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Altered serotonin receptor expression is associated with depression-related behavior in the R6/1 transgenic mouse model of Huntington's disease

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Dysregulation of the serotonergic signaling system has been implicated in the pathology of mood disorders including depression, and various rodent models of disrupted serotonergic signaling display depression-related behavioral phenotypes. Depression is a common neuropsychiatric feature of preclinical Huntington's disease (HD) but the underlying changes in the HD brain contributing to the development of depression are unknown. Using the R6/1 transgenic mouse model of HD, we show that pre-motor symptomatic HD mice display sex-specific depressive-related behaviors on the forced-swim (FST), tail-suspension (TST) and novelty-suppressed feeding (NSFT) tests while having muted responses to acute anti-depressant administration. The baseline behaviors of HD mice were similar to the behavioral phenotypes of serotonin (5-HT) receptor and transporter null mutants, and gene expression of specific serotonin receptors were subsequently found to be reduced in the hippocampus and cortex of HD mice. Female HD mice had an additional deficit in cortical expression of serotonin transporter (SerT). Environmental enrichment normalized the FST behavioral response of female HD mice corresponding with increased gene expression of specific 5-HT receptors in the hippocampus and cortex. Our findings implicate altered serotonergic signaling as the basis for the development of depression during the preclinical stages of HD.

INTRODUCTION

Psychiatric manifestations are a major component of the early symptoms of Huntington's disease (HD), among which depression is commonly reported during the early, pre-motor symptomatic stages (1,2). The prevalence of depression within the HD population has been estimated to range between 30 and 50% (1,3,4) and can develop before the earliest cognitive changes are noticeable. The reasons for the increased incidence of depression in HD remain unclear.

HD shares certain pathology with depression. Weight loss and elevated cortisol levels (5–7) are common features of depression that have also been observed in HD patients. Dysregulation of the serotonergic signaling system has been implicated as a major factor in mood disorders including depression. There have been a few reports of reduced serotonin (5-HT) receptor binding in post-mortem HD brains (8–11). Disruption of serotonergic signaling results in altered depression-related behaviors as evident from observations of 5-HT receptor and transporter null mutants (12–16).

The R6 transgenic mouse lines are the established working models of HD (17). The often used R6/2 line displays faster and more aggressive disease progression (mice show symptoms between 5 and 6 weeks of age) and is more representative of early onset HD (18). The R6/1 line develops cognitive deficits from 12 to 14 weeks of age followed by motor impairment (19–22) and mirrors 95% of HD patients who develop symptoms in adulthood.

Environmental enrichment (EE) is a laboratory paradigm that offers increased cognitive stimulation to experimental subjects and has been shown to have beneficial effects in various models of neurological disorders (reviewed in 23). EE has been shown to delay onset and slow disease progression of R6/1 HD mice (24) and rescues hippocampal-specific cognitive deficits (20). The beneficial effects have been associated with increased hippocampal neurogenesis and more complex dendritic morphology of the hippocampal neurons (25). Interestingly, EE has also been suggested to correct depression-related behaviors of various rodent models of depression (26–29).

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Behavioral analysis of the R6/1 transgenic mouse model was undertaken to test for the presence of depression-related behaviors, following which gene expression of various serotonin receptors (htr1A, htr1B, htr2A and htr2C) and the serotonin transporter (SerT) was investigated. We hypothesized that altered gene expression in the brains of 12-week-old, pre-motor symptomatic R6/1 HD transgenic mice was associated with altered behavioral responses on the forced-swim (FST), tail-suspension (TST) and novelty-suppressed feeding (NSFT) tests which are commonly used to study the rodent models of depression. Only female HD mice displayed altered behavioral responses on the forced-swim, tail-suspension and novelty-suppressed feeding tests. The immobility times of male HD mice on the TST and FST were unresponsive to acute administration of anti-depressant drugs, whereas female HD mice selectively responded to the selective serotonin-reuptake inhibitor (SSRI) sertraline on the FST. The analysis of serotonin receptor expression in the hippocampus and cortex revealed a general reduction in mRNA levels of serotonin 1A (htr1A), 1B (htr1B) and 2A (htr2A) receptors. However, female HD mice had a further reduction in hippocampal htr1B and cortical serotonin transporter (SerT) mRNA levels when compared with male HD mice. EE normalized the behavioral response of female HD mice on the FST while having no effect on female wild-type (WT) or male mice. The female HD-specific effect of enrichment corresponded with specific increases in hippocampal htr1A and htr1B expression and cortical htr2C expression. Taken together, the development of female-specific depression-related behaviors is associated with reductions in gene expression of specific components of the serotonergic signaling system.

RESULTS

HD mice have normal body weights, motor co-ordination and serum corticosterone levels

Twelve-week-old HD mice did not differ from WT mice in body weight, rotarod performance and serum corticosterone levels. The average body weight of male mice was greater than female mice ($F_{(1,51)} = 74.117$, $P < 0.001$) but HD mice did not differ in weight when compared with WT mice ($F_{(1,51)} = 2.709$, $P = 0.106$) (Fig. 1A). On the accelerating rotarod, which is a measure of motor co-ordination ability, male and female mice performed to similar levels ($F_{(1,18)} = 0.161$, $P = 0.694$) (Fig. 1B), whereas HD mice performed just as well as WT mice on this task ($F_{(1,18)} = 0.295$, $P = 0.595$). Levels of corticosterone had been demonstrated to increase with disease progression in the R6/2 mouse model as well as in HD patients (30). However, at 12 weeks of age, serum corticosterone levels did not differ between R6/1 HD and WT mice ($F_{(1,42)} = 0.0508$, $P = 0.823$). There was no difference between the sexes ($F_{(1,42)} = 0.0439$, $P = 0.835$) (Fig. 1C).

Behavioral phenotyping of depression-related behaviors in HD mice

Forced-swim test. There were no overall sex differences in FST performance ($F_{(1,68)} = 2.574$, $P = 0.113$) but there was a significant genotype effect ($F_{(1,68)} = 21.005$, $P < 0.001$) (Fig. 1D). In addition, there was a significant sex–genotype interaction ($F_{(1,68)} = 6.400$, $P = 0.014$). *Post hoc* analysis showed that female HD mice recorded significantly greater FST immobility time than female WT mice ($P < 0.001$), but there was no difference within the male group ($P = 0.140$). Male WT mice were more immobile than female WT mice ($P = 0.006$) but there was no sex difference within the HD group ($P = 0.507$).

Tail-suspension test. HD mice recorded less immobility time on the TST than WT mice ($F_{(1,71)} = 14.586$, $P < 0.001$), and female mice were more immobile than male mice ($F_{(1,71)} = 17.501$, $P < 0.001$) (Fig. 1E). There was also a significant genotype–sex interaction ($F_{(1,71)} = 4.304$, $P = 0.042$). Female HD mice were significantly less immobile than female WT mice ($P < 0.001$) but no difference was observed between male WT and HD mice ($P = 0.170$). Within the WT group, female mice spent more time immobile than male mice ($P < 0.001$) but there was no sex difference within the HD group ($P = 0.118$).

Novelty-suppressed feeding test. Following food deprivation, both female and male mice lost ~15% of their body weights and there was no difference between WT or HD mice with regard to weight loss (data not shown). There was an overall genotype effect ($F_{(1,37)} = 5.67$, $P = 0.023$) on the latency to feed, and *post hoc* analysis showed that female HD mice recorded a significantly longer latency period ($P = 0.033$) than their WT counterparts but there was no difference within the male group ($P = 0.266$) (Fig. 1F). There were no differences between the genotypes or sexes in the amount of food consumed post-NSFT (data not shown).

Effects of acute anti-depressant administration on FST and TST performance of female mice

The effects of acute administration of sertraline and desipramine on female FST performance were examined (Fig. 2A). Two-way ANOVA revealed a genotype effect ($F_{(1,48)} = 19.945$, $P < 0.001$) on FST performance of female mice and sertraline ($F_{(1,48)} = 9.500$, $P = 0.004$) but not desipramine ($F_{(1,43)} = 0.0000125$, $P = 0.997$) administration. In agreement with the basal phenotyping results, saline-treated HD control mice recorded significantly greater immobility time than saline-treated WT mice ($P = 0.002$). Sertraline significantly reduced FST immobility time of female HD ($P = 0.025$) and WT ($P = 0.046$) mice.

Similar to the findings on the FST, the overall TST performance of female mice was significantly affected by genotype ($F_{(1,31)} = 8.557$, $P = 0.007$) and altered by sertraline ($F_{(1,31)} = 12.280$, $P = 0.002$) but not desipramine administration ($F_{(1,32)} = 1.491$, $P = 0.232$) (Fig. 2B). Saline-treated HD mice recorded reduced TST immobility times compared with saline-treated WT mice ($P = 0.015$). In contrast to the FST results, sertraline did not significantly alter TST performance of female HD mice ($P = 0.117$), despite reducing the mean immobility time of WT mice ($P = 0.002$). Desipramine also reduced TST immobility time of WT mice ($P = 0.019$) but did not alter HD performance ($P = 0.650$).

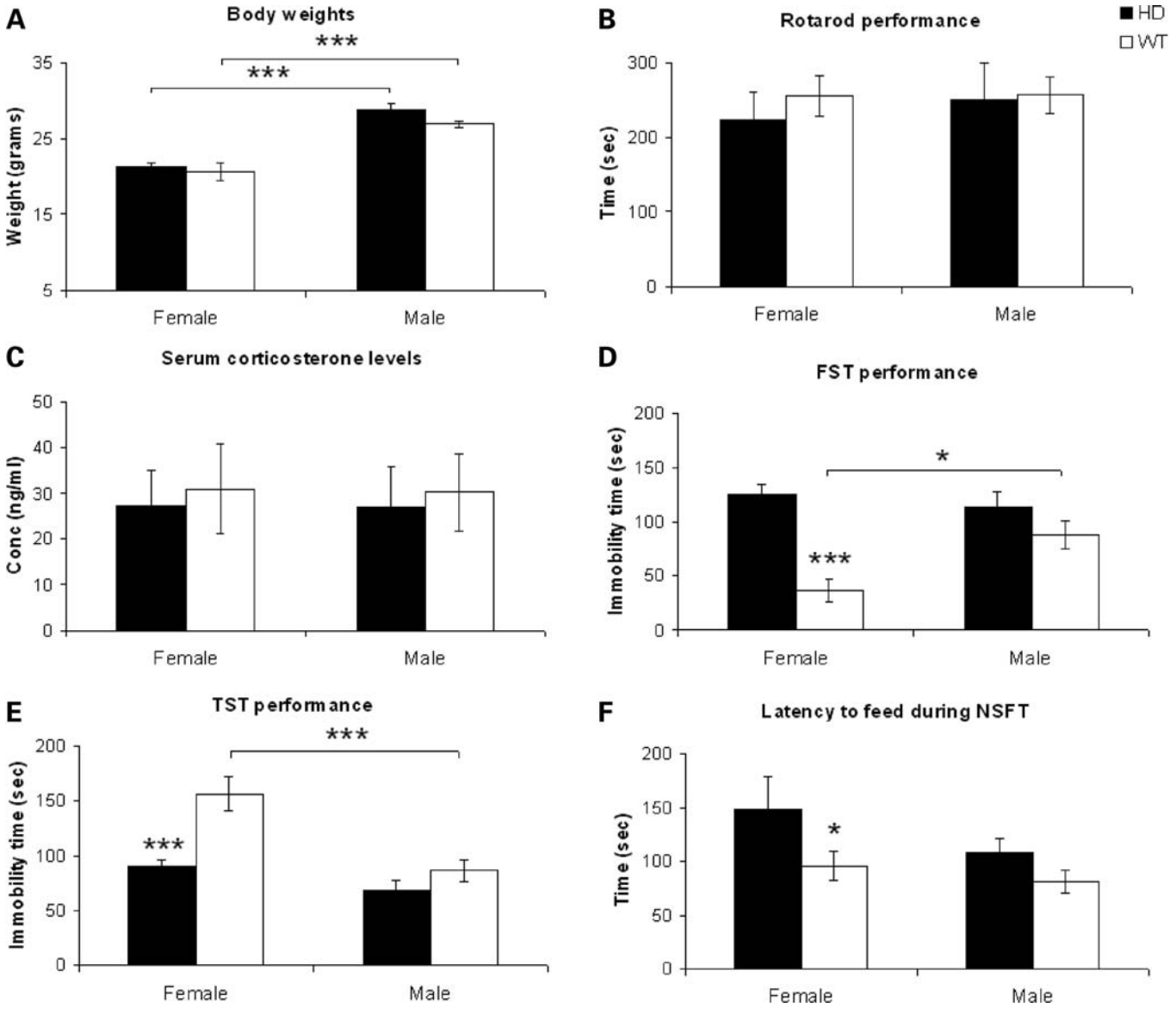

Figure 1. Pre-motor symptomatic female HD mice have altered depression-related behaviors. There were no differences between HD and WT mice of both sexes in (**A**) average body weights (weight/grams—female HD: 21.3 ± 0.4, female WT: 20.6 ± 1.2; male HD: 26.9 ± 0.5, male WT: 28.8 ± 0.8), (**B**) rotarod performance (latency to fall/s—female HD: 224.0 ± 37.8, female WT: 254.8 ± 26.8; male HD: 250.0 ± 50.0, male WT: 256.2 ± 24.8) and (**C**) serum corticosterone levels (corticosterone concentrations/ng per ml—female HD: 27.3 ± 7.7, female WT: 31.0 ± 9.8; male HD: 27.0 ng/ml ± 8.7, male WT: 30.1 ng/ml ± 8.4). Female HD mice ($n = 21$) recorded longer immobility times than female WT mice ($n = 12$) on the FST (**D**) but no difference was detected in the male group (HD $n = 14$; WT $n = 22$) (FST immobility times/s—female HD: 124.6 ± 10.9, female WT: 36.4 ± 14.4; male HD: 113.1 ± 14.0, male WT: 87.7 ± 12.1). On the TST (**E**), female ($n = 17$) but not male ($n = 20$) HD mice were less immobile than their respective WT comparisons (female $n = 12$; male $n = 24$) (TST immobility times/s—female HD: 89.3 ± 10.0, female WT: 148.1 ± 11.9; male HD: 68.3 ± 8.7, male WT: 85.7 ± 9.0). During the NSFT (**F**), female HD mice ($n = 7$) recorded increased latency to feed on the NSFT than female WT ($n = 12$) but there was no difference in the male group (HD $n = 9$; WT $n = 10$) (latency to feed/s—female HD: 148.9 ± 30.1, female WT: 95.8 ± 13.6; male HD: 107.3 ± 13.6, male WT: 81.2 ± 10.0). HD, R6/1 transgenic mice; WT, wild-type mice; FST, forced-swim test; TST, tail-suspension test; NSFT, novelty-suppressed feeding test. Error bars represent mean ± SEM ($^{*}P < 0.05$, $^{***}P < 0.001$, *post hoc* Bonferonni *t*-test).

Effects of acute anti-depressant administration on FST and TST performance of male mice

Genotype was a significant determinant of male FST performance ($F_{(1,54)} = 13.010$, $P < 0.001$) as was sertraline treatment ($F_{(1,54)} = 6.837$, $P = 0.012$). However, desipramine treatment did not have a significant overall effect ($F_{(1,45)} = 0.869$, $P = 0.357$). Interestingly, there were significant genotype–drug interactions for both sertraline ($F_{(1,54)} = 4.942$, $P = 0.031$) and desipramine ($F_{(1,45)} = 16.524$, $P < 0.001$). TST immobility times of male WT mice were significantly reduced by sertraline ($P = 0.001$) and desipramine ($P = 0.025$) but sertraline had no effect on male HD mice ($P = 0.785$), and desipramine-treated male HD mice surprisingly recorded increased immobility times ($P = 0.002$).

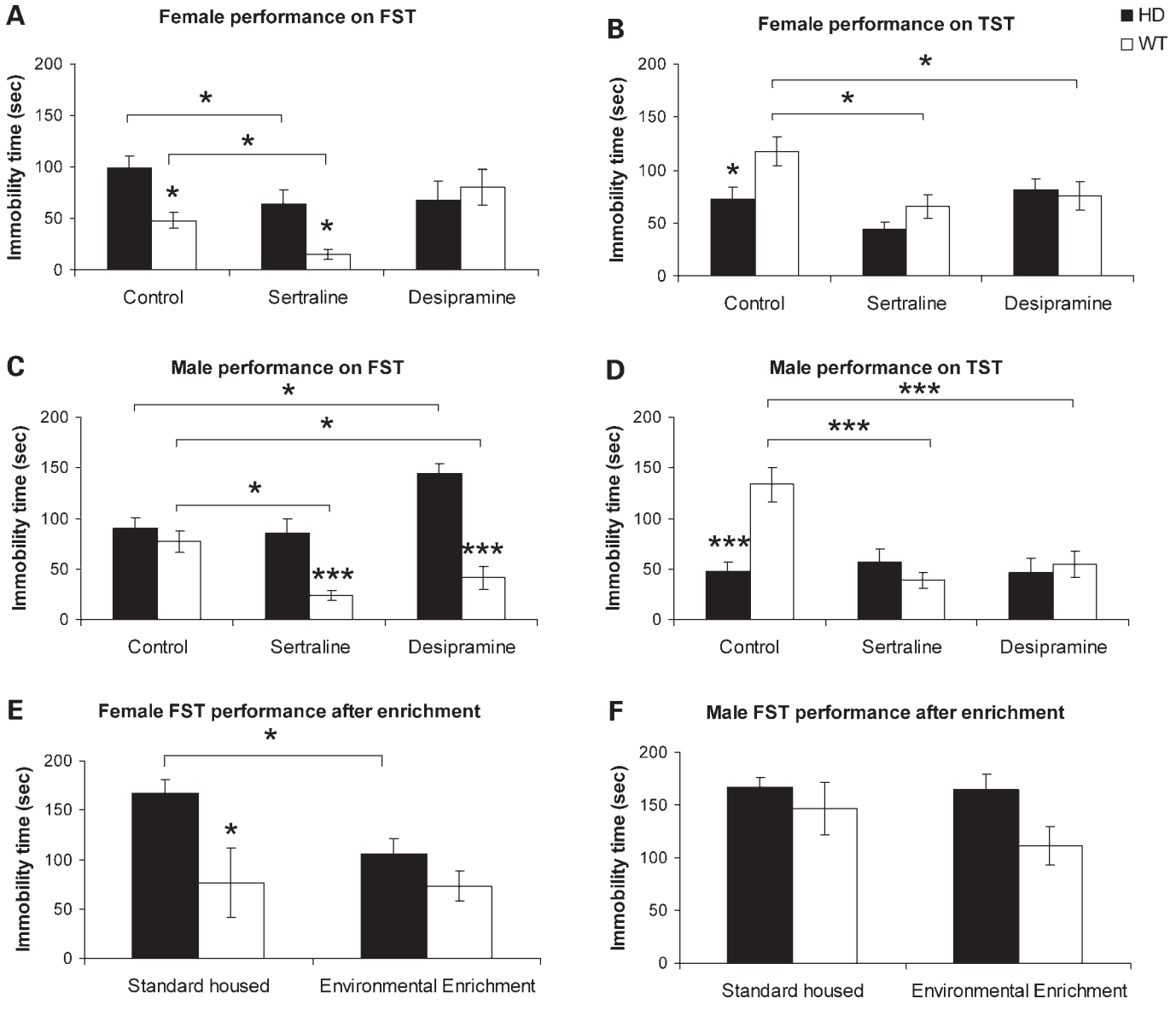

Figure 2. Behavioral effects of acute anti-depressant administration and EE. Acute administration of sertraline reduced immobility time of female HD ($n = 14$) and WT ($n = 11$) mice on the FST but there was no effect of desipramine (HD $n = 10$; WT $n = 10$) (**A**). Immobility times of female WT mice on the TST were reduced by sertraline ($n = 9$) and desipramine ($n = 10$) but female HD behavior was unaltered (**B**). Male WT mice had reduced immobility times on the FST following sertraline ($n = 13$) and desipramine ($n = 9$) administration (**C**). Sertraline ($n = 9$) and desipramine ($n = 11$) also reduced male WT immobility times on the TST (**D**). Four weeks of EE reduced immobility time of female HD mice ($n = 7$) on the FST (**E**) but did not alter female WT ($n = 5$) or male HD ($n = 9$) and WT ($n = 7$) performance (**F**). HD, R6/1 transgenic mice; WT, wild-type mice; FST, forced-swim test; TST, tail-suspension test. Error bars represent mean immobility times ± SEM (*$P < 0.05$, ***$P < 0.001$, Bonferonni *t*-test).

Genotype ($F_{(1,29)} = 7.148$, $P = 0.013$) and sertraline ($F_{(1,29)} = 10.978$, $P = 0.003$) or desipramine ($F_{(1,31)} = 6.890$, $P = 0.014$) treatments were all significant factors impacting on the overall performance of male mice on the TST (Fig. 2D). As expected, saline-treated HD controls recorded significantly lower TST immobility times than saline-treated WT mice ($P < 0.001$). Sertraline ($P < 0.001$) and desipramine ($P < 0.001$) significantly reduced the TST immobility times of male WT mice but both drugs had no effect on HD performance.

Environmental manipulations rescue the depressive phenotype of female HD mice

The experimental paradigm of EE enhances levels of mental and physical activity and exerts an 'anti-depressive' effect on various rodent lines (31–33). We investigated whether EE would alter the behavioral phenotype of HD mice on the FST.

There was an overall genotype effect ($F_{(1,22)} = 9.007$, $P = 0.007$) but not an environmental effect ($F_{(1,22)} = 2.618$, $P = 0.122$) observed in the female group (Fig. 2E). However,

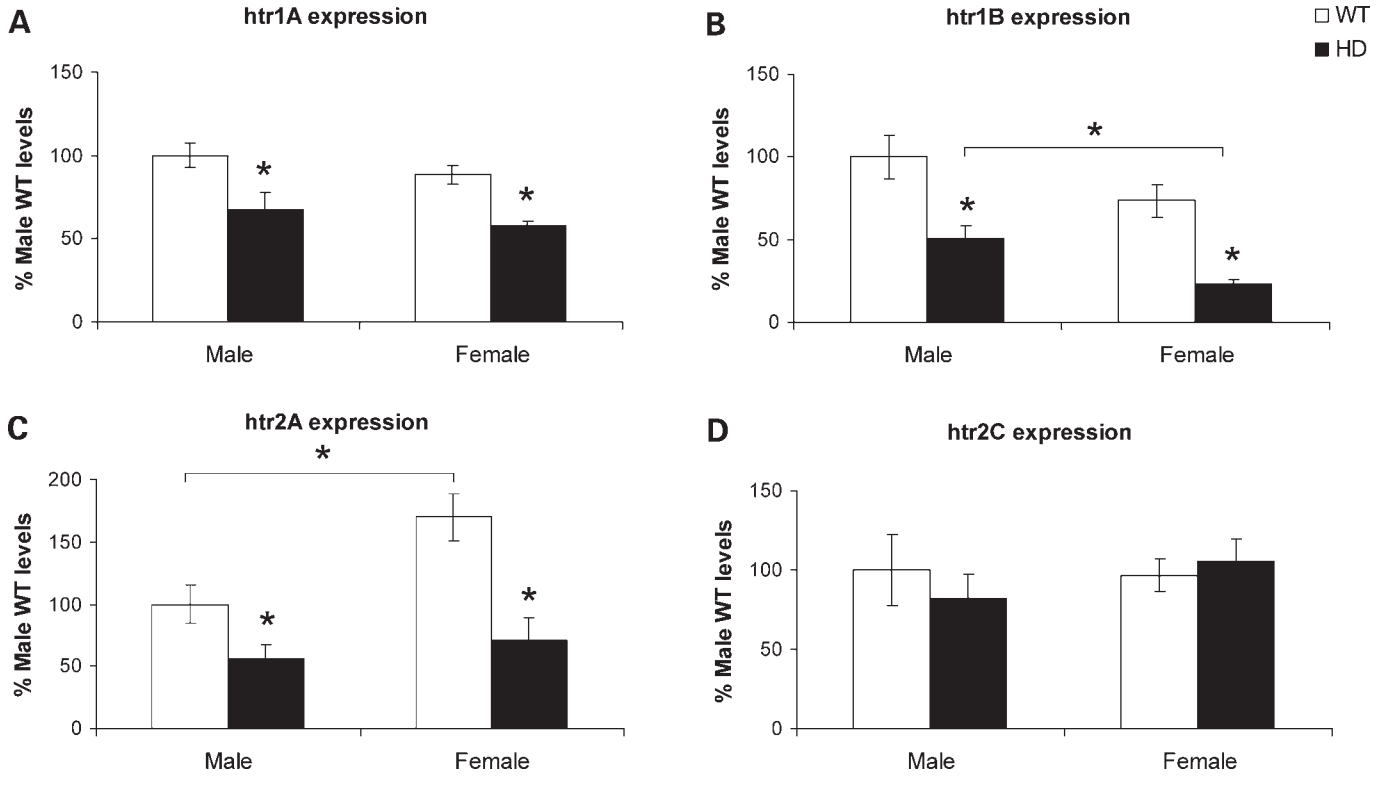

Figure 3. Sex differences in hippocampal expression of serotonin receptors. Expression of htr1A (**A**), htr1B (**B**) and htr2A (**C**) in the hippocampus was quantified by real-time PCR and revealed a HD-specific sex difference in htr1B mRNA levels as well as an increased expression of htr2A in female WT mice compared with male WT mice that was not detected within the HD group. No differences in htr2C expression were observed between the genotypes or sexes (**D**). Fold-change normalized to and presented as a percentage of male WT levels. HD, R6/1 transgenic mice; WT, wild-type mice. Error bars represent mean expression levels ± SEM (*$P < 0.05$, Bonferonni *t*-test).

post hoc analysis showed that while standard-housed (SH) female HD mice were significantly more immobile than SH WT mice ($P = 0.006$), EE reduced mean FST immobility time of female HD mice ($P = 0.032$) but did not alter the mean immobility time of female WT mice ($P = 0.908$). There was no significant effect of genotype ($F_{(1,32)} = 3.137$, $P = 0.087$) or environment ($F_{(1,32)} = 3.609$, $P = 0.067$) on FST performance of male mice (Fig. 2F).

Changes in gene expression associated with behavioral differences

Altered behaviors on the FST, TST and NSFT have been observed in 5-HT_{1A}, 5-HT_{1B} and 5-HTT null mutants (12–14,16). Therefore, the behavioral phenotype of the HD mice might reflect a disruption of serotonergic signaling due to a change in 5-HT receptor expression. The expression of the various 5-HT receptors was examined since both the basal behavioral phenotype of female HD mice and the EE-induced correction of that phenotype could be due to specific changes in the expression of 5-HT receptors that occur in the female, but not male, HD brain.

Sex differences in the expression of Type 1 and Type 2 5-HT receptors in hippocampus

There were overall genotype differences in hippocampal expression of htr1A ($F_{(1,19)} = 20.785$, $P < 0.001$) and htr1B ($F_{(1,19)} = 22.129$, $P < 0.001$). In addition, overall sex differences were observed in htr1B expression ($F_{(1,19)} = 9.155$, $P = 0.009$). Htr1A expression was significantly reduced in male (−33%, $P = 0.004$) and female (−42%, $P = 0.007$) HD mice (Fig. 3A) but there were no sex differences within the WT ($P = 0.221$) or HD ($P = 0.327$) groups. Htr1B expression was also significantly decreased in male (−50%, $P = 0.004$) and female (−77%, $P = 0.005$) HD mice (Fig. 3B). There was also significantly less htr1B mRNA in the female than in the male HD hippocampus (−27%, $P = 0.037$). A similar difference within the WT groups did not reach statistical significance (−27%, $P = 0.064$).

There was also a genotype difference in htr2A expression ($F_{(1,19)} = 19.250$, $P < 0.001$) but no significant overall sex difference ($F_{(1,19)} = 3.494$, $P = 0.083$). Htr2A expression was reduced in both male ($P = 0.041$) and female ($P = 0.002$) HD mice (Fig. 3C). Female WT mice had greater expression levels than male WT mice ($P = 0.037$) but this sex difference was not detected within the HD group. There were no differences in hippocampal htr2C expression between the genotypes ($F_{(1,19)} = 0.122$, $P = 0.732$) or sexes ($F_{(1,19)} = 0.622$, $P = 0.442$) (Fig. 3D).

Differential modulation of hippocampal Type 1 and Type 2 5-HT receptor expression by EE

The effect of EE on the expression of the various serotonin receptors was examined. There was a significant

environmental effect on htr1A expression in the female hippocampus ($F_{(1,19)} = 96.729$, $P < 0.001$; Fig. 4A) that was absent in the male mice ($F_{(1,19)} = 1.129$, $P = 0.304$; Fig. 4B). EE female WT ($P = 0.002$) and HD ($P = 0.045$) mice had significantly greater levels of htr1A mRNA than their respective SH groups. There was also a significant environmental effect on htr1B expression in the female hippocampus ($F_{(1,19)} = 7.781$, $P = 0.018$; Fig. 4C) that was not observed in males ($F_{(1,19)} = 0.319$, $P = 0.005$; Fig. 4D). EE female HD mice had significantly greater htr1B expression than their SH counterparts ($P = 0.042$) but there was no significant increase in enriched female WT mice.

There was no overall environmental effect on hippocampal htr2A expression ($F_{(1,19)} = 11.076$, $P = 0.005$; Fig. 4E) but there was a significant gene–environment interaction ($F_{(1,19)} = 5.885$, $P = 0.031$). EE reduced htr2A expression in female WT mice ($P = 0.018$) but did not alter HD levels. There was no environmental modulation of htr2A expression in the male hippocampus ($F_{(1,19)} = 0.0965$, $P = 0.761$; Fig. 4F). Hippocampal expression of htr2C was unchanged by EE.

No sex differences in cortical expression of Type 1 and Type 2 5-HT receptors

Expression of serotonin receptors in the cortex has also been implicated in the pathogenesis of depression. To determine whether changes in serotonin receptor expression in the HD brain were localized to the hippocampus and if the behavioral changes induced by EE were hippocampus-specific, expression of the serotonin receptors was also investigated. There were strong genotype differences in the expression of htr1A ($F_{(1,19)} = 105.996$, $P < 0.001$; Fig. 5A), htr1B ($F_{(1,19)} = 15.144$, $P = 0.001$; Fig. 5B) and htr2A ($F_{(1,19)} = 106.497$, $P < 0.001$; Fig. 5C) in the HD cortex. Levels of htr2C mRNA were unaltered in the HD cortex ($F_{(1,19)} = 2.011$, $P = 0.177$; Fig. 5D). Expression of all four receptors did not differ between the sexes.

Cortical expression of Type 1 and Type 2 5-HT receptors is unaltered by EE

There was no effect of EE on cortical expression of htr1A in female ($F_{(1,19)} = 0.718$, $P = 0.410$; Fig. 6A) and male ($F_{(1,19)} = 0.00335$, $P = 0.955$; Fig. 6B) mice. Similarly, there was also no environment effect on htr1B expression in female ($F_{(1,19)} = 0.481$, $P = 0.499$; Fig. 6C) and male ($F_{(1,19)} = 0.313$, $P = 0.584$; Fig. 6D) mice.

Levels of htr2A mRNA in the cortex were unchanged by enrichment in female ($F_{(1,19)} = 1.877$, $P = 0.191$; Fig. 6E) and male ($F_{(1,19)} = 0.283$, $P = 0.602$; Fig. 6F) mice. Htr2C expression also remained unaltered in female ($F_{(1,19)} = 2.653$, $P = 0.124$; Fig. 6G) and male ($F_{(1,19)} = 0.0148$, $P = 0.905$; Fig. 6H) mice.

Gene expression of 5-HT transporter (SerT) is unchanged in the HD hippocampus and cortex

The serotonin transporter has a key role in the regulation of serotonergic transmission and is also implicated in the pathogenesis of depression (34–36). Various SerT polymorphisms alter the effectiveness of anti-depressant treatments (reviewed in 37), and SerT knockout mice display altered depression-related behaviors to those displayed by female HD mice (16). Hence, we investigated whether the HD behavioral phenotype and muted response to acute anti-depressant administration were due to a change in SerT expression in the HD brain. There were no significant sex differences in SerT expression found in the hippocampus ($F_{(1,19)} = 0.329$, $P = 0.574$; Fig. 7A) or in the cortex ($F_{(1,19)} = 1.890$, $P = 0.189$; Fig. 7B), even though SerT expression in the hippocampus of female WT mice trended to be greater than male WT levels (+53%, $P = 0.073$). Despite HD mice having reduced hippocampal SerT expression when compared with WT levels, there was no overall genotype difference ($F_{(1,19)} = 2.750$, $P = 0.117$) but there was a genotype difference in cortical SerT expression ($F_{(1,19)} = 5.503$, $P = 0.033$). There were no environmental effects on hippocampal SerT expression in female ($F_{(1,19)} = 0.174$, $P = 0.682$; Fig. 7C) and male ($F_{(1,19)} = 0.138$, $P = 0.715$; Fig. 7D) mice. There were also no environment-mediated alterations in SerT expression in the female ($F_{(1,19)} = 2.594$, $P = 0.128$; Fig. 7E) or male ($F_{(1,19)} = 0.635$, $P = 0.437$; Fig. 7F) cortex.

DISCUSSION

Depression is the most common psychiatric symptom diagnosed in HD patients during the preclinical stages (1,2,4); however, the exact reasons for this frequent co-morbidity remain unclear. Disruption of serotonergic signaling results in altered depression-related behaviors that are more prominent in females (12,13,38). Here, we detected altered depression-related behaviors in pre-motor symptomatic female HD mice that correspond with reduced expression of specific serotonin receptors, as well as the serotonin transporter, in the cortex and hippocampus. The presence of altered depression-related behaviors together with reduced serotonin receptor expression is consistent with other models of depression and supports the hypothesis that decreased serotonergic neurotransmission underlies the development of depressive-like behaviors in HD.

At an age when HD mice show no differences in anxiety-related behavior on the elevated plus maze and light–dark chamber (20), the presence of altered depression-associated behaviors was most striking in female HD mice. The three tests we have employed model different aspects of depressive-like behavior with the FST and TST modeling the experience of helplessness akin to ‘despair’ by exposure to an inescapable stressful situation and the NSFT modeling stress-induced anxiety.

The FST (39) and TST (40) are routinely used to assess the effects of anti-depressants in mice, to test for potential anti-depressant compounds (41–46) while their use in studies of depression-related behaviors in various rodent models (12,47) remains controversial and highly debatable. It was surprising to observe the conflicting behaviors displayed on the FST and TST by female HD mice. Taken independently, the TST result would have suggested an ‘anti-depressive’ phenotype. However, together with our findings of reduced

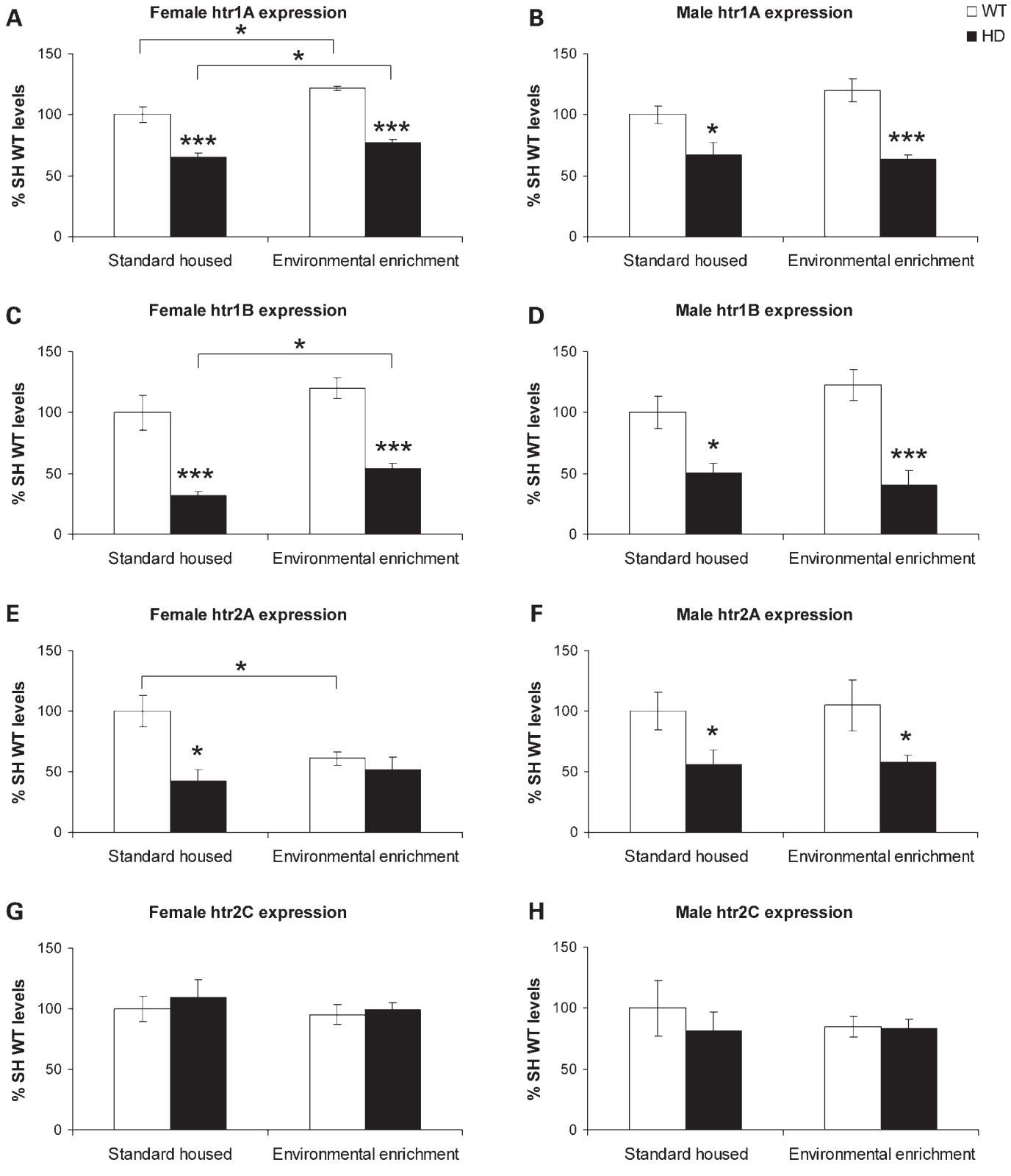

Figure 4. Hippocampal expression of serotonin receptors in female mice is altered by EE. Quantification of htr1A expression in the hippocampus by real-time PCR in female (**A**) and male (**B**) mice revealed a female-specific effect of EE. A specific up-regulation of htr1B expression restricted to female HD mice (**C**) was not observed in male mice (**D**). Enrichment reduced htr2A expression in female WT mice (**E**) but did not alter expression in male mice (**F**). EE did not alter hippocampal expression of htr2C in female (**G**) and male (**H**) mice. Fold-change normalized to and presented as a percentage of the respective SHWT group. HD, R6/1 transgenic mice; WT, wild-type mice. Error bars represent mean expression levels ± SEM ($^{*}P < 0.05$, $^{***}P < 0.001$, Bonferonni *t*-test).

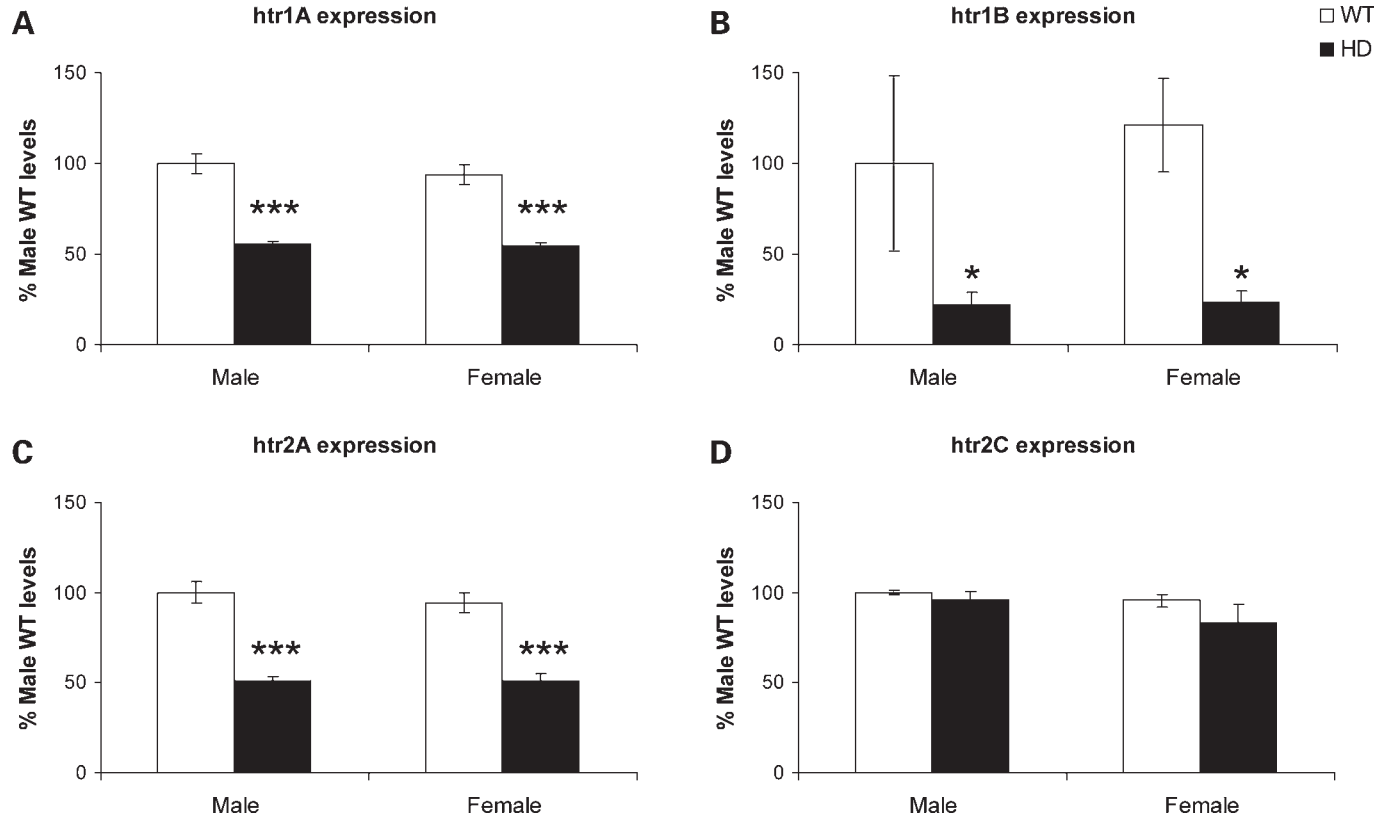

Figure 5. No sex differences in cortical expression of serotonin receptors. Expression of htr1A was reduced in both male ($P < 0.001$) and female ($P < 0.001$) HD cortices (**A**). Similarly, cortical levels of htr1B mRNA were reduced in male ($P = 0.014$) and female HD mice ($P = 0.016$) (**B**). Cortical expression of htr2A was also significantly reduced in male ($P < 0.001$) and female ($P < 0.001$) HD mice (**C**). No differences in cortical htr2C expression were detected between the sexes (**D**). Fold-change normalized to and presented as a percentage of male WT levels. HD, R6/1 transgenic mice; WT, wild-type mice. Error bars represent mean expression levels ± SEM ($^{*}P < 0.05$, $^{***}P < 0.001$, Bonferonni *t*-test).

htr1A gene expression in the hippocampus and cortex of HD mice, this behavioral response was consistent with that observed of the serotonin receptor 1A knockout mouse line (12,13). Interestingly, the combination of the opposing FST and TST behaviors we observed are similar to the behavioral patterns and sex differences that have been reported in the serotonin receptor 1B and serotonin transporter knockout lines (13,14,16). Therefore, the results of the behavioral examination and gene expression studies strongly suggest that altered expression of different components of the serotonergic signaling system (specifically htr1A, htr1B and SerT) underlie the altered depression-related behavioral phenotype of the HD mice. These findings are consistent with, and extend, previous reports of reduced serotonin 1A and 1B receptor binding in post-mortem HD brains (11) as well as reduced serotonin 1A receptor binding in the hippocampal and cortical regions of late-stage R6/2 transgenic mice (48). Interestingly, htr1B expression in the female HD hippocampus was significantly reduced when compared with male HD levels, making it the prime candidate in mediating the sex difference in the HD-depression phenotype.

The concurrent up-regulation of hippocampal htr1A and htr1B expression with a correction of the behavioral response of female HD mice on the FST by EE is further evidence that both receptors modulate depression-associated behaviors. A disruption of serotonin receptor function is also likely to contribute to our observations of the absence of the acute effects of serotonergic-based anti-depressants on HD mice, mirroring the results of a previous study which reported that fluoxetine (another SSRI) did not have any effect on serotonin receptor-1A knockouts (12). The hippocampus-specific enrichment effects are intriguing since significant reductions in the expression of those same serotonin receptors were also detected in the HD cortex but were unchanged by EE. Although modification of cortical serotonin receptor expression does not appear to be necessary for the correction of female HD behavior on the FST, the possible contribution of decreased serotonergic signaling in the cortex to other aspects of the overall HD phenotype, such as the development of cognitive deficits, cannot be ruled out and warrants further investigation.

The hypothalamic-pituitary adrenal axis regulates circulating levels of the hormone corticosterone in response to stress which were found to be within the normal range based on our basal serum corticosterone measurements. However, the different behavioral responses of HD mice might reflect more than just depressive-like behavior. It is possible that HD mice respond differently to stress so as an extension of our findings, future studies examining corticosterone levels following acute stress (e.g. immediately after FST exposure) or after dexamethasone

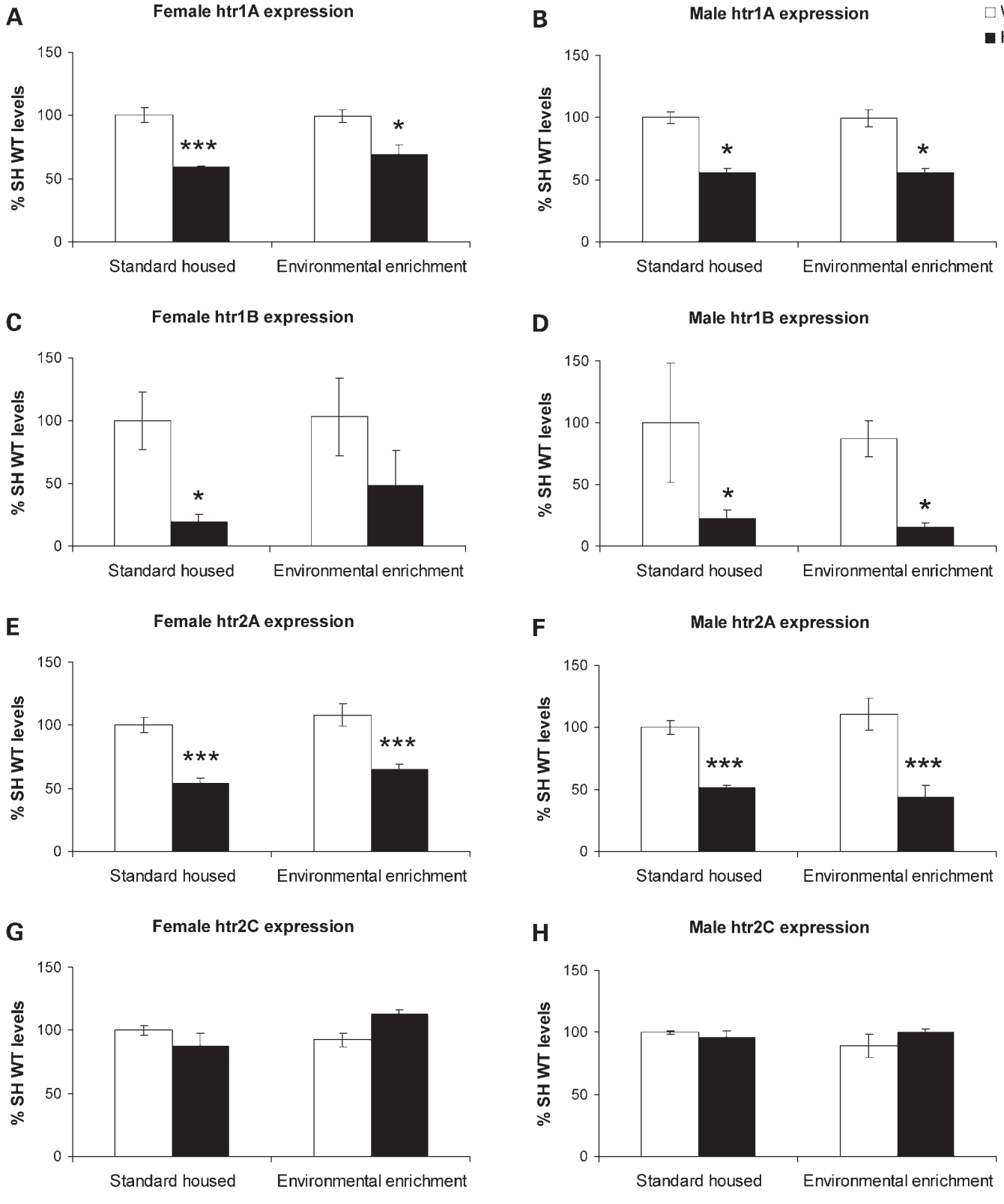

Figure 6. Cortical serotonin receptor expression is unaltered by EE. Htr1A expression was unaltered by EE in the female (**A**) and male (**B**) cortices. Similarly, enrichment did not change expression of htr1B (**C** and **D**) and htr2A (**E** and **F**) in WT and HD mice of both sexes. Levels of htr2C mRNA remained unaltered as well (**G** and **H**). Fold-change normalized to and presented as a percentage of the respective SHWT group. HD, R6/1 transgenic mice; WT, wild-type mice. Error bars represent mean expression levels ± SEM ($^{*}P < 0.05$, $^{***}P < 0.001$, Bonferonni *t*-test).

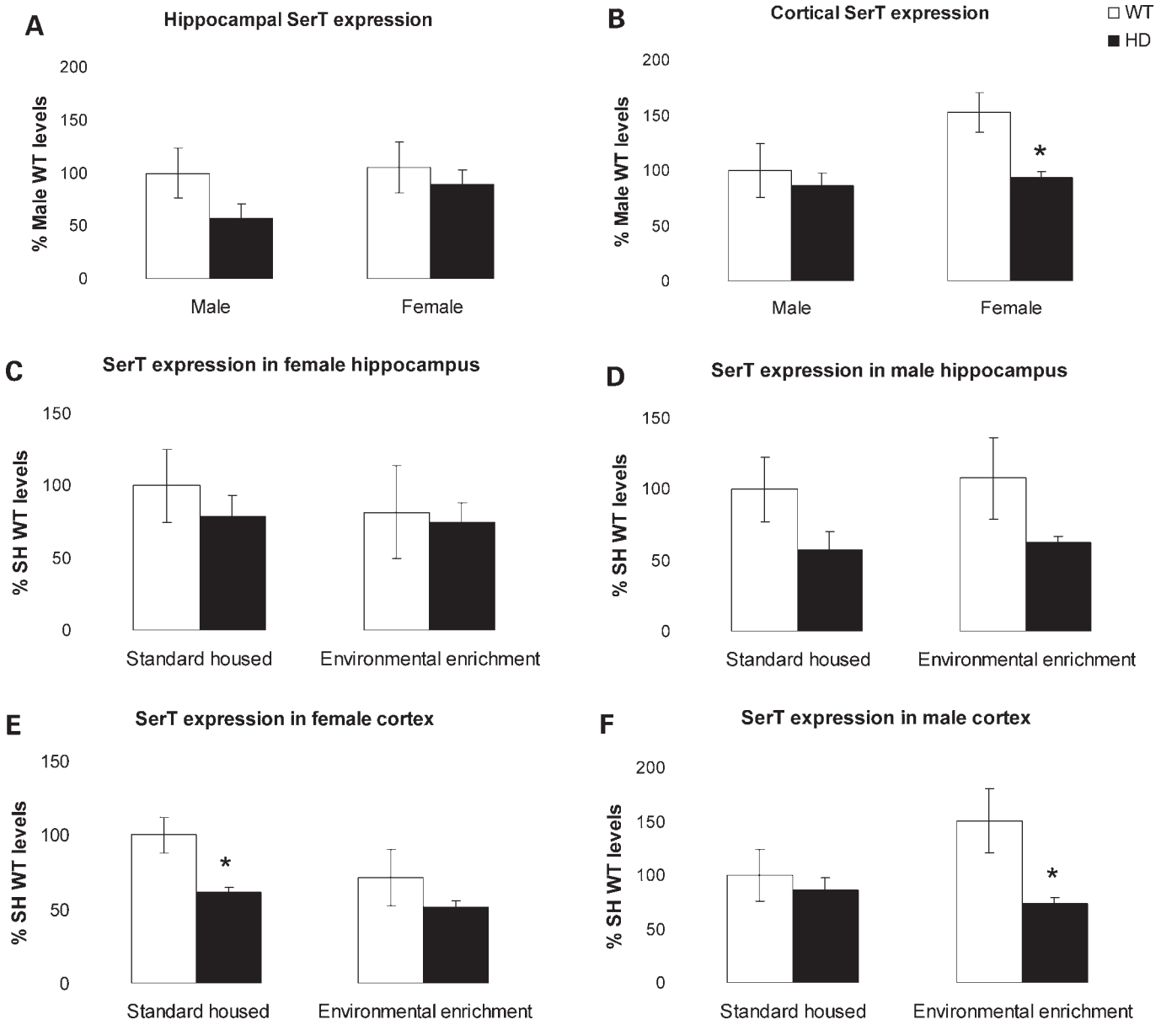

Figure 7. Expression of serotonin transporter in the hippocampus and cortex. Levels of SerT mRNA in the hippocampus (**A**) and cortex (**B**) of male and female WT and HD mice. SerT expression in the male HD hippocampus was not significantly reduced ($P = 0.108$). SerT expression trended toward being greater in the female than male WT cortex ($P = 0.073$) and is reduced in the female HD cortex ($P = 0.020$). There were no effects of EE on SerT expression in the female (**C**) and male (**D**) hippocampus. There were also no significant effects of EE on SerT mRNA levels in the female (**E**) and male (**F**) cortices. SerT, serotonin transporter. Fold-change normalized to and presented as a percentage of male WT levels (A and B) or the respective SHWT group (C–F). HD, R6/1 transgenic mice; WT, wild-type mice. Error bars represent mean expression levels ± SEM. (*$P < 0.05$, Bonferonni *t*-test).

administration could reveal altered neuroendocrine responses in HD mice. The different behavioral responses of male and female HD mice on the three tests could also be due to the effects of sex steroids which have been established to modulate stress response (49). Examining the neuroendocrine response of male and female HD mice to various forms of stressors would address that issue further.

The female HD-specific increased latency to feed in the NSFT is similar to the behavioral response of 5-HT_{1A} knockout mice (50) and is consistent with our findings of reduced htr1A mRNA levels in the HD brain. There are two possible interpretations of this finding. First, the increased latency to begin eating could be due to heightened avoidance of the center of the open field. However, there was no evidence that HD mice tended to avoid the center of the NSFT test arena more than WT mice (Supplementary Material, Fig. S1A) nor were the HD mice showing signs of hyper-anxiety in an open-field arena test (Supplementary Material, Fig. S1B). The second interpretation of this behavior is female HD mice are unable to suppress a fearful behavioral response to overcome the feeling of hunger. This is the more likely rationale for this HD behavioral response. One caveat

of conducting this test is that serotonin signaling has been shown to be involved in food intake, in particular through 5-HT_{2C} receptor signaling (51–53). However this is also unlikely to be compromising our findings since htr2C levels were unaltered in the HD brain. Overall, the implications of our identification of sexual dimorphism in depression-associated behaviors align with evidence that females are twice as likely to develop depression in the general population and point toward potential sex differences within HD patients. Hence, our results should provide the impetus for future clinical studies to investigate whether such a sexual dichotomy exists in the incidence of clinical depression in the HD population.

Mental and physical activities are the key modulators of brain function and exert a strong influence over the development of various disorders of the central nervous system (reviewed in 23). The importance of environmental modifiers in clinical HD was further emphasized in a study of a large Venezuelan population of HD kindred which established that environmental factors accounted for ~60% of variance in age of onset beyond that explained by CAG repeat length (54). The significant environmental effect on the development of HD symptoms was first demonstrated with the experimental paradigm of EE that delayed the onset and progression of motor symptoms (24,55) and slowed cognitive decline in the R6/1 HD line (20). We now show that EE also normalizes the depression-related behavioral phenotype of female HD mice on the FST by restoring it to WT levels, hence effectively ameliorating the depression-associated behavior in HD and confirming the 'anti-depressive' effects of EE recently demonstrated in other rodent models (27,32).

Epidemiological studies implicate environmental factors in the development of depression (56–60). Increased levels of physical activity and exercise alleviate depressive mood and reduce clinical depression scores (61–65). Those findings are mirrored by the reported 'anti-depressive' effects of wheel-running in rodents (66,67). Given the strong evidence that physical activity is 'anti-depressive' and the feasibility of using exercise as a means to improve depressive mood of HD patients, it would be of great interest to investigate whether voluntary wheel-running, which delays the onset of cognitive and motor symptoms of R6/1 HD mice (21), would also correct the behavioral phenotype of the female HD mice.

Interestingly, sertraline, but not desipramine, showed an anti-depressant effect by reducing the immobility times of female HD mice on the FST. This evidence of a greater effectiveness of an SSRI over a tricyclic anti-depressant in female HD mice is consistent with clinical evidence (68) and warrants further investigation as it would potentially impact on the treatment of depression in HD. However, it also raises an interesting question as to the role of sex hormones in modulating an anti-depressant response since the presence of female sex hormones is known to enhance and even accelerate the response to SSRIs (69–71).

Our findings in the R6/1 transgenic model agree with a previous study describing reduced 5-HT_{1A} receptor density in the hippocampus and cortex of late-stage R6/2 transgenic mice (48) and several other rodent models of depression (72). In addition, they are also consistent with reports of reduced 5-HT_{1A} and 5-HT_{1B} receptor binding in post-mortem HD brains (11) and depressed patients (73–75). Taken together, this study provides direct experimental evidence supporting the hypothesis that depression is an intrinsic symptom of HD and not a mere epiphenomenon, and that the R6/1 HD transgenic mouse line is a valid model for studying HD-related depression.

The development of depression in presymptomatic HD patients may reflect significant changes that have occurred in the cerebral cortex as part of a genetic predisposition for depression, in particular a disruption of serotonergic signaling due to a reduction in the expression of the Type 1 serotonin receptors within the hippocampus.

MATERIALS AND METHODS

Mice

R6/1 transgenic mice (HD) and WT littermates (17) were bred from the colony maintained at the Howard Florey Institute (HFI). Genotype was determined by polymerase chain reaction (PCR) with genomic DNA from toe biopsy, and animals were randomly allocated into groups of four per cage in standard mouse boxes. Mice that underwent EE were housed in the larger cages (15 × 28 × 38 cm) from 8 to 12 weeks with objects and toys of various sizes and material that were changed weekly. In addition, enriched mice were also placed into large 120 l boxes three times a week with additional objects to interact with (23). All behavioral tests described in this paper were performed on mice at 12 weeks of age. All experiments were approved and performed in accordance with the guidelines of the HFI Animal Ethics Committee and the National Health and Medical Research Council (NHMRC).

Basal serum corticosterone levels

For the analysis of basal serum corticosterone levels, mice naïve to behavioral testing were killed by cervical dislocation between 8.00 and 10.00 a.m. Blood was collected via cardiac puncture, left to clot at room temperature for 30 min and centrifuged at 1000*g* for 15 min. Serum was then collected and corticosterone quantified with an immunoassay according to the manufacturer's specifications (DE3600, R&D Systems, MN, USA).

Forced-swim test. Mice were individually placed into a beaker of water (23–25°C) for a total of 300 s. Total immobility time of each mouse after the first 60 s was manually scored by an experienced experimenter blind to genotype and housing condition.

Tail-suspension test. Mice were suspended on a Bioseb apparatus (Bioseb, Chaville, France) for a total of 300 s and immobility time over the final 240 s was automatically recorded. Mice that climbed up onto the suspension hook were excluded from subsequent analysis.

Novelty-suppressed feeding test. Mice were food deprived for 48 h prior to testing but allowed 2 h of feeding after an initial

24 h period. Water was available *ad libitum*. Body weights pre- and post-deprivation were recorded. The NSFT was conducted in a room with the lighting level at 1200 and 350 Lux in the middle of test arena (80 × 80 × 80 cm). The test arena was lined with 2 cm of bedding material, and a single food pellet was placed on a piece of filter paper in the center. Individual mice were placed into a random corner of the test arena, and the latency to grasp and feed on the food pellet within a 5 min time frame was recorded. Upon the initiation of feeding or reaching the time limit, mice were immediately removed and placed back into their home cages where they were allowed to feed on a single food pellet of pre-determined weight for 5 min. The amount of food consumed in that time was recorded.

Acute anti-depressant administration

Sertraline (Pfizer Inc., CT, USA) was dissolved in distilled water with 1:100 Tween-20 and desipramine (Sigma-Aldrich, NSW, Australia) was dissolved in distilled water. Both drugs were administered (20 mg/kg) by intraperitoneal (i.p.) route with control animals injected with equivalent volumes of vehicle. Drugs were administered 30 min before TST and 60 min before FST.

Real-time PCR for quantification of mRNA expression

Mice were killed by cervical dislocation for fresh dissection of the hippocampus and cortex which were snap frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated using Qiagen RNeasy extraction kits (Qiagen, NSW, Australia) and stored at −80°C. RNA concentration and quality were determined using an Agilent Bioanalyzer 2100. cDNA was reverse transcribed from 1 μg of total RNA per sample using Applied Biosciences Reverse Transcription kits (PE Applied Biosystems, Foster City, CA, USA). The reverse transcription PCR conditions were 25°C—10 min, 48°C—30 min and 95°C—5 min. cDNA products were stored at −20°C. Quantitative real-time PCR was performed using SYBR Green PCR master mix (Sigma-Aldrich) on a PE ABI Prism® 7500 Light Cycler system (PE Applied Biosystems). All primer pairs were optimized for working concentrations before use and primer sequences are as follows. Serotonin 1A receptor (htr1A) F: CCC CAA CGA GTG CAC CAT, R: GCG CCG AAA GTG GAG TAG AT; Serotonin 1B receptor (htr1B) F: CAC CAA CCT CTC CCA CAA CT, R: CCA GAG AGG CGA TCA GGT AG; Serotonin 2A receptor (htr2A) F: CAC TGT GAA GCG AGG CAT AA, R: AAG CCG GAA GTT GTA GCA GA; Serotonin 2C receptor (htr2C) F: TGC CAT CGT TTG GGC AAT A, R: CGT CCC TCA GTC CAA TCA CA; Serotonin transporter (SerT) F: CTT CAG CCC CGG ATG GTT, R: GTG GAC TCA TCA AAA AAC TGC AAA; Cyclophilin F: CCC ACC GTG TTC TTC GAC A, R: CCA GTG CTC AGA GCT CGA AA. Cyclophilin was used as the endogenous housekeeping gene as it is not altered in the R6/1 mouse line at this age (Zajac, unpublished data). Real-time PCR conditions were 50°C—2 min, 95°C—10 min, followed by 40 cycles of 95°C—15 s and 60°C—1 min. All reactions were performed on five individual subjects per group, each in triplicate. Melt curve analyses were performed to ensure that only one reaction product was obtained.

Data analysis and statistics

Statistical analyses were performed using Sigmastat 2.03.0 (SPSS Inc.). Body weight and basal corticosterone levels of male and female mice were analyzed separately for genotypic differences by one-way ANOVA. Results of the initial FST, TST and NSFT were analyzed with a two-way ANOVA for the effects of sex and genotype. Following the observation of sex differences, subsequent male and female data sets collected from the acute anti-depressant and EE studies were analyzed separately with two-way ANOVA. For real-time PCR, technical replicates that returned with a 0.5 cycle difference from the mean were discarded from analysis. Statistical analyses were performed on relative fold-changes determined by the $2^{-\Delta\Delta Ct}$ method. Mean fold-change of the various groups was normalized to either male WT levels for the analysis of sex differences or SH WT levels for the analysis of enrichment effects before analysis with two-way ANOVA. All *post hoc* analysis was performed with Bonferroni *t*-test. The level of statistical significance for all analyses was set at $\alpha = 0.05$.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. The author(s) declare that, except for income received from primary employers, no financial support or compensation has been received from any individual or corporate entity for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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