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Regular Article

The influence of the HPG axis on stress response and depressive-like behaviour in a transgenic mouse model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disease caused by a CAG tandem repeat mutation encoding a polyglutamine tract expansion in the huntingtin protein. Depression is among the most common affective symptoms in HD but the pathophysiology is unclear. We have previously discovered sexually dimorphic depressive-like behaviours in the R6/1 transgenic mouse model of HD at a pre-motor symptomatic age. Interestingly, only female R6/1 mice display this phenotype. Sexual dimorphism has not been explored in the human HD population despite the well-established knowledge that the clinical depression rate in females is almost twice that of males. Female susceptibility suggests a role of sex hormones, which have been shown to modulate stress response. There is evidence suggesting that the gonads are adversely affected in HD patients, which could alter sex hormone levels. The present study examined the role sex hormones play on stress response in the R6/1 mouse model of HD, in particular, its modulatory effect on the hypothalamic–pituitary–adrenal (HPA) axis and depression-like behaviour. We found that the gonads of female R6/1 mice show atrophy at an early age. Expression levels of gonadotropin-releasing hormone (GnRH) were decreased in the hypothalamus of female HD mice, relative to wild-type female littermates, as were serum testosterone levels. Female serum estradiol levels were not significantly changed. Gonadectomy surgery reduced HPA-axis activity in female mice but had no effect on behavioural phenotypes. Furthermore, expression of the oestrogen receptor (ER) α gene was found to be higher in the adrenal cells of female HD mice. Finally, administration of an ERβ agonist diarylpropionitrile (DPN) rescued depressive-like behaviour in the female HD mice. Our findings provide new insight into the pathogenesis of sexually dimorphic neuroendocrine, physiological and behavioural endophenotypes in HD, and suggest a new avenue for therapeutic intervention.

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Introduction

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by an autosomal dominant genetic mutation in the *huntingtin* gene (The Huntington's Disease Collaborative Research Group, 1993). Depression is an early-onset symptom (Duff et al., 2007; Kirkwood et al., 2001) and has estimated prevalence of approximately 50% (Gargiulo et al., 2009; Paulsen et al., 2001, 2005; Shiwach, 1994). Despite this, aetiology of depression in HD is not well understood.

The hypothalamic–pituitary–adrenal (HPA) axis is the endocrine system responsible for stress adaptation via the production of glucocorticoids. Its hyperactivity is the most consistently found biological abnormality in clinical depression (Lok et al., 2012; Pariante and Lightman, 2008; Stetler and Miller, 2011). In HD patients, hyperactivity of the HPA-axis has also been observed (Aziz et al., 2009; Bjorkqvist et al., 2006), although analysis of this phenotype is sparse. Abnormally increased HPA-axis function has also been observed in mouse models of HD (Bjorkqvist et al., 2006; Du et al., 2012). Prolonged exposure to elevated glucocorticoid has been found to be damaging to the brains of humans (Colla et al., 2007; Crochemore et al., 2005), rodents (Sapolsky et al., 1988) and monkeys (Sapolsky, 1990). This shared pathophysiology suggests that HPA-axis dysregulation may be a convergent mechanism contributing to depression in these two diseases.

In the general population, it has long been noted that females are twice as likely as males to develop clinical depression (Breslau et al., 1995; Kendler et al., 1993) as well as generalised anxiety disorder, which is often co-morbid with depression (Hoyer et al., 2001). The female sex hormone, especially the most potent oestrogen, 17β-estradiol (E2) has received much attention for its ability to influence stress adaptation (Young and Altemus, 2004). Abnormal decrease in oestrogen levels is linked to depression and altered regulation of the HPA-axis in females. Depressed women have been found to have reduced E2 (Schuschke et al., 2000). The hypothalamic–pituitary–gonadal (HPG) axis regulates the development and function of the gonads and in depressed patients this axis was found to be diminished (Baischer et al.,

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1995; Meller et al., 2001). Correspondingly, depression rates increase in women who are undergoing menopause (Freeman, 2010; Harsh et al., 2009). Supplementing E2 has had some success in treating post-partum (Dennis et al., 2008; Rasgon et al., 2007) and post-menopausal depression (Baksu et al., 2009; Studd and Panay, 2009). In rodent models, OVX surgery resulted in a heightened sensitivity to stress-induced depression-like behaviour as measured by the forced-swim test (FST) (Bekku and Yoshimura, 2005; Bekku et al., 2006; Nakagawasai et al., 2009). E2 supplementation was found to be anxiolytic in the FST and the tail-suspension test (Dhir and Kulkarni, 2008). These behavioural differences correlated with changes in HPA-axis regulation: aged rats with diminished E2 exhibited increased corticosterone response to ether stress and ACTH challenge whilst chronic treatment with E2 corrected the phenotype (Ferrini et al., 1999; Lo et al., 2006). Interestingly, the HPG-axis is also curtailed in HD patients (Markianos et al., 2005, 2007; Van Raamsdonk et al., 2007b) as well as in rodent models of HD (Hannan and Ransome, 2012; Papalexi et al., 2005; Van Raamsdonk et al., 2007b). Whilst there has only been a few studies examining the role sex hormones in HD, this evidence suggests that changes to E2 levels, subsequently leading to heightened HPA-axis activity, could contribute to depression in HD.

Despite the extremely high prevalence of depression in the HD population, only very recently have studies examined whether sex differences exist. Investigating antidepressant usage in prodromal HD patients, Rowe and colleagues found that more female HD patients were prescribed antidepressants than males (Rowe et al., 2012), an indicator of higher rates of depression. This finding is further endorsed by another study which found that female HD patients had significantly higher rates of past and current depression than male patients (Zielonka et al., 2012). But whether sex hormones are involved is yet to be determined. The only clinical study looking at sex hormones in female HD patients measured serum testosterone levels but conspicuously not E2 levels (Markianos et al., 2007).

We have previously described a female-specific depression-related behavioural phenotype in the R6/1 transgenic mouse model of HD (Pang et al., 2009; Renoir et al., 2011, 2012), which also displayed HPA-axis hyperactivity (Du et al., 2012). Here, we investigated whether the sex-dimorphism of the phenotypes is due to modulatory effects of sex hormones. To our knowledge, this is the first study to directly examine the role of sex hormones in the context of depression in a model of Huntington's disease. We hypothesised that the HPG-axis would be dysfunctional in female R6/1 mice and that this would result in altered sex-hormone regulation of the HPA-axis, leading to its hyperactivity and subsequently depression-like behavioural phenotypes.

Methods

Animals

R6/1 transgenic mice and wild-type (WT) littermates (female only) were bred from a colony maintained at the Florey Institute of Neuroscience and Mental Health. Genotype was determined by polymerase chain reaction (PCR) with genomic DNA from tail biopsies. CAG repeat length was sequenced for all studs used for breeding purposes using the Roche FastStart PCR Master (Roche Applied Science, Indianapolis, USA) and then repeat numbers were determined by the University of Melbourne Pathology. Animals were randomly weaned into groups of 4–5 per cage (15 × 30 × 12 cm), other than ensuring that a mix of genotypes was present in each cage. All experiments were conducted at either 8 or 12 weeks of age. All mice were housed together in a room with 12 hour light/dark cycle and had access to food and water ad libitum. All experiments were approved by the Florey Institute Animal Ethics Committee in accordance to the guidelines of the National Health and Medical Research Council (NHMRC) Australia.

Gonadal weight measurements

Mice were killed via cervical dislocation. Ovaries were dissected out, surrounding fat removed and immediately weighed. Weight reported for each individual mouse is the total weight of the two ovaries in grammes. Ovary weights were measured at 8 and 12 weeks of age.

Serum estradiol measure

Serum E2 levels of 12-week-old mice were measured. The phase of the estrus cycle for each mouse was determined by using the Diff Quick staining reagent (Thermo Fisher Scientific, Scoresby, VIC, Australia) according to the manufacturer's instructions. Samples were collected in the proestrus phase, when E2 levels are at their peak. Mice were anaesthetised using isoflurane (4% initially then 2% to maintain anaesthesia). Blood was collected via cardiac puncture. Blood samples were put into 1.5 ml Eppendorf tubes and left at room temperature for 30 min to allow coagulation. They were then centrifuged at room temperature (1090 rcf for 15 min) and serum was collected and frozen immediately at −20 °C. Samples were outsourced to Monash Institute of Medical Research (Clayton, Vic, Australia), Endocrinology and Immunophysiology Laboratory and analysed via radioimmunoassay.

Serum testosterone measure

Serum testosterone concentrations were quantified in 12-week-old mice using enzyme-linked immune sorbent assay (EIA) (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer's instructions.

DEX–ACTH challenges

For the DEX–ACTH challenge, 12-week-old mice were given i.p. administration of dexamethasone (DEX) (0.1 mg/kg body weight; Sigma-Aldrich, St. Louis, MO, USA) between 0800 and 1000 H. Six hours after DEX administration, mice received ACTH (i.p., 500 μg/kg body weight; ProSpec, Rehovot, Israel). Thirty minutes post-ACTH injection, mice were killed and trunk blood was collected for corticosterone analysis.

Quantification of corticosterone

Serum corticosterone concentrations were quantified using EIA (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

OVX

Mice were subjected to OVX surgeries at 8 weeks of age to allow time for healing and for the ovary-derived endogenous E2 to be cleared when experiments were carried out at 12 weeks of age. Mice were anaesthetised with isoflurane (4% initially, then 2% to maintain anaesthesia). Two incisions were made on the dorsal side above the location of the ovaries. Ovaries were removed and incisions were sutured. Betadine ointment was used on the incisions and a single injection of meloxicam (1–3 mg/kg) was given. Mice were placed under a heat lamp individually until fully awake and moving freely. Mice were then re-housed together with original cage-mates and were given soy-free diet (SF06-053, Specialty Feeds, Glen Forrest, WA, Australia) for the duration of their housing. Sham surgeries were identical to OVX surgeries except that the ovaries were left intact.

Locomotor activity

Locomotor activity was tested during the light phase, mostly in the morning. Mice were acclimatised to the experimental room for 1 h

prior to testing. The room had light levels at 15–30 lx. Mice were placed in the middle of the arena and activities were recorded for 15 min. The arenas were cleaned thoroughly with ethanol between mice. The arena used is the Med Associates Inc. Activity Test Chambers (ENV-510) with an infrared photobeam detector (ENV-256T) and the software used is the Med Associates Inc. Activity Monitor Version 6.02 (Med Associates Inc., St Albans, Vermont).

Forced-swim test (FST)

FST was performed between 9 and 11 am to control for diurnal variations. It involves placing each individual mouse into a beaker of water (24–26 °C) for 5 min. Immobility time after the first minute in the water was measured by an experienced experimenter blind to the identity of the mice.

Diarylpropionitrile (DPN) administration

The ERβ agonist DPN was administered according to the protocol of Walf et al. (2008, 2009). Briefly, mice were injected subcutaneously with a single dose of DPN (0.1 mg/kg) or vehicle 44 h prior to the FST.

RNA extraction

Mice were killed via cervical dislocation and the brains were removed for micro-dissection of the hypothalamus. Adrenal glands were also removed. All tissues were snap frozen in liquid nitrogen and stored at −80 °C until further use. Tissue was disrupted using a sonicator and RNA was isolated using RNeasy RNA Mini kits (Qiagen, Melbourne, Victoria) according to the manufacturer's instructions. Extracted RNA was stored at −80 °C until further use. The quality and concentration of final elutes were determined using an Agilent 2100 Bioanalyser (Australian Genomic Research Facility Ltd., Melbourne, Victoria).

Reverse transcription

RNA was reverse transcribed into cDNA using SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, Mulgrave, Victoria). The reaction was performed in a GeneAmp PCR System Model 2400 (Perkin-Elmer Life Sciences, Emeryville, CA, USA). The reverse transcription conditions are as follows: 25 °C for 10 min, 42 °C for 60 min and 85 °C for 5 min. cDNA products were stored at −20 °C until further use.

Real-time quantitative PCR

Hypothalamic oestrogen receptor (ER) α and β expressions, were measured using real-time quantitative PCR using the Applied Biosystems 7500 Fast Real-time PCR system sequence detection software version 1.4 (Applied Biosystems, Foster City, CA, USA). This process quantifies the amount of PCR product once every cycle of amplification via interpolation of the fluorescent cyanine dye SYBR Green (S-4438, Sigma, Castle Hill, NSW), which increase fluorescence ($\lambda_{max} = 522$ nm) in the presence of double stranded DNA. Cyclophilin was used as an endogenous control on the same plate to detect pipetting differences or signs of contamination as well as to ensure cDNA quality. Primers were designed by first obtaining the nucleotide sequence from the NCBI database and forward and reverse primers were designed using Primer Express version 1.5. The primers were produced by Sigma (Genosys, Castle Hill, NSW). For gonadotropin-releasing hormone (GnRH), the forward sequence was: 5′-AGC ACT GGT CCT ATG GGT TG-3′ and the reverse sequence was: 5′-GGG CCA GTG CAT CTA CAT CT-3′. For ERα, the forward sequence was: 5′-AAG GGC AGT CAC AAT GAA CC-3′ and the reverse sequence was: 5′-GCC AGG TCA TTC TCC ACA TT-3′. For ERβ, the forward sequence was: 5′-CTG TGC CTC TTC TCA CAA GGA-3′ and the reverse sequence was: 5′-TGC TCC AAG GGT AGG ATG GAC-3′. Gene expression levels of target genes are expressed as a fraction of cyclophilin expression level.

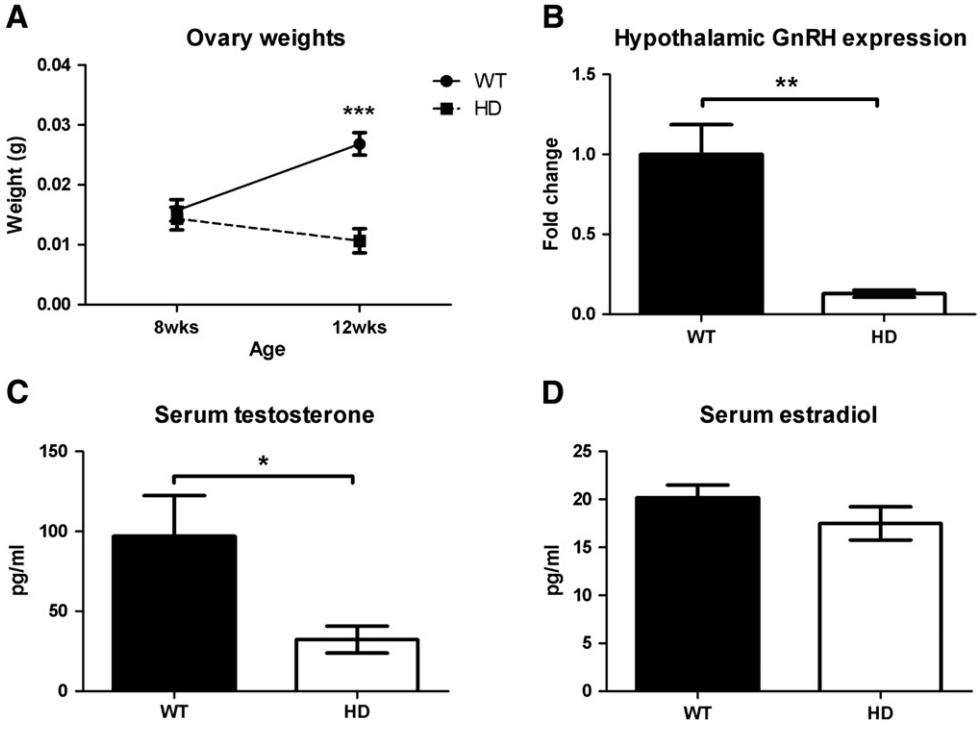


Fig. 1. HPG-axis. Female ovarian weight of HD mice is comparable to WT mice at 8 weeks with WT having a mean of 0.0157 ± 0.0018 g and HD a mean of 0.01436 ± 0.00189 g. However, by 12 weeks of age, ovaries of HD mice become significantly smaller than WT, with WT having a mean of 0.0268 ± 0.0019 g and HD a mean of 0.0107 ± 0.002 g. Two-way ANOVA, ** $p < 0.01$, *** $p < 0.001$, n = 6–9 per group (A). GnRH gene expression at 12 weeks was significantly reduced (WT − 1 ± 0.187, HD − 1 ± 0.023). Student's *t*-test, **$p < 0.01$ n = 4 per group (B). Serum testosterone level was significantly lower in HD mice (32.07 ± 8.403 pg/ml) compared to WT mice (97.09 ± 25.37 pg/ml). Student's *t*-test, n = 6 per group (C). Serum E2 level was measured during the proestrus phase. No differences in levels were detected between WT (20.168 ± 1.34 pg/ml) and HD (17.498 ± 1.74 pg/ml) animals. Student's *t*-test, n = 13 WT and 11 HD (D).

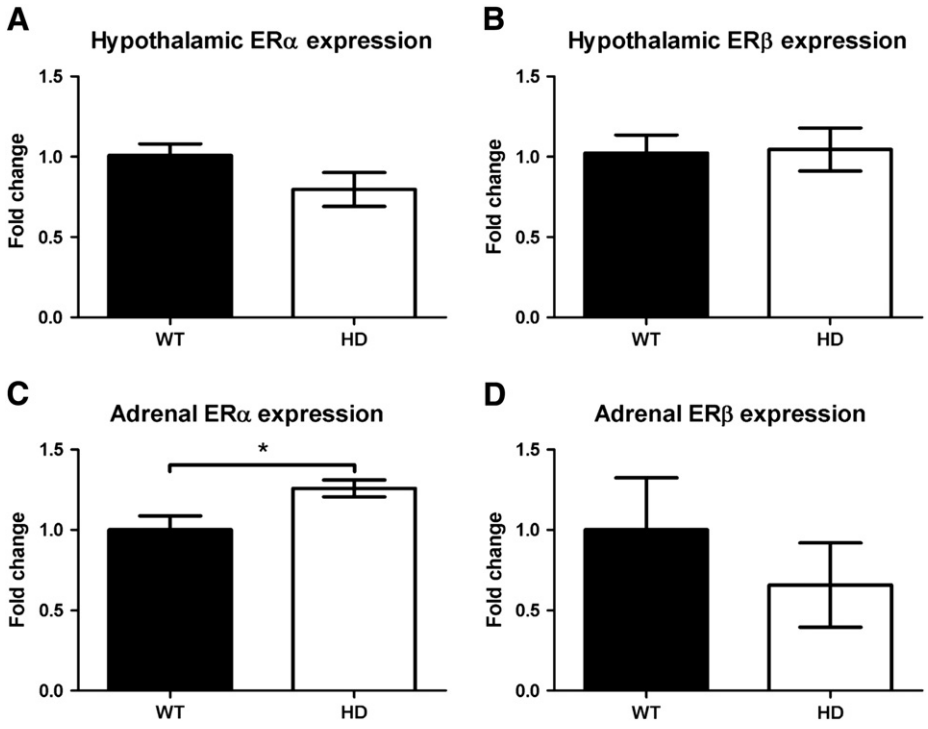


Fig. 2. ERα and ERβ gene expression in female R6/1 mice. Expression of both ERα (WT−1 ± 0.07; HD − 0.79 ± 0.11) (A) and ERβ (WT − 1 ± 0.11; HD − 1.02 ± 0.13) (B) is found to be unchanged in the hypothalamus. In the adrenal gland, ERα expression is significantly higher in R6/1 mice (1.26 ± 0.05) than WT mice (1 ± 0.09) (C) whilst no differences in ERβ expression were observed between R6/1 (0.66 ± 0.26) and WT mice (1 ± 0.32) (D). Student's *t*-tests, $^{*}p < 0.05$, n = 4–6 per group.

Statistics

Where appropriate, Student's *t*-test or 2-way ANOVA was used to analyse statistical significance between experimental factors. Significance was deemed to be got when $p < 0.05$. For the 2-way ANOVA, where significance was reached ($p < 0.05$), Bonferroni post-hoc tests were used to analyse comparisons between groups. Statistics were analysed using GraphPad Prism® 5 for windows (version 5.04).

Results

Ovary atrophy in R6/1 female mice

Ovary wet weights were taken at 8 and 12 weeks of age. Two-way ANOVA revealed a significant genotype effect ($F_{(1,40)} = 12.94$, $p = 0.0009$) and significant genotype × age interaction ($F_{(1,40)} = 9.232$, $p = 0.0042$) but no significant effect of age ($F_{(1,40)} = 2.308$, $p = 0.1365$). Bonferroni post-hoc comparison showed that whilst female ovaries did not differ in weight at 8 weeks of age ($t_{(31)} = 0.5601$, $p > 0.05$), those of the HD mice became significantly smaller than the WT at 12 weeks of age ($t_{(9)} = 3.827$, $p < 0.001$) (Fig. 1A).

Reduced hypothalamic gonadotropin-releasing hormone (GnRH) gene expression in R6/1 female mice

At 12 weeks of age, gene expression analysis shows that hypothalamic gene expression of GnRH was significantly reduced in female R6/1 mice (Student's *t*-test, $t_{(6)} = 4.82$, $p = 0.0029$) compared to controls (Fig. 1B).

Reduced serum testosterone level in R6/1 female mice

Serum testosterone level in 12-week-old mice was measured. Student's *t*-test showed that there was a significant genotype difference ($t_{(10)} = 2.421$, $p = 0.036$) (Fig. 1C).

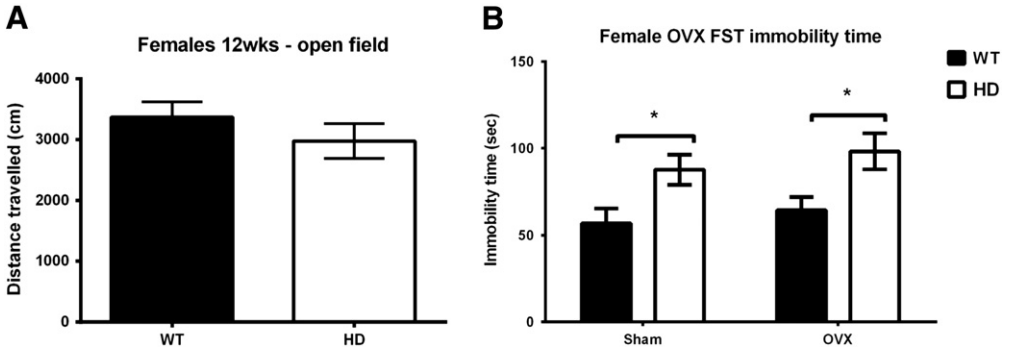


Fig. 3. Exploration and immobility time in female mice. (A) No differences were observed in the distance moved between WT (mean − 3372.64 ± 247 cm) and R6/1 mice (mean − 2975.71 ± 286.8 cm) in the open field (n = 14 and 10 respectively, unpaired *t*-test $p = 0.3073$). (B) In both treatment groups, HD mice exhibited higher immobility time than WT mice (WT sham − 56.9 ± 8.51, HD sham − 87.6 ± 8.58, WT OVX − 64.5 ± 7.50, HD OVX − 98.2 ± 10.42). No effect of OVX was observed. Two-way ANOVA, $^{*}p < 0.05$, n = 10–12 per group.

No alteration in serum E2 level in R6/1 female mice

The disruption of the HPG-axis and the link between E2 with depression, particularly in light of its ability to influence HPA-axis activity, led us to quantify the serum E2 levels in female R6/1 mice at 12 weeks of age. Student's *t*-test showed that there was no genotype difference ($t_{(22)} = 1.234$, $p = 0.2301$) (Fig. 1D).

ERα and β gene expression

Hypothalamic ERα gene expression was examined in 12-week-old female R6/1 mice but was found to be unchanged at baseline compared to WT mice (Student's *t*-test, $t_{(6)} = 1.631$, $p = 0.1541$) (Fig. 2A). Likewise, ERβ expression was found to be unaltered in the hypothalamus (Student's *t*-test, $t_{(8)} = 0.1262$, $p = 0.9027$) (Fig. 2B). In the adrenal gland, ERα expression was found to be significantly higher in R6/1 mice compared to WT (Student's *t*-test, $t_{(6)} = 2.512$, $p = 0.0458$) (Fig. 2C) whilst ERβ gene expression did not alter between genotypes (Student's *t*-test, $t_{(10)} = 0.8207$, $p = 0.4309$) (Fig. 2D).

Manipulation of sex steroids and its effects on stress response and HPA-axis regulation

Depression-related behaviour after OVX

No baseline differences in exploration are seen at 12 weeks of age between WT and HD mice ($t_{(22)} = 1.045$, $p = 0.3073$) (Fig. 3A). Four weeks after ovariectomy, at 12 weeks of age (Fig. 3B), FST revealed a significant genotype difference, with HD mice showing elevated immobility time compared to WT mice ($F_{(1,41)} = 12.79$, $p = 0.0009$). However, there was no effect due to treatment ($F_{(1,41)} = 1.021$, $p = 0.3183$) nor genotype × treatment interaction ($F_{(1,41)} = 0.02767$, $p = 0.8687$). Bonferroni post-hoc tests found that HD mice displayed significantly higher immobility times compared to WT mice in both the sham treated ($t_{(21)} = 2.443$, $p < 0.05$) and OVX mice ($t_{(20)} = 2.614$, $p < 0.05$).

Female DEX–ACTH induced serum corticosterone

The DEX–ACTH test is used to test adrenal function. There were significant genotype ($F_{(1,25)} = 4.748$, $p = 0.039$) and treatment ($F_{(1,25)} = 17.27$, $p = 0.0003$) effects but no genotype × treatment interaction ($F_{(1,25)} = 0.3709$, $p = 0.548$). Bonferroni post-hoc tests showed that OVX caused reductions in corticosterone levels for both WT ($t_{(13)} = 2.563$, $p < 0.05$) and HD ($t_{(12)} = 3.3$, $p < 0.01$) mice. Despite overall genotype difference, WT and HD corticosterone levels within each treatment group did not differ significantly (Fig. 4).

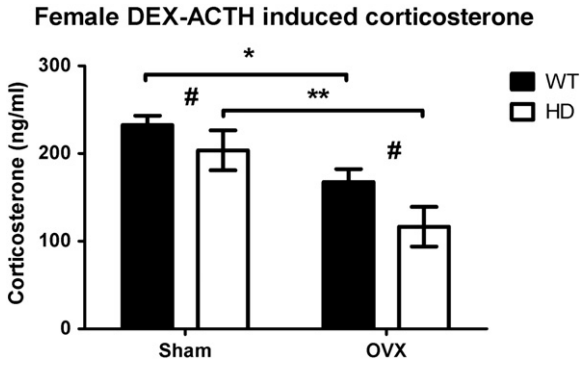


Fig. 4. Effect of gonadectomy on HPA-axis response. Challenging the HPA-axis directly using the DEX–ACTH test, we observed significant treatment effect ($p < 0.001$) suggesting that OVX lowers corticosterone response to ACTH stimulation for both WT (sham — 232.44 ± 10.70 ng/ml, OVX — 167.54 ± 14.70 ng/ml) and HD (sham — 203.71 ± 22.73 ng/ml, OVX — 116.53 ± 22.66 ng/ml) mice. A significant genotype effect ($p < 0.05$) suggests HD mice have reduced corticosterone response compared to WT. # signify significant overall genotype effect, $p < 0.039$, $^{*}p < 0.05$, $^{**}p < 0.01$, n = 6–8 per group.

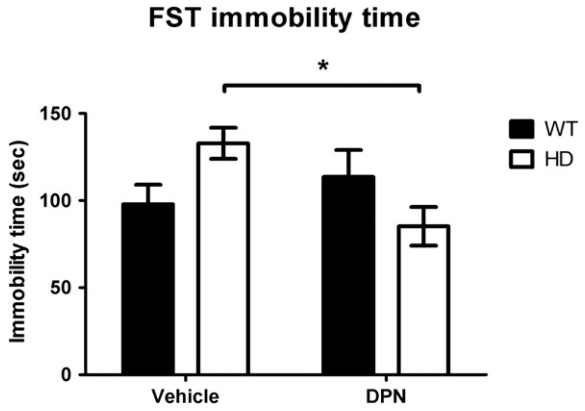


Fig. 5. Forced swim test immobility time after DPN or vehicle treatment. DPN caused a significant decrease in the immobility time of HD mice (vehicle — 132.88 ± 8.92 s, DPN — 85.23 ± 11.09 s) ($p < 0.05$) whilst not altering immobility time in WT mice (vehicle — 97.89 ± 11.17 s, DPN — 113.6 ± 15.36 s). $^{*}p < 0.05$, n = 8–13 per group.

Reduced immobility after DPN injection

Two-way ANOVA showed that an acute injection of the ERβ agonist DPN 44 h prior to the FST resulted in a significant treatment × genotype interaction ($F_{(1,36)} = 6.495$, $p = 0.0152$) but no significant treatment ($F_{(1,36)} = 1.65$, $p = 0.2072$) or genotype effects ($F_{(1,36)} = 0.0708$, $p = 0.7916$). Bonferroni post-hoc test showed that the DPN treatment resulted in a significant reduction of immobility time in HD mice compared to vehicle treated mice ($t_{(19)} = 2.741$, $p < 0.05$) (Fig. 5).

Discussion

The cause of depression in HD is ill understood despite its high prevalence. However, evidence suggests that alterations to sex-hormone regulation may contribute to its aetiology. Following our group's previous findings of depression-like behaviour in female R6/1 mice and of disrupted HPG-axis in male R6/1 mice, we now report significant abnormalities of the HPG-axis in female R6/1 mice at early stages of disease progression. Furthermore, manipulating sex hormones through OVX was able to decrease adrenal response to ACTH stimulation in both WT and R6/1 mice, reducing corticosterone release. However, behaviourally, R6/1 mice were unaffected by OVX and retained their depression-like phenotype. This finding suggests a partition between HPA-axis function and depression-related behavioural phenotypes but confirms the role E2 plays in enhancing stress responses in females. Interestingly, boosting ERβ signalling via DPN reduced depression-like behaviour in the R6/1 mice. These results show that E2 effects permeate both behavioural and physiological aspects of stress response in HD and are a potential diagnostic and therapeutic target.

HPG-axis in the R6/1 mouse model of HD

In both HD patients (Markianos et al., 2005, 2007; Van Raamsdonk et al., 2007b) and various rodent models of HD (Hannan and Ransome, 2012; Papalexi et al., 2005; Van Raamsdonk et al., 2007a), the HPG-axis has been observed to be abnormal; although in both the R6/2 and YAC128 mouse models, this was examined at advanced stages of disease progression. As depression occurs early in HD, scrutinising the HPG-axis at earlier stages is warranted to ascertain whether dysfunctions of the HPG-axis contribute to its manifestation. Our group has previously found that male R6/1 mice, at the pre-motor symptomatic age of 12 weeks, showed significantly reduced serum testosterone levels compared to WT (Hannan and Ransome, 2012). Concurring with this finding, this study established that at the same age, female R6/1 mice display abnormalities in the HPG-axis — namely atrophy of the gonads and reduced GnRH gene expression in the hypothalamus. Ovarian

atrophy in the R6/1 mice deserves further scrutiny as it has to our knowledge never been examined in HD models or humans. The early onset nature of HPG-axis abnormalities in the R6/1 mice corresponds temporally to both the depression-like phenotypes and the abnormally enhanced HPA-axis activities observed previously in the female R6/1 mice (Du et al., 2012; Pang et al., 2009), suggesting that HPG-axis abnormality may contribute to these phenotypes.

GnRH is a key regulatory gene of the HPG-axis which is synthesised and released in the hypothalamus. In the rapidly deteriorating R6/2 mouse model of HD, GnRH neuron number in the hypothalamus is reduced (Papalexi et al., 2005). However, GnRH has not been examined in the R6/1 model, particularly at an early stage of disease progression. It is known that GnRH possesses anxiolytic effects independent of their endocrine influences (Umathe et al., 2008). Reduced expression of GnRH in the R6/1 mice, therefore, may contribute to the depression-like phenotypes in addition to the developmental effects on the gonads and subsequently sex hormone production. The HPA-axis in female R6/1 mice has been found by our group to be hyperactive at 12 weeks of age (Du et al., 2012). Glucocorticoid action, via HPA-axis activation, has been found to repress GnRH release, leading to diminished E2 production in the ovaries (Kalantaridou et al., 2004). It is possible that the hyperactivity of the HPA-axis in female R6/1 mice may contribute to reduced GnRH expression but this requires further investigation. Future studies can tackle this link via manipulation of corticosterone signalling by the use of GR antagonism (i.e. mifepristone) or adrenalectomy surgery. The anxiolytic effect of GnRH itself can be explored by the use of GnRH agonists.

Testosterone levels in female HD patients have been found to be reduced (Markianos et al., 2007) whilst oestrogen levels have not been examined in patients. Previously, our group has found reduced testosterone in male R6/1 mice by 12 weeks of age (Hannan and Ransome, 2012). Likewise, the current study shows that testosterone is also significantly reduced in female mice at the same age. This agrees with the reduced GnRH expression and atrophy of the ovaries. Although studies of testosterone influence on depression in women are scant, reduced testosterone is associated with increased rates of depression (Zarrouf et al., 2009). A large cohort study reported lower salivary testosterone levels in non-medicated women and men with depression compared to controls and that the use of SSRIs is associated with increased levels of testosterone (Giltay et al., 2012). This may indirectly be due to E2 effects as E2 is metabolised from testosterone. Serum E2 levels of depressed women have been found to be lower than normal (Young et al., 2000). Depression rates also increase in women during menopause, when oestrogen levels drop (Llaneza et al., 2012; Sandilyan and Dening, 2011). However, despite R6/1 mice displaying reduced GnRH gene expression, reduced ovary weights and reduced testosterone level, serum E2 level was not significantly reduced compared to WT. The lack of change could mean that certain compensatory mechanisms which ensure stable E2 levels in the R6/1 mice are invoked by the abnormality of the HPG-axis. Such mechanisms may include adrenal compensation, which occurs in both mice and humans (Edery et al., 1982; Piltonen et al., 2002). However, more germane to the aims of the present study, the presence of depression-like phenotype and HPA-axis hyperactivity in the absence of altered E2 level suggests that any influence of E2 does not depend on its level per se, but more likely occurs downstream and reliant on their receptors and effects post-binding.

The two types of ERs, α and β, have been shown to have opposing actions in the regulation of the HPA-axis. ERβ activation can suppress some of ERα's excitatory and anxiogenic influences (Handa et al., 2009; ter Horst, 2010). An imbalance of these two receptors can potentially lead to altered stress response and depression-related phenotypes. Indeed, analysis of the hypothalamus of depression patients showed that ERα gene expression level was elevated (Wang et al., 2008). However, we found gene expression levels of both receptors in the hypothalamus of R6/1 mice to be unaltered. This suggests that the potential influence of ERs may be mediated via extra-hypothalamic regions given that ERs are expressed in a range of HPA-axis regulatory sites. Our group has recently found that abnormal hyperactivity of the HPA-axis specifically in R6/1 female mice was due to the dysfunction of the adrenal gland (Du et al., 2012). A recent study examining cynomolgus monkeys found that application of ERα agonist levormeloxifene increased cortisol production by the adrenals (Wood et al., 2012). Our finding of adrenal-specific increase in ERα expression in female R6/1 adrenal glands compared to WT mice once more points to the adrenal gland as an early centre of dysfunction in the female R6/1 mice and that altered oestrogen modulation may play a part in its dysfunction.

Manipulation of sex hormones

OVX has been used extensively to examine the effects of oestrogens on depression-related phenotypes. After OVX, mice have been found to display increased immobility time in the FST, which was subsequently restored by treatment with E2 (Bekku and Yoshimura, 2005; Bekku et al., 2006; Estrada-Camarena et al., 2011; Nakagawasai et al., 2009). However, other studies have found that the presence of ovarian hormone may in fact increase susceptibility to stress-induced depression (Deecher et al., 2008). LaPlant and colleagues found that OVX mice displayed decreased susceptibility to chronic unpredictable stress-induced anxiogenic effects, as measured by the FST and elevated-plus maze (LaPlant et al., 2009). Others found that females responded to the stress imposed by the elevated-plus maze with higher corticosterone concentrations than males, but this sex difference is diminished by OVX (Aoki et al., 2010). Similarly, in OVX rats, administration of either estradiol benzoate or the ERα agonist propyl pyrazole triol increased baseline corticosterone concentration more than two fold (Serova et al., 2010).

It was somewhat surprising, therefore, to find that OVX had no effect on the FST behaviour of both WT and R6/1 mice in the present study. The overall genotype difference remained, with R6/1 mice having higher immobility times in both sham surgery and OVX groups; confirming the finding of our previous study (Pang et al., 2009). Open field test shows no difference in motor ability in R6/1 mice at 12 weeks of age. Interestingly, Lagunas and colleagues as well as Laplant and colleagues found that OVX alone did not elicit depression-like behaviour in mice, as measured by the FST (Lagunas et al., 2010; LaPlant et al., 2009). In both cases, a stress paradigm was required to elicit a depression-like behaviour in OVX mice. Lagunas and colleagues submitted mice to different lengths of oestrogen deprivation. Depression-like behaviour was elicited in the long-term (20 weeks after OVX) mice, not in the short-term (6 weeks after OVX) mice, and then only after chronic unpredictable stress (Lagunas et al., 2010). Chronic unpredictable stress alone in gonadally intact mice did not increase depression-like behaviour on the FST, suggesting that OVX increased the susceptibility to stress. The duration of E2 deprivation appears critical in determining the extent of depression-related phenotype.

The DEX–ACTH test is used to analyse adrenal function. It was found previously by our group (Du et al., 2012) that female R6/1 mice displayed a hyper-sensitivity to ACTH stimulation, releasing significantly more corticosterone than WT mice. In vitro, E2 was found to upregulate basal corticosterone as well as increasing pregnenolone, a corticosterone precursor, in rodent adrenal cells, whereas testosterone reduced corticosterone and pregnenolone levels (Nowak et al., 1995). As mutant huntingtin is expressed highly in the adrenal glands in a variety of rodent HD models including the R6/1 mice (Moffitt et al., 2009; Sathasivam et al., 1999), it is possible that the female-specific phenotype in the R6/1 mice is the result of the oestrogen-induced sensitivity to stress compounded with the cellular changes caused by the HD mutation. Our finding that OVX significantly reduces ACTH-induced corticosterone release in both WT and R6/1 mice agrees with previous finding of oestrogen as a promoter of corticosterone release in the adrenals. There is also a significant overall genotype effect, suggesting that corticosterone release is lower in R6/1 mice compared to WT. This

finding is consistent with the notion that E2 facilitates HPA-axis responses. Interestingly, in the sham surgery group, the corticosterone level of HD mice is not significantly higher than the WT, as was previously found in mice not subjected to any form of surgery (Du et al., 2012). It seems that the sham surgery procedure reduced the exaggerated corticosterone response in the female R6/1 mice and that OVX was even more effective in doing so. It is possible that this long-term desensitisation is due to cytokine influences on the HPA-axis as has been previously shown in studies looking at adaptation to lipopolysaccharide challenges (Borges et al., 2007; Valles et al., 2002), with evidence suggesting reduced adrenal sensitivity to ACTH as an adaptive change to increases in cytokines, as would occur after surgery (Grinevich et al., 2001). It would be interesting to examine cytokine profiles and HPA-axis states in sham-operated and OVX mice to analyse the effect of surgery on HPA-axis regulation. The significant reduction of corticosterone post-OVX suggests that the presence of E2 can potentially contribute to the female-specific depression. The lack of behavioural difference after OVX in spite of HPA-axis changes points to a dissonance between the HPA-axis and depression-related behavioural phenotypes. It would be interesting to measure basal and stress-induced corticosterone levels in OVX mice to thoroughly analyse the effect of surgery on HPA-axis regulation as well as to see the long-term effect of OVX on HD progression in the R6/1 mice.

ERβ agonism rescued depression-like behaviour

The increase in ERα mRNA expression found in the adrenal of R6/1 mice suggests an abnormal increase in adrenal activity as discussed earlier. Unlike ERα, which increased corticosterone response to acute stress, activation of ERβ reduced corticosterone response in rats (Liu et al., 2012). In mice, ERβ KO resulted in anxiety and depression-like behaviours, as well as reduced serotonin in various brain regions (Imwalle et al., 2005; Krezel et al., 2001), effects not seen in ERα KO mice (Krezel et al., 2001). Indeed, in ERβ KO mice, the antidepressant effect of E2 in the FST was absent (Rocha et al., 2005). Testosterone has been found to possess antidepressant effects both in humans (Howell and Shalet, 2001; Kaminetsky, 2005) and rodents (Edinger and Frye, 2005; Frye and Seliga, 2001). A metabolite of testosterone, 5α-androstane-3β,17β-diol (3β-diol), is found to suppress the HPA-axis via binding exclusively to ERβ and this has been proposed as the mediator of testosterone's antidepressant effects (Lund et al., 2004, 2006; Pak et al., 2005). Therefore, the significant reduction in testosterone seen in female R6/1 mice may reflect significantly reduced 3β-diol levels and reduced ERβ signalling. The ERβ agonist DPN infused into the central nucleus of the amygdala of rats reduced anxiety-like behaviour and stress-induced corticosterone levels, counteracting the anxiogenic effects of GR agonism of the same area (Weiser et al., 2010). A recent study found that two ERβ agonists, selective oestrogen receptor modulator (SERM)-beta1 and SERM-beta2, are both able to reduce depression-like behaviour in the FST in mice as well as increase neurogenesis in rats (Clark et al., 2012). Previous study has shown that chronic daily DPN injections have anxiolytic effects in female mice (Oyola et al., 2012). In fact, a single acute injection of ERβ agonist DPN was able to reduce depression and anxiety-like behaviours in mice (Walf et al., 2008, 2009). With this evidence, and with little literature on the use of ERα antagonists and their effects on depression-related phenotypes, we used DPN as a treatment on female R6/1 mice.

Here, we show that a single application of DPN was able to significantly reduce the immobility time in R6/1 female mice by approximately 35%. Having only effects on the R6/1 females whilst not affecting WT behaviour, this result suggests that there is a specific dysregulation of ERβ signalling which contributes to the depression-like phenotype and renders the R6/1 mice more sensitive to the benefits of ERβ agonism. Whilst further investigation is warranted on the potential benefits of ERβ agonism, for example, using other behavioural tests of depression-related behaviours such as the saccharin-preference test, as well as the mechanism through which it functions, this novel finding suggests a new avenue of treatment. The possibility of using ERα antagonists also warrants further exploration as well as selective ER modulators and how all these can work in conjunction with antidepressants.

Conclusions

Our work provides the first examination of the influences of E2 on depression-like behaviour and HPA-axis regulation in an animal model of HD. We found that, despite alterations to the HPG-axis, E2 does not seem to make a major contribution to the depression-like behavioural phenotype in female R6/1 mice. However, the excising of E2 via OVX decreased HPA-axis reactivity in both WT and R6/1 mice. The expression of the depression-like behavioural phenotype in R6/1 females is likely to be due to a compound of abnormal adrenal sensitivity on top of neurological changes in the brain caused by the HD mutation. These findings demonstrate the importance of peripheral organs in HD and furthermore suggest that special attention is warranted with respect to sexually dimorphic endophenotypes and depression, as well as their therapeutic implications.

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