Molecular Neurobiology (2019) 56:394–405
https://doi.org/10.1007/s12035-018-1085-x

Role of HPA and the HPG Axis Interaction in Testosterone-Mediated Learned Helpless Behavior

Birgit Ludwig[1] · Bhaskar Roy[1] · Yogesh Dwivedi[1]

Received: 12 February 2018 / Accepted: 11 April 2018 / Published online: 28 April 2018

Abstract

Affective disorders show sex-specific differences in prevalence, symptoms, and complications. One hypothesis for this discrepancy is the interaction between the hypothalamic-pituitary-adrenal (HPA) axis and hypothalamic-pituitary-gonadal (HPG) axis. The present study investigates the influence of androgen on the behavioral phenotype and explores how it interacts with HPA axis genes. Gonadectomized (GDX) and GDX rats treated with testosterone propionate (T) were tested for learned helplessness (LH) behavior and compared with tested controls (TC). Prefrontal cortex was used for analyses of HPG- axis-related genes (androgen receptor, (*Ar*); estrogen receptor-β (*Er-β*)) and HPA axis-related genes (corticotropin-releasing hormone, (*Crh*); glucocorticoid receptor, (*Nr3c1*); corticotropin-releasing hormone receptor 1, (*Crhr1*); corticotropin-releasing hormone receptor 2, (*Crhr2*); FK506 binding protein 5, (*Fkbp5*)). Promoter-specific CpG methylation in the *Crh* gene was determined by bisulfite sequencing. Chromatin immunoprecipitation (ChIP) assay was used for determining ER-β binding on the proximal promoter region of *Crh* gene. Serum testosterone levels confirmed a testosterone-depleted GDX group, a group with supraphysiological levels of testosterone (T) and another group with physiological levels of testosterone (control (C)). Unlike GDX rats, T group exhibited significantly higher LH score when compared with any other group. *Crh* and *Fkbp5* genes were significantly upregulated in GDX group compared with controls, whereas *Er-β* showed a significant downregulation in the same group. Methylation analysis showed no significant differences in-between groups. ChIP assay was unable to determine a significant change in ER-β binding but revealed a notable contrast in *Crh* promoter occupancy between T and GDX groups. Altogether, the present study reveals an increased susceptibility to depression-like behavior due to chronic supraphysiological level of androgen via HPA axis inhibition.

Keywords Hypothalamic-pituitary-adrenal axis · Hypothalamic-pituitary-gonadal axis · Stress · Depression · Testosterone · Androgen receptor

Abbreviations

HPA	Hypothalamic-pituitary-adrenal axis
HPG	Hypothalamic-pituitary-gonadal axis
GDX	Gonadectomized
T	Testosterone propionate
LH	Learned helplessness
TC	Tested controls
Ar	Androgen receptor
ER-β	Estrogen receptor-β
Crh	Corticotropin-releasing hormone
Nr3c1	Glucocorticoid receptor
Crhr	Corticotropin-releasing hormone receptor
Fkbp5	FK506 binding protein 5
ChIP	Chromatin immunoprecipitation
PFC	Prefrontal cortex
PND	Postnatal day
ELISA	Enzyme-linked immunosorbent assay
ET	Escape test
IS	Inescapable shock
DTT	Dithiothreitol
qPCR	Quantitative polymerase chain reaction
Gapdh	Glyceraldehyde 3-phosphate dehydrogenase
cDNA	Complementary DNA
PBS	Phosphate buffered saline
PI	Proteasomal inhibitors

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12035-018-1085-x) contains supplementary material, which is available to authorized users.

✉ Yogesh Dwivedi
ydwivedi@uab.edu

[1] Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, SC711 Sparks Center, 1720 7th Avenue South, Birmingham, AL 35294, USA

RPM	Revolution per minute
TE	Tris-ethylenediaminetetraacetic acid
LiCl	Lithium chloride
FST	Forced swim test
AAS	Anabolic androgenic steroid
3-β-diol	5-Alpha-androstane 3beta,17beta diol
ERE	Estrogen receptor elements
cAMP	cAMP-response elements
AP-1	Activator protein-1
PVN	Periventricular nucleus

Introduction

The origins of depressive symptoms and the corresponding *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*) diagnoses are still subject of scientific discussion, but it has been established that molecular changes at genetic, epigenetic, and gene expression levels reflect phenotypic traits and states of disease [1, 2]. One of the most vulnerable systems to genetic polymorphisms and life adversities in relation to mood disorders is the HPA axis [3]. Sex-related differences in HPA-stress reactivity might also be one of the reasons behind differences in the vulnerability of psychiatric disorders such as mood disorders and anxiety. The complex interaction between the HPA axis and the HPG axis has been studied intensively over the past decades because their interaction seems to be the biological correlate of sex-specific differences in the responsiveness to stress [4, 5]. The prevalence of mood disorders in women is two times higher than in men and the effect of this gender difference seems to depend on factors such as age and hormonal state [6]. Mood disorders are the most prevalent disorder associated with suicide. In contrast with the higher prevalence of mood disorders in women [7], suicide rates are significantly higher in men compared with women [8]. It is evident that not only biological factors contribute to the individual's risk of suicide but also stressful life events, interpersonal problems, use of psycho-active substances such as cannabis, social isolation, and feelings of hopelessness are important risk factors in suicidal behavior [9].

Several studies have been conducted to elucidate the association between male sex hormones and suicide and provide a biological hypothesis for the sex differences in suicidal behavior [10–14]. The significant higher rate of completed suicides in men, points towards an androgen hypothesis that could not be proven until now. The lack of definitive findings between plasma testosterone and suicidal behavior has been suggested to be due to circadian alterations with the sex hormones feedback system [15]. Testosterone, similar to cortisol, shows significant diurnal variations [16] that may not be controllable by statistic measures. The expression of androgen-binding receptors might be stable enough to support this hypothesis as well as refine the model of the HPG axis. ER-α, which shows higher affinity for estradiol, fails to show associations with suicide [17]. Estrogen receptor-beta (ER-β), which responds to testosterone metabolites, seems to be associated with mood disorders in women [18, 19]. Based on findings of testosterone levels and psychiatric disorders, Walther et al. [20] suggested that a hypermethylation of the androgen receptor (*Ar*) gene might be leading to a concomitant downregulation of AR and depression symptoms in men, but there are no clinical studies with psychiatric populations yet [20].

Molecular studies investigating the interaction between the HPA and HPG axes found a consistent suppression of the HPA axis by androgens [4, 21, 22], and it has been suggested that this suppression is mediated through the binding of dimerized ER-β with a palindromic estrogen response element present on corticotropin releasing hormone (*Crh*) promoter when complexed with testosterone metabolite 5α-androstane-3β, 17β-diol (3-β-diol) [5]. Additionally, this regulation could be subjected to epigenetic interferences originating from close HPA/HPG interaction in the brain [5]. An explanatory overview of testosterone metabolism relevant in this context can be found in Fig. 1. HPA/HPG axes interaction and their regulation by steroids have not been studied in relation to depression-like behavior in the rodent model. To do so, we tested whether gonadectomy (GDX) or supplementation with testosterone can alter depression-like behavior and explored possible mechanisms behind this dynamic interaction. We used a learned helplessness (LH) paradigm to test depression-like symptoms in a rodent model. To the best of our knowledge there is no study so far showing the effects of GDX/testosterone-treatment on the behavioral outcome of the learned helplessness paradigm. To examine the possible underlying mechanisms, we examined expression of genes associated with the HPA/HPG axes such as *Ar*, *Er-β*, *Fkbp5*, glucocorticoid receptor (*Nr3c1*), corticotropin-releasing hormone (*Crh*), CRH receptor 1 (*Crhr1*), and receptor 2 (*Crhr2*) in the prefrontal cortex (PFC) of these rats. We chose PFC because this brain area plays a critical role in emotion regulation, cognition, neuroendocrine stress responses, and synaptic plasticity [23]. In addition, the genes associated with HPA as well as HPG genes are highly expressed in this brain area [24, 25].

We hypothesize that exogenous administration of testosterone at supraphysiological dose will lead to depressive-like symptoms in rats, which will be mediated through interaction of ERβ with HPA axis genes in brain areas relevant to emotion regulation and cognition.

Materials and Methods

Animals

Thirty male Long Evans rats (PND 25) were obtained from Envigo Laboratories (Indianapolis, IN, USA) and were housed in individual cages (3/cage) under standard

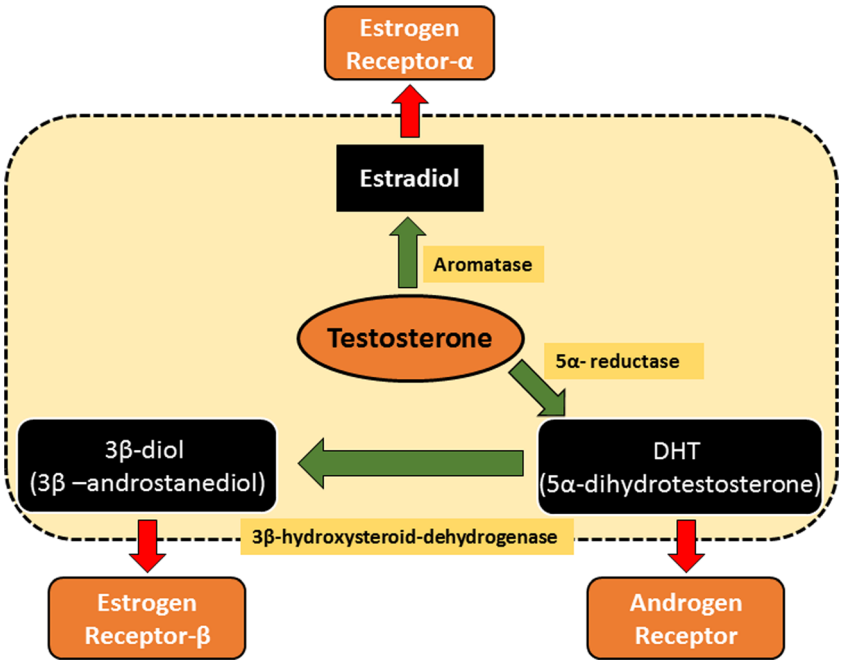

Fig. 1 Schematic simplified pathway for testosterone metabolism

laboratory conditions (temperature 21 ± 1 °C, humidity 55 ± 5%, 12-h light/dark cycle). All rats received ad libitum food and water. After 5 days of acclimation, they were randomly divided into three groups: GDX with daily injections of testosterone propionate (Sigma-Aldrich, St. Louis, MS, USA) (T; $n = 10$), GDX with subsequent daily injections of vehicle only (corn oil) (GDX; $n = 10$) and naïve controls (C; $n = 10$). Experimental procedures were approved by the IACUC of the University of Alabama at Birmingham, and all procedures were conducted in strict adherence with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The overall animal procedure has been represented with a schematic diagram in Fig. 2a.

Surgical Procedures

The animals were anesthetized with isoflurane (5% induction, 1–3% maintenance) and vitals such as respiratory rate were constantly monitored to minimize the occurrence of cardio-respiratory failure. Prior to incision, rats were administered carprofen (5 mg/kg, s.c.) and buprenorphine (0.05–0.1 mg/kg, s.c.) (Henry-Schein, Melville, NY, USA). Using aseptic technique, the rat abdomen was shaved and cleaned with betadine and a single transverse incision in the caudal abdomen was made; the testicular fat pad on the one side was being pulled through the incision using a blunt forceps. A hemostat was placed below the testes and epididymis across the testicular cord, then a ligature was placed below the hemostat and the testes and epididymis were removed with a scissors. The incisions were closed with monocryl sutures (Ethicon, Somerville, NJ, USA). The rats were monitored, and the body temperature was regulated during and after the procedure by a warming pad. A second subcutaneous dose of carprofen (5 mg/kg) was administered as an analgesic 24 h after the surgery.

Testosterone Injections

A daily dose of 7.5 mg/kg testosterone propionate (Sigma-Aldrich, St. Louis, MS, USA) in 0.1 ml of corn oil was subcutaneously injected to ten GDX rats (T group) over the period of 28 days. The dose was chosen in accordance with previous experiments in order to attain supraphysiological levels of serum testosterone in T group [26]. Ten other GDX rats were administered equal volume of corn oil only. Body weights were assessed every 2 weeks, and the daily dose was adjusted according to the weight.

Behavioral Testing

The behavioral testing started on post-natal day (PND) 60, 29 days after surgery and conducted during the light cycle. The procedures to induce depression-like behavior were undertaken according to our previous publications [27, 28]. A total of 30 rats: 5 T rats, 5 C rats, and 5 GDX rats were restrained only (TC) whereas 5 T rats, 5 C rats, and 5 GDX rats (IS) were restrained and given a total of 100 tail shocks ("inescable shocks") delivered for 5 s at the rate of 1.0 mA 1 day before their escape latency was measured in the shuttle box (Fig. S1).

The shuttle box consisted of two equal-sized compartments (18 × 18 × 30 cm) that are separated by a small gate (6 cm

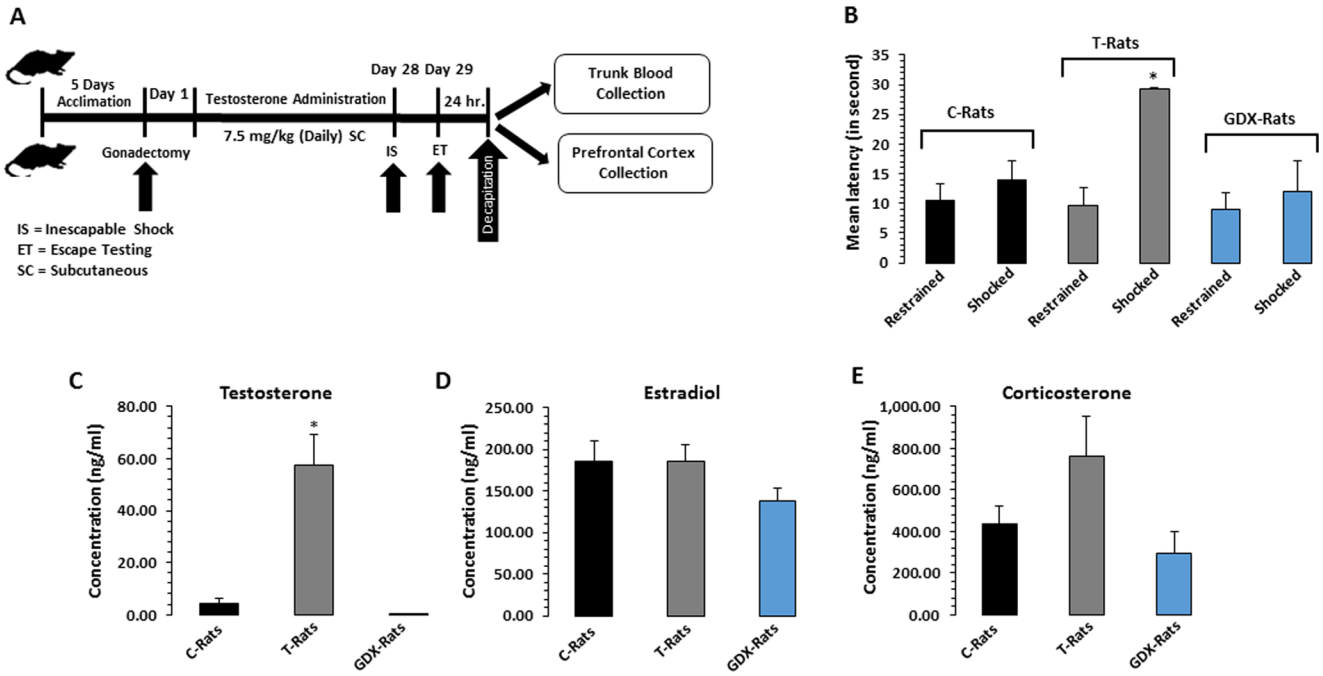

Fig. 2 Learned helplessness model of gonadectomized rats treated with testosterone and their serum level estimation of testosterone, estradiol, and corticosterone. **a** Schematic diagram of the timeline followed as part the testosterone treatment to induce learned helplessness model in gonadectomized rat. **b** The bar diagrams present the escape latencies in control (C rats), GDX (GDX rats), and T (T rats) groups. Shocked T rats show significantly higher escape latency in the shuttle test, compared with restrained controls ($p = 0.001$). Data is represented as ± SEM. **c** Serum concentration (ng/ml) of testosterone in control vs. GDX group ($p = 0.095$) and control vs. T group ($p = 0.002$) are shown with the bar diagram. **d** In a similar way, serum concentration of estradiol (ng/ml) was also compared in the control vs. GDX group ($p = 0.17$) as well as the control vs. *T* group ($p = 0.99$) of rats. **e** Representative bar diagram demonstrates the serum level concentration of corticosterone in GDX ($p = 0.33$) and T rats ($p = 0.16$) as compared with the control group. Data is represented as ± SEM. (*$p \leq 0.05$)

wide and 7 cm high). There is also a grid floor, through which electric current was applied, and a signaling light at the top of both compartments went on. Performance was analyzed according to the behavior during 30 shuttle escape test (ET) trials. Each trial started with a light stimulus of 5 s, announcing a subsequent footshock of maximum 30 s duration at 0.6 mA. The escape latency was recorded as the time needed to shuttle into the other compartment after the onset of the footshock. Animals showing escape latency of ≥ 20 s qualified as learned helpless (LH). Total time of testing for LH behavior was ~20–30 min depending on the behavioral outcome. A total of three experiments over the course of 3 weeks were performed, and a mean latency was calculated for each animal and each group.

Tissue Collection

Twenty-four hours after the ET, rats were decapitated and trunk blood was collected (08:00–12:00). Brains were removed and flash-frozen, then stored at − 80 °C until further dissections. Subsequently, the prefrontal cortex was dissected and 10 μm sections were cut on a cryostat (Leica CM1950; Leica, Wetzlar, Germany), mounted on slides and stored in − 80 °C until further analyses.

ELISA-Based Testosterone, Estradiol, and Corticosterone Analyses in Serum

Serum from trunk blood was isolated and stored at − 80 °C until the assays were performed. Serum levels of testosterone, estradiol, and corticosterone were assessed using enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, MA, USA), according to the manufacturer's instructions. Data are presented as concentration values (ng/mL).

RNA Isolation and qPCR Based Gene Expression Analysis in Rat PFC

RNA was isolated using TRIzol® (Life Technologies, USA) as described earlier [27]. RNA purity was determined by measuring the optical density with an absorbance ratio of 260/280 (NanoDrop 2000c, Thermo-Scientific, Waltham, MA, USA). All samples had 260/280 ratio > 1.80.

One microgram total RNA was reverse transcribed using M-MLV Reverse Transcriptase (Invitrogen, Grandsland, NY, USA) and oligo (dT) primer. The oligo dT primer annealing step was carried out at 5 μM concentration in presence of 1 mM dNTPs by incubating the reaction at 65 °C for 5 min. The reaction was quenched by holding at 4 °C for more than

2 min. The reaction was mixed with 1× first-strand synthesis buffer, 0.01 mM DTT, 2 U of RNaseOut, and 200 U of M-MLV Reverse Transcriptase and incubated at 37 °C for 50 min. Finally, the reaction was inactivated at 70 °C. Relative abundance of transcripts were measured with a quantitative real-time PCR machine (Stratagen MxPro3005, La Jolla, CA, USA) using 1× EvaGreen qPCR mastermix (Applied Biological Material Inc., Canada) in combination with 0.8 μM each of gene-specific forward and reverse primers (Table 1). Fortyfold diluted raw cDNA was used as template for qPCR amplification using a thermal parameter of initial denaturation at 95 °C for 10 min followed by a repeating 40 cycles of denaturation at 95 °C for 10 s, primer annealing at 60 °C for 15 s, and an extension of amplicon at 72 °C for 20 s. Possibility of primer dimer formation and secondary product amplification was ruled out by running template-free samples. Relative gene expression levels were normalized with *Gapdh* and fold-change value was determined following Livak's ΔΔCt calculation method (Livak and Schmittgen [29]).

Bisulfite Sequencing Based Promoter Methylation Analysis of *Crh* Gene

Genomic DNA was extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). Concentration and purity of isolated genomic DNA were determined using Nanodrop (NanoDrop 2000c, Thermo-Scientific, Waltham, USA). A total amount of 300 ng was used for bisulfite conversion using the EZ-DNA Methylation-Direct™ Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. Methylation-specific (MSP) nested PCR primers (Table 1) were used to specifically amplify (GeneAmp PCR System 9700, Applied Biosystems, Waltham, MA, USA) the target region within the *Crh* gene promoter and sequenced using the 3730xl DNA Analyzer (ABI Life Technologies, Grand Island, NY, USA). Sequencing results were viewed and interpreted using the Chromas program (Technelysium, DNA Sequencing Software, Australia).

Chromatin-Immunoprecipitation-Based Transcriptional Analysis of *Crh* Gene Promoter Via ER-β Binding

Endogenous binding of ER-β to the estrogen response element present on upstream region of rat *Crh* promoter was studied following antibody-mediated chromatin immunoprecipitation (ChIP) assay. Briefly, ~ 15 mg of frozen PFC was homogenized in ice-cold phosphate-buffered saline (PBS). Resulting menaced tissue homogenate was used for 1% formaldehyde-based chemical cross-linking for 15 min at room temperature. The cross-linking reaction was quenched by adding 125 mM glycine with an additional incubation period of 5 min at room temperature. Cross-linked tissue homogenate was washed twice with ice-cold PBS and lysed with ice-cold cell lysis buffer supplemented with protease and proteasomal inhibitors (PI) for 15 min on ice bath. The lysed suspension was again homogenized to avoid any cellular clumps and subsequently centrifuged to decant out any extracellular debris. Finally, chromatin was solubilized and extracted by incubating on ice for 30 min with PI supplemented nuclear lysis buffer. Released chromatin fraction was sonicated to get chromatin fragments of 200–600 bp. Insoluble material from sheared chromatin was cleared with a brief centrifugation at 14 k RPM for 20 min. Equal amount of diluted chromatin samples devoid of 10% fraction (input) was used in immunoprecipitation with ER-β antibody (Abcam, Cambridge, MA, USA) pre-conjugated protein A/G magnetic beads for an overnight period. Immunoenriched chromatin-bead complex sequentially washed with low salt buffer twice, high salt buffer once, lithium chloride (LiCl) buffer once, and lastly Tris-EDTA (TE) buffer twice. After washing, the DNA-protein complex was uncoupled from the beads using freshly prepared elution buffer and reverse cross-linked at 65 °C for 4 h with vigorous shaking. The similar steps were followed for preparing input fraction DNA. Both the immunoprecipitated and input fraction DNA was then purified following phenol/chloroform/isoamyl alcohol method. Finally, immunoprecipitated DNA was subjected to relative quantification with EvaGreen (Applied Biological Material Inc., Richmond, BC, Canada) dye-based chemistry. Amplification in qPCR system was done using DNA sample collected after immunoprecipitation as well as from input control. The primers used for amplifying the identified estrogen response element on the *Crh* promoter are provided in Table 1.

Results

Effect of Chronic Testosterone Administration on Escape Latency Test

The effect of chronic testosterone treatment on the rat's performance on the escape latency test is shown in Fig. 2b. Shocked T rats showed significantly higher escape latencies in the shuttle test compared with restrained controls ($p = 0.001$). The latency of shocked GDX rats was lower than the latency of the restrained control rats, but it did not reach a statistically significant level ($p = 0.74$). The group differences were not present for the TC groups that were only restrained (GDX vs. T: $p = 0.68$; T vs. C: $p = 0.83$).

Table 1 Oligo sequences used in gene expression, methylation and ChIP assays

Genes	Forward (5′-3′)	Reverse (5′-3′)
Ar primer pair	TGA GAT CCC GTC CTC ACT	AGC GAG CGG AAA GTT GTA GT
Crh primer pair	CAA GCT CAC AGC AAC AGG AA	ATT TTG TCC TAG CCA CCC CT
Crhr1 primer pair	ATG TTC GTC TGC ATT GGC TG	TGC CAA ACC AGC ACT TTT CA
Fkbp5 primer pair	TGG CTG TAG TAA GTC GGT CA	CAA CTC CGG GAA ACA AGT GA
Crhr2 primer pair	GGA TGA CAA GCA GAG GAA GT	AGC ACT AGG AAA AGC AGG AA
Er-β primer pair	AGT GGC CAT GTT GGT TCC TA	AAA TCC ACA AGC CCC TCT GT
MSP primer pair 1	TTT GGA TAA TTT TAT TTA AG	TTG AGT TTT TTT ATA TTA GAG TTT G
MSP primer pair 2 (nested)	GTT AAT GGA TAA GTT ATA AGA AGT TTT T	TTA TAT TAG AGT TTG GAG TGA GAT
Crh-ChIP primer pair	TCA GTA TGT TTT CCA CAC TTG G	TTT CAA CAC TGA ATC TCA CAT CC

Effect of Chronic Testosterone Administration on Serum Levels of Testosterone, Estradiol, and Corticosterone

The serum testosterone level is shown in Fig. 2c. It was found that the serum testosterone level of shocked T rats was significantly higher compared with shocked C rats ($p = 0.0023$), validating the reliability of our testosterone treatment. The serum testosterone levels of shocked GDX rats were found to be lower than the serum levels of shocked C rats but did not reach significance ($p = 0.095$). Serum levels of estradiol and corticosterone are shown in Fig. 2d, e. No significant differences were found between the groups.

Gene Expression Analysis in the PFC of Testosterone (T)-Treated Rats

Transcript level expression of HPA axis genes (*Crhr1*, *Crhr2*, *Nr3c1*, *Crh*, and *Fkbp5*) and steroid receptor genes *Ar and Er-β* were analyzed in the PFC of shocked T, GDX, and C rats. *Gapdh* transcription levels were used as normalizer, which did not significantly differ between the three groups ($p = 0.19$).

One-way ANOVA followed by Bonferroni corrections revealed significant differences in-between groups for expression of *Er-β* ($p = 0.003$), *Ar* ($p = 0.005$), and *Crh* ($p = 0.004$). *Fkbp5* failed to show a significance ($p = 0.085$) when tested for one-way ANOVA. No significant in-between group differences were found for *Nr3c1, Crhr1*, and *Crhr2*.

When tested individually, *Ar* expression was significantly up-regulated in the GDX rats ($p = 0.045$) and downregulated in the T rats; however, this downregulation could not reach a statistically significant level ($p = 0.07$). Contrasting results were shown for the expression of *Er-β*, a significant downregulation in the GDX rats ($p = 0.036$) and a trend towards upregulation in the T rats that was very close to statistical significance ($p = 0.059$). Unlike *Er-β* gene, *Crh* expression showed a significant upregulation in the GDX rats ($p = 0.017$) and nonsignificant downregulation in the T rats ($p = 0.53$) when compared with controls. Chaperone protein *Fkbp5* also showed a similar pattern with a significant upregulation in the GDX group ($p = 0.02$) and a nonsignificant downregulation in the T group ($p = 0.96$). *Crhr1* (GDX vs. C: $p = 0.14$; T vs. C: $p = 0.25$), *Crhr2* (GDX vs. C: $p = 0.57$; T vs. C: $p = 0.78$), and *Nr3c1* (GDX vs. C: $p = 0.76$; T vs. C: $p = 0.28$) showed no significant differences in between groups. The data is presented in Fig. 3.

Methylation Status of *Crh* Gene Promoter

We analyzed the methylation of the *Crh* promoter region to understand the influence of testosterone on the HPA axis regulation. Methylation-specific primers (Fig. 4a) targeted to amplify specific CpG sites on bisulfite converted gDNA were used to determine methylation status of *Crh* promoter. The methylation of each of the 11 CpG sites was assessed using Chromas software, comparing the status of the different groups (T, GDX, and C) to each other. The bisulfite sequencing of amplified PCR product did not detect any methylation associated changes in *Crh* promoter. The nine detectable CpG methylation sites were compared individually between C rats vs. T rats and C rats vs. GDX rats, and their methylation status is presented in Fig. 4b. The percent of methylation and related statistical analyses identified from this comparison are presented in Table S1. Additionally, the methylation status of individual CpG site as identified from each animal considering all three comparing groups are presented as checker table in Fig. S2.

In Vivo Binding of ER-β on Rat *Crh* Gene Promoter

To determine the regulatory effect of ER-β on *Crh* gene expression, we performed a ChIP assay (the respective primer binding sites on the *Crh* promoter spanning the two estrogen response elements are shown schematically in Fig. 5a) in the PFC of all escape latency-tested rats. The assay did not show a significant change in binding between ER-β and *Crh* gene promoter. However, the data demonstrated a contrasting

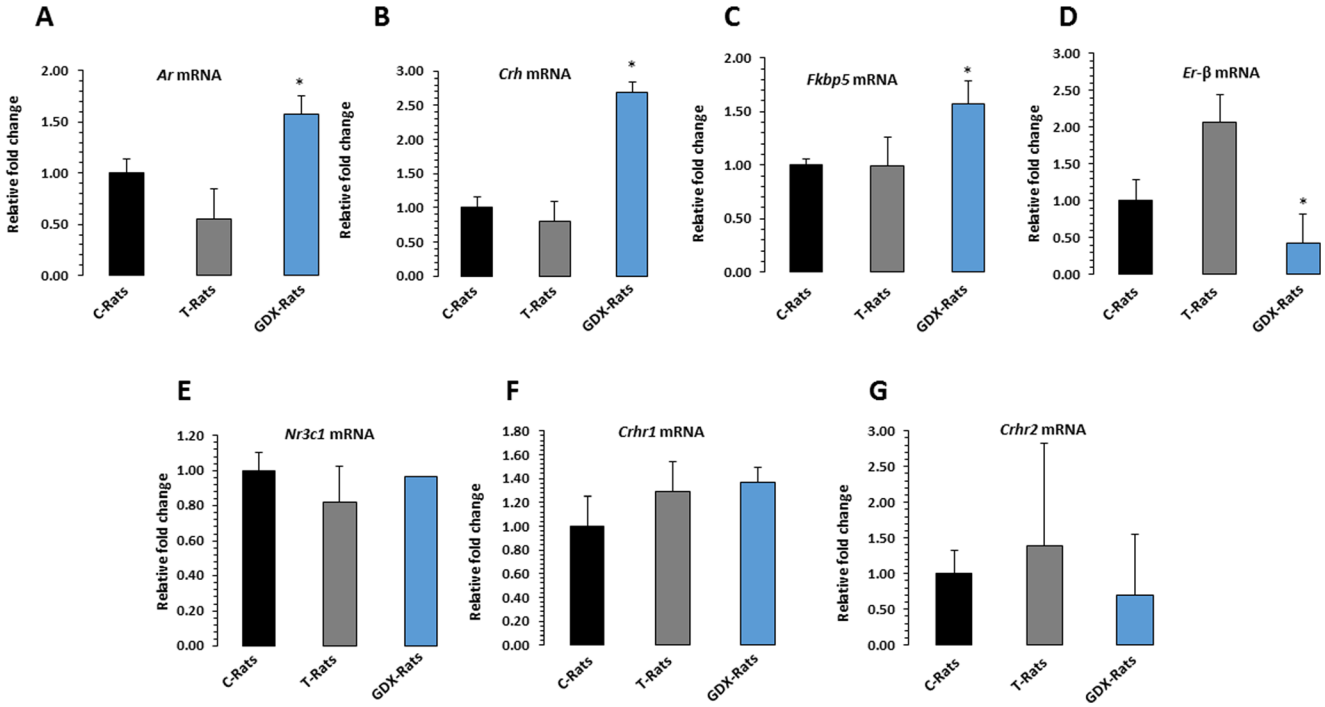

Fig. 3 mRNA-related expression of HPA- and HPG axis genes in the prefrontal cortex of C, GDX, and T rats as determined by qPCR. **a** mRNA expression of *Ar* gene did not differ significantly between *T* and *C* groups ($p = 0.07$). mRNA expression differed significantly between the GDX and C groups ($p = 0.045$). **b** mRNA expression of *Crh* gene did not differ significantly between the T and C groups ($p = 0.53$). mRNA expression differed significantly between the GDX and C groups ($p = 0.017$). **c** mRNA expression of *Fkbp5* gene did not differ significantly between the T and C groups ($p = 0.96$). mRNA expression differed significantly between the GDX and C groups ($p = 0.019$). **d** mRNA expression of *Er-ß* gene did not differ significantly between the T and C groups ($p = 0.059$). mRNA expression differed significantly between the GDX and C groups ($p = 0.036$). **(E)** mRNA expression of *Nr3c1* gene neither significantly differed between the T and C groups ($p = 0.28$) nor between the GDX and C groups ($p = 0.76$). **f** mRNA expression of *Crhr1* gene neither significantly differed between the T and C groups ($p = 0.25$) nor between the GDX and C groups ($p = 0.14$). **g** mRNA expression of *Crhr2* gene neither significantly differed between the T and C-grousp ($p = 0.78$) nor between GDX group and C group ($p = 0.57$). Data is represented as ± SEM.

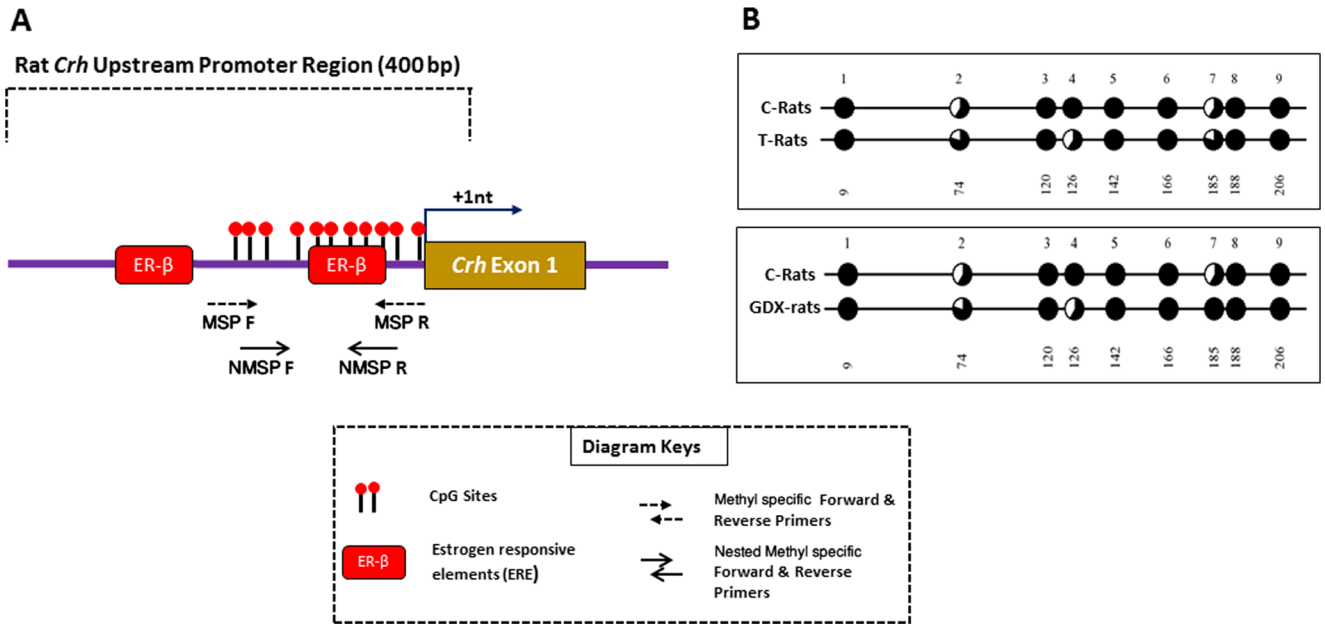

Fig. 4 Methylation of *C*rh promoter region. **a** The schematic diagram is showing the 400-bp upstream region of *Crh* gene with relative binding sites for ER-β. The in silico identified that the 11 CpG sites are indicated with ball and stick drawing relative to the 1st exon of *Crh* gene. The primer binding positions for promoter methylation analysis are also indicated with line diagram. **b** The diagram represents the nine detectable CpG sites on *Crh* promoter and their group-wise collective methylation status compared between C vs. T and C vs. GDX rats

promoter interaction profile of ER-β on *Crh* gene between GDX and T rats. The results showed an increased binding of ER-β on *Crh* promoter in T rats ($p = 0.09$). On contrary, a notable decrease in ER-β binding on *Crh* promoter ($p = 0.15$) was identified in GDX rats (Fig. 5b). For the GDX group of samples, the depleted binding of ER-β on *Crh* promoter was matched with the significant low expression of *Er-β* as well as high level of *Crh* gene expression assuming a negative or inhibitory effect of ER-β on *Crh* gene transcription.

Discussion

The effect of the chronic testosterone injections as well as the effect of the gonadectomy was established by measuring serum levels of testosterone, estradiol, and corticosterone. T group (T rats) had significantly higher, physiologically relevant levels of testosterone than the control group (C rats). On the other hand, GDX group (GDX rats) had significantly lower levels of testosterone than the control group. No significant differences were found for estradiol levels, which suggest that the exogenous testosterone was not aromatized to estradiol but was metabolized to a different metabolite. It is well known that testosterone has the potential to be aromatized, and it holds a moderate affinity to bind to the aromatase enzyme compared with other androgens. Interestingly, in a recent study, it has been demonstrated that both testosterone propionate and dihydrotestosterone propionate inhibit HPA axis [30]. The corticosterone concentration followed a similar pattern of distribution such that it was higher in the T group but lower in the GDX group although they did not reach significance in between groups, which could be due to a relatively small sample size and a relatively high variation in corticosterone levels among individual rats. Although our data is not significant, it is pertinent to mention that high exogenous doses of dihydrotestosterone can decrease corticosterone levels [31]. A recent study also found that testosterone and corticosterone levels were negatively correlated in psychologically stressed rats, which is in line with previous results, showing an inhibition of the HPA axis by high levels of testosterone [32]. A recent publication found increased cortisol response to stress in healthy men who received exogenous testosterone compared with placebo. The treated subjects also showed an increased negative effect in anticipation to the stressor compared with the subjects receiving placebo. Although the study designs between this study and the present study differ (application of testosterone: topical gel vs. sq injection, long-term vs. short-term treatment), it appears important to mention the results since they translate our findings to clinical aspects [33].

Using the escape-latency test, depression-like behavior was assessed. The T rats that were exposed to inescapable shocks showed significantly stronger depression-like behavior than any other tested group. Interestingly, T rats having not undergone inescapable shocks (TC), showed significantly less depressive-like symptoms compared with the T rats that received inescapable shocks (IS). This suggests that in order to reach the threshold of depression-like behavior, both chronic administration of testosterone and exposure to traumatic events (inescapable shocks) are required. The GDX group had the lowest escape-latency scores but not significantly lower than the control group. There were no significant differences between the other groups. So far, there is no study examining the effect of gonadectomy/testosterone treatment on the behavioral outcome of the learned helplessness paradigm. There is one study, which compared the outcomes of learned helplessness between female and male rats. Interestingly, female rats did not show learned helplessness behavior. On the other hand, male rats did show helplessness behavior but there was no difference between GDX and sham rats with normal testosterone levels [34]. These results were replicated in our study as we also did not find any significant difference in escape latency between GDX and control rats.

The forced swim test (FST) is another well-established behavioral paradigm to test for depression-like behavior. Most studies have previously shown that the absence of gonadal hormones induces anxiety-like behavior as well as depression-like behavior [35, 36]. In the forced swim test paradigm, replacement of testosterone induces antidepressant-like effects [37, 38]. Filova et al. [39] used the same behavioral approach but did not find any significant differences between the GDX group and the group supplemented with testosterone [39]. There are several explanations for the contrasting findings of this study. First of all, a different behavioral test was used in our study and although they are thought to measure the same phenotype and although LH and FST are shown to be positively correlated with each other [40], there might be factors within the experiment that influence the outcome in certain cases. In the LH paradigm, the subjects are exposed to pain, whereas FST does not include any experiences of pain. It has been suggested that testosterone decreases the perception of pain [41] and increases the pain threshold [42]. If pain threshold is the deciding factor for the motivation to escape quickly, which translates to low depression score, then subjects with high testosterone level would have a very low perception of pain, and they would therefore not be motivated to escape quickly, showing depression-like symptoms in the LH paradigm.

Another explanation could be that there is no consistent correlation between testosterone levels and depression-like symptoms but that depression-like symptoms are found on both ends of the spectrum. Data from chronic administration of supraphysiological doses of testosterone revealed anxiogenic-like behavior in the elevated plus maze test [43]. There are several publications suggesting that suicide attempts correlate with high levels of testosterone [10–12, 20]. Recent

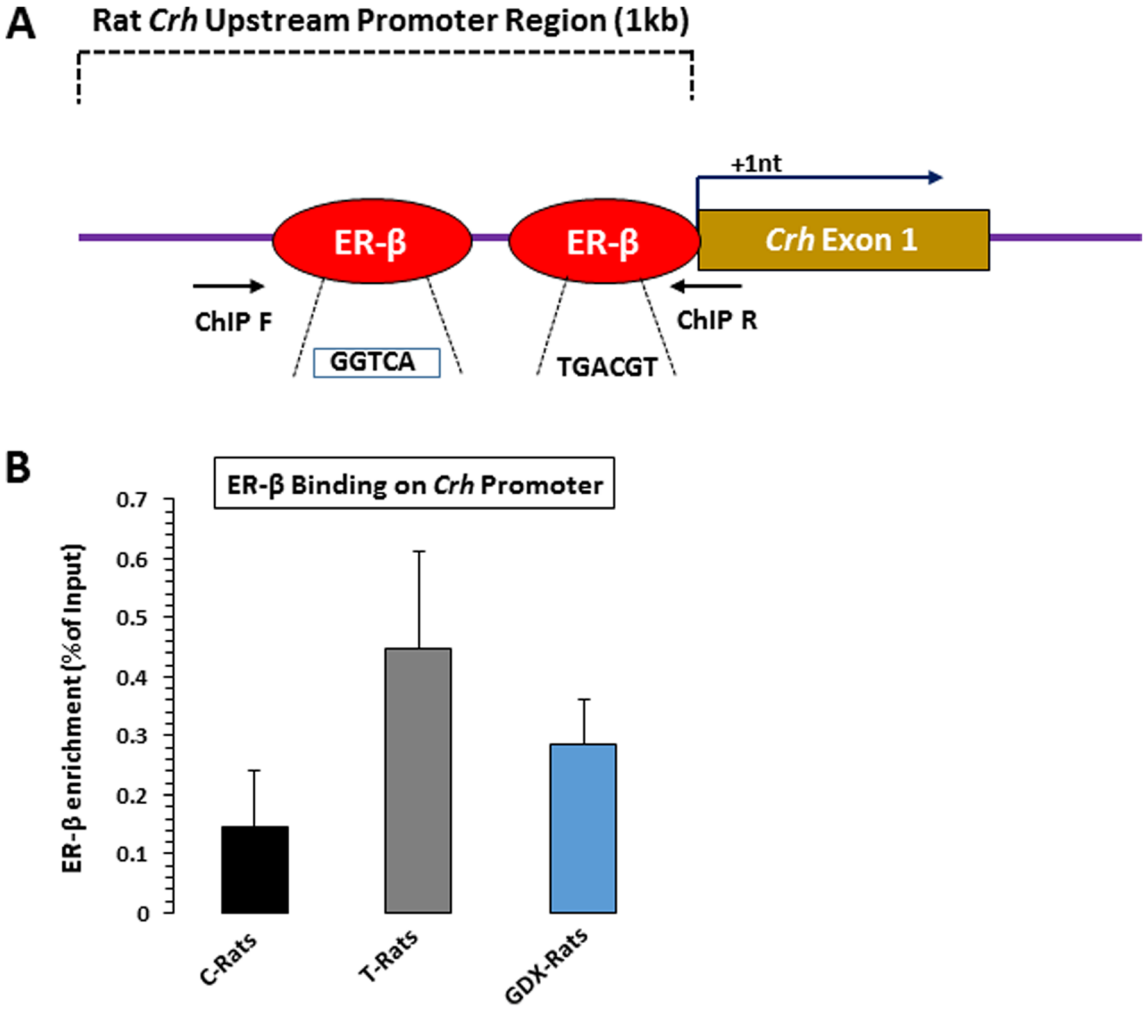

Fig. 5 ChIP Assay for ER-β binding on *Crh* Promoter in rat PFC. **a** Schematic diagram representing two ER-β binding sites on upstream promoter region of rat *Crh* gene. The diagram also represents the two binding sites with their corresponding consensus motifs. The ChIP-specific primer annealing sites are also presented in the promoter region of *Crh* gene relative to the transcriptional start site (not to scale). **b** Binding of ER-β on *Crh* gene promoter demonstrated the differential promoter occupancy in T and GDX rats as compared with C rats ($n = 3$/group). Data is represented as ± SEM

studies found rats treated with supraphysiological doses of testosterone show depression-like symptoms, operationalized by the tail suspension test [44] and the forced swim test [45]. This is also highly relevant regarding long-term anabolic androgenic steroid (AAS) abuse. There seems to be an association between depressive symptoms and long-term abuse of AAS [46, 47]. Altogether, it appears that long-term treatment with supraphysiological doses of testosterone induces depression-like symptoms in rats that were additionally exposed to uncontrollable stressors. This hypothesis has to be further explored and the results have to be replicated both for LH and other behavioral tests.

To examine the interaction of HPG and HPA axis, expression levels of various HPA and the HPG axis genes were assessed. We found that levels of HPA axis genes (*Crh* and *Fkbp5*) were upregulated in PFC of GDX rats. This is consistent with the previous findings showing similar results [4, 22, 48]. We were not able to replicate the findings of re-establishing inhibition of the HPA axis by supplementing GDX rats with testosterone. A potential explanation might be that supraphysiological levels of testosterone affect a different downstream pathway than supplemental testosterone doses do. It has been shown that androgen replacement therapy increased *Crhr2* level which was hypothesized to be due to *Crh* levels modifying the *Crhr2* expression on the cell surface [49]. In our study, no significant difference was detected when testing *Crhr2* expression in-between groups. The different study design (androgen replacement vs. supra-physiological levels of testosterone) might explain this discrepancy.

Androgens are known to decrease and increase *Ar* expression by altering mRNA stability [50]. Since the half-life of an mRNA is crucial to determine how long mRNA can be translated into proteins, this posttranscriptional regulation is one of the most rapid ways to regulate gene expression. It has been

suggested that the direction of regulation of *Ar* by androgens is divergent and cell-specific [51]. In most tissues, androgen action is downregulating *Ar* mRNA [52], which is in line with our findings. GDX rats showed a significant upregulation of *Ar* mRNA, whereas T rats failed to show significant downregulation but revealed a trend in this direction ($p = 0.07$).

We hypothesized that exogenous testosterone might also be metabolized to 5alpha-androstane 3beta,17beta diol (3-β-diol) and consequently binds to ER-β. To test this hypothesis, we assessed the expression of *Er-β* and found a significant upregulation in the GDX rats and a trend towards downregulation in the T rats. Handa et al. [53] suggest that treatment with 3-beta-diol leads to an upregulation of *Er-β* [53]. We further hypothesized that the lack of 3-β-diol in GDX rats leads to a downregulation of *Er-β* expression and the lack of inhibition activated the stress-response and caused the concomitant upregulation of *Crh* gene expression. Previous studies support this hypothesis [53–55].

Steroid hormones, such as testosterone and its metabolites, control HPA axis gene by regulating its gene transcription (through protein-protein interaction or by binding directly to response elements) [56]. The classic pathway for agonist-bound ER-β would be to bind to palindromic estrogen receptor elements (EREs) in the promoter region in order to activate transcription. Interestingly, there are five interspersed perfect half palindromic EREs in the *Crh* promoter region [57]. There are several other pathways that have been investigated in relation to the promoter region of *Crh*, i.e., cAMP-response elements (CRE) and activator protein-1 (AP-1) activity. Recent data suggests that AP-1 sites are not the predominant sites that regulate the *Crh* gene through ER-β binding [58]. Contradictory data findings exist concerning the quality of action of ER-β on *Crh* expression. Chen et al.'s [59] findings suggest a stimulatory role of ER-β on *Crh* expression, which might be mediated through functional EREs in the promoter region of *Crh* [59]. Handa et al. [54] on the other hand suggest that the androgen metabolite 3-β-diol confers inhibitory effects on *Crh* through its binding to ER-β [54]. These effects might also depend on the brain region and could be shown for the periventricular nucleus of the hypothalamus (PVN) by the same group [60]. Our results suggest that the action conveyed through ER-β might be inhibitory. ChIP-based qPCR data revealed a decreased binding of ER-β on *Crh* proximal promoter region which corresponds well with downregulated of *Er-β* expression seen in the GDX rats. This might have resulted in upregulated *Crh* expression with an assumption of ER-β being repressive on the *Crh* promoter. Similarly, a change in ER-β binding on *Crh* promoter was also noticed in T rats. An increased interaction between ER-β and Crh promoter could possibly be indicating the underlying cause of decreased *Crh* gene transcription in T rats.

Most publications report a hyperactivation of the HPA system in depression-like behavior and according to that, an upregulation of *Crh* mRNA expression in depression-like rodent models [61]. There are several potential explanations why contrary to previous findings, depression-like behavior goes hand in hand with a nonsignificant downregulation of HPA axis genes in our sample and why resilient behavior in GDX rats correlates with a significant upregulation of HPA axis genes. First of all, there is the possibility that LH does not measure depressive-like behavior but anxiety-like behavior and that GDX rats showing lower escape latency is a reflection of their lower threshold of stress (with a concomitant HPA activation) whereas the T rats show a higher threshold of stress and thus do not react adequately to the stress stimuli in the escape latency test (with a concomitant blunted HPA response). Another possible explanation for the present data could be that supraphysiological levels of testosterone decrease the pain perception, thus T rats are not as motivated to escape and show a decreased HPA response compared with GDX rats [41, 42]. Last but not least, HPA axis activation might not be a neurobiological correlate of depressive-like behavior. This last hypothesis is contradicting the consensus of the scientific community based on a myriad of previous findings.

Also, there are additional pathways of testosterone metabolites that might explain the behavioral findings. Instead of testosterone being metabolized to 3-β-diol, it might have been metabolized to 5 alpha-Androstan-3 alpha, 17 beta-diol (3α-diol), which is a positive allosteric modulator of $GABA_A$-receptors [62], conveying sedative and anxiolytic effects. One limitation of this study is the small-sample size of the shocked rats, and the fact that only one behavioral paradigm was used to test for depressive-like behavior. Another shortcoming of the current study is the lack of generalizability of these findings to other brain regions. Further research in other physiologically relevant regions, such as the paraventricular nucleus of the hypothalamus is needed to further elucidate the molecular mechanism of HPG-HPA interaction.

Conclusion

Altogether, our study suggests that HPA inhibition by long-term androgen treatment in supraphysiological doses leads to less resilience and a higher susceptibility to depression-like symptoms. This appears to be associated with altered expression of HPA axis genes in conjunction with *Crh* regulation by ER-β. Further studies, concerning the influence of steroids on the susceptibility to depression and the underlying changes in gene transcription are needed to further elucidate the complex interaction between depressive phenotype, HPA axis, and sex hormones.

Funding Information The research was supported by grants from the National Institute of Mental Health (R01MH082802, 1R01MH101890, R01MH100616, 1R01MH107183-01) to Dr. Dwivedi. The sponsoring agency had no role in study design, collection, analysis, interpretation of data, and in the writing of the manuscript.

Compliance with Ethical Standards

Research Involving Animals This research involves the use of animals. The study was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Cohen-Woods S, Craig IW, McGuffin P (2013) The current state of play on the molecular genetics of depression. Psychol Med 43(4): 673–687. https://doi.org/10.1017/S0033291712001286
2. Krishnan V, Nestler EJ (2008) The molecular neurobiology of depression. Nature 455(7215):894–902. https://doi.org/10.1038/nature07455
3. Murgatroyd C, Spengler D (2011) Epigenetic programming of the HPA axis: early life decides. Stress 14(6):581–589. https://doi.org/10.3109/10253890.2011.602146
4. Handa RJ, Nunley KM, Lorens SA, Louie JP, McGivern RF, Bollnow MR (1994) Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. Physiol Behav 55(1):117–124
5. Handa RJ, Weiser MJ (2014) Gonadal steroid hormones and the hypothalamo-pituitary-adrenal axis. Front Neuroendocrinol 35(2): 197–220. https://doi.org/10.1016/j.yfrne.2013.11.001
6. Faravelli C, Alessandra Scarpato M, Castellini G, Lo Sauro C (2013) Gender differences in depression and anxiety: the role of age. Psychiatry Res 210(3):1301–1303. https://doi.org/10.1016/j.psychres.2013.09.027
7. Arsenault-Lapierre G, Kim C, Turecki G (2004) Psychiatric diagnoses in 3275 suicides: a meta-analysis. BMC Psychiatry 4:37. https://doi.org/10.1186/1471-244X-4-37
8. Moller-Leimkuhler AM (2003) The gender gap in suicide and premature death or: why are men so vulnerable? Eur Arch Psychiatry Clin Neurosci 253(1):1–8. https://doi.org/10.1007/s00406-003-0397-6
9. Serafini G, Pompili M, Innamorati M, Rihmer Z, Sher L, Girardi P (2012) Can cannabis increase the suicide risk in psychosis? A critical review. Curr Pharm Des 18(32):5165–5187
10. Sher L, Grunebaum MF, Sullivan GM, Burke AK, Cooper TB, Mann JJ, Oquendo MA (2014) Association of testosterone levels and future suicide attempts in females with bipolar disorder. J Affect Disord 166: 98–102. https://doi.org/10.1016/j.jad.2014.04.068
11. Sher L, Grunebaum MF, Sullivan GM, Burke AK, Cooper TB, Mann JJ, Oquendo MA (2012) Testosterone levels in suicide attempters with bipolar disorder. J Psychiatr Res 46(10):1267–1271. https://doi.org/10.1016/j.jpsychires.2012.06.016
12. Sher L (2017) Commentary: CSF and plasma testosterone in attempted suicide. Front Public Health 5:92. https://doi.org/10.3389/fpubh.2017.00092
13. Zhang J, Jia CX, Wang LL (2015) Testosterone differs between suicide attempters and community controls in men and women of China. Physiol Behav 141:40–45. https://doi.org/10.1016/j.physbeh.2015.01.004
14. Tripodianakis J, Markianos M, Rouvali O, Istikoglou C (2007) Gonadal axis hormones in psychiatric male patients after a suicide attempt. Eur Arch Psychiatry Clin Neurosci 257(3):135–139. https://doi.org/10.1007/s00406-006-0686-y
15. Perez-Rodriguez MM, Lopez-Castroman J, Martinez-Vigo M, Diaz-Sastre C, Ceverino A, Nunez-Beltran A, Saiz-Ruiz J, de Leon J et al (2011) Lack of association between testosterone and suicide attempts. Neuropsychobiology 63(2):125–130. https://doi.org/10.1159/000318085
16. Long N, Nguyen L, Stevermer J (2015) PURLS: It's time to reconsider early-morning testosterone tests. J Fam Pract 64(7):418–419
17. Giegling I, Rujescu D, Mandelli L, Schneider B, Hartmann AM, Schnabel A, Maurer K, De Ronchi D et al (2008) Estrogen receptor gene 1 variants are not associated with suicidal behavior. Psychiatry Res 160(1):1–7. https://doi.org/10.1016/j.psychres.2007.05.007
18. Ostlund H, Keller E, Hurd YL (2003) Estrogen receptor gene expression in relation to neuropsychiatric disorders. Ann N Y Acad Sci 1007:54–63
19. Graae L, Karlsson R, Paddock S (2012) Significant association of estrogen receptor binding site variation with bipolar disorder in females. PLoS One 7(2):e32304. https://doi.org/10.1371/journal.pone.0032304
20. Walther A, Rice T, Kufert Y, Ehlert U (2016) Neuroendocrinology of a male-specific pattern for depression linked to alcohol use disorder and suicidal behavior. Front Psychol 7:206. https://doi.org/10.3389/fpsyt.2016.00206
21. Williamson M, Viau V (2008) Selective contributions of the medial preoptic nucleus to testosterone-dependant regulation of the paraventricular nucleus of the hypothalamus and the HPA axis. Am J Physiol Regul Integr Comp Physiol 295(4):R1020–R1030. https://doi.org/10.1152/ajpregu.90389.2008
22. Bingaman EW, Magnuson DJ, Gray TS, Handa RJ (1994) Androgen inhibits the increases in hypothalamic corticotropin-releasing hormone (CRH) and CRH-immunoreactivity following gonadectomy. Neuroendocrinology 59(3):228–234
23. Goto Y, Yang CR, Otani S (2010) Functional and dysfunctional synaptic plasticity in prefrontal cortex: roles in psychiatric disorders. Biol Psychiatry 67(3):199–207. https://doi.org/10.1016/j.biopsych.2009.08.026
24. McKlveen JM, Myers B, Flak JN, Bundzikova J, Solomon MB, Seroogy KB, Herman JP (2013) Role of prefrontal cortex glucocorticoid receptors in stress and emotion. Biol Psychiatry 74(9):672–679. https://doi.org/10.1016/j.biopsych.2013.03.024
25. Marrocco J, McEwen BS (2016) Sex in the brain: hormones and sex differences. Dialogues Clin Neurosci 18(4):373–383
26. Cooper SE, Wood RI (2014) Androgens and opiates: testosterone interaction with morphine self-administration in male rats. Neuroreport 25(7):521–526. https://doi.org/10.1097/WNR.0000000000000125
27. Smalheiser NR, Lugli G, Rizavi HS, Zhang H, Torvik VI, Pandey GN, Davis JM, Dwivedi Y (2011) MicroRNA expression in rat brain exposed to repeated inescapable shock: differential alterations in learned helplessness vs. non-learned helplessness. Int J Neuropsychopharmacol 14(10):1315–1325. https://doi.org/10.1017/S1461145710001628
28. Timberlake MA 2nd, Dwivedi Y (2015) Altered expression of endoplasmic reticulum stress associated genes in hippocampus of learned helpless rats: relevance to depression pathophysiology. Front Pharmacol 6:319. https://doi.org/10.3389/fphar.2015.00319
29. Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec; 25(4):402–8. https://doi.org/10.1006/meth.2001.1262
30. Handa RJ, Kudwa AE, Donner NC, McGivern RF, Brown R (2013) Central 5-alpha reduction of testosterone is required for testosterone's inhibition of the hypothalamo-pituitary-adrenal axis response to restraint stress in adult male rats. Brain Res 1529:74–82. https://doi.org/10.1016/j.brainres.2013.07.021
31. Toufexis DJ, Wilson ME (2012) Dihydrotestosterone differentially modulates the cortisol response of the hypothalamic-pituitary-adrenal axis in male and female rhesus macaques, and restores circadian secretion of cortisol in females. Brain Res 1429:43–51. https://doi.org/10.1016/j.brainres.2011.10.024
32. Stephens MA, Mahon PB, McCaul ME, Wand GS (2016) Hypothalamic-pituitary-adrenal axis response to acute psychosocial

stress: effects of biological sex and circulating sex hormones. Psychoneuroendocrinology 66:47–55. https://doi.org/10.1016/j.psyneuen.2015.12.021
33. Knight EL, Christian CB, Morales PJ, Harbaugh WT, Mayr U, Mehta PH (2017) Exogenous testosterone enhances cortisol and affective responses to social-evaluative stress in dominant men. Psychoneuroendocrinology 85:151–157. https://doi.org/10.1016/j.psyneuen.2017.08.014
34. Dalla C, Edgecomb C, Whetstone AS, Shors TJ (2008) Females do not express learned helplessness like males do. Neuropsychopharmacol 33(7):1559–1569. https://doi.org/10.1038/sj.npp.1301533
35. Wainwright SR, Lieblich SE, Galea LA (2011) Hypogonadism predisposes males to the development of behavioural and neuroplastic depressive phenotypes. Psychoneuroendocrinology 36(9):1327–1341. https://doi.org/10.1016/j.psyneuen.2011.03.004
36. Frye CA, Edinger KL, Lephart ED, Walf AA (2010) 3alpha-androstanediol, but not testosterone, attenuates age-related decrements in cognitive, anxiety, and depressive behavior of male rats. Front Aging Neurosci 2:15. https://doi.org/10.3389/fnagi.2010.00015
37. Frye CA, Walf AA (2009) Depression-like behavior of aged male and female mice is ameliorated with administration of testosterone or its metabolites. Physiol Behav 97(2):266–269. https://doi.org/10.1016/j.physbeh.2009.02.022
38. Wainwright SR, Workman JL, Tehrani A, Hamson DK, Chow C, Lieblich SE, Galea LA (2016) Testosterone has antidepressant-like efficacy and facilitates imipramine-induced neuroplasticity in male rats exposed to chronic unpredictable stress. Horm Behav 79:58–69. https://doi.org/10.1016/j.yhbeh.2016.01.001
39. Filova B, Malinova M, Babickova J, Tothova L, Ostatnikova D, Celec P, Hodosy J (2015) Effects of testosterone and estradiol on anxiety and depressive-like behavior via a non-genomic pathway. Neurosci Bull 31(3):288–296. https://doi.org/10.1007/s12264-014-1510-8
40. Pare WP (1994) Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats. Physiol Behav 55(3):433–439
41. Choi JC, Chung MI, Lee YD (2012) Modulation of pain sensation by stress-related testosterone and cortisol. Anaesthesia 67(10):1146–1151. https://doi.org/10.1111/j.1365-2044.2012.07267.x
42. Pednekar JR, Mulgaonker VK (1995) Role of testosterone on pain threshold in rats. Indian J Physiol Pharmacol 39(4):423–424
43. Selakovic D, Joksimovic J, Obradovic D, Milovanovic D, Djuric M, Rosic G (2016) The adverse effects of exercise and supraphysiological dose of testosterone-enanthate (TE) on exploratory activity in elevated plus maze (EPM) test—indications for using total exploratory activity (TEA) as a new parameter for ex. Neuro Endocrinol Lett 37(5):383–388
44. Joksimovic J, Selakovic D, Jakovljevic V, Mihailovic V, Katanic J, Boroja T, Rosic G (2017) Alterations of the oxidative status in rat hippocampus and prodepressant effect of chronic testosterone enanthate administration. Mol Cell Biochem 433:41–50. https://doi.org/10.1007/s11010-017-3014-0
45. Matrisciano F, Modafferi AM, Togna GI, Barone Y, Pinna G, Nicoletti F, Scaccianoce S (2010) Repeated anabolic androgenic steroid treatment causes antidepressant-reversible alterations of the hypothalamic-pituitary-adrenal axis, BDNF levels and behavior. Neuropharmacology 58(7):1078–1084. https://doi.org/10.1016/j.neuropharm.2010.01.015
46. Eklof AC, Thurelius AM, Garle M, Rane A, Sjoqvist F (2003) The anti-doping hot-line, a means to capture the abuse of doping agents in the Swedish society and a new service function in clinical pharmacology. Eur J Clin Pharmacol 59(8–9):571–577. https://doi.org/10.1007/s00228-003-0633-z
47. Perry PJ, Kutscher EC, Lund BC, Yates WR, Holman TL, Demers L (2003) Measures of aggression and mood changes in male weightlifters with and without androgenic anabolic steroid use. J Forensic Sci 48(3):646–651
48. Viau V, Lee P, Sampson J, Wu J (2003) A testicular influence on restraint-induced activation of medial parvocellular neurons in the paraventricular nucleus in the male rat. Endocrinology 144(7):3067–3075. https://doi.org/10.1210/en.2003-0064
49. Weiser MJ, Goel N, Sandau US, Bale TL, Handa RJ (2008) Androgen regulation of corticotropin-releasing hormone receptor 2 (CRHR2) mRNA expression and receptor binding in the rat brain. Exp Neurol 214(1):62–68. https://doi.org/10.1016/j.expneurol.2008.07.013
50. Ing NH (2005) Steroid hormones regulate gene expression posttranscriptionally by altering the stabilities of messenger RNAs. Biol Reprod 72(6):1290–1296. https://doi.org/10.1095/biolreprod.105.040014
51. Yeap BB, Krueger RG, Leedman PJ (1999) Differential posttranscriptional regulation of androgen receptor gene expression by androgen in prostate and breast cancer cells. Endocrinology 140(7):3282–3291. https://doi.org/10.1210/endo.140.7.6769
52. Burnstein KL, Maiorino CA, Dai JL, Cameron DJ (1995) Androgen and glucocorticoid regulation of androgen receptor cDNA expression. Mol Cell Endocrinol 115(2):177–186
53. Handa RJ, Sharma D, Uht R (2011) A role for the androgen metabolite, 5alpha androstane 3beta, 17beta diol (3beta-diol) in the regulation of the hypothalamo-pituitary-adrenal axis. Front Endocrinol (Lausanne) 2:65. https://doi.org/10.3389/fendo.2011.00065
54. Handa RJ, Weiser MJ, Zuloaga DG (2009) A role for the androgen metabolite, 5alpha-androstane-3beta,17beta-diol, in modulating oestrogen receptor beta-mediated regulation of hormonal stress reactivity. J Neuroendocrinol 21(4):351–358. https://doi.org/10.1111/j.1365-2826.2009.01840.x
55. Lund TD, Munson DJ, Haldy ME, Handa RJ (2004) Dihydrotestosterone may inhibit hypothalamo-pituitary-adrenal activity by acting through estrogen receptor in the male mouse. Neurosci Lett 365(1):43–47. https://doi.org/10.1016/j.neulet.2004.04.035
56. Ni X, Nicholson RC (2006) Steroid hormone mediated regulation of corticotropin-releasing hormone gene expression. Frointiers in Bioscience: a Journal and Virtual Library 11:2909–2917
57. Vamvakopoulos NC, Chrousos GP (1993) Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression. Potential implications for the sexual dimophism of the stress response and immune/inflammatory reaction. J Clin Invest 92(4):1896–1902. https://doi.org/10.1172/JCI116782
58. Miller WJ, Suzuki S, Miller LK, Handa R, Uht RM (2004) Estrogen receptor (ER)beta isoforms rather than ERalpha regulate corticotropin-releasing hormone promoter activity through an alternate pathway. J Neurosci 24(47):10628–10635. https://doi.org/10.1523/JNEUROSCI.5540-03.2004
59. Chen XN, Zhu H, Meng QY, Zhou JN (2008) Estrogen receptor-alpha and -beta regulate the human corticotropin-releasing hormone gene through similar pathways. Brain Res 1223:1–10. https://doi.org/10.1016/j.brainres.2008.05.043
60. Hiroi R, Lacagnina AF, Hinds LR, Carbone DG, Uht RM, Handa RJ (2013) The androgen metabolite, 5alpha-androstane-3beta,17beta-diol (3beta-diol), activates the oxytocin promoter through an estrogen receptor-beta pathway. Endocrinology 154(5):1802–1812. https://doi.org/10.1210/en.2012-2253
61. Swaab DF, Bao AM, Lucassen PJ (2005) The stress system in the human brain in depression and neurodegeneration. Ageing Res Rev 4(2):141–194. https://doi.org/10.1016/j.arr.2005.03.003
62. Reddy DS, Jian K (2010) The testosterone-derived neurosteroid androstanediol is a positive allosteric modulator of GABAA receptors. J Pharmacol Exp Ther 334(3):1031–1041. https://doi.org/10.1124/jpet.110.169854