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Research Report

Motor function and dopamine release measurements in transgenic Huntington's disease model rats

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ABSTRACT

Huntington's disease (HD) is a fatal, genetic, neurodegenerative disorder characterized by deficits in motor and cognitive function. Here, we have quantitatively characterized motor deficiencies and dopamine release dynamics in transgenic HD model rats. Behavioral analyses were conducted using a newly-developed force-sensing runway and a previously-developed force-plate actometer. Gait disturbances were readily observed in transgenic HD rats at 12 to 15 months of age. Additionally, dopamine system challenge by ip injection of amphetamine also revealed that these rats were resistant to the expression of focused stereotypy compared to wild-type controls. Moreover, dopamine release, evoked by the application of single and multiple electrical stimulus pulses applied at different frequencies, and measured using fast-scan cyclic voltammetry at carbon-fiber microelectrodes, was diminished in transgenic HD rats compared to age-matched wild-type control rats. Collectively, these results underscore the potential contribution of dopamine release alterations to the expression of motor impairments in transgenic HD rats.

1. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat on the gene that encodes the huntingtin protein (htt) (The Huntington's Disease Collaborative Research Group, 1993). This expanded CAG repeat results in the expression of an expanded polyglutamine segment at the N-terminal region of the protein, ultimately resulting in the overt psychological and physiological syndrome associated with HD, which includes cognitive dysfunction, psychiatric deficiency, choreic movements, and gait disturbances (Bates et al., 2002).

The discovery of the causative mutation of HD led to the initial development of the R6 line of CAG-triplet-repeat transgenic mice (Mangiarini et al., 1996). R6/2 mice, the most commonly employed HD model, carry exon 1 of the HD gene with ~150

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Abbreviations: aCSF, artificial cerebral spinal fluid; AMPH, amphetamine; DA, DA; DAT, DA transporter; FSCV, fast scan cyclic voltammetry; HD, Huntington's disease; htt, huntingtin; WT, wild-type

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CAG repeats and develop an overt HD-like phenotype with rapid onset (Mangiarini et al., 1996): subtle motor and cognitive deficits develop at 4–5 weeks of age, overt motor symptoms develop at 9–11 weeks of age, and animals typically die at 12–16 weeks of age. Importantly, R6/2 mice, as well as other mice from the R6 line, also exhibit extensive atrophy and neuronal degeneration in the striatum as well as the formation of aggregates and intra-nuclear inclusion bodies (Davies et al., 1997, 1999; Kosinski et al., 1999; McGowan et al., 2000; Scherzinger et al., 1997), resembling, to some extent, the neuropathology found in HD patients.

Occurring shortly thereafter was the development of other genetically altered mouse lines, including transgenic, knock-in, and virally-inserted variants, that model human HD in different ways and to various extents (reviewed in Levine et al., 2004; Menalled, 2005; Menalled and Chesselet, 2002; Wang and Qin, 2006). For example, yeast artificial chromosome-128 (YAC128) mice, which express the full length IT15 gene with 128 CAG repeats, showed a less aggressive disease progression compared to R6/2 mice: hyperkinesis at 3 months, hypokinesis at 6 months, and significant motor impairment at 12 months (Slow et al., 2003).

The recent development of transgenic HD (HDtg) rats (von Hörsten et al., 2003) has provided an additional HD model amenable to studies that require a larger animal than that of a mouse. HDtg rats carry a fragment of the huntingtin gene with 51 CAG repeats (under the control of the endogenous rat promoter) and are, therefore, similar to R6/2 mice in that they express a truncated form of huntingtin. However, compared to R6/2 mice and to most other HD model mice, the onset rate of the abnormal behavioral phenotype, which is influenced by CAG repeat length and other factors, is more gradual in the HDtg rat: no easily recognized motor symptoms at 5 months, a decline in spatial learning and working memory at 10 months, and progressive impairments of hind- and fore-limb coordination and balance on the accelorod at 10–15 months (von Hörsten et al., 2003). Moreover, polyglutamine aggregate recruitment sites form at 6–9 months and striatal atrophy is evident at 12 months (Nguyen et al., 2006). HDtg rats gain weight more slowly than their WT controls, being about 20% lighter by the age of 24 months (von Hörsten et al., 2003).

Recent evidence has suggested that alterations in striatal dopamine (DA) regulation influence the expression of phenotypic behaviors in R6/2 mice (Hickey et al., 2002). The striatum, which is densely innervated with dopaminergic terminals, is among the first brain regions to show degeneration in HD patients (Bates et al., 2002). Previous studies, conducted in mice and rats that model HD, have revealed substantial impairment in the ability of striatal presynaptic terminals to release DA. For example, DA release, induced by malonate infusion and measured using microdialysis sampling, was diminished in R6/1 mice compared to age-matched wild-type (WT) control mice (Petersén et al., 2002). Additionally, microdialysis sampling measurements have revealed diminished extracellular concentrations and blunted amphetamine-induced increases in the striata of awake R6/2 and YAC128 mice (Callahan and Abercrombie, 2011). Furthermore, a decrease in electrically-evoked DA release, obtained using fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes, has also been found in brain slices from R6/2 (Johnson et al., 2006) and R6/1 (Ortiz et al., 2011) mice as well as in anesthetized rats that had undergone treatment with 3-nitropropionic acid (3NP), a neurotoxin that induces striatal cell loss (Kraft et al., 2009). It is important to note also that these HD models have also shown deficiencies in either gait or locomotion (Bolivar et al., 2004; Brooks et al., in press; Carter et al., 1999; Fowler et al., 2009; Johnson et al., 2006; Kraft et al., 2009; Lüesse et al., 2001; Naver et al., 2003) and that R6/2 mice have previously shown a blunted behavioral response to pharmacological challenge with cocaine and methamphetamine, inhibitors of DA uptake (Hickey et al., 2002; Johnson et al., 2006). Collectively, these results suggest that, at least in these models of HD, DA release impairments play a role in the expression of the overt behavioral phenotype.

The purpose of this study was to investigate the behavioral and neurochemical characteristics of HDtg rats. Our data suggest that DA release, evoked in striatal brain slices by application of electrical stimulus pulses and measured using FSCV, is decreased overall in HDtg rats compared to WT control rats. Additionally, DA content, measured in striatal homogenates, is the same. Behaviorally, HDtg rats exhibit impairments in gait as well as a blunted response to amphetamine injection. Collectively, these data underscore differences in neurochemical profile and phenotype onset between HDtg rats and the R6 mouse line.

2. Results

2.1. *Behavioral measurements*

A newly-developed force-sensing runway was used to analyze gait force production in WT, heterozygous (htHDtg), and homozygous (hmHDtg) HDtg rats (Fig. 1). Data were analyzed for differences in runway force variation between WT, htHDtg, and hmHDtg rats (Fowler et al., 2009). This force variation, caused by changes in the force of the rat against the runway as it places and lifts its feet while ambulating ("trotting"), is plotted in kilograms (vertical axis) versus time in seconds (horizontal axis; see Figs. 1A and B). To quantitatively analyze the consistency of the forces produced by the rats' gait during ambulation, a parameter, known as the "mid-run force range," was identified (Fig. 1C). As shown in Fig. 1B, this parameter is identified first by locating the mid-run time point, the time which is half of the total run time. Timing the duration of the run begins when the rat first touches the force-sensing floor and ends when the rat is completely off the floor in the goal box. Once the mid-run time point is identified, the difference between the maximum and minimum force readings within the time window at 0.25-s prior to and 0.25-s after mid-run time point were obtained for every run. These values, which reflect maximum force production during a run, were then averaged for the rats within each genotype to obtain the bar graph in Fig. 1C.

Rats that were 12 to 18 months old were used here because HDtg rats are expected to have developed hind- and fore-limb coordination deficiencies within the 10 to 15-month age range (von Hörsten et al., 2003), yet the rats were young enough so that they could adequately ambulate. Raw data traces (Fig. 1A) demonstrate the presence of a progressive gait slowing of an hmHDtg rat compared to a WT rat. The appearance of this gait slowing and accompanying lower force production is likely not induced