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Exercise attenuates neuropathology and has greater benefit on cognitive than motor deficits in the R6/1 Huntington's disease mouse model ☆

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disease caused by a mutation within the huntingtin gene that induces degeneration within the striatal nuclei, progressing to widespread brain atrophy and death. The neurodegeneration produces symptoms that reflect a corticostriatal disconnection syndrome involving motor, cognitive and psychiatric disturbance. Environmental enrichment has been demonstrated to be beneficial to patients with neurological disorders, with exercise being central to this effect. Rodent studies have confirmed exercise-induced neurogenesis and increased growth factor levels in the brain and improved behavioural function. The present study sought to determine whether an extended regime of exercise could retard disease progression in the R6/1 mouse model of HD. The study was designed specifically with a translational focus, selecting behavioural assessments with high clinical predictive validity. We found that exercise improved gait function in both control and HD mice and selectively improved performance in the R6/1 mice on a motor coordination aspect of the balance beam task. Exercise also retarded the progression of cognitive dysfunction on water T-maze procedural and reversal learning probes presented serially to probe cognitive flexibility. In addition, exercise reduced striatal neuron loss in the R6/1 mice but increased striatal neuronal intra-nuclear inclusion size and number relative to non-exercised R6/1 mice which demonstrated increased numbers of extra-neuronal inclusions, suggesting that the functional effects were striatally mediated. These results confirm and extend those from previous studies that demonstrate that HD may be amenable to exercise-mediated therapeutics, but suggest that the impact of such interventions may be primarily cognitive.

Introduction

Huntington's disease (HD) is a neurodegenerative disease caused by a single mutation in the gene that codes for the protein huntingtin (The Huntington's Disease Collaborative Research Group, 1993). HD is primarily characterised by the insidious and progressive neurodegeneration of the medium spiny neurons of the caudate nucleus and cortical atrophy with abnormalities in other brain regions occurring as the disease progresses (Aylward et al., 1998; Rosas et al., 2002, 2005; Tabrizi et al., 2009, 2011; van den Bogaard et al., 2011; Vonsattel et al., 1985). The disease induces motor, cognitive and psychiatric symptoms, which ultimately result in death around 15 years from onset.

There is considerable evidence that exercise or a more active lifestyle has a beneficial effect on the symptoms and prognosis of several disease states including Alzheimer's disease (Verghese et al., 2003; Abbott et al., 2004; Rovio et al., 2005; Podewils et al., 2005; Larson et al., 2006), and Parkinson's disease (Cruise et al., 2011; Gobbi et al., 2009; Muller and Muhlack, 2010; Nocera et al., 2010). These studies demonstrate an exercise-mediated improvement in the daily functioning of patients, sometimes after several years of follow-up studies. In addition to these palliative effects measured through cognitive and motor assessments, there is evidence that exercise may also slow the rate of disease neuropathology (Cruise et al., 2011; Gobbi et al., 2009; Muller and Muhlack, 2010; Nocera et al., 2010). To date only a single randomised controlled physical activity study in HD patients has been performed and demonstrated marked functional benefit with relatively little intervention (Khalil et al., 2013).

A small number of animal studies using mouse models of HD found that exercise may ameliorate some aspects of motor and cognitive dysfunction (Pang et al., 2006; van Dellen et al., 2008; Wood et al., 2011). R6/1 mice exposed to voluntary wheel running from a young age were found to demonstrate less body clasping when suspended by the tail, a delayed onset of motor dysfunction in a static beam test and produced a greater number of spontaneous alternations in a cognitive spatial alternation test, but wheel running had no effect on other transgene-induced functional deficits (Pang et al., 2006; van Dellen

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et al., 2008). In other studies with normal mice (in both middle and old age) (Berchtold et al., 2010; van Praag et al., 1999, 2005), and mouse models of neurological dysfunction (Griesbach et al., 2009; Nichol et al., 2009) exercise was found to enhance learning. However, a study in mice bred to produce high levels of wheel running failed to see this effect (Rhodes et al., 2003). Studies on exercise and cognitive function use probes of hippocampus-mediated spatial learning in HD mouse lines (Pang et al., 2006; Potter et al., 2010). However, this type of probe has little relevance to the primary striatal neuropathology of HD. Hence, although there is some evidence to suggest that exercise in HD mouse lines may be of potential benefit to the clinical population, previous studies have typically used behavioural tests with low face validity and little translational relevance.

The present study was designed to determine whether life-long daily access to voluntary exercise was able to provide benefit to HD mice in a clinically relevant way that could be developed as a model therapeutic system. Hence, we sought to design a study with an emphasis on high predictive (translational) validity, such that the tests used in the animal study were correlates of those commonly used in clinical assessments of HD patients, and presently being used in the HD exercise study being run in Cardiff University (Khalil et al., 2013). The tests were chosen to be sensitive probes of cortico-striatal dysfunction, the principal cause of functional decline in HD. We also sought to determine the underlying mechanism of any beneficial effect of exercise in the HD mice by measuring striatal dopamine and BDNF activation, as both have been implicated in HD pathology (Augood et al., 1997; Giampa et al., 2013; Glass et al., 2000; Zuccato et al., 2001, 2008), and by a detailed stereological analyses of the striatum to determine atrophy, neuron numbers and inclusion pathology.

Material and methods

Animals

The R6/1 HD mouse line (Mangiarini et al., 1996) was chosen for the present study as it develops marked pathology over the course of 7 months and has been used in previous exercise studies (Pang et al., 2006; van Dellen et al., 2008). At the onset of the study, 67 mice were allocated to the experiment with 3 male mice subsequently being removed, two at 3 months of age and 1 at four months of age: one wildtype control mouse developed a cataract, and one R6/1 exercise group mouse developed an anal prolapse with a second mouse from this group demonstrating excessive epileptic type of seizures. With the removal of the 3 mice, 64 R6/1 mice congenic to a C57BL/6j background were used: Group 1 = wildtype control (7 males and 8 females); Group 2 = R6/1 control (9 males and 8 females); Group 3 = wildtype exercise (9 males and 8 females); Group 4 = R6/1 exercise (7 males and 8 females). The R6/1 mice in this study carried between 120 and 125 CAG repeats with a mean of 123 and were bred, tail-tipped and genotyped in-house. The mice had ad libitum access to food and water throughout the study period. The mice were housed at an ambient room temperature of 21 ± 1 °C at a humidity of 60 ± 1%. All experiments were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 and local ethical review. The defined humane endpoint for the mice was 20% body weight loss, hence although some mice may live to 11 months in other studies, 7 months of age was taken as the final testing time point as a significant number of mice approached the humane endpoint. At the end of the experiment of the original 64 mice that began testing 52 mice remained, 9 non-exercised R6/1 mice and 3 exercised mice having died or been euthanized due to the predefined endpoints.

Exercise administration

Exercise was administered daily (14 h/day, 5 days/week), from post-weaning (5 weeks of age) by individual housing of the mice overnight (17.30–09.30) in cages (34 cm × 28 cm × 1 cm) fitted with an ENV-044 tabulating running wheel, connected wirelessly to an DIG-804 interface hub and laptop computer running the SOF-861 wheel analysis software (Med Associates, St. Albans, VT, USA). By day, the mice were returned to their grouped housing conditions in the same sized cages to prevent the development of abnormal behaviours caused by continual social isolation. Non-exercised mice were treated in the same way as the R6/1 mice but without access to a running wheel to be consistent with previous studies (Pang et al., 2006; van Dellen et al., 2008).

Behavioural testing

The mice were tested at regular monthly intervals (every 4 weeks) from 8 weeks of age through to 28 weeks of age throughout their lives on a number of tests of motor function and tested twice on a water T-maze attentional shift task at an early (9 weeks) and a late (22 weeks) disease time point. For the motor tests data analyses were taken from data points between 4 and 7 months of age to control for sex differences in wheel running levels in the R6/1 mice (see below). Behavioural tests were selected for their high translational value and consequently have clinically relevant human correlates (rotarod and gait analysis ≡ kinetic and kinematic gait assessments; grip strength ≡ dynamometry; motor activity ≡ self report and direct accelerometer based measures of daily physical activity; balance beam ≡ clinical balance tests and force plate measure; water T-maze ≡ procedural learning/attentional probe). All tests were performed blind to genotype.

Body weight

Body weight was measured weekly to assess the general health of the animals. Body weight is also an indicator of disease development in HD, as mice that are gene carriers tend to lose weight relative to their wildtype littermates.

Rotarod

Motor coordination was tested on the accelerating version of the rotarod test using a standard apparatus (Ugo Basile, Varese, Italy), as described previously (Brooks et al., 2004, 2012c, 2012d, 2012f). During test sessions, the mice are allowed three trials, with data from the final 2 being collected.

Balance beam

An elevated bridge (balance beam) apparatus was used to measure balance and motor coordination as described previously (Brooks et al., 2012a). A tapered balance beam (1.5 cm to 0.5 cm) with a ledge running its length (Schallert, 2006) was used to determine whether the mice had a balance impairment. The balance beam was 100 cm in length and angled at 17° with the start point at the low end and a goal box at the high end. The start point was 15 cm from one end of the beam, and the end point was 10 cm from the other. The beam was also fitted with a ledge that ran the full length at 2 cm below the height of the running surface, and protruded 0.5 cm on either side of the beam to prevent the animals from falling, and to aid in the identification of foot-slips. Mice were trained to run the beam prior to testing. During testing, each animal was given a trial run, and then two experimental runs from which the data was collected. At the onset of testing the mouse was placed on the extreme end of the beam (low end), facing away from the beam. The mouse must turn towards the beam in order to run it, and reach the goal box. The time it took the mouse to turn on the end of the beam was taken as a measure of motor coordination. The running/walking was then timed from the start point to the end