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.

Keywords

dopamine; Huntington's disease; microelectrodes; voltammetry; behavior; transgenic

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

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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 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; Scherzinger et al., 1997; Davies et al., 1999; Kosinski et al., 1999; McGowan et al., 2000), 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, knock-out, and virally-inserted variants, that model human HD in different ways and to various extents (reviewed in Menalled and Chesselet, 2002; Levine et al., 2004; Menalled, 2005; 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 mouse strains 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 (Carter et al., 1999; Lüesse et al., 2001; Naver et al., 2003; Bolivar et al., 2004; Johnson et al., 2006; Fowler et al., 2009; Kraft et al., 2009; Brooks et al., 2011) 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 1B). 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 solely by aging, as illustrated by the raw data traces (Fig 1A): little difference in the consistency of gait (rhythm and force) was seen in 12-month-old WT rats compared to 18-month-old WT rats. Mid-run force range is a parameter that is predicted to be greater in animals exhibiting a normal gait and decreased in animals with a gait disturbance, based on previous work in which R6/2 mice were found to have an altered gait (lower force production and decreased rhythmicity) compared to age-matched WT control mice (Fowler et al., 2009). As shown in Fig. 1C, the hmHDtg rats exhibited significantly lower force variation than the WT rats, with the htHDtg rats falling only slightly below the WT rats at this age (ANOVA: $F_{[2,13]} = 4.526$, $p < 0.05$; Tukey post hoc: WT versus hmHDtg, $p < 0.05$).

The gait data prompted us to determine if HDtg rats showed a different behavioral response to AMPH compared to respective WT controls. AMPH enters neurons either by lipophilic diffusion through the membrane or by passage through the dopamine transporter, in which case it causes allosteric translocation of the transporter, thereby inducing the reverse transport of DA to the extracellular space (Fischer and Cho 1979; Liang and Rutledge 1982). HDtg rats and WT control rats were treated with 2.5 mg/kg AMPH on 4 different occasions 4-5 days apart (Fig. 2). Two age groups were used: twenty-two 9-month-old rats divided among WT, htHDtg, and hmHDtg, and seventeen 20-26-month-old rats (average ages by genotype: WT, 20.0 months; htHDtg, 22.1 months; hmHDtg, 22.0 months). Behavioral data (in the form of force variations recorded with 10 ms resolution) were measured with a force-plate actometer and analyzed with Fourier analysis to quantify the head movement frequency at peak power for each rat. Group mean power spectra for the WT, htHDtg, and hmHDtg rats at the younger age exhibited the near 10 Hz rhythm characteristic of focused stereotypy. Among the older rats, the WT and htHDtg genotypes displayed the same pattern, but the older hmHDtg rats' spectral function was characteristic of locomotor stimulation (Fowler et al., 2003), not focused stereotypy. The frequency in Hz of the peak location in the power spectrum was determined for each rat, and these data were entered into a two-way analysis of variance (2 age levels by 3 genotypes). A significant age effect ($F[1,33] = 21.696$, $p < 0.001$), genotype effect ($F[2,33] = 3.581$, $p < 0.05$), and age-by-genotype interaction ($F[2,33] = 3.312$, $p < 0.05$) were indicated.

2.2 Striatal DA Release

It has been previously shown that stimulated striatal DA release is impaired in R6/1 and R6/2 mice (Johnson et al. 2006, Ortiz et al. 2011). To determine if release is also decreased in the older HDtg rats, DA was electrically evoked using a bipolar stimulus electrode and measured using FSCV at carbon-fiber microelectrodes in brain slices harvested from WT and HDtg rats (average ages: WT, 21.3 months; HDtg, 21.2 months). Slices were harvested from the older rats because, based on data obtained from R6/2 and R6/1 mice, the greatest impairment in release would be expected to occur in older animals. Raw data traces of DA release evoked using either a single electrical stimulus pulse (Fig. 3A) or a series of 120 electrical stimulus pulses administered at 120 Hz (Fig. 3B) are shown. The more intense stimulation parameter was used to determine if the difference in release would become more robust. Cyclic voltammograms (CVs), included above the stimulated release plots, verify the presence of evoked DA. Overall, DA release was diminished in HDtg rats (Fig. 3C, two-way ANOVA, main genotype effect on DA release, ($F[1,12] = 5.40$, $p < 0.05$). This analysis did not support an interaction between number of pulses and genotype, nor a main effect of number of pulses on DA release.

If mechanisms involving DA reserve pool depletion, similar to R6/2 mice, were involved in HDtg rats, we would expect a frequency dependent effect. Therefore, the 120 stimulation pulse regimen was applied at frequencies of 20, 30, 40, 50, 60, and 120 Hz and DA release was again measured using FSCV (Fig. 4). Although no effect of stimulation frequency was noted for either HDtg or WT rats, a significant main effect of genotype (WT higher than HDtg) on DA release was indicated (two-way ANOVA, $p < 0.005$, $F[1,30] = 11.43$, $n = 4$ HDtg rats and 3 WT rats).

2.3 Striatal Catecholamine Content

The content of DA and two of its metabolites, HVA and DOPAC, were measured by HPLC with electrochemical detection in striatal lysates (Table 1). There was not a significant difference in content between HDtg and WT rats ($p > 0.05$).

3. Discussion

We describe here behavioral measurements, obtained using a newly-developed force-sensing runway and a force plate actometer, and neurochemical measurements of stimulated DA release, obtained in acutely dissociated brain slices. The behavioral measurements revealed that HDtg rats suffer from impairments in gait. Moreover, HDtg rats, particularly in the 20- to 26-month age group, were resistant to the development of focused stereotypy following injection with AMPH. Not surprisingly, DA release was impaired in rats within this age group. The content of DA and two of its main metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), measured in striatal lysates, did not differ significantly between genotypes.

Impairments in DA release, evoked in striatal brain slices by single and multiple electrical stimulus pulse regimens, have been found previously in R6/2 (Ortiz et al. 2010; Johnson et al. 2006) and R6/1 (Ortiz et al. 2011) mice. It appeared, if only by visual inspection, that release was more impaired in HDtg rats when applying long trains of electrical stimulus pulses compared to when applying single electrical stimulus pulses. Previous work, in which long pulse trains (120 pulses, 20 to 60 Hz) were applied in brain slices, has suggested that reserve pool DA is depleted in R6/2 mice, potentially through a decrease in the number of vesicles (Ortiz et al. 2010). It is thought by some that the DA reserve pool is mobilized under periods of intense synaptic activity (reviewed recently in Denker and Rizzoli, 2010). To approximate this activity in brain slices from R6/2 mice and WT control mice, a similar multiple-pulse stimulation regimen (120 stimulus pulses) was applied and DA release was measured. The results obtained in HDtg rats are similar to those in R6/2 mice in that a significant genotype effect on DA release, in which release is depressed in the transgenic animals, is found throughout a range of stimulation frequencies. However, the data did not indicate a frequency-dependent alteration in DA release in HDtg rats between, whereas in R6/2 mice DA release was significantly decreased at 50 and 60 Hz in R6/2 mice compared to age-matched WT mice. Moreover, the fact that we found no difference in striatal DA content between HDtg and WT rats suggests that reserve pool DA stores, which make up 80 to 90% of the DA-containing vesicles within neurons (Rizzoli and Betz, 2005), are not diminished. Collectively, these results do not support DA reserve pool depletion as a mechanism of dopamine release impairment in HDtg rats at the age groups tested.

It is well known that the neurodegenerative processes of Huntington's disease predominantly target the striatum, and this property of the disease is recapitulated in both rat (von Hörsten et al. 2003) and mouse models (e.g., YAC128 model: Tang et al., 2007). The striatum serves as the main interface between the cerebral cortex and striatal targets in the basal ganglia, thereby influencing the translation of cortical plans into actions by coordinating the expression of selected motor responses while concurrently suppressing unwanted responses (Cooper et al. 2003). Therefore, alterations in DA system function, particularly changes in the nigrostriatal pathway, may impact the control of intentional movement. The dorsolateral caudate region of the mammalian brain receives its dopaminergic innervation almost exclusively from the nigrostriatal pathway (Gerfen 1984). The gait data are congruent with a previous report that R6/2 mice exhibit gait abnormalities in the absence of pharmacological challenge (Fowler et al., 2009). Therefore, the fact that we observed impaired DA release in HDtg rats, similar to other rodents that model HD, is consistent with the concept that nigrostriatal pathway dysfunction influences the ability of these rats to ambulate normally.

Lesioning of the striatum in normal rats results in altered sensitivity to the behavioral effects of the indirect-acting DA agonist AMPH (Kelly et al.,1975; Moore, 1977). Near total depletion of striatal DA produced by intranigral injection of the neurotoxin 6-

hydroxydopamine (6-OHDA) abolished both locomotor and stereotypy responses to AMPH (Creese and Iversen, 1975), thereby suggesting that normal rats' AMPH response is dependent on the nigrostriatal DA system. Therefore, loss of striatal function in HDtg rats would also be expected to affect the behavioral response to AMPH. In normal Sprague Dawley rats lower doses (0.5 to 1.8 mg/kg) of AMPH increase locomotor activity compared to saline treated controls. However, in a higher dosing range, extending from about 2.0 mg/kg to about 6.0 mg/kg, Sprague Dawley rats exhibit a complete cessation of locomotion, and, while immobile, the rats make rapid rhythmic vertical and horizontal head movements (Fowler et al., 2003). This higher-dose manifestation of AMPH's behavioral effects is known as focused stereotypy. Prior to the development of the force-plate actometer, focused stereotypy was assessed with a subjective rating scale approach (Creese and Iversen, 1975); however, the force-plate method eliminates the need for a rating scale and shows that focused stereotypy is characterized by a precisely-regulated near-10-Hz rhythm of force oscillations induced in the force plate by the rats' head movements (Fowler et al., 2001, 2003, 2007).

The significant genotype effect revealed by two-way ANOVA reveal that HDtg rats were less sensitive to injection with AMPH (2.5 mg/kg, ip) than corresponding age-matched WT control rats. Moreover, AMPH treatment reveals a gene-dosing effect in 20- to 26-month-old HDtg rats; the number of copies of the mutant HD gene impacts the behavioral phenotype, potentially through differential effects on the DA system. The significant age-dependent effect also emphasizes the progressive nature of phenotype onset on this HD model. The sensitivity to amphetamine roughly correlates with the onset of the overt phenotype: little difference in response is noted at 9 months of age progressing to a substantial difference by 20-26 months of age.

In conclusion, we have used powerful behavioral assays to reveal substantial motor deficiencies in a transgenic rat model of HD. HDtg rats, similar to R6/2 mice, suffer from gait impairments and have a blunted response to amphetamine injection. Moreover, striatal DA release is impaired even though DA content is unchanged. These results suggest that dopamine release impairments contribute to phenotypical motor impairments in HDtg rats, similar to other HD model rodents.

4. Experimental Procedure

4.1 Materials

d-Amphetamine sulfate (AMPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Prior to administration to rats, AMPH was dissolved in saline (0.9% NaCl, 2.5 mg/mL).

4.2 Transgenic HDtg rats

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Kansas. Behavioral and neurochemical measurements were collected from transgenic HDtg rats and wild-type (WT) littermate control rats, which are on a Sprague-Dawley background. The initial development and evaluation of this transgenic rat line has been described previously (von Hörsten et al. 2003). A colony of rats were housed in the University of Kansas Animal Care Unit under constant temperature (70 ± 2 °C), humidity ($50 \pm 20\%$), and light/dark cycle (lights on at 6:00 AM, lights out at 6:00 PM). Rats were provided with food and water ad libitum except for food-restricted rats used in the force-sensing runway. Prior to testing on the force-sensing runway, rats were switched from continuous food availability in the home cage to 3-hr access to food daily. During this time, the rats were weighed daily for 1 week to ensure that 3-hr access to food is sufficient to maintain body weight. The colony consisted of homozygous HDtg (hmHDtg), heterozygous

HDtg (htHDtg), and wild-type (WT) control rats. For breeding, selected heterozygotes from the colony were paired. Genotyping was accomplished by real-time polymerase chain reaction (RT-PCR) at Erlangen University, Germany and confirmed at the University of Kansas Genomics Facility using an RT-PCR genotyping protocol that distinguishes among hmHDtg, htHDtg, and WT rats. Behavioral experiments in which animals were injected with AMPH were conducted using hmHDtg, htHDtg, and WT rats at 9 and 20 to 26 months of age. Gait disturbance experiments were conducted on rats 12 to 15 months of age. Measurements of DA release and DA content were collected using 20- to 26-month-old homozygous HDtg rats and age-matched WT control rats. Measurements were collected at this age because is it considered to be near end stage with the most severe phenotype. To enhance the statistical power and help mitigate the effects of the limited number of rats available at this age due to attrition, DA release measurements were collected at four arbitrarily selected locations in the dorsolateral caudate of slices.

4.3 Behavioral measurements

The force-plate actometer and methods of data analysis were used as previously described (Fowler et al. 2001). Briefly, two equations drawn from physics are used to compute the coordinate (x,y) position of the subject's center of force based on the force measurements at each of the four corners of the force plate:

$$x = (X1f1 + X2f2 + X3f3 + X4f4) / (f1 + f2 + f3 + f4) \quad \text{and} \quad y = (Y1f1 + Y2f2 + Y3f3 + Y4f4) / (f1 + f2 + f3 + f4),$$

where lower case represents variables, and upper case represents fixed constants. Upper case X and Y values are the positions of the four force transducers mechanically coupled to the load plate. In this coordinate system, the center of the plate is x=0, y=0. The f's symbolize the force measurements from each of the 4 force transducers. All of the variable quantities in the formulas vary as a function of time, but the "(t)" notation has been omitted for convenience of simplified presentation. The load plate used here had dimensions of 42 cm × 42 cm and was supported by four Model 31 Sensotec (Columbus, OH) force transducers. The force-plate actometer provides high temporal and spatial resolution behavioral data. Data described in this manuscript were collected at a rate of 100 samples/s. The spatial resolution of center of force is about 1 mm for a rat. Quantification of the rhythmic movements during focused stereotypy is derived from the Fz force (i.e., the sum of the forces registered by the 4 transducers at a given time sample). Calibration procedures have been developed and published (Fowler et al., 2001). The actometer used here was enclosed in a sound- and light-attenuating chamber.

Behavioral activity measurements in the force-plate actometer were collected from each rat in four separate 90 minute sessions, spaced 3 or 4 days apart. Rats were given an ip injection of d-amphetamine sulfate at a dosage of 2.5 mg/kg immediately prior to the start of each session. From our previous experience (Fowler et al., 2003), this amphetamine dose is just at the threshold for producing focused stereotypy in rats upon first administration, but invariably induces focused stereotypy in all rats by the 4th injection. Focused stereotypy is a syndrome in which the rats exhibit repetitive head movements at a frequency of 10 Hz and complete cessation of locomotion (Fowler et al, 2003). Only data from the fourth session were analyzed in order to allow the rats to become sensitized to the effects of amphetamine. Collected z-axis force data were converted to force power spectra by Fourier analysis as previously described (Fowler et al, 2001; Fowler et al., 2007).

Measurements of gait were collected using a force-sensing runway, a behavioral instrument that operates using identical principles as the force-plate actometer. Similar measurements of gait exhibited by R6/2 mice and age-matched WT control mice had been collected

previously with a conventional force-plate actometer (Fowler et al., 2009). However, the longer gait distance required when collecting measurements from rats necessitated the use of a modified apparatus. The force sensing runway apparatus has an elongated plate (0.8 m long by 0.15 m wide) with a clear, elongated Plexiglas enclosure. At one end of the apparatus is a chamber that serves as the start area. At the other end of the apparatus is a chamber that contains a sweet, condensed milk reward. During a typical measurement trial, the rat was placed in the starting chamber and allowed to ambulate along the force sensing runway to reach the reward in the chamber at the opposite end. The rats were given access to the reward for a period of 5 s, after which they were placed in their home cage for one minute prior to being placed in the starting chamber for another trial. Each rat was subjected to 10 trials per day for 6 to 8 days. Data were analyzed for differences in runway force variation between WT, htHDtg, and hmHDtg rats (Fowler et al., 2009). This analysis is illustrated in Fig. 1. The mid-run time point was identified, and this allowed the location of a 0.5-s wide time window centered on the midpoint of the run. Within this time window the difference between the maximum force and minimum force was calculated yielding the dependent variable "mid-run force range." This variable quantitates the force output differences among WT, htHDtg, and hmHDtg rats.

4.4 Brain Slice Preparation

Rats, anesthetized by isoflurane inhalation, were decapitated and brains were removed and placed in artificial cerebrospinal fluid (aCSF) chilled to about 0 °C and continuously saturated with 95% O_2/5% CO_2. The aCSF consisted of NaCl (126 mM), KCl (2.5 mM), NaH_2PO_4 (1.2mM), $CaCl_2$ (2.4mM), $MgCl_2$ (1.2mM), $NaHCO_3$ (25mM), HEPES (20mM), and D-Glucose (11mM), adjusted to a pH of 7.4. Cerebella were sliced off with a razor blade and brains were longitudinally cut down the center into two halves. The striatum was removed from one of the halves of each brain and immediately frozen on dry ice and stored at −80°C for future neurotransmitter content analyses by HPLC. The other brain half was mounted on a Teflon block using Krazy Glue®. The block was fastened in the bay of the vibratome slicer (Leica Microsystems, Bannockburn, IL, USA) and 300 μm thick coronal striatal brain slices were made. During this slicing operation the brain was maintained in ice-cold aCSF. For the collection of voltammetry recordings, each brain slice was placed in the superfusion chamber and maintained at a constant temperature of 34° C and supplied with a continuous flow (2 mL/min) of oxygenated aCSF. Slices were allowed to equilibrate in the superfusion chamber for 60 minutes prior to obtaining measurements.

4.5 DA release in brain slices

The fabrication of carbon-fiber microelectrodes and their application in collecting single pulse and multiple pulse stimulation data were accomplished using procedures similar to those previously described (Ortiz et al. 2010). The potential applied to the carbon-fiber microelectrode was held constant at −0.4 V. The potential was then linearly increased to +1.0 V and then decreased back to −0.4 V at a scan rate of 300 V/s. DA release was evoked by the local application of either a single electrical stimulus pulse or a continuous series of 120 electrical stimulus pulses applied at frequencies of 20, 30, 40, 50, 60, and 120 Hz. Each stimulus pulse was biphasic, 4 ms in total duration, and applied at a current of 350 μA. The CV application rate was either 10 CVs/s when used in conjunction with the 120-pulse stimulus or 60 CVs/s when used in conjunction with the single-pulse stimulus. A headstage amplifier (UNC chemistry department electronics design facility, Chapel Hill, NC) was interfaced with a computer via a breakout box and custom software provided by R. M. Wightman and M.L.A.V. Heien, University of North Carolina, Chapel Hill. An Ag/AgCl reference electrode was used. The carbon-fiber was inserted to a depth of 100 μm below the brain slice surface within the dorsolateral caudate-putamen region of the striatum between the prongs of a bipolar stimulation electrode (Plastics One, Roanoke, VA). This brain region

receives its dopaminergic input from the substantia nigra pars compacta (SNpc) via the nigrostriatal pathway (Gerfen, 1984). The current was then measured at the peak oxidation potential for DA (about +0.6V vs. Ag/AgCl reference electrode). DA release was measured at four separate locations in the dorsolateral caudate and averaged for each slice in order to enhance statistical power. Working electrodes were calibrated with DA standards of known concentration (1 to 2 μM) in a flow cell, at a flow rate of 2 mL/min and at room temperature ($23 \pm 2°C$), before and after each use. The averages of the pre- and post-calibration measurements were used as calibration factors.

4.6 DA content measurement

Determinations of the content of DA and its metabolites in striatal brain homogenates were conducted using high performance liquid chromatography (HPLC) as previously described (Kraft et al. 2009). Briefly, dissected striata that had been previously stored at $-80°C$ were homogenized in ice-cold 0.2M perchloric acid and then centrifuged ($11,000 \times g$, 20 minutes) and the supernatant filtered (0.22 μm; Fisher Scientific, Pittsburgh, PA). The supernatant was loaded in aliquots of 10 μL onto a 150×3.2 mm MD-150 column (ESA, Inc., Chelmsford, MA, USA) with a mobile phase consisting of NaH_2PO_4 (86 mM), octansesulfonic acid (1.7 mM), and acetonitrile (10%), adjusted to a pH of 3.0. A flow rate of 0.4 mL/min was used. The analyte was detected using a two-channel Coulochem II electrochemical detector (ESA, Inc., Chelmsford, MA, USA). Concentrations of DA, HVA, and DOPAC are expressed in units of μg/g of brain tissue.

4.7 Data Analysis

For statistical comparisons involving DA release and DA content data, GraphPad Prism statistical software (GraphPad Software, Inc., La Jolla, CA) was used. For statistical comparisons of behavioral data, Systat (Systat Software, Inc., Chicago, IL) was used. Data are expressed as a mean +/− SEM. Comparisons were assessed by Student's t-test (unpaired) or ANOVA, where appropriate. A probability level of 5% ($p < 0.05$) was considered significant.

Acknowledgments

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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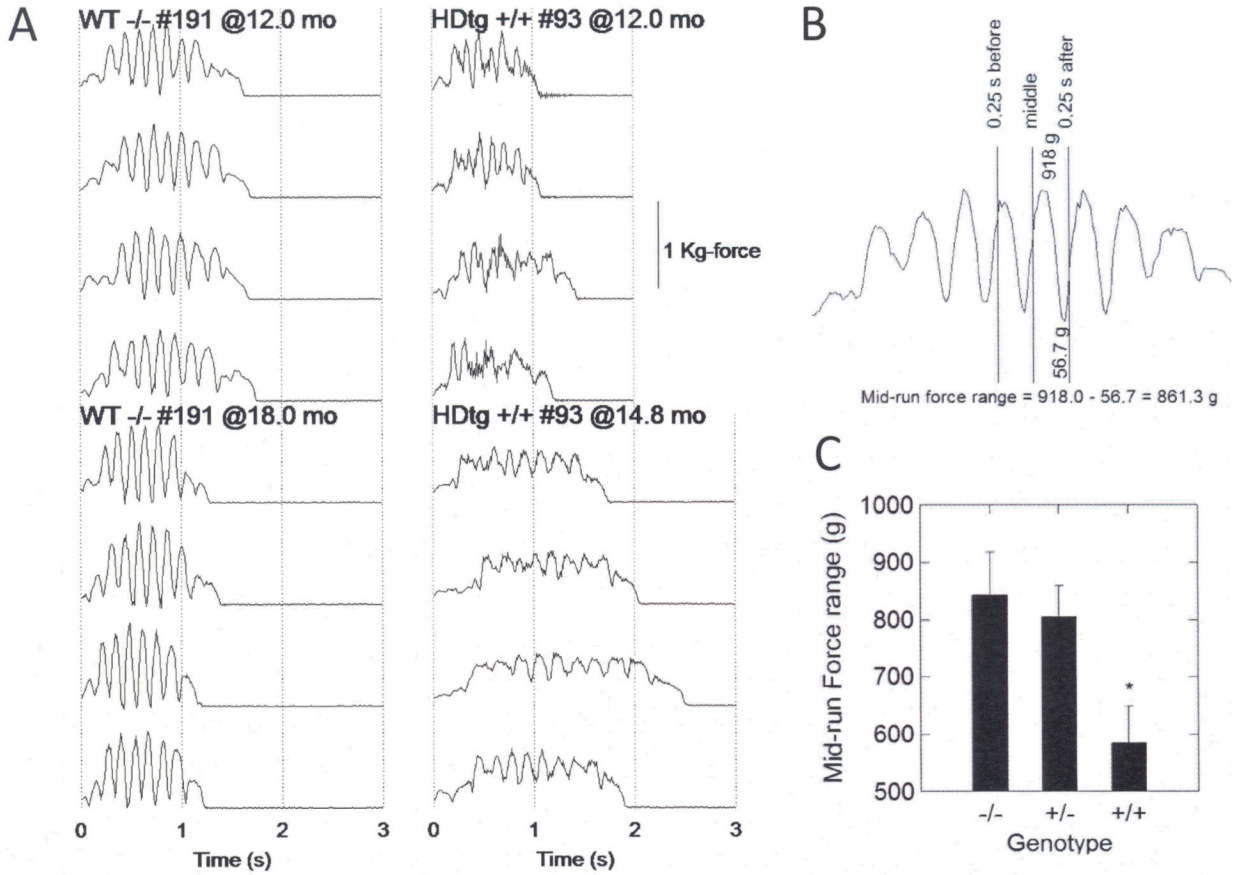
Figure 1. Homozygous HDtg rats express progressive gait disturbances
A, Raw data traces indicate the presence of a gait dysrhythmia and decreased force production of an hmHDtg (+/+) rat at 12 and 14.8 months of age. Traces from WT (−/−) rats are also included for comparison. B, Diagram showing how force-time waveforms from single runs in the force-sensing runway were treated quantitatively to obtain the dependent variable called "mid-run force range" for each rat. C, Force amplitude during runway locomotion expressed as the mid-run force range for the 10th trial of the last training session for each of 16 male rats, 12 to 15 months old. The values of n per group were: WT (−/−), 5; htHDtg (+/−), 6; and hmHDtg (+/+), 5 (* $p < 0.05$, t-test). Error bars indicate ± SEM.
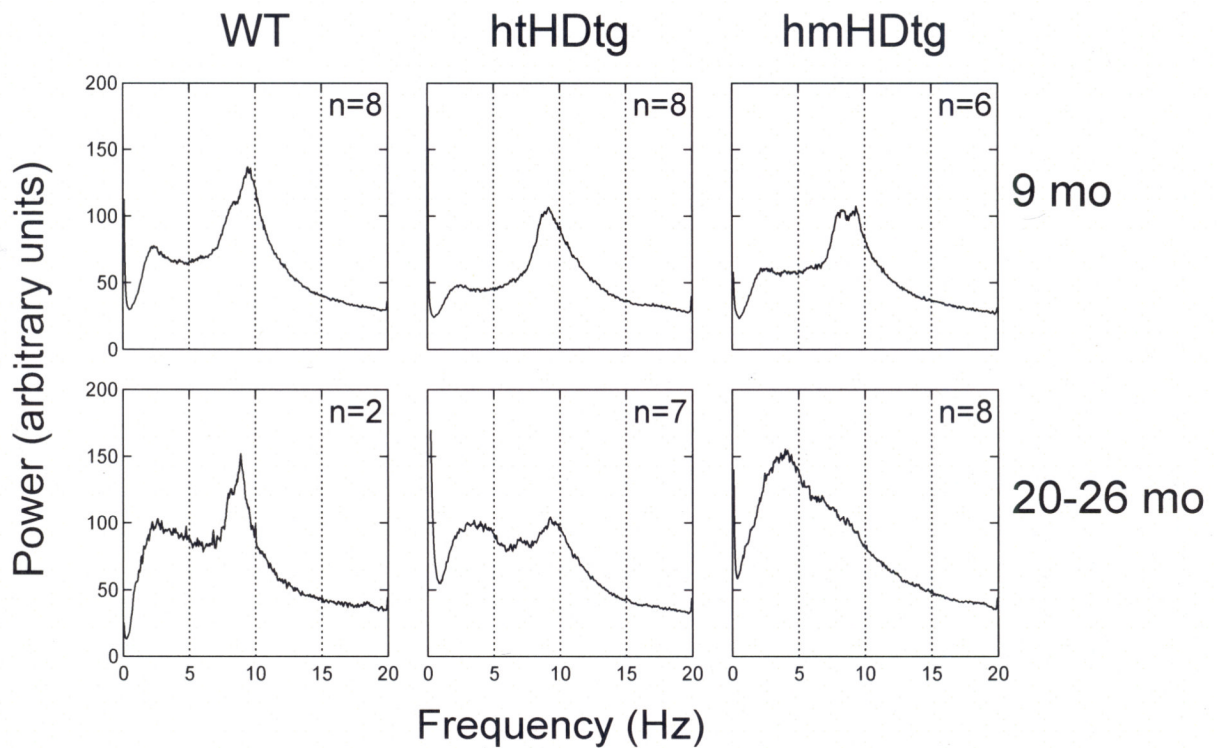
Figure 2. The expression of focused stereotypy is decreased in transgenic HD rats compared to WT control rats

Group average power spectra for WT (−/−), htHDtg (+/−), and hmHDtg (+/+) rats for the indicated ages are shown. The force-plate force variation, upon which Power was based, was expressed as a percent of body weight. These data are for 1 hr of behavior collected 30 min after the 4th exposure to 2.5 mg/kg d-AMPH sulfate. A significant age effect ($F_{[1,33]} = 21.696$, $p < 0.001$), genotype effect ($F_{[2,33]} = 3.581$, $p < 0.05$), and age-by-genotype interaction ($F_{[2,33]}=3.312$, $p < 0.05$) were indicated by two-way ANOVA. Values of n per group are indicated in the figure.
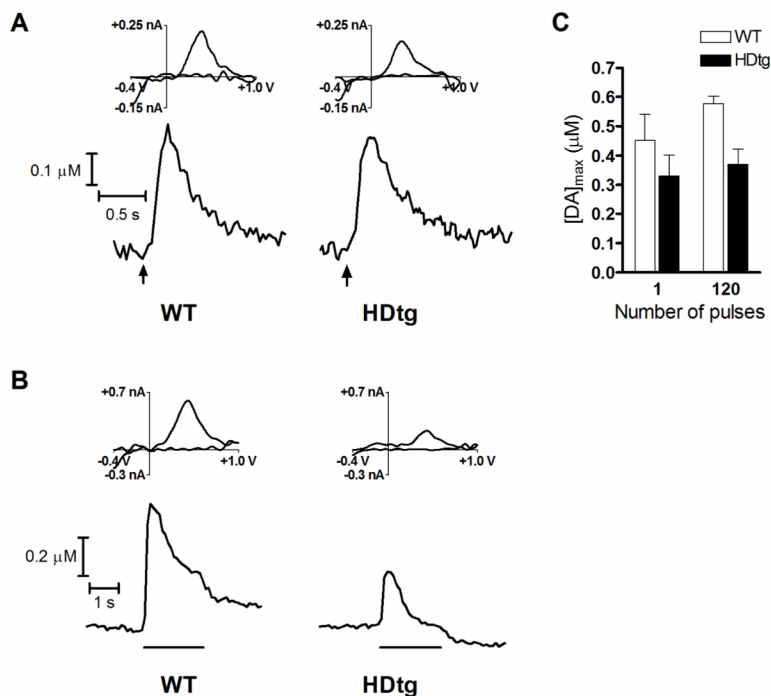
Figure 3. Stimulated DA release in brain slices from late-stage HDtg rats and WT rats
A and B, Raw data traces shown in which DA release was evoked by a single electrical stimulus pulse (A) or a series of 120 electrical stimulus pulses (B). Stimulus pulse applications are denoted by arrows (A) or lines (B) underneath respective plots. Cyclic voltammograms, provided above each plot, confirm the presence of DA. C, Values averaged over multiple animals and plotted. DA release in slices from HDtg rats was diminished compared to WT controls. A significant genotype effect ($F[1,12] = 5.40$, $p < 0.05$, n = 4 WT and 5 HDtg rats) was indicated by two-way ANOVA.
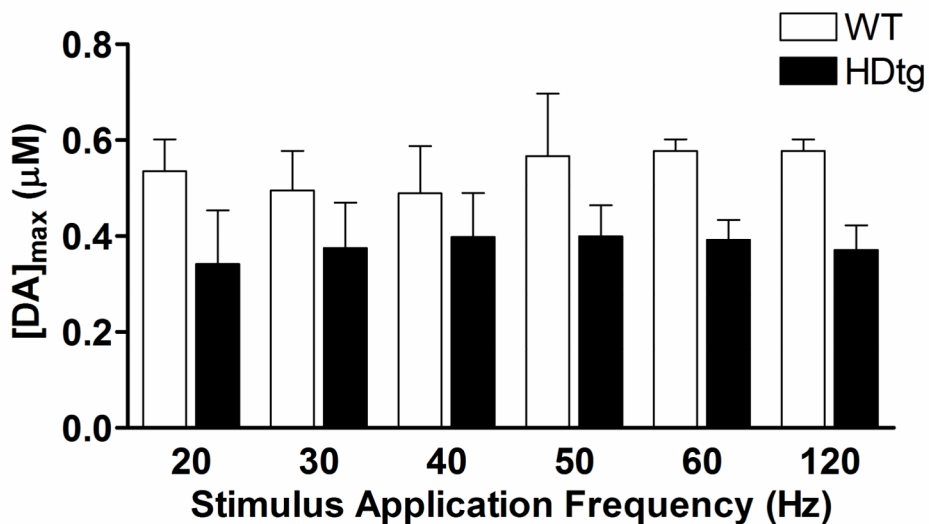
Figure 4. DA release stimulated at different frequencies in brain slices from late-stage HDtg and WT rats

DA release was stimulated at a series of application frequencies and measured by FSCV. Stimulation frequency had little effect on release; however, release was significantly impaired in HDtg rats compared to WT controls (two-way ANOVA, $p < 0.005$, $F[1,30] = 11.43$, n = 3 WT rats and 4 HDtg rats).

Table 1
The content of DA, DOPAC, and HVA does not differ between WT and HDtg rats

Striatal brain lysates from 20- to 26-month-old HDtg rats and aged-matched WT control rats were analyzed by high performance liquid chromatography (HPLC) with electrochemical detection. Content is expressed in µg of neurochemical/g of wet striatal brain tissue. No significant difference in content values between HDtg and WT rats was noted. Abbreviations: DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid.

	WT		HDtg	
	Mean ± SEM	n	Mean ± SEM	n
DA	33.1 ± 8.4 µg/g	4	45.0 ± 11.8 µg/g	5
DOPAC	13.7 ± 4.5 µg/g	5	22.4 ± 2.3 µg/g	5
HVA	10.8 ± 1.2 µg/g	3	12.8 ± 3.3 µg/g	5