Neurobiology of Disease xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

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

Christina Mo [a,b], Terence Y. Pang [a,1], Mark I. Ransome [a,b,1], Rachel A. Hill [a,b], Thibault Renoir [a,*,2], Anthony J. Hannan [a,b,2]

[a] Florey Institute of Neuroscience and Mental Health, Kenneth Myer Building, University of Melbourne, Parkville, Australia
[b] Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Australia

ARTICLE INFO

Article history:
Received 18 November 2013
Revised 24 April 2014
Accepted 4 May 2014
Available online xxxx

Keywords:
Huntington's disease
Corticosterone treatment
Stress hormone
Short-term memory
HPA axis
Cell proliferation
TrkB

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., 2009; Marder et al., 2013; 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

* Corresponding author at: Florey Institute for Neuroscience and Mental Health, Kenneth Myer Building, University of Melbourne, VIC 3010, Melbourne, Australia.
E-mail addresses: thibault.renoir@unimelb.edu.au, tibo.renoir@gmail.com (T. Renoir).
Available online on ScienceDirect (www.sciencedirect.com).
[1] TY Pang and MI Ransome should be regarded as joint second authors.
[2] T Renoir and AJ Hannan should be regarded as joint last authors.

http://dx.doi.org/10.1016/j.nbd.2014.05.004
0969-9961/

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 (>190 mg/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 (10 am–12 pm, 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

Please cite this article as: Mo, C., et al., High stress hormone levels accelerate the onset of memory deficits in male Huntington's disease mice, Neurobiol. Dis. (2014), http://dx.doi.org/10.1016/j.nbd.2014.05.004