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Research report

Use of a force-sensing automated open field apparatus in a longitudinal study of multiple behavioral deficits in CAG140 Huntington's disease model mice

Stephen C. Fowler*, Nancy A. Muma

Department of Pharmacology and Toxicology, Pharmacy School, University of Kansas, Lawrence, KS 66045, USA

HIGHLIGHTS

- A force plate actometer quantified behavior of CAG140 HD model mice aged 6–65 week.
- From 11 week, HD mice were hypoactive, and tremor-like movements emerged at 65 week.
- At 39 week, gait parameters (stride rate and stride length) were normal.
- Lengthening of wall rear durations was observed at 52 and 65 week.
- Zygosity effects were seen for several behaviors, but not for distance traveled.

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ABSTRACT

Behavioral testing of mouse models of Huntington's disease (HD) is a key component of preclinical assessment for potential pharmacological intervention. An open field with a force plate floor was used to quantify numerous spontaneous behaviors in a slowly progressing model of HD. CAG140 (+/+, +/−, −/−) male and female mice were compared in a longitudinal study from 6 to 65 weeks of age. Distance traveled, wall rears, wall rear duration, number of low mobility bouts, in-place movements, number of high velocity runs, and gait parameters (stride rate, stride length, and velocity) were extracted from the ground reaction forces recorded in 20-min actometer sessions. Beginning at 11 weeks, HD mice (both +/− and +/+) were consistently hypoactive throughout testing. Robust hypoactivity at 39 weeks of age was not accompanied by gait disturbances. By 52 and 65 weeks of age the duration of wall rears increased and in-place tremor-like movements emerged at 65 weeks of age in the +/+, but not in the +/− HD mice. Taken together, these results suggest that hypoactivity preceding frank motor dysfunction is a characteristic of CAG140 mice that may correspond to low motivation to move seen clinically in the premanifest/prediagnostic stage in human HD. The results also show that the force plate method provides a means for tracking the progression of behavioral dysfunction in HD mice beyond the stage when locomotion is lost while enabling quantification of tremor-like and similar in-place behaviors without a change in instrumentation. Use of force plate actometry also minimizes testing-induced enrichment effects when batteries of different tests are carried out longitudinally.

1. Introduction

It has been over 2 decades since publication of the seminal report establishing the genetic cause of Huntington's disease (HD): a CAG triplet repeat expansion in the gene that codes for the huntingtin protein [1]. Building on this genetic discovery, researchers have developed at least 56 distinct genetic mouse models of the disease [2]. These genetic models have led to a substantial and ongoing effort to identify phenotypic traits (behavioral, neurochemical, neurophysiological, neuropathological) that allow the charting of disease course and set the context for preclinically assessing the potential efficacy of pharmacological treatments. In the behavioral domain of this modeling effort, one of the most frequently used phenotyping tools is the open field test [2,3]. The central purpose of the work reported here was to show how a force plate actometer [4] can be used as an open field that, in addition to affording measurement of locomotor activity, it can be used concurrently to measure several other behavioral parameters with high poten-

* Corresponding author. Fax: +1 785 864 5219.
E-mail addresses: scfowler@ku.edu (S.C. Fowler), nmuma@ku.edu (N.A. Muma).

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tial relevance to the expression of HD-like symptoms in mice. These additional measures include number of wall rears, duration of wall rears, frequency of occurrence of bouts of non-locomotion episodes, frequency of occurrence of long, straight runs, selected gait parameters (e.g., stride rate, stride length, and run velocity), and in-place behaviors. These latter behaviors, such as tremor or grooming, occur during periods marked by the absence of locomotion. Recording of such behaviors with a force plate actometer is made possible by the instrument's capacity to sense movement in the vertical direction even when no locomotion is occurring [4].

Force plate actometry has been successfully used to quantify several HD-like behavioral abnormalities in the fast progressing, short-life span R6/2 transgenic mouse model of HD [5] and was successfully used to demonstrate the value of disrupting the binding of calmodulin to mutant huntingtin in the R6/2 mouse model [6]. However, the force plate measurement approach has not heretofore been used to examine any slowly progressing, relatively long-lived HD mouse models, mainly created with a CAG triplet repeat expansion in a full length gene for huntingtin (the mouse used in this study is known in the literature as CAG140, KI140, CAG140 KI, or Q140; for a summary, see [2]). Such knock-in models are genetically and phenotypically more like human HD than the transgenic mouse models and appear to be preferred in preclinical research on potential pharmacological or dietary HD interventions [2,7–9].

For the work presented here we chose the "CAG140" mouse [10]. This model was first developed with a CAG triplet repeat expansion of about 140, but the number of CAG repeats in the offspring is variable and has shown some shrinkage, yet still retains its CAG140 name and its HD-like behavioral phenotype [2,11]. The CAG140 mouse has been reported to be hyperactive in the open field at 1 month of age [10], whereas, others have reported hypoactivity at this age [8,9]. However, at about 2 months and older, hypoactivity and/or diminished frequency of rearing appear to be a consistent finding in both heterozygous and homozygous CAG140 mice of both sexes [8–12]. Gait disturbance in the form of shortened stride was reported for 12 month-old CAG140 homozygous mice [10], but, in contrast, Rising et al. [12] did not detect gait alterations in CAG140 mice (neither homozygous nor heterozygous) during monthly testing conducted for 18 months. With respect to in-place behaviors, Hickey et al. [7] observed that at 20–26 months of age homozygous CAG140 mice exhibited clearly discernable "tremor or shakiness" in their home cages.

Neuropathology studies have established abnormalities in several brain loci in CAG140 mice (striatum, nucleus accumbens, olfactory tubercle, cerebral cortex) that are prominently damaged in human HD [10,11,13]. In addition, electrophysiological methods using CAG140 mice have revealed disruption of corticostriatal circuitry function [14], as well as, dysfunction in synaptic transmission in medium-sized spiny neurons [15], a neuronal population that is particularly vulnerable to loss in HD. Thus, the HD-like phenotype of the CAG140 mouse is well documented at several levels of analysis and appears suitable for pushing forward refinements in behavior methodology pertinent to HD preclinical research.

2. Materials and methods

2.1. Animals, husbandry, and genotyping

Three breeding pairs of CAG 140 knock-in heterozygote C57BL/6J mice were obtained from Drs. Marie-Francoise Chesselet and Michael Levine (David Geffen School of Medicine University of California Los Angeles). This knock-in mouse model was generated with a full-length mouse/human chimeric huntingtin gene with 140CAG repeats in exon 1 [10]. We generated 11 L from these breeding pairs which resulted in 36 females (8−/−, 19+/−, 9+/+) and 37 males (11−/−, 17+/−, 9+/+) that were used for behavioral testing. Mice were genotyped by PCR amplification of DNA extracted from tail biopsies. As previously reported, the CAG repeat length is unstable in successive generations in this knock-in model similar to other models resulting in variability in CAG repeat length. Quantitation of CAG triplet repeat length was performed by Laragen, Inc., Culver City, CA 90,232. The results, based on 54CAG140 mice (homozygous and heterozygous combined), indicated a mean CAG triplet repeat expansion of 135.8, a standard deviation of 6.9, with a range of 122-147. Mice were housed in a temperature-, humidity-, and light-controlled room (12 h light/dark cycle, lights on 0600 h) and food and water were available ad libitum. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as approved by the University of Kansas Institutional Animal Care and Use Committee.

2.2. Behavioral methods

2.2.1. Apparatus

The apparatus was a force plate actometer with a square floor [4] measuring 42 cm on each side. A mouse was confined to the load plate by a clear polycarbonate cage with 30 cm high walls. The cage was suspended 2 mm above the load plate so that the mass of the cage itself would not be sensed by the force transducers that supported the load plate. This size of the load plate was sufficiently large to allow mice to express their running behavior in uninterrupted sequences of locomotion suitable for gait analysis, but small enough to eliminate galloping. Note that a square 42 cm on a side has a diagonal measurement of 59.4 cm. The 4 force transducers that supported the load plate at its corners were sampled 100 times/s (i.e., temporal resolution of 0.01 s). Force resolution was 0.2 g-force, and spatial resolution was about 2 mm. A Pascal program written in house directed the timing and data-logging processes via a LabMaster interface. Additional Pascal algorithms were used to extract the macrobehavioral variables (e.g., distance traveled), and a scrolling graphics program written in Visual Basic was used to extract selected gait parameters (e.g., velocity of runs, stride length and stride rate) from the data stream.

2.2.2. Procedure

A total of 73 mice (19−/−, 36+/−, and 18+/+, as mentioned in Section 2.1) were run in the force plate actometer located in a quiet, dimly lit (~2 lux) room. Mice were evaluated once at each age in 20-min recording sessions. The ages at testing were 6, 11, 26, 39, 52, and 65 weeks. Data were collected during the light phase of the daily 12-h light/dark cycle. Between recording sessions the actometer floor and walls were wiped down with 70% ethanol solution.

2.2.3. Behavior quantitation

2.2.3.1. Distance traveled. Distance traveled was defined as the sum of the distances of adjacent *x*–*y* pairs of center of force locations taken every 0.50 s for each mouse. This time spacing between center of force locations was used in order to emphasize movements reflecting locomotion and to decrease the contribution to distance traveled of small high frequency movements that are detected when the temporal spacing is 0.01 s.

2.2.3.2. Force variability. Force variability was defined as the root mean square of vertical force variations sampled at 100 samples/s by analog-to-digital conversion. In order to eliminate any influences of body weight on the force variable, force was expressed as a percent of body weight with the 100% offset removed. See Fig. 1A1 for a 7.00-s (700-samples) example of the resulting vertical force time series for two runs. The first run in Fig. 1A1 begins at the left most dashed vertical line; the second run begins at about 4.8 s. The