kloet reported differences in water maze performance after removal of the adrenal cortex but not after removal of the adrenal medulla (Oitzl and de Kloet, 1992). Similarly, it was previously reported that immobility in the FST is not affected by removal of the adrenal medulla, provided that several weeks have intervened between surgery and behavioral testing (Veldhuis et al., 1985). More studies however are warranted in order to further assess the role and possible sex-differentiated effects of aldosterone and catecholamines in the behavioral tests used herein.
In overall, present data showed that anxiety- and depressive-like behavioral profile was mainly modulated in males following adrenalectomy and corticosterone replacement. These results confirm and extend our previous results regarding sex differences in tests of anxiety and depression. Interestingly, our previous findings (Kokras et al., 2011) showed that antidepressant treatment reduced anxiety levels in both sexes, affecting hypothalamic targets and also reducing corticosterone in males whereas extra-hypothalamic targets were affected in females and importantly without alteration in corticosterone levels. Given that herein, males and females were not equally affected by manipulations in peripheral corticosterone as mentioned above, we could hypothesize the existence of two sex-oriented mechanisms of stress response: a predominantly male-type response, which seems to be associated with peripheral endogenous corticosterone and, a female-type behavioral response, which seems to be less dependent on this corticosterone influence. It could be further postulated that such hypothesized sex-dependent types of stress response might link to sex differences in the occurrence and magnitude of anxiety and depressive phenomenology. Further studies with the use of appropriate models for females are warranted, in order to shed more light in the current hypothesis, and in the pathophysiology of affective disorders in men and women.

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References


