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Research report

Progression of behavioural despair in R6/2 and Hdh knock-in mouse models recapitulates depression in Huntington's disease

Alessandro Ciamei[a], Peter J. Detloff[b], A. Jennifer Morton[a,*]

[a] Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Site, CB2 3DY Cambridge, UK
[b] Department of Biochemistry and Molecular Genetics, University of Alabama, Birmingham, AL 35294, USA

HIGHLIGHTS

- We studied forced swim test behaviours in R6/2 and Hdh knock-in mice.
- In both lines, increased despair (floating) was shown at an early stage of disease progression.
- In both lines, early-stage floating levels decreased as the mice aged.
- Increased swimming and climbing behaviours were observed in late-stage HD mice.

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ABSTRACT

In Huntington's disease (HD) depression is observed before the disease is diagnosed, and is likely to be a component of the disease, rather than a consequence. Depression in HD patients does not progress in parallel with other symptoms; rather it peaks at early- to mid-stages of the disease and declines thereafter. In mice, depressive-like behaviours can be measured as an increase in behavioural despair (floating) observed in the forced swim test (FST). Floating in the FST is modulated differently by antidepressants with different mechanisms of action. Drugs that increase levels of serotonin inhibit floating by promoting horizontal swimming, whereas drugs that increase levels of noradrenaline inhibit floating by enhancing vertical swimming (climbing). We compared the FST behavioural profiles of two different allelic series of HD mice, a fragment model (R6/2 mice carrying 120, 250, or 350 CAG repeats), and a knock-in model (Hdh mice carrying 50, 150, or 250 CAG repeats). The FST behavioural profile was similar in both lines. It was characterized by an early-stage increase in floating, and then, as the mice aged, floating decreased, whereas active behaviours of swimming and climbing increased. Our results show that, as with depression in HD patients, floating in HD mice does not progress linearly, suggesting that, at the late stages of the disease, an increase in serotonergic and noradrenergic activity might contribute to lower floating levels in HD mice. If similar compensatory changes occur in humans, this should be taken into account when considering the treatment of depression in HD patients.

1. Introduction

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by an expanded CAG repeat in the *HD* gene [1] associated with profound neuronal loss in the striatum and cortex [1,2]. HD results in severe motor impairment and cognitive decline. Psychiatric disturbances are also frequently observed in HD, with depression being the most common complaint [3,4]. Depression occurs in between 30% and 50% of cases, and may contribute to a further worsening of the quality of life in HD patients (and of their carers) [3–6]. Whilst a depressed condition in HD patients might be ascribed to both the stress and frustration originating from having the disease, and from the awareness of its fatal nature, biological factors are also likely to play an important role in the aetiology of depression in HD [3]. Indeed, symptoms of depression are among the earliest symptoms that can be found in HD patients, being detectable up to 10 years before the onset of motor symptoms [6], often before the patient becomes aware of his own condition. One of the most controversial aspects of depression in HD is that, contrarily to what is observed when other neuropsychological symptoms such as apathy are considered, the occurrence and severity of depressive symptoms do not correlate with disease

* Corresponding author. Tel.: +44 01223 334057; fax: +44 01223 333840.
E-mail address: ajm41@cam.ac.uk (A.J. Morton).

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progression [7]. Rather, they are common in early- and mid-stage patients, but then become less prevalent in late-stage patients [3,7]. This finding has been interpreted with caution given that a progressive acceptance by the patients of their own condition, as well as a progressive loss in self-awareness caused by dementia, might interfere or mask the depressive symptoms [3,7,8].

Most animal research in the field of depression has used the forced swim test (FST) to investigate the occurrence of depressive-like behaviours in rodents [9–11]. In the FST, animals are released into an inescapable glass cylinder filled with water. After initial engaging in escape-seeking behaviours, rodents start to float passively on the water surface, apparently giving up to any escape-oriented active behaviour. It has been shown that when mice or rats are treated with antidepressants that increase brain levels of serotonin, they display a decrease in floating in the FST that is counterbalanced by an increase in horizontal swimming (swimming). On the other hand, antidepressants increasing the brain levels of noradrenaline (NA), decrease floating and increase the time spent by the animals trying to climb up the glass cylinder to escape (climbing) [11].

The FST has been widely used to study depressive-like behaviours in several mouse models of HD [8,12–18]. Despite some discrepancies that are likely to be due to differences in the strategy of data collection adopted (e.g. continuous behavioural scoring vs time sampling techniques), or to the genetic background of the mice used, the FST behavioural profiles of the lines tested this far (R6/1 [12,16,18], YAC128 [14,15], N171-82Q [15], Hdh-111Q [17]) seem to converge on an increased level of behavioural despair (i.e. increased floating), occurring when overt motor symptoms are still absent. This is thought to model the early depressive symptoms observed in HD patients. However, when longitudinal studies were carried out in mouse models of HD (YAC128 [14,15] and N171-82Q [15]), no signs of a decrease in basal levels of despair were detected that could recapitulate the decrease in depressive symptoms displayed by HD patients.

R6/2 mice express the N-terminal fraction of the human *HTT* gene and are one of the most widely used mouse models of HD [19–21]. Their cognitive and motor deficits have been well characterized [21,22]. We have generated an allelic series of R6/2 mice with CAG repeats ranging between 110 and 400 [22]. Mice from the allelic series are characterized by a paradoxical inverse correlation between the age of onset of the phenotype and the CAG repeat length, such that increasing the length of the CAG repeat expansion causes a delay in the onset of the disease [22], including motor function. In one of our previous studies, we observed that when 250 CAG R6/2 mice are tested in a water-based swimming task (a double-dissociation water maze task) at 8 weeks and 12 weeks of age (i.e. early and mid-late stages of phenotype progression), 8-week old R6/2 mice tended to spend more time floating than 12-week old mice [23]. This result was unexpected, since the mice were capable of swimming well at 12 weeks of age. Although the water maze task is not suitable for an accurate measure of behavioural despair in mice, we thought that in R6/2 mice it was possible that depressive-like behaviours might not progress in parallel with the disease, similarly to what observed in HD patients.

The longitudinal behavioural profile of R6/2 mice in the FST has not been investigated in detail previously. Thus, in a first set of experiments, we performed a detailed, longitudinal characterization of the FST behavioural profile of R6/2 mice carrying 110, 250, and 350 CAG repeats. We then extended our longitudinal analysis of FST behaviours to the Hdh line of knock-in mice carrying repeat expansions of 50, 150, and 250 CAG in exon 1 of the mouse *Htt* gene [24–26]. Hdh knock-in mice are known to display a positive correlation between disease onset and length of the CAG expansion, as it is typically observed in HD patients [24,26].

2. Materials and methods

2.1. Animals

R6/2 mice were taken from colonies established at the University of Cambridge, UK [22]. Hdh knock-in mice, congenic to C57BL6/J, were generated at the University of Birmingham, AL, USA [27,28], and colonies were established at the University of Cambridge, UK. Mice were housed in single sex, mixed genotype groups of 10 mice. WT littermates were used as controls. Genotyping and repeat length measurements were performed by Laragen, Los Angeles, USA. All studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Throughout the experiments, mice were housed within a 12-h light/dark cycle (lights on at 7:30 AM and off at 7:30 PM) in a temperature-controlled (19–21 °C) and humidity-controlled ($45 \pm 10\%$) environment. Dry food, mash food, and water through lowered water bottle spouts, were available ad libitum. All mice were tested only once.

2.1.1. R6/2 mice

All the mice tested were generated by backcrossing R6/2 mice onto CBA × C57Bl/6 F1 mice, as previously described [22]. Small groups of WT mice were taken for testing from each of the lines tested, and the data thus obtained were pooled together to make a single WT group. Three groups of R6/2 mice were used carrying CAG repeats of different length. Mean (±SEM) CAG repeat lengths were: 109.8 ± 0.5 CAG for the 110 CAG repeat group, 252.7 ± 1.1 CAG for the 250 CAG repeat group, and 345.2 ± 0.7 for the 350 CAG repeat group. Mice from the 110 CAG repeat group were tested at 6 weeks, 8 weeks and 12 weeks of age. Mice from the 250 CAG repeat group were tested at 6 weeks, 8 weeks, 12 weeks, and 16 weeks of age, whereas mice from the 350 CAG repeat group were tested at 6 weeks, 8 weeks, 12 weeks, 16 weeks and 24 weeks of age. Differences in the number of age groups were dictated by the different survival rates of the lines of R6/2 mice tested [22]. For example, we tested more time points in the 350 CAG repeat group than in the 250 CAG repeat group because the 350 CAG repeat mice live longer. Group sizes are indicated in Supplementary content (Table 1).

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2015.05.010

2.1.2. Hdh knock-in mice

All the mice tested were generated by backcrossing Hdh HET male mice onto female C57Bl/6 mice. Small groups of WT littermate mice were taken for testing from each of the lines tested, and the data thus obtained pooled together in a single WT group. Three lines of Hdh knock-in mice were used carrying 50 (Q50; 50 ± 0.1 CAG), 150 (Q150; 147.6 ± 0.9 CAG), and 250 (Q250; 252.1 ± 2.8 CAG) CAG repeats. In contrast to what is observed in R6/2 mice, many months are needed in order to observe significant differences in the behavioural phenotype of the lines of Hdh mice tested. For this reason, we chose to test the mice at ages that could be considered representative of the early, mid, and late stages of the disease in one of the lines tested (i.e. Q250 mice; see Section 3.2). Male and female mice from all groups were tested at 3, 6, and 12 months of age. Group sizes are indicated in Supplementary content (Table 2).

Supplementary Table 2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2015.05.010

2.2. Forced swim test

The FST was carried out using a 5 L transparent glass beaker (15 cm in diameter) filled with 3.5 L of water at a temperature of 25 °C. This arrangement prevented the mice from finding