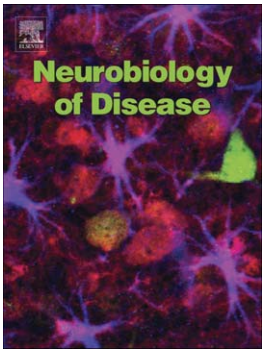

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High stress hormone levels accelerate the onset of memory deficits in male Huntington's disease mice

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Abstract

Huntington's disease (HD) is a neurodegenerative disorder caused by a tandem repeat mutation in the huntingtin gene. Lifestyle factors, such as lack of activity may contribute to the variability in the age of disease onset. Therefore, better understanding of environmental modifiers may uncover potential therapeutic approaches to delay disease onset and progression.

Recent data suggest that HD patients and transgenic mouse models show a dysregulated stress response. In this present study, we elevated stress hormone levels through oral corticosterone (CORT) treatment and assessed its impact on the development of motor impairment and cognitive deficits using the R6/1 transgenic mouse model of HD. We found that CORT consumption did not alter rotarod performance of R6/1 HD or wild-type (WT) littermates. However, the onset of hippocampal-dependent Y-maze deficits was accelerated in male R6/1 mice by 5 days of CORT treatment, whereas short term memory of WT and female R6/1 mice was unaffected.

We then further investigated the male HD susceptibility to CORT by measuring TrkB activation, BDNF and glucocorticoid receptor expression as well as the level of cell proliferation in the hippocampus. CORT treatment increased the levels of phosphorylated TrkB in male R6/1 mice only. There were no effects of CORT on hippocampal BDNF protein or mRNA levels; nor on expression of the glucocorticoid receptors in any group. Hippocampal cell proliferation was decreased in male R6/1 mice and this was further reduced in CORT-drinking male R6/1 mice. Female mice (WT and R6/1) appeared to be protected from the impacts of CORT treatment in all our hippocampal measures. Overall, our data demonstrate that treatment with corticosterone is able to modulate the onset of HD symptomatology.

We present the first evidence of a male-specific vulnerability to stress impacting on the development of short-term memory deficits in HD. More generally, we found that female mice were protected from the detrimental effects of CORT treatment on a variety of hippocampus-based measures. Hippocampal plasticity and memory in HD may be more susceptible to the impacts of stress in a sex-dependent manner. We propose clinical investigations of stress as a key environmental modifier of HD symptom onset.

Introduction

Huntington's disease (HD) is a dominantly inherited neurodegenerative condition caused by a trinucleotide repeat expansion in the HD gene (Huntington's Disease Collaborative Research Group, 1993). Cognitive, psychiatric and motor symptoms generally appear in adulthood (with an average age at onset of 40 years) (Myers, 2004). While the length of the trinucleotide repeat expansion is the strongest predictive factor for age of onset of disease symptoms, non-genetic factors appear to exert a large influence (Wexler et al., 2004). Thus, efforts have been focused on uncovering the potential non-genetic factors which modify the trajectory of this devastating disease. Understanding the contribution of environmental modulators to disease onset and progression may uncover novel therapeutic approaches to managing this disease.

A handful of environmental modulators have been correlated with age of onset in HD patients based on retrospective questionnaire studies (Buruma et al., 1987; López-Sendón et al., 2011; Marder et al., 2013; Marder et al., 2009; Simonin et al., 2013; Trembath et al., 2010). For example, Trembath et al. found that a premorbid lifestyle of activities lacking in physical or intellectual challenge (a passive lifestyle) was correlated with an earlier age of onset in HD, independent of CAG repeat length. HD patients in the most passive tertile showed a mean onset of 4.6 years earlier than the least passive tertile (Trembath et al., 2010). In support of this, a randomized, controlled pilot trial in 25 HD patients suggested functional benefits of a moderate 8-week home-exercise program on motor symptoms (Khalil et al., 2013). Simonin et al. reported an association of an earlier onset (mean of 3.6 years) with higher daily caffeine

intake (>190mg/day) in HD patients, but not with the consumption of alcohol or smoking (Simonin et al., 2013). These studies provide evidence that lifestyle factors may have a substantial influence on age of onset in HD patients. However there are many non-genetic factors that remain to be investigated.

Animal studies allow control of genetic and environmental and have also demonstrated that environmental interventions can modulate the onset of the HD phenotype. In the clinically relevant R6/1 transgenic mouse model, which harbours an exon 1 fragment expressing the human HD mutation (Mangiarini et al., 1996), enriched housing through cognitive and somatosensory stimulation has been shown to delay the onset of motor deficits (Spires et al., 2004; van Dellen et al., 2000). Environmental enrichment also ameliorated the depression-related behavioural phenotype (Du et al., 2012) and cognitive deficits (Nithianantharajah et al., 2008) in R6/1 mice. 'Brain training' through cognitive enrichment (Wood et al., 2011), caloric restriction (Duan et al., 2003) and supplementation with essential fatty acids (Clifford et al., 2002) have also shown benefits on HD pathology in transgenic mouse models. Furthermore, voluntary exercise through wheel-running delayed the onset of specific motor symptoms (Pang et al., 2006; van Dellen et al., 2008) and cognitive deficits (Harrison et al., 2013; Pang et al., 2006) as well as correcting the depressive-like phenotype (Renoir et al., 2012) in HD mouse models. The rescue of HD deficits by environmental enrichment and exercise was associated with enhanced hippocampal neurogenesis and increased levels of brain-derived neurotrophic factor (BDNF) mRNA and protein levels in the hippocampus or striatum (Lazic et al., 2006; Pang et al., 2006; Spires et al., 2004; Zajac et al., 2009).

Stress is an indelible facet of life, and this is particularly true for HD gene-positive individuals who have to cope with the psychological aspects of being diagnosed with an incurable condition (Downing et al., 2012; Ho et al., 2009). The hypothalamus-pituitary-adrenal axis (HPA axis) is the key biological system that regulates the behavioural and physiological response to stress. It culminates in the elevated production and secretion of glucocorticoid stress hormones such as cortisol (corticosterone in rodents, CORT) by the adrenal glands. The HPA axis activity in HD patients appears dysregulated with elevated levels of urinary cortisol levels and its positive correlation with disease progression (Bjorkqvist et al., 2006). It is also reported that the diurnal rhythm of cortisol is disrupted (Aziz et al., 2009). The R6 transgenic mouse models also mirror these abnormalities in elevated baseline corticosterone levels (Bjorkqvist et al., 2006) and sustained elevation of CORT levels after an acute stressor (Du et al., 2012).

Oral CORT exposure has been shown to reduce hippocampal levels of brain-derived neurotrophic factor (BDNF) (Gourley et al., 2008b). However, the effect of CORT treatment on levels of the major BDNF receptor, tropomyosin-related kinase B (TrkB) is unclear and depend on the animal models used, time points examined and the corticosterone dose (Kutiyanawalla et al., 2011; Suri and Vaidya, 2013). Hippocampal cell proliferation is reduced by exogenous CORT treatment (Brummelte and Galea, 2010; Hodes et al., 2012). These molecular and cellular changes induced by CORT-treatment are partly regulated by the activation of glucocorticoid receptors (Schaaf et al., 2000; Suri and Vaidya, 2013; Wong and Herbert, 2005). We previously reported that the impairment of hippocampal-based memory performance in female R6/1 mice was impaired by an acute bout of confinement stress, while

WT mice were unaffected (Mo et al., 2013). That was the first evidence that stress could be a key non-genetic modifier of HD symptom onset.

Therefore, in order to further study the impact of stress on HD pathogenesis, we passively administered CORT (25 mg/L) in the drinking water to investigate the effect of elevated CORT levels on the development of the motor and cognitive deficits in R6/1 HD mice. We found that CORT treatment accelerated the onset of Y-maze memory impairment in male HD mice only. Consistent with this, the levels of hippocampal cell proliferation were lowest in CORT-drinking male HD mice and CORT treatment increased the levels of activated TrkB, but had no effects in female R6/1 mice or WT controls. Collectively, our data suggest a male HD-specific vulnerability of hippocampal-based measures and cognitive performance to corticosterone treatment.

Results

Effects of the HD mutation and chronic CORT treatment on weight gain

Weekly weight monitoring showed an overall age*genotype interaction for weight gain in both female ($F_{(2.49, 30.26)}$ = 14.52, p<0.001) (**Fig.1A**) and male ($F_{(2.52, 27.44)}$ = 43.40, p<0.001) animals (**Fig.1B**). HD mice showed a reduced rate of weight gain compared to their WT controls. Pair-wise analysis for both sexes showed significantly impaired gain in HD compared to WT mice from the earliest measured time point (7 weeks of age, WT vs. HD, p<0.05).

There was no overall effect of CORT treatment in females ($F_{(1, 85)}$ =0.528, p=0.470) or males ($F_{(1, 76)}$ = 0.594, p=0.443). However, there was an age*genotype*CORT interaction ($F_{(2.52, 27.44)}$ =3.61, p=0.020) in the male dataset (**Fig. 1B**) and post-hoc pair-wise comparisons revealed that WT CORT-drinking male mice showed reduced weight gain only during the first week of treatment (10.0% Water vs. 4.9% CORT, p=0.001). In contrast, CORT reduced weight gain of male HD mice in the second (7.7% Water vs. 3.0% CORT, p=0.013) and third (13.8% Water vs. 8.1% CORT, p=0.006) weeks of treatment with a trend for reduction in the first week (p=0.088).

Effects of the HD mutation and chronic CORT treatment on rotarod performance

Motor abnormalities are a classical symptom in HD and a standard test for motor coordination in mice is the accelerating rotarod test. As expected, a significant age*genotype interaction was found in female ($F_{(5.27, 28.34)}$ = 8.47, p<0.001; Fig.2A) and male ($F_{(4.71, 11.76)}$ = 2.63, p=0.031; **Fig. 2B**) animals, indicating a decline in HD performance with age in both sexes. Post-hoc analysis revealed deficits in motor coordination began at 10 weeks of age in male HD (p=0.002) and 12 weeks of age in female HD mice (p<0.001). CORT treatment did not have any overall effect in female ($F_{(1, 43)}$ = 0.10, p=0.754) or male ($F_{(1, 20)}$ = 0.01, p=0.939) animals.

Effects of the HD mutation and 5 days of CORT treatment on short-term spatial memory

We recently reported that male R6/1 mice display a Y-maze deficit from 8 weeks of age (Mo et al., 2013) and female R6/1 mice from 10 weeks of age (unpublished data). We therefore began CORT treatment at 6 weeks of age and tested mice at 7 weeks of age to assess whether the 5-day CORT treatment would accelerate the onset of the Y-maze deficit in HD animals. At this age, there was no difference between groups in CORT consumption **(Supp fig. 1)**. There was also no effect of CORT treatment on plasma coticosterone levels at the time we did the behavioural experiments (10am-12pm, data not shown). There was no effect of genotype ($F_{(1, 38)}$ = 0.16, p=0.691) or CORT treatment ($F_{(1, 38)}$ = 0.19, p=0.664) on the novel arm preference index of female mice (**Fig. 3A**). However, there was a significant genotype*CORT interaction ($F_{(1, 38)}$ = 4.22, p=0.047) in male animals (**Fig. 3B)** with post-hoc analysis indicating that CORT-drinking male R6/1 (but not WT) mice showed a reduced novel arm preference (p=0.032) compared to water-drinking HD animals.

Notably, this reduced novel arm preference in CORT-drinking HD males was not due to any effect on locomotor activity since the total distance travelled in the Y-maze was not influenced by CORT treatment during the training trial (**Supp Fig. 2**) or the testing trial (males $F_{(1, 38)}$ = 1.18, p=0.285, females $F_{(1, 38)}$ = 1.10, p=0.301) (**Fig. 3C, D**). Female R6/1 mice explored more than WT mice in the 5-min trial ($F_{(1, 38)}$ = 5.24, p=0.028) (**Fig. 3C**).

The latency to leave the home arm of the Y-maze was not significantly affected by CORT treatment during the 10-min training trial (**Supp Fig. 2C, D**) or during the 5-min testing trial

(female: $F_{(1, 38)}$ = 0.33, p=0.568, male: $F_{(1, 38)}$ = 1.96, p=0.170) (**Fig. 3E, F**). However, there was an effect of genotype in female animals during the 5-min testing trial only ($F_{(1, 38)}$ = 4.13, p=0.049), showing that R6/1 mice were quicker to approach the centre of the maze compared to WT mice.

Effects of the HD mutation and 5 days of CORT treatment on key elements of the stress response axis

Shrunken adrenal glands are a physiological effect of treatment with CORT-drinking, as previously reported in WT male rodents (Donner et al., 2012; Karatsoreos et al., 2010; Magariños et al., 1998). In female animals, measurements of adrenal gland weights revealed no effect of 5 days of CORT treatment ($F_{(1, 26)}$ = 1.19, p=0.285) but a significant effect of genotype ($F_{(1, 26)}$ = 7.15, p=0.013), showing higher adrenal weights in female HD mice compared to WT controls (**Fig. 4A**). In male animals, there was no effect of the HD mutation ($F_{(1, 18)}$ = 1.04, p=0.320) but an overall effect of CORT ($F_{(1, 18)}$ = 58.68, p<0.0001) with a marked reduction in the adrenal weights of CORT-drinking male mice (**Fig. 4B**).

The Y-maze is a hippocampal-dependent task, so we investigated whether 5 days of CORT treatment altered the expression levels of the glucocorticoid (GR) and mineralocorticoid (MR) receptors in the hippocampus (**Fig. 5**). GR mRNA levels were not affected by CORT treatment in females ($F_{(1, 19)}$ = 0.10, p=0.754) or males ($F_{(1, 20)}$ = 0.02, p=0.877). There was no genotypic difference in both groups (females: $F_{(1, 19)}$ = 0.05, p=0.822, males $F_{(1, 20)}$ = 0.16, p=0.694) (**Fig. 5A, B**).

In contrast, genotype differences in MR mRNA levels were detected for both female (**Fig 5C**; $F_{(1, 21)}$ = 4.61, p=0.044) and male (**Fig 5D**; $F_{(1, 20)}$ = 15.81, p<0.0001) groups, with levels reduced to 78% of controls in female HD (**Fig. 5C**) and 72% in male HD (**Fig. 5D**). There were no overall CORT-treatment effects on MR expression in both groups (females: $F_{(1, 21)}$ = 0.86, p=0.363, males: $F_{(1, 20)}$ = 0.433, p=0.518).

Effects of the HD mutation and 5 days of CORT treatment on hippocampal BDNF and TrkB levels as well as hippocampal cell proliferation

High levels of exogenous CORT treatment reduce mRNA and protein levels of the neurotrophin, brain-derived neurotrophic factor (BDNF) in the hippocampus (Jacobsen and Mørk, 2006). In R6/1 HD mice, reduced levels of hippocampal BDNF were previously associated with impaired learning (Giralt et al., 2009). We therefore quantified hippocampal BDNF levels after 5 days of CORT treatment. BDNF mRNA levels in female mice were not affected by CORT treatment ($F_{(1, 20)}$ = 0.70, p=0.410) or genotype ($F_{(1, 17)}$ = 1.83, p=0.194) (**Fig. 6A**). In the male group, there was no effect of CORT treatment ($F_{(1, 18)}$ = 0.57, p=0.458) but there was a genotype effect ($F_{(1, 18)}$ = 7.20, p=0.015) (**Fig. 6B**). Analysis of BDNF protein levels by ELISA showed a genotype effect in both females ($F_{(1, 20)}$ = 15.72, p<0.0001) and males ($F_{(1, 19)}$ = 6.45, p=0.020), but there were no treatment effects (Females: $F_{(1, 20)}$ = 0.70, p=0.410, males: $F_{(1, 19)}$ = 0.191, p=0.667) or significant genotype*treatment interactions (**Fig. 6C, D**).

Exogenous CORT treatment has also been shown to impact on TrkB protein (Hodes et al., 2012; Kutiyanawalla et al., 2011). We investigated the levels of full length (FL) TrkB, its truncated form as well as the active phosphorylated TrkB receptor (pTrkB) in the hippocampus after 5 days of CORT administration (**Fig. 7**). In female mice, analyses of FL TrkB data showed no overall effect of genotype ($F_{(1, 20)}$ = 0.03, p=0.855) or CORT treatment ($F_{(1, 20)}$ = 0.01, p=0.922) (**Fig. 7A**). The pTrkB/TrkB ratio was also unaffected by CORT treatment ($F_{(1, 20)}$ = 2.05, p=0.168) (**Fig. 7C**). However, there was an overall effect of genotype ($F_{(1, 20)}$ = 13.78, p=0.001) with HD water-drinking mice showing 47% ± 0.04 of the pTrkB/TrkB ratio compared to WT Water controls. Representative blots are shown in **Figure 7E**. Analyses of pTrkB levels alone also revealed an effect of genotype ($F_{(1, 20)}$ = 12.93, p=0.002, data not shown).

In male mice, analyses of FL TrkB data showed no effect of genotype ($F_{(1, 19)}$ = 0.31, p=0.581) or CORT treatment ($F_{(1, 19)}$ = 0.90, p=0.355) (**Fig. 7B**). Analyses of the pTrkB/TrkB ratio also revealed no significant effects of genotype ($F_{(1, 19)}$ = 0.22, p=0.644) or CORT ($F_{(1, 19)}$ = 1.16, p=0.296), however there was a genotype*CORT interaction ($F_{(1, 19)}$ = 5.78, p=0.026) (**Fig. 7D)**. LSD post-hoc analysis revealed a higher pTrkB/TrkB ratio in R6/1 HD CORT-drinking mice compared to HD water-drinking controls (p=0.027). **Figure 7F** shows representative blots. Analyses of pTrkB levels alone did not reveal any significant effects or interactions (data not shown). There were no significant effects or interactions for analysis of truncated TrkB levels in males or females (data not shown).

CORT treatment in rodents has been shown to reduce hippocampal cell proliferation (Murray et al., 2008) and we have previously reported reduced proliferation in the hippocampal sub-granular zone (SGZ) of male R6/1 mice by 13 weeks of age (Ransome and Hannan, 2013). Using the endogenous mitotic marker, Ki-67, we investigated if 5 days of CORT affected the density of proliferative cells in the dentate gyrus (**Fig. 7**). In females, there was no effect of CORT ($F_{(1, 26)}$ = 0.80, p=0.379) or genotype ($F_{(1, 26)}$ = 0.42, p=0.522) (**Fig.7C**). In contrast, in male animals there was both an effect of CORT ($F_{(1, 32)}$ = 5.20, p=0.029) and genotype ($F_{(1, 32)}$ = 5.21, p=0.029) (**Fig. 7D**). Indeed, the male HD CORT-drinking group had the lowest density of proliferative cells in the SGZ at 7 weeks of age. Following post-hoc analyses in males, only the HD CORT group (14519 ± 2054 cells/mm^3) was significantly different (p=0.003) when compared to WT control animals (25653 ± 3489 cells/mm^3) (**Fig. 7D**).

Discussion

Preclinical studies of HD transgenic mouse models (Hockly et al., 2002; van Dellen et al., 2000) have corroborated with clinical evidence that the age of onset of HD symptoms is not solely predicted by the CAG repeat mutation but is subject to modulation by various lifestyle factors (Buruma et al., 1987; López-Sendón et al., 2011; Marder et al., 2009; Simonin et al., 2013; Trembath et al., 2010; Wexler et al., 2004). Here we report for the first time that treatment with corticosterone (CORT) accelerated the onset of short-term memory impairment in male R6/1 HD mice. This dose of CORT did not impair memory in WT controls suggesting that HD males are hypersensitive to corticosterone treatment. Altered levels of cell proliferation in CORT-treated male HD mice may be a candidate for this vulnerability, whereas TrkB activation could be a compensatory response. Finally, we observed protection against the effects of CORT administration at this dose in female mice.

Corticosterone treatment accelerated the onset of Y-maze memory deficits in male HD mice

Cognitive dysfunction, including impaired short-term memory, is a prominent symptom in HD (Meudell et al., 1978; Scholz and Berlemann, 1987). Short-term memory deficits in the Y-maze test develop at 8 weeks of age in male R6/1 HD mice (Mo et al., 2013) and we now report that 5 days of oral CORT treatment accelerated this deficit in male HD mice. The dose of CORT used in our study was moderate (Karatsoreos et al., 2010) and WT mice were spared from any CORT-induced Y-maze deficits. Also, the HD susceptibility to CORT-treatment appears to be male-specific since female mice were unaffected. This CORT-induced impairment was not due to a difference in exploration of the maze or latency to first

leave the home arm (**Fig. 3, Supp fig. 2**). It was also unrelated to anxiety, as measured in the light-dark box (**Supp fig. 3B**).

Further cognitive testing would be required to determine if our findings are specific to the Y-maze task. In this regard, we attempted the hippocampal-dependent novel location recognition test (NLRT) but compared to previous reports (Nithianantharajah et al., 2008) unexpectedly found that 7 week old R6/1 mice were impaired in the NLRT (**Supp fig. 4**).It was therefore unsuitable to test the potential genotype-treatment interaction after 5 days of CORT since baseline performance differed. Assessments using more complex cognitive tasks such as Morris water-maze, radial arm maze or Barnes maze may not be suitable since they are innately stressful (Harrison et al., 2009) and would also be inappropriate for our 5-day treatment window.

Interestingly, the CORT-induced Y-maze deficit was observed in male but not female HD mice despite an equal dose of CORT between the sexes. Sex differences are often reported in the stress literature and evidence for a protective effect of estrogens is discussed when the stressor only impairs male animals (Conrad et al., 2004; Luine and Frankfurt, 2013). Altered levels of sex hormones as part of HD pathophysiology may offer a possible explanation for the male HD-specific Y-maze result seen in the present study. Testicular atrophy and low serum testosterone concentrations have been reported in R6/1 HD mice (Hannan and Ransome, 2012) and HD patients (Markianos et al., 2005; Van Raamsdonk et al., 2007). In contrast, female R6/1 mice show no change in circulating estradiol concentrations compared to wild-type controls (Du & Hannan, unpublished data) although data on estradiol levels in female HD patients is not yet available. The decline in serum testosterone in male HD patients was associated with dementia and correlated with HD functional rating scores

(Markianos et al., 2005). In general, declining androgen levels have been linked to impaired memory function (Bussiere et al., 2005; Janowsky et al., 2000). High levels of glucocorticoids also act to impair hippocampal processes (McEwen et al., 2012), as we have shown in the reduced levels of cell proliferation in the present study. Thus, we propose that male HD mice (and possibly patients) are subject to a 'double hit' (pathological reduction in androgens coupled with hypersensitivity to glucocorticoids) which is detrimental to cognitive processing. We therefore assessed key elements of hippocampal neuroplasticity after 5 days of CORT treatment.

Five days of corticosterone treatment did not change hippocampal glucocorticoid receptor gene expression

There was no effect of 5 days of CORT-drinking on GR or MR gene expression in the hippocampus (**Fig. 5**). Instead, robust alterations have been reported by others after higher doses or chronic exposure to exogenous CORT (Sapolsky et al., 1984; Tornello et al., 1982; Wu et al., 2013). It is well-established that GR and MR are key regulators of the physiological stress response (Reul and De Kloet, 1985). Therefore, it is most likely that the short duration of treatment at this dose in our study may not be sufficient to elicit changes in hippocampal GR and MR gene expression, despite the obvious peripheral effects on adrenal gland weights in male mice. Consistent with that, a 6-day CORT treatment in rats was insufficient to cause a change in hippocampal GR mRNA levels in spite of reduced adrenal weights (Young et al., 1995).

Five days of corticosterone treatment increased hippocampal TrkB activation in male HD mice, independent of BDNF levels

BDNF and its high affinity receptor TrkB are implicated in various aspects of brain function, cellular plasticity and cognitive processing (Li et al., 2012). Glucocorticoids regulate BDNF/TrkB signalling at many levels (Suri and Vaidya, 2013). CORT and glucocorticoid administration generally reduces hippocampal TrkB protein and mRNA levels (Gourley et al., 2008b; Hodes et al., 2012; Kutiyanawalla et al., 2011; Yi et al., 2012), however, increases in TrkB have also been reported (Roskoden et al., 2004; Schaaf et al., 1997; Vellucci et al., 2002). These studies employed higher doses and/or many weeks of administration (2 – 7 weeks). The relatively short exposure period (5 days) and moderate dose (4.7 mg/kg/day) may explain why the levels of FL TrkB and truncated TrkB levels were unaltered in the present study (**Fig. 7**).

In contrast to the absence of CORT effects on TrkB protein levels, 5 days of CORT treatment increased the pTrkB/TrkB ratio, specifically in male R6/1 HD mice (**Fig.7C, D**). TrkB receptor phosphorylation (activation) is usually through binding of BDNF, leading to downstream phosphorylation cascades that promote neuronal survival and differentiation (Barnabé-Heider and Miller, 2003). We found that TrkB activation was enhanced by CORT treatment independent of changes to BDNF levels (**Fig. 6**) and FL and truncated TrkB levels (**Fig. 7A, C**). An increase in pTrkB levels unrelated to BDNF was also reported by Jeanneteau and colleagues after acute administration of the synthetic glucocorticoid, dexamethasone, in juvenile rats and *in vitro* (Jeanneteau and Chao, 2013; Jeanneteau et al., 2008). The glucocorticoid-induced increase in pTrkB was through genomic glucocorticoid receptor (GR) signalling and may provide a mechanism for the neuroprotective effects of elevated glucocorticoids (Jeanneteau et al., 2008). Whether this can be extended to the adult

hippocampus has yet to be proven. In an oral CORT study, the level of pTrkB as well as downstream ERK1/2 were decreased in the male mouse hippocampus after 20 days of oral CORT administration (6.9 mg/kg/day) (Gourley et al., 2008a). However, reductions in pTrkB after prolonged CORT treatment may be secondary to an overall reduction in hippocampal BDNF levels (Gourley et al., 2008b).

It is tempting to relate Y-maze performance and TrkB activation since the CORT-induced changes were both specific to male R6/1 mice (**Fig. 7D**). However, the results are difficult to reconcile. BDNF-induced activation of TrkB signalling in the hippocampus is crucial for spatial memory acquisition and consolidation (Mizuno et al., 2003; Yamada and Nabeshima, 2003) but we showed impaired spatial memory with increased pTrkB in CORT-drinking R6/1 males.

On the other hand, TrkB is expressed in neural progenitor cells of the dentate gyrus and its signalling regulates adult hippocampal neurogenesis (Choi et al., 2009, Li et al., 2008). For example, one of the downstream activators of TrkB is the phosphoinositide 3-OH kinase (PI3K)/Akt pathway (Patapoutian and Reichardt, 2001), which also regulates adult hippocampal proliferation (Peltier et al., 2007). An increase in TrkB phosphorylation may be a result of compensation for the low levels of cell proliferation in CORT-drinking male R6/1 mice (**Fig. 8**).

Five days of corticosterone treatment reduced hippocampal cell proliferation in male animals

The memory impairment found in the Y-maze test and the increased hippocampal pTrkB levels in CORT-drinking male R6/1 mice suggest that the HD mutation in males may cause changes that interfere with the response to CORT. In support of male-specific effects, 5 days of CORT-drinking reduced the density of hippocampal SGZ proliferating cells in male animals (WT and R6/1) but not female animals (**Fig. 8**), corroborating with a rat restraint stress study (Hillerer et al., 2013). Untreated male R6/1 mice exhibited a reduced proliferative cell population in the dentate gyrus by 7 weeks of age. We previously reported this sex difference in cell proliferation by 13 weeks of age in R6/1 mice (Ransome and Hannan, 2013). As a result of both genotype and CORT effects, CORT-drinking male HD mice showed a 43% reduction in proliferation after 5 days of treatment, compared to water-drinking WT controls. The HD mutation reduced proliferative capacity in the dentate gyrus, which was then exacerbated by CORT exposure. This exemplifies the notion of a HD vulnerability to stress-induced effects.

With respect to the functional implications of this reduction in hippocampal cell proliferation, another study which showed that stress almost halved progenitor proliferation reported no effect on subsequent differentiation and long-term neuronal survival in the adult hippocampus (Malberg and Duman, 2003). This is not surprising since only a subset of the mitotic pool differentiated into mature neurons with synaptic integration (Espósito et al., 2005). Therefore, while the Ki-67(+) experiment reflects our behavioural results, the male HD-specific deficit in short-term memory could also be explained by other cellular impairments, such as changes in spine morphology (Conrad et al., 1996; Gourley et al., 2013), which are known to correlate with synaptic plasticity.

Altered hippocampal measures and adrenal gland weights in R6/1 mice at 7 weeks of age

Regardless of CORT treatment, we report some HD differences in the levels of hippocampal and adrenal gland measures in R6/1 male and female mice (summarized in **Table 1**). The levels of hippocampal MR gene expression were reduced in female and male HD mice at 7 weeks of age (**Fig. 5C, D**). There is no data available on MR levels in HD mice or patients. We found a ~20% reduction in the mRNA levels of MR in the R6/1 HD hippocampus at a young age (7 weeks of age). The majority of MRs are occupied under baseline conditions and regulate the circadian rhythm of plasma CORT secretion (Reul and De Kloet, 1985). This abnormal reduction in MR gene expression may contribute to the exaggerated diurnal CORT levels in the blood and urine of HD patients and mice (Aziz et al., 2009; Bjorkqvist et al., 2006). Inefficient clearance of serum CORT and disrupted circadian rhythm activity could also help explain abnormal CORT regulation in HD. GR expression levels and function in R6/1 mice do not differ from WT levels at 12 weeks of age (Du et al., 2012), corroborating with the present findings at 7 weeks of age.

Abnormal BDNF signalling is a prominent pathology of HD (Zuccato and Cattaneo, 2007). The HD mutation reduced hippocampal BDNF protein in R6/1 mice of both sexes at 7 weeks of age (**Fig. 6C, D**). This corroborates with other studies which found a deficit in BDNF levels in HD transgenic mice at a pre-motor symptomatic age (Hermel et al., 2004; Peng et al., 2008; Zajac et al., 2009; Zuccato et al., 2001). In contrast, quantification of mRNA levels showed reductions only in male R6/1 mice (**Fig. 6A, B)**. In female R6/1 mice, the reduction in BDNF protein but not mRNA compared to controls may reflect natural biological variance or a mismatch between mRNA and protein levels as reported previously in the mouse hippocampus (Adlard et al., 2005). Several studies have suggested that mRNA turnover rate,

transcriptional changes, protein secretion and protein degradation may contribute to their relative differences (Tropea et al., 2001; Vogel and Marcotte, 2012). Indeed, the correlation between mRNA and protein can be as low as 40% due to the many processes that regulate transcription, translation and other aspects of RNA/protein metabolism (Vogel and Marcotte, 2012).

Levels of the full length and truncated isoforms of TrkB were normal in R6/1 mice up to 6 months of age (Brito et al., 2013; Gharami et al., 2008). Our findings support these previous data and there were no deficits in truncated or full length TrkB in R6/1 mice at 7 weeks of age (**Fig. 7A, B**). However, the HD mutation markedly decreased the pTrkB/TrkB ratio in female HD animals (47 ± 0.04 % of WT control levels) (**Fig. 7C**). In contrast, male R6/1 HD mice did not show significant deficits in pTrkB levels compared to WT controls (**Fig. 7D**). Others have reported that striatal pTrkB levels in R6/1 mice are reduced to half the levels of WT controls at a late stage of the disease (6 months of age) (Gharami et al., 2008), however they did not investigate potential sex differences. Estradiol levels regulate the levels of pTrkB in the hippocampus of female mice (Spencer-Segal et al., 2011) through the estrogen receptor (ER)α and ERβ (Spencer-Segal et al., 2012). Further work investigating estrogenic signalling in the hippocampus of female R6/1 mice may help elucidate the mechanism mediating the R6/1 sex difference in TrkB activation.

We also reported an increase in wet adrenal gland weight in only female R6/1 mice. This was apparent at both 7 weeks (**Fig. 4**) and 14 weeks of age (**Supp fig. 5**) compared to WT controls. This increase in adrenal weight may be related to the prolonged secretion or

impaired clearance of stress hormone in the adrenal glands and depressive-like phenotype seen in only female R6/1 mice (Du et al., 2012; Pang et al., 2009). However, the increase in wet weight may not be explained by adrenal hypertrophy since there were no differences in the cross-sectional area or cell density of the zona fasciculata, the adrenal region which produces glucocorticoids (Du et al., 2012). There were also no differences in the area or thickness of the adrenal cortex (Du et al., 2012) or medulla (data not reported). Increased adrenal gland weight is seen in the more aggressive R6/2 HD mouse model, along with morphological hypertrophy (Bjorkqvist et al., 2006). Bjorkqvist and colleagues found hyperplasia of the R6/2 adrenal cortex and pituitary lobe as a result of excessive ACTH and corticosterone, but did not analyse by sex (Bjorkqvist et al., 2006). Measuring corticosterone blood levels during CORT treatment would be required to better correlate our behavioural observation to HPA axis function. However because mice drink overnight (in their active dark cycle), collecting blood at relevant time-points had been proven challenging. Relevant to our data, we found no effect of CORT treatment on plasma corticosterone levels at the time we did the behavioural experiments or collect the tissue (10am-12pm, data not shown).

Decline in motor coordination in HD mice was unaffected by CORT treatment

Rotarod performance in R6/1 mice, started to decline compared to WT controls at 12 weeks of age in females, and 10 weeks in males (**Fig. 2**). Similar to our findings, Gharami and colleagues reported sex differences in rotarod performance and body weight where male R6/1 mice showed an earlier progression in both measures compared to females (Abbott and Nelson, 2000; Gharami et al., 2008). Chronic CORT treatment beginning from 6 weeks of age had no effect on R6/1 or WT motor performance. The absence of chronic effects did not appear to be due to physiological habituation to CORT-drinking, as measured by gross

adrenal gland weights (**Supp fig. 5**). Eight weeks of CORT-drinking reduced adrenal gland weights in animals at 14 weeks of age, except WT female mice. This corroborates with the lack of CORT effects seen in WT female mice throughout the study.

Motor function in general could be less sensitive to the effects of chronic elevations of stress hormone. Support for this comes from the distribution of stress receptors, which are more concentrated in regions related to learning, memory and emotional processing (Reul and De Kloet, 1985). In addition, the dose of CORT in the present study was moderate in its physiological effects compared to other stressors used in the literature. For example, chronic restraint water immersion is able to impair rotarod performance in WT mice (Mizoguchi et al., 2002). Therefore, we cannot rule out that more severe stressors may be able to accelerate or exacerbate motor symptoms, as well as memory deficits in HD.

In conclusion, we have shown that treatment with the stress hormone corticosterone could bring forward the onset of memory decline in male HD mice. By using a moderate dose, we were able to highlight the behavioural, cellular and molecular vulnerability of the male HD hippocampus to stress-induced impairments. This vulnerability to stress treatment combined with the additional psychological burden experienced by HD patients (Bombard et al., 2012; Downing et al., 2012) may contribute to the higher rates of depression and suicide in HD individuals compared to the general population (Paulsen et al., 2005; Schoenfeld et al., 1984). If stress is confirmed by clinical investigations to be a negative environmental modulator in HD, improved counselling and therapies to promote resilience may help prevent an accelerated cognitive decline in this stress susceptible group. Future studies investigating the impact of more severe stressors on other HD symptoms such as cognitive deficits would further reveal the extent to which stress contributes to HD onset and progression.

Materials and Methods

Animals

R6/1 hemizygote males (Mangiarini et al., 1996) originally from the Jackson Laboratory (Bar Harbour, ME, USA) were bred with CBA x C57BL/6 (CBB6) F1 females to establish the R6/1 colony at the Florey Neuroscience Institutes. Female and male R6/1 offspring and their WT littermates were genotyped from tail and toe biopsies using polymerase chain reaction (PCR). CAG trinucleotide repeat length sequencing of samples from HD mice showed a mean length of 136.4±3.3 repeats. Mice were weaned at 3 weeks of age into standard housed (15x30x12 cm), single-sex groups (n=4-5) of mixed genotype. All mice had access to food and water *ad libitum* and were maintained under a 12-hour light/dark cycle (0700 light on). Behavioural testing occurred during the light phase. Body weight was monitored weekly, beginning one day prior to the start of corticosterone administration (see below) at 6 weeks of age. All experiments were approved and performed in accordance with the guidelines of the Florey Neuroscience Institutes Animal Ethics Committee and the National Health and Medical Research Council (NHMRC).

Oral corticosterone treatment

Passive delivery of CORT in drinking water avoids injection stress and induces elevations relevant to circadian rhythm fluctuations (Birt et al., 2001). We expected an exaggerated response to stress in HD mice compared to WT controls so we chose a concentration of CORT (25 mg/L) that has moderate physiological and behavioural effects (Karatsoreos et al., 2010). This dose elevates circulating CORT to levels comparable to restraint stress (> 130

ng/ml) during active drinking (Gourley et al., 2008b; Stone and Lin, 2008). Corticosterone (4-pregnen-11-beta 21-diol-3 20-dione 21-hemisuccinate, Steraloids Inc, Rhode Island, USA) was dissolved in drinking water under basic conditions (pH>12) for 4-6 hours at 4°C. After warming to room temperature, the pH was returned to neutral (pH 7.2-7.4). CORT solution was used within 72 hours and kept in the dark stored at 4°C to retard degradation (Gourley et al., 2008b). Consumption of CORT was measured in a behaviourally-naïve, single-housed cohort of mice. Daily measurements of CORT were taken over five days for each mouse and expressed as mg of CORT per kg body weight per day.

Behavioural testing

CORT-treated mice had continual access to CORT in place of drinking water from 6 weeks to 14 weeks of age. One cohort of mice was assessed weekly from 6 weeks of age for motor function and a separate cohort was tested for Y-maze memory performance at 7 weeks of age.

Accelerating rotarod

The rotarod is a motorized revolving cylinder (0.3cm diameter, 0.2cm longitudinal ridges) upon which mice tread at accelerating revolutions per minute (7650, Ugo Basile, Comerio, Italy). The revolutions accelerated from 4 to 40 per minute (rpm) over 300 seconds and the latency to fall off was recorded as a measure of motor coordination (Spires et al., 2004). Mice were acclimatized to the apparatus 24-hours before the first test at 6 weeks of age. Acclimatization consisted of placing each mouse on the cylinder for 2-mins at 4 rpm followed by acceleration to 20 rpm over 2-mins. During acclimatization, mice were

immediately replaced on the cylinder if they fell off. For all testing trials, clasping the cylinder for 3 full rotations was equivalent to a fall. Baseline testing at 6 weeks of age (prior to CORT treatment) was followed by weekly testing until 14 weeks of age.

Y-maze

The Y-maze short-term spatial memory test is based on a rodent's innate exploratory drive and is sensitive to the effects of stress (Conrad et al., 2004). The maze is a Y-shaped apparatus with 3 identical arms (10x30x17 cm) with visual cues extending above each and a centre area. A training trial begins when the mouse is placed in the home arm and allowed to explore the maze for 10-mins with one arm blocked off at the junction to the centre area (novel arm). After a 1-hour interval, the mouse is returned to home arm for the testing trial when the entire maze is accessible for 5-mins.

Intact spatial exploratory memory performance is represented by a preference for exploring the novel arm. A novel arm preference index is calculated for the testing trial: time spent in the novel arm / [average time spent in other two arms], where a preference of 1 indicates no preference (Etkin et al., 2006; Mo et al., 2013). Time spent in each arm, total distance travelled and latency to leave the home arm during the training and testing trials were analysed live by Ethovision software (Noldus Information Technology Inc., Netherlands) from a camera mounted on the ceiling.

Adrenal gland weights

After 5 days of CORT treatment, mice were killed by cervical dislocation and the adrenal glands were immediately dissected and weighed. Due to effects of the HD mutation and CORT treatment on body weight, we expressed adrenal gland weight as a ratio of body weight: adrenals(mg)/body weight(g).

Tissue preparation for hippocampal gene expression and protein analyses

A cohort of behaviourally naïve mice at 7 weeks of age (5 days of CORT) were killed by cervical dislocation (9am – 12pm) and the hippocampus was immediately dissected on an ice-cold surface and snap frozen for storage at -80 °C. For each mouse, one hippocampus was used for mRNA expression analysis for BDNF, glucocorticoid and mineralocorticoid receptors, and the other for ELISA analysis for BDNF. For the gene expression studies, tissue samples were disrupted using a Diaganode Bioruptor (UCD-300; Life Research, VIC, Australia). Total RNA was isolated using Qiagen RNeasy MINI extraction kits in accordance with the manufacturer's instructions (Qiagen, VIC, Australia) and included the optional DNase1 treatment step. RNA concentrations and A260/280 ratios were determined with a Nanodrop (2000c Thermo Scientific, Wilmington, DE, USA).

Determining glucocorticoid receptors and BDNF mRNA levels with real-time PCR

Relative quantification of mRNA levels was determined as previously described (Pang et al., 2009). 1000ng of RNA per sample was reverse transcribed into cDNA using Superscript VILO cDNA Synthesis kit (Invitrogen, Life Technologies, VIC, Australia). Two separate reactions of no enzyme and no RNA served as study controls. Reverse transcription PCR was performed in a thermal cycler (Takara Shuzo, Tokyo, Japan) at 25 °C for 10-min, 48 °C for 30-min and 95 °C for 5-min. The cDNA products were stored at −20 °C until subsequent use.

Target gene primer sequences were designed using Primer 3. Primer sequences (Sigma Genosys, Castle Hill, NSW) are as follows (5' to 3'): GR forward: AGGCCGCTCAGTGTTTTC TA, GR reverse: TACAGCTTCCACACGTCAGC; MR forward: GGCTTCTGGGTGTCACTATGG, MR reverse: CACAGATAGTTGTGTTGTCCTTCCA; BDNF coding forward: GCGCCCATGAAAGAAGTAAA, BDNF coding reverse: TCGTCAGACCTCTCGAACCT; Reference gene: Cyclophilin forward: CCCACCGTGTTCTTCGACA, Cyclophilin reverse: CCAGTGCTCAGAGCTCGAAA. Optimal primer dilutions and amplification efficiencies were determined prior to commencement of real-time PCR studies. Real-time PCR was performed on a7500 Fast Real-time PCR system (Applied Biosystems, VIC, Australia) using SYBR green (S4438; Sigma-Aldrich, NSW, Australia). Each sample was plated in triplicate and negative PCR controls were included in the study. The real-time PCR conditions were 50 °C for 2-min, 95 °C for 10-min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1-min. Dissociation curve analysis was also performed. The expression levels of target genes were determined using the comparative Ct (ΔΔCt) method, and normalized to the mean expression level of the wild-type control group.

BDNF ELISA for protein quantification

The amount of total BDNF protein in the hippocampus was assessed by enzyme-linked immunosorbent assay (ELISA) and expressed in terms of total hippocampal protein. The hippocampus was sonicated (Sonics & Materials, Inc., CT, USA) on ice in 200µl lysis buffer containing 1M Tris pH 7.5, 50mM EDTA, 0.1M EGTA, 50% Glycerol, 10% Triton X-100, 0.5M NaF, 50mM Na Pyrophosphate, 0.1M Na3V04, 1% Dithiothreitol and Protease inhibitor cocktail (Sigma-Aldrich, MO, USA). Homogenates were spun at 20,000 x g at 4 °C for 20-mins and total protein in the supernatant was quantified using the Bradford Protein Assay (BCA, Thermo-Scientific, Rockford, IL, USA) from the absorbance at 562nm (Benchmark Plus Microplate Reader, Bio-Rad, CA, USA). Samples were stored at -20 °C until ELISA analyses using the E-max BDNF ELISA kit (Promega, Madison, WI, USA). Samples were analysed in triplicate following the manufacturer's instructions. The final concentration of BDNF was expressed as the total amount of hippocampal protein extracted (pg/mg protein).

Western blot analysis

Western blot analysis was performed as previously described (Hill et al., 2010). Briefly, sample volume required for 50 µg of protein was added along with an equal volume of loading buffer (0.4M Tris pH 6.8, 37.5% glycerol, 10% SDS, 1% 2 mercaptoethanol, 0.5% bromphenol blue, dH_2O). Samples were then denatured for 10 minutes at 95°C before SDS-PAGE (15% or 10% acrylamide gel, 120 V, 1.5 hours) and transferred to a nitrocellulose membrane. The membrane was then incubated with primary antibody overnight at 4°C. Primary antibodies were rabbit anti-TrkB (H-181, 1:1000, Santa Cruz Biotechnology) which

produced two bands at approximately 140 kDa (full length TrkB) and 95kDa (truncated TrkB), rabbit anti-phosphorylated TrkB (phospho-Tyr705, 1:1000, Signalway Antibody, Pearland, TX, USA) which produced a band at approximately 140 kDa, or mouse anti-β-actin (1:5000, Sigma-Aldrich, Castle Hill, NSW, Australia). The next day, the membrane was incubated with either anti-rabbit or anti-mouse IgG HRP-linked secondary antibodies (Cell Signalling, Danvers, MA, USA). Images were captured using a Luminescence Image Analyzer (Fuji film LAS-4000, FujiFilm Life Science, Stamford, CT, USA), and analyzed using Multi Gauge software (FujiFilm Life Science). BDNF levels were normalized against levels of the housekeeping gene, β-actin. Data are expressed relative to the average value of the male control group (water-treated).

Hippocampal cell proliferation quantification

Tissue processing

Mice treated with 5 days of CORT and controls (7 weeks of age) were killed with an intraperitoneal overdose of 0.2 ml sodium pentobarbitone (325 mg/ml). After responsive reflexes were abolished, mice were intracardially perfused with approximately 100 ml of phosphate-buffered saline (PBS) followed by 100ml of 4% paraformaldehyde fixative in 0.1M PBS (pH 7.3). The brain was removed and post-fixed for 24-hours followed by storage in 30% sucrose solution until they were no longer buoyant (2-3 days) at 4 °C. Brains were embedded in molds of optimal cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetek, CA, USA) and frozen using isopentane and dry ice for storage at -80 °C. The length of hippocampus was coronally sectioned on a Leica CM3050 S cryostat (Leica Biosystems,

NSW, Australia) at 40µm 1/6 series and collected free-floating in cryoprotectant solution (25% v/v ethylene glycol, 25% glycerol in 0.1M PBS) for storage at -20 °C.

Immunohistochemistry

Staining for Ki-67 was performed on 1/6 series of free-floating hippocampal sections. Washes were performed using 0.1M PBS (pH 7.3) and incubations were at room temperature unless otherwise stated. Hippocampal hemi-sections were washed (2 x 15-min) and quenched for 20-mins using 1% hydrogen peroxide in PBS followed by 3 x 15-min washes. Sections were incubated for 1-hour with blocking solution consisting of 5% normal donkey serum (Merck Millipore, MA, USA) and 0.1% Triton X-100 (Sigma-Aldrich, WI, USA) in 0.1M PBS before overnight incubation with rabbit anti-Ki-67 (1:200) (Thermo Scientific, Rockford, IL, USA) in blocking solution. After 3 x 15-min washes, sections were incubated with biotinylated rabbit antisheep (Vector laboratories, CA, USA) 1:400 in blocking solution for 2-hours. Following 3 x 15-min washes, sections underwent a 2-hour incubation with Vectastain ABC Elite solution (Vector Laboratories, CA, USA) 1:100 in 0.1% PBS. Sections were then developed after 3 x 15-min washes with diaminobenzidine (DAB) chromagen and chromophore solution (Dako Laboratories, CA, USA) until optimal Ki-67 to background staining was visualized under a microscope. Sections were then mounted on superfrost slides (Fisher Scientific, PA, USA), air-dried and coverslipped using DPX (Fisher Scientific Ltd., Leicestershire, UK).

Cell counting

Counts of Ki-67-immunopositive (+) cells were performed on blind-coded slides. Only Ki-67(+) cells in the subgranular zone were counted, which was defined as the region approximately 2 cell widths (16 μm) either side of the junction between the hilus and granule cell layer (Ransome and Turnley, 2008). Ki-67(+) cells were visualized using a light microscope (Olympus BX61, Center Valley, PA) and counted under an Olympus UPlan FL N 60x objective lens in 7-9 unilateral sections per mouse. The length of the SGZ was measured using Image Pro Plus software (Media Cybernetics Inc., Rockville, MD, USA) so that the total volume of the SGZ could be estimated by multiplying the measured SGZ length, the width of the SGZ (36 μm), and the section thickness (40 μm). The number of Ki-67(+) cells was expressed as mean density counts (total count/volume of SGZ).

Statistical Analysis

Analyses of variance (ANOVAs) were used to examine main effects and interactions. Repeated measures ANOVAs were used to analyse weight gain and rotarod performance. Violations of sphericity were corrected using the Greenhouse-Geisser correction. To determine specific group differences in case of significant main effects (or interaction), ANOVAs were followed by Fisher's least significant difference (LSD) or Bonferroni post-hoc tests. In all cases, the significance level was set at $p<0.05$. Statistical analyses were performed using SPSS statistics Version 21.0 (IBM, Armonk, NY, USA) and GraphPad Prism 5 (GraphPad software, Inc., LA Jolla, CA).

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Conflict of Interest

The authors 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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Figure Legends

Figure 1. Effects of HD mutation and chronic CORT treatment on weight gain.

As expected, all animals put on weight over time. However, HD mice showed reduced weight gain in both females (**A**) and males (**B**) compared to WT controls. This effect of the HD mutation worsened with age as suggested by significant interactions between genotype and age for both sexes. CORT treatment did not have any effect on weight gain in female mice (**A**) but reduced weight gain in male animals (**B**). This reduction in males was actually dependent on both the genotype and the age, since CORT-drinking only affected WT males in the first week of treatment while HD males were affected during the first 3 weeks of treatment. Finally, both WT- and HD- CORT groups recovered to the same rates of water controls thereafter. Weight gain is expressed as a percentage of weight at 6 weeks of age, recorded prior to commencement of CORT treatment (Baseline). * $p<0.01$ WT Water vs WT CORT, # $p<0.05$ HD Water vs HD CORT. Values represent means ± SEM, n=20-23 females and n=20-23 males per group.

Figure 2. The effects of CORT treatment on rotarod performance.

There was a progressive motor coordination deficit in HD mice which developed at 12 weeks of age in female HD mice (**A**) and 10 weeks of age in male HD mice (**B**). CORT treatment beginning at 6 weeks of age had no effect on female or male rotarod performance in HD or WT animals. Baseline refers to the time point prior to the start of CORT treatment at 6 weeks of age. Latency to fall from rotarod was tested once per week with a maximum time set at 300 sec. ** $p<0.01$ and ***$p<0.001$ for genotype difference. Values represent means ± SEM, n=11-13 females and n=9-12 males per group.

Figure 3. Effect of 5-day treatment with CORT on Y-maze memory.

At 7 weeks of age, water-drinking female HD (**A**) and male HD (**B**) mice showed a similar novel arm preference to WT mice. However, male HD mice treated with 5 days of CORT did not perform in the Y-maze test compared to water controls (**B**). The distance travelled in the 5-min testing trial of the Y-maze was similar between male groups (**D**) but there was a genotype effect in females with HD mice travelling a slightly longer distance (**C**). There was no significant effect of CORT in first latency to approach the centre of the maze during the testing trial (**E, F**). Novel arm preference index = Time spent in the Novel arm/[average of time spent in other two arms], where an index of 1 indicates no preference. Values represent means ± SEM, n=9-12 females and n=10-11 males per group. ** $p<0.01$ HD Water vs HD CORT, # $p<0.05$ for overall effect of genotype.

Figure 4. Adrenal gland weights after 5 days of CORT treatment at 7 weeks of age.

Adrenal gland weights were increased in female R6/1 mice compared to WT controls at 7 weeks of age (**A**). In males, there was no difference between genotypes but 5 days of CORT-drinking markedly reduced adrenal weights (**B**). Normalized adrenal values: Wet adrenal weight (mg)/body weight(g). Values represent means ± SEM, n=6-8 females and 5-6 males per group. * $p<0.05$, # $p<0.001$ for overall effects of genotype and CORT, respectively.

Figure 5. Hippocampal glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA levels after 5 days of CORT administration.

Levels of GR were similar to controls in both females (**A**) and males (**B**). Levels of MR were reduced in female HD mice (**C**) and male HD mice (**D**) at 7 weeks of age compared to WT controls but there was no effect of CORT treatment. Values represent means ± SEM of levels normalized to WT water controls. GR n=6 males, n=5-6 females, MR n=6 males, n=6-7 females. * $p<0.05$ and *** $p<0.001$ for overall effect of genotype.

Figure 6. Hippocampal BDNF mRNA and protein levels after 5 days of CORT.

Female HD mice showed reduced BDNF protein at 7 weeks of age (**C**) but not significantly altered mRNA levels relative to WT controls (**A**). HD males showed reduced hippocampal mRNA (**B**) and protein levels (**D**) compared to WT animals. Five days of CORT treatment had no effect on BDNF protein or mRNA levels in any group. Values represent means ± SEM. BDNF mRNA levels were normalized to WT water controls, BDNF protein n=6-7 females, n= 5-6 males per group. BDNF mRNA n=5-6 females, n=5-6 males per group. * $p<0.05$ and *** $p<0.001$ for overall effect of genotype.

Figure 7. Hippocampal TrkB protein levels after 5 days of CORT treatment

Full length TrkB protein levels were no different between WT and R6/1 HD mice at 7 weeks of age in females (**A**) or males (**B**). Five days of CORT treatment also had no effect. In contrast, the ratio between phosphorylated TrkB (pTrkB) and TrkB was markedly reduced in female R6/1 mice compared to WT controls. However there was no effect of CORT (**C**).

CORT treatment increased pTrkB/TrkB ratio in male R6/1 mice only (**D**). Representative blots are shown for females (**E**) and males (**F**). Truncated TrkB levels were also assayed and showed no significant effects or interactions (data not shown). Values represent means ± SEM. All protein was expressed as a ratio of β-actin and then normalized to the mean value of WT water controls. n=6 females and n= 5-6 males per group. ## $p<0.01$ for overall effect of genotype and * $p<0.05$ for post-hoc analysis between HD Water and HD CORT groups. FL TrkB: full length, pTrkB: phosphorylated TrkB, Tr TrkB: truncated TrkB.

Figure 8. Hippocampal SGZ cell proliferation after 5 days of CORT treatment.

Representative photomicrographs of Ki-67-immunostained coronal sections (40μm) of the dentate gyrus in female (**A**) and male (**B**) mice. Blinded quantification of Ki-67-immunopositive (+) cell densities in the dentate gyrus subgranular zone (SGZ) of female (**C**) and male (**D**) mice. Male R6/1 mice showed reduced Ki-67(+) cell densities at 7 weeks of age. Male WT and Male HD counts are reduced by 5 days of CORT treatment. Counts are similar between groups in females. Scale bar = 100 μm. Values represent means ± SEM, n=7-8 per group for females, n=8-10 per group for males. *$p<0.05$ for overall effect of CORT, \$ $p<0.05$ for overall effect of genotype.

Figure 9. Summary of oral CORT effects on motor and cognitive measures in R6/1 mice.

CORT treatment (25mg/L) from 6 weeks to 14 weeks of age had no effect on the onset or progression of rotarod deficits in male or female R6/1 mice. Weight gain was reduced in male R6/1 mice compared to WT controls at 7 weeks of age and this was further suppressed by CORT treatment in the initial few weeks. The onset of a Y-maze deficit was accelerated to 7 weeks of age by 5 days of CORT treatment. Y-maze performance in female R6/1 and WT mice was unaffected. Further molecular and cellular measures were investigated at 7 weeks of age (see **Table 1**).

Table 1. Summary of the results from measures taken at 7 weeks of age after 5 days of CORT treatment.

🡻 indicates reduced, 🡹 indicates increased and – indicates no change compared to WT or water-drinking controls. '**HD**' indicates the effect was specific to R6/1 HD mice and not found in WT littermates. All molecular measures refer to hippocampus.

Supplementary figure 1. Amount of CORT consumed by WT and HD mice after 5 days of treatment.

The average consumption of 25 mg/L CORT (in mg/kg/day) over 5 days did not differ between groups. There was no effect of genotype in average dose of CORT over 5 days in females (WT 4.61 ± 0.31 vs. HD 4.78 ± 0.31 mg/kg/day, p=0.874, Mann Whitney test) (**A**) or males (WT 4.39 ± 0.37 vs. HD 4.87 ± 0.20, p=0.178 mg/kg/day, Mann Whitney test) (**B**). Values represent means ± SEM, n=5-6 per group for females, n=6-7 per group for males.

Supplementary figure 2. Effect of corticosterone on Y-maze exploration in the 10-min training trial

Distance travelled (**A, B**) and Latency to leave the Home arm (**C, D**) during the 10-min training trial of the Y-maze test at 7 weeks of age. There are no genotype or CORT effects in exploration and time to reach the centre of the maze in males or females. n=9-12 females and n=10-11 males per group. Values represent means ± SEM.

Supplementary figure 3. The effects of one week of CORT treatment on light-dark box anxiety.

CORT treatment had no effect on time spent in the light (**B**) or distance travelled (**C**) in male mice. However, 7 days of CORT increased time spent in the light compartment in WT and HD female mice (**A**) without affecting total distance travelled in the arena (**D**). n=10-12 per group for females, n=9-10 per group for males. Values represent means ± SEM. ** $p<0.01$ for overall effect of CORT.

Supplementary figure 4. Male and female R6/1 mice were impaired in the novel location recognition test at 7 weeks of age.

In the hippocampal-dependent, novel location recognition test, novel preference analyses (**A**) revealed an effect of genotype ($F_{1, 45}$ = 10.67, p=0.002), indicating that R6/1 mice were impaired at baseline age (7 weeks of age). Therefore the cognitively impairing effects of CORT on HD could not be assessed using this test. There was no effect of sex ($F_{1, 45}$ = 0.36, p=0.549) or genotype*sex interaction ($F_{1, 45}$ = 1.02, p=0.317). Analyses of distance travelled data (**B**) again showed an effect of genotype ($F_{1, 45}$ = 4.64, p=0.037), but also a significant overall effect of sex ($F_{1, 45}$ = 15.13, p<0.001). There was no genotype*sex interaction ($F_{1, 45}$ = 1.94, p=0.17). Preference for novel location = Time spent investigating [Novel location / (Familiar + Novel locations)] x 100. Females n= 16-18, males n=6-9, ** p<0.01 for overall effect of genotype, ^^^ p<0.001 for overall effect of sex.

Supplementary figure 5. The effects of 8-week CORT treatment on adrenal gland weights.

At 14 weeks of age, adrenal gland weights were increased in female R6/1 HD mice compared to WT controls (**A**). Eight weeks of CORT-drinking reduced adrenal gland weights in female R6/1 mice, but not female WT mice. In males (**B**), chronic CORT-drinking reduced adrenal weights, regardless of genotype. Normalized adrenal values: Wet adrenal weight (mg)/body weight(g). n=11-15 per group for females, n=6-8 per group for males. Values represent means ± SEM. ### p<0.001 for overall effect of CORT, ** p<0.01 for Water HD vs CORT HD post-hoc comparisons.

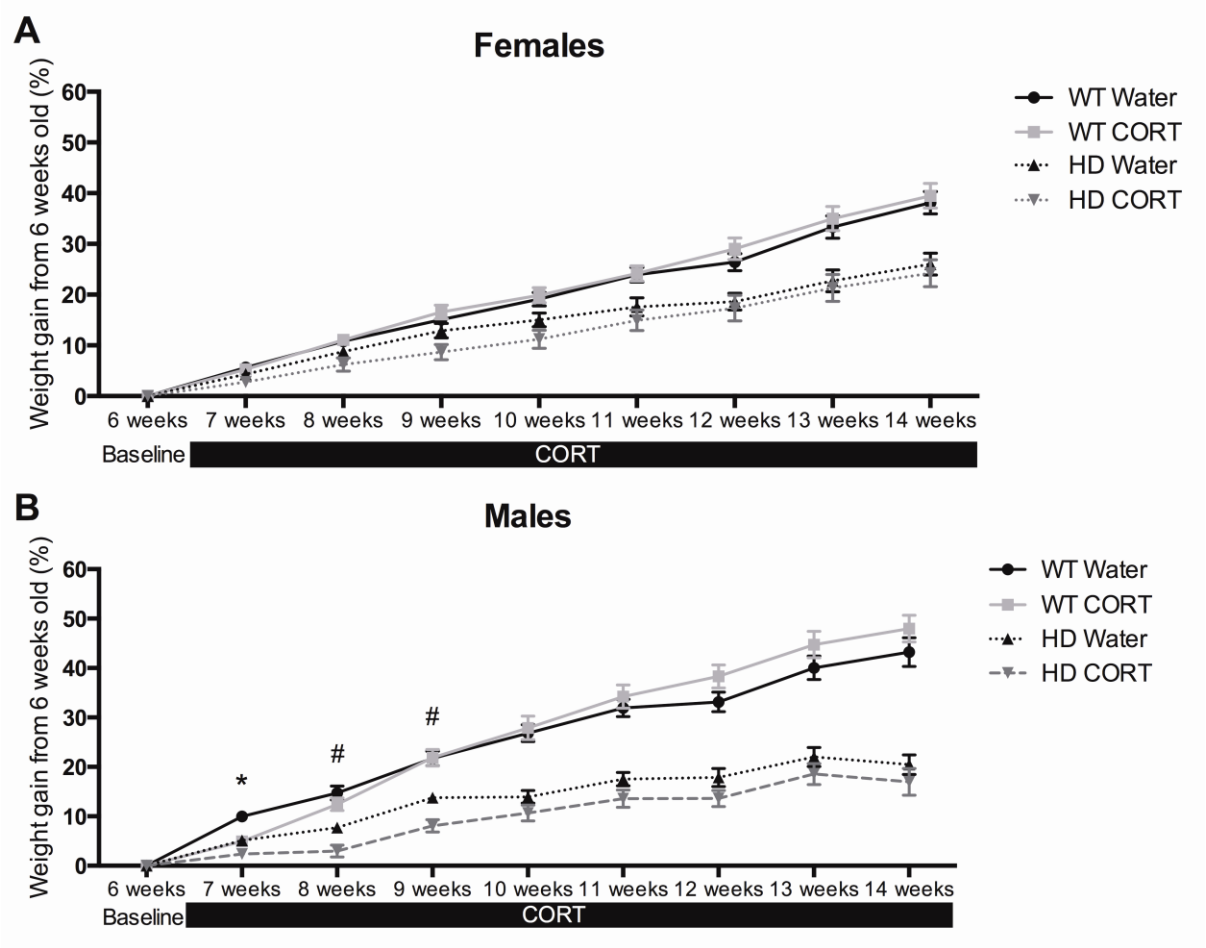
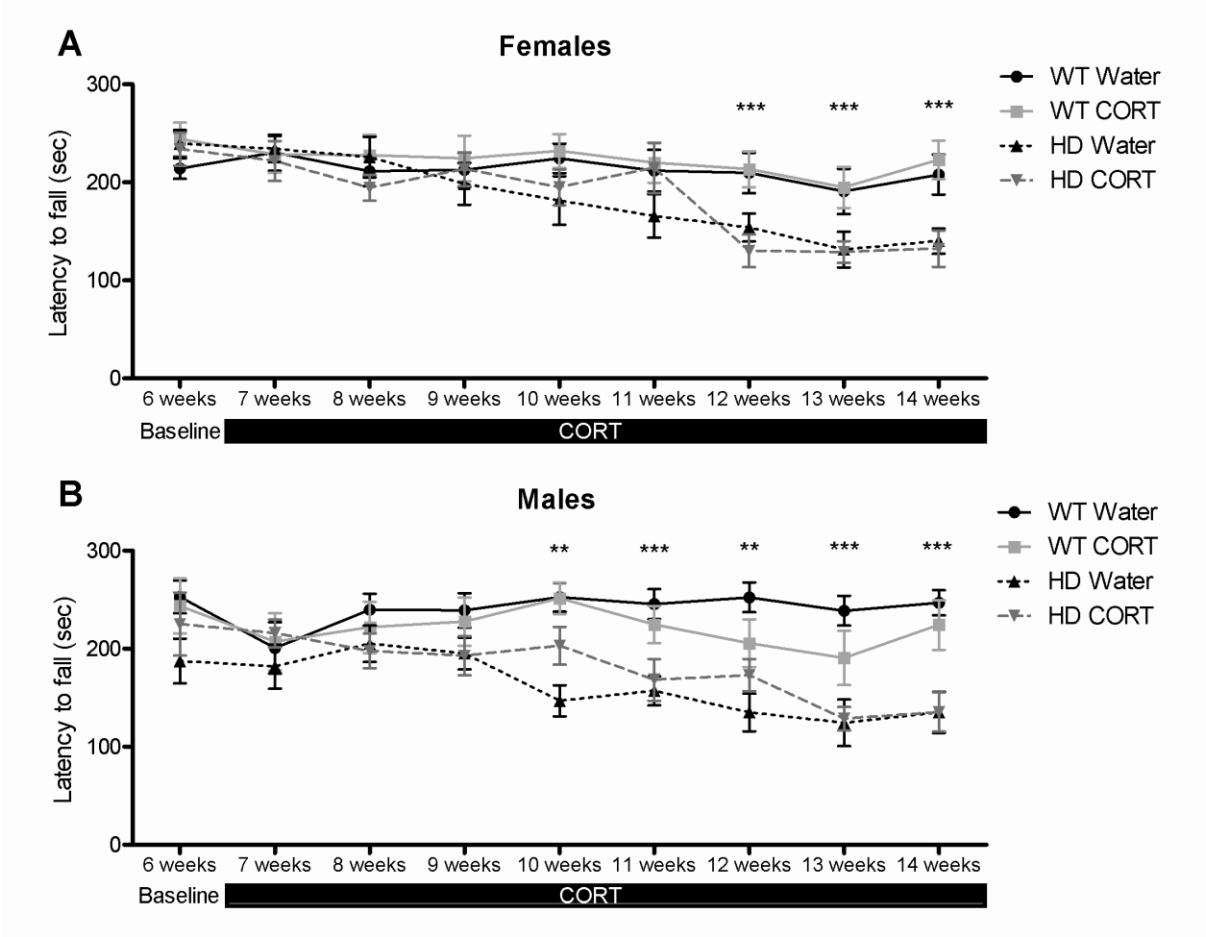


Figure 1



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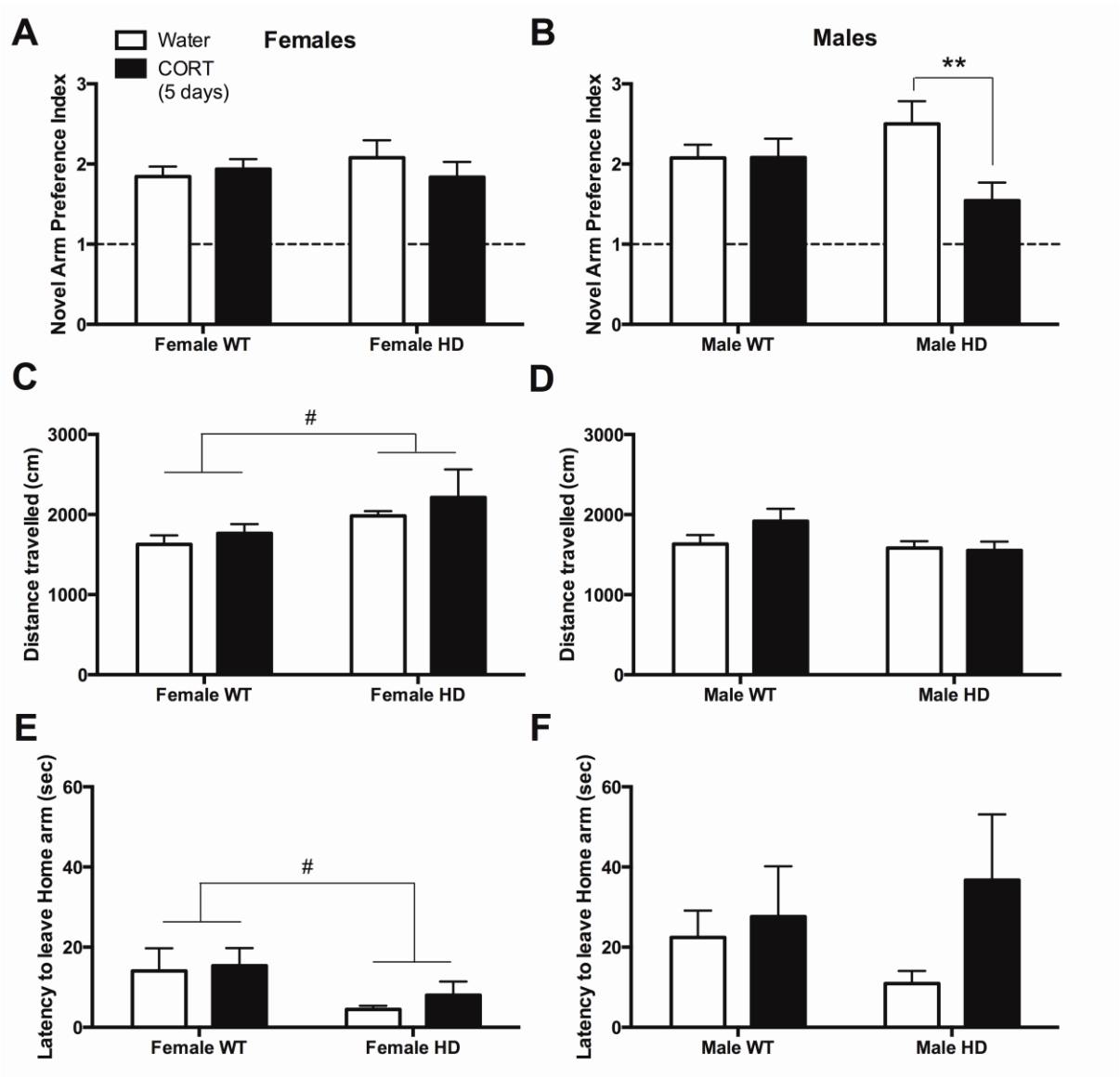

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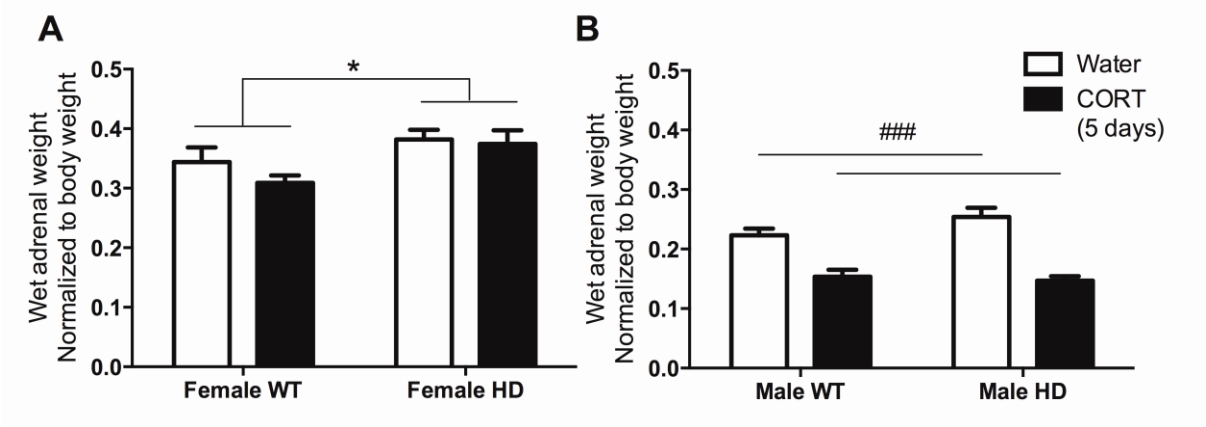


Figure 4

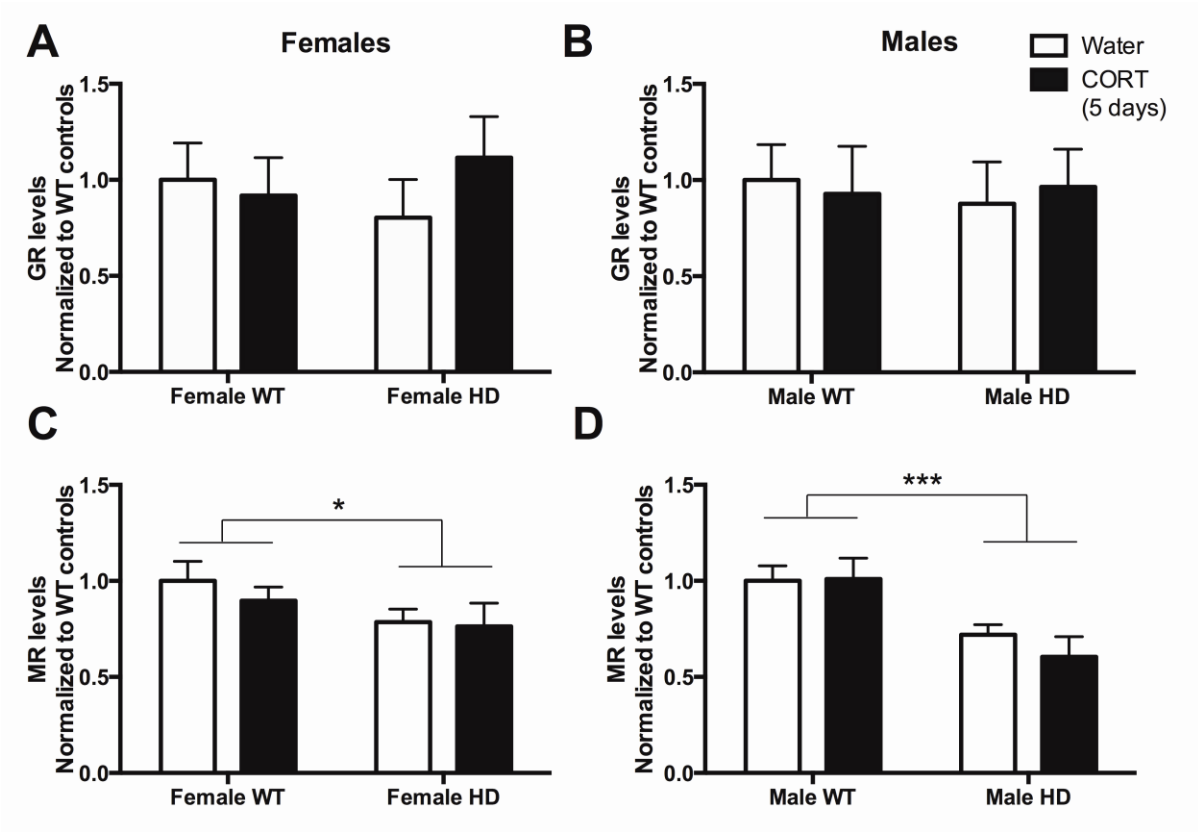
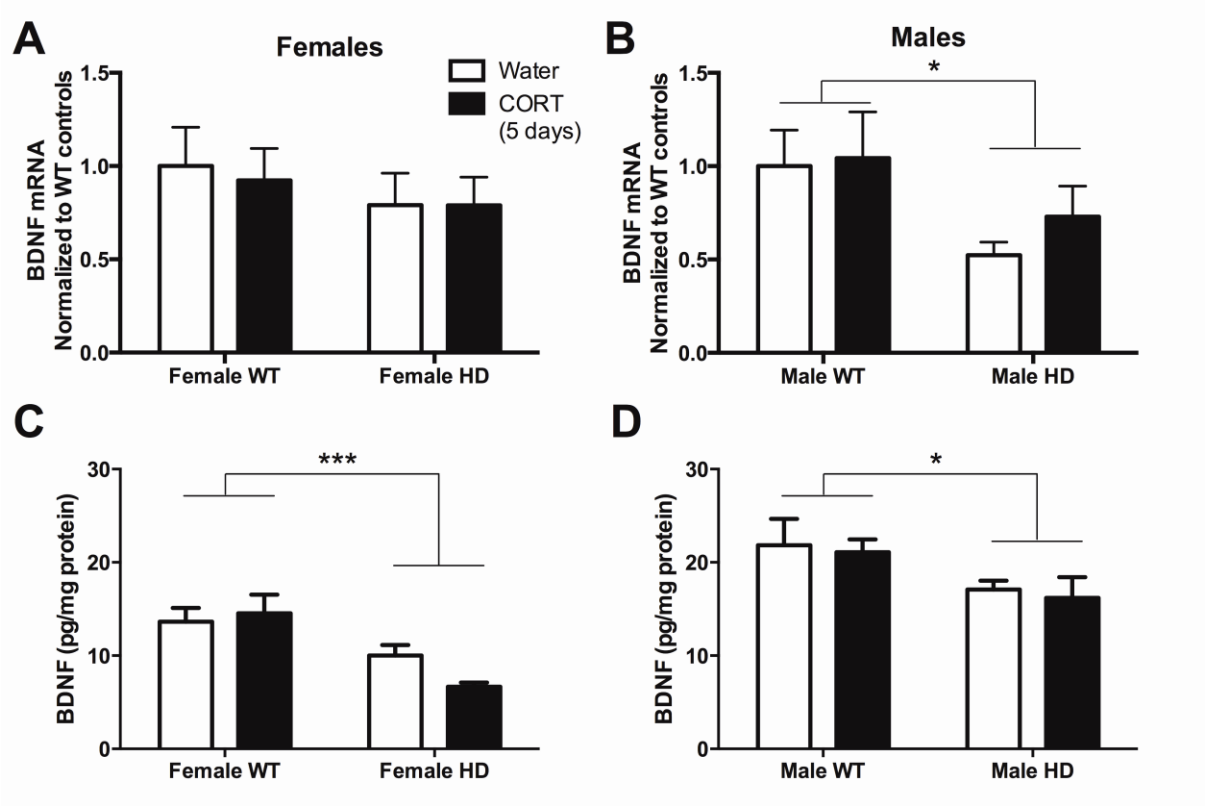


Figure 5



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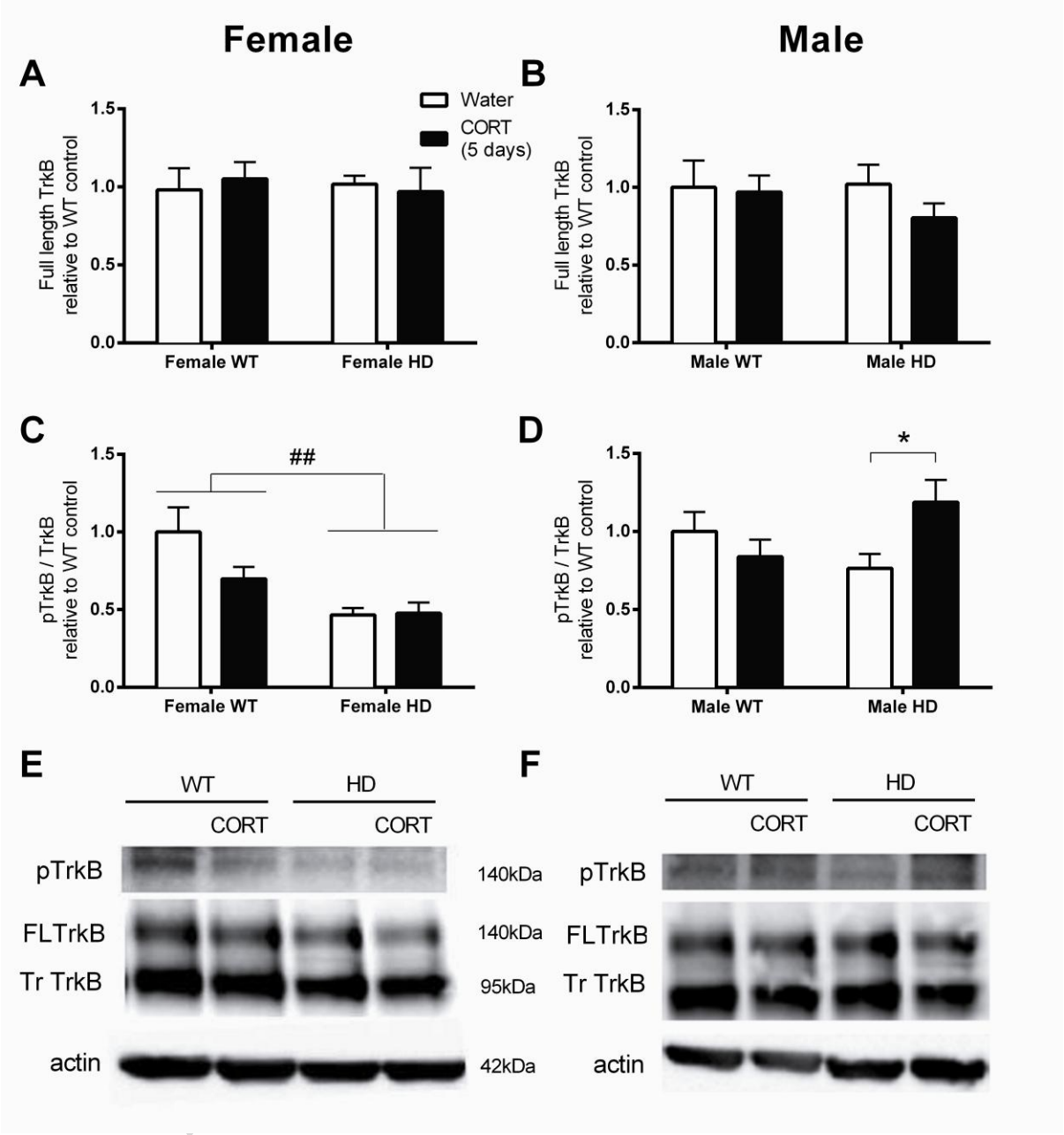

Figure 7

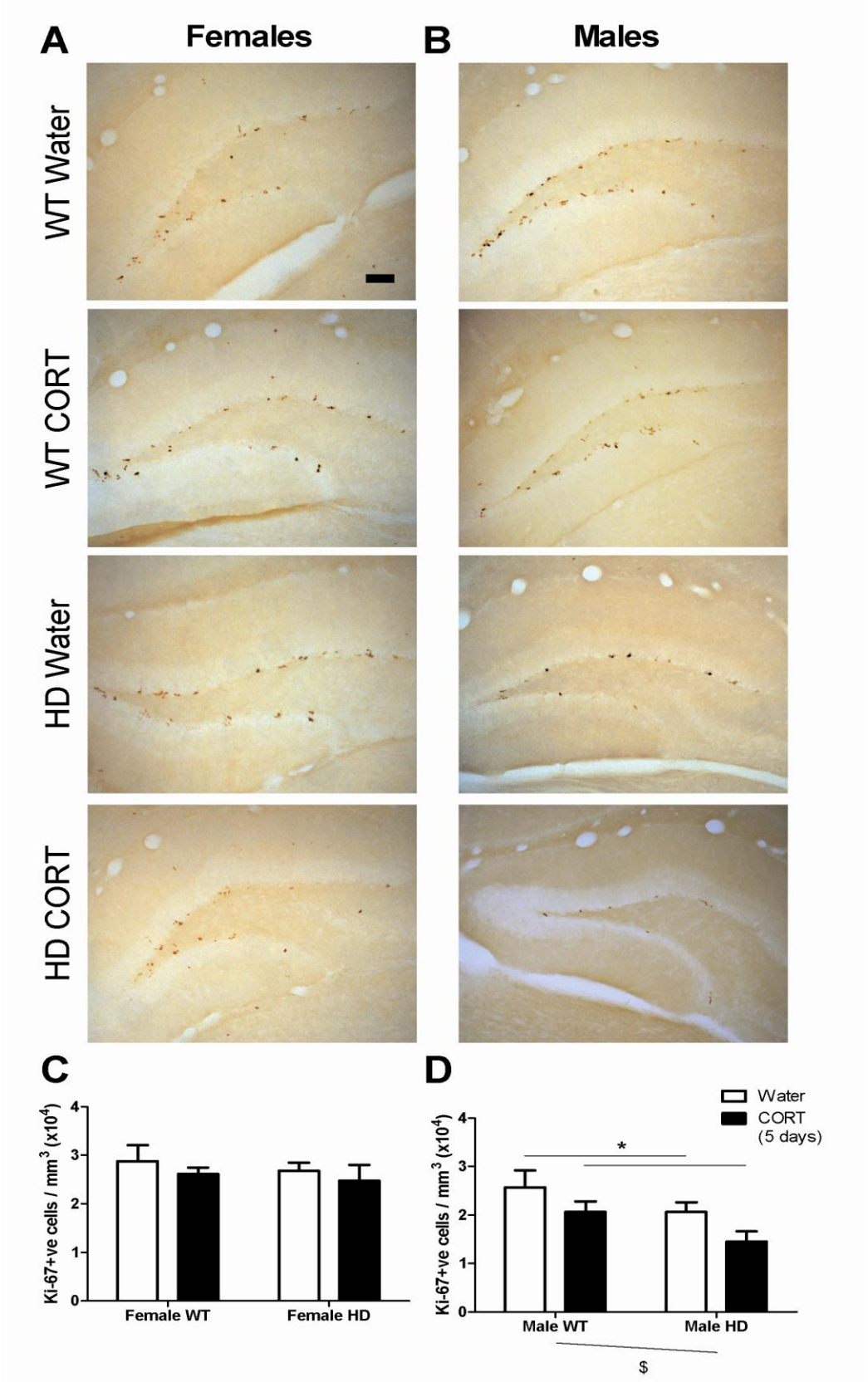

Figure 8

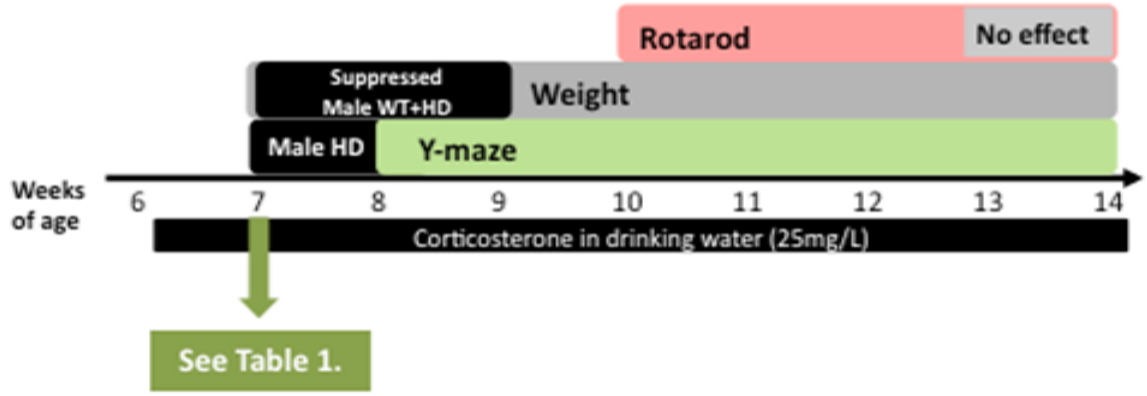

Figure 9

Table 1. Summary of the results from measures taken at 7 weeks of age after 5 days of CORT treatment.

	HD mutation 7 weeks of age		5 days of CORT	
	Female	Male	Female	Male
Y-maze	–	–	–	↓HD
Mineralocorticoid receptor	↓	↓	–	–
Glucocorticoid receptor	–	–	–	–
BDNF total mRNA	–	↓	–	–
BDNF total protein	↓	↓	–	–
pTrkB/TrkB	↓	–	–	↑HD
Hippocampal cell proliferation	–	↓	–	↓
Adrenal gland weights	↑	–	–	↓

↓ indicates reduced, ↑ indicates increased and – indicates no change compared to WT or water-drinking controls. '**HD**' indicates the effect was specific to R6/1 HD mice and not found in WT littermates. All molecular measures refer to hippocampus.

Highlights:

- CORT treatment accelerated short-term memory deficits in male HD mice only

- Baseline hippocampal BDNF and mineralocorticoid receptor mRNA were lower in HD mice

- Impaired hippocampal cell proliferation in male HD mice was further reduced by CORT

- CORT treatment increased the levels of pTrkB in male HD mice only