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Research report

Novel ethological endophenotypes in a transgenic mouse model of Huntington's disease

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HIGHLIGHTS

- We describe protocols to assess ethologically-relevant behaviors in R6/1 HD mice.
- Olfactory and nest-building tests were sensitive to early decline in HD mice.
- Male vocalization testing revealed a late-stage sexual deficit in HD mice.
- Species-specific assays may improve modeling of the functional impact of disease.

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ABSTRACT

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disorder with a characteristic triad of cognitive, affective and motor symptoms. Transgenic HD mice show excellent construct and face validity for many of these symptoms, however the decline in some facets of every day activity in humans is difficult to model. One approach is the assessment of species-relevant behaviors. Here we described three ethologically appropriate tests in the mouse—olfactory sensitivity, nest-building and sexually-motivated vocalizations. In R6/1 HD mice, olfactory and nest-building tests were sensitive to early dysfunctions induced by the HD mutation. Male vocalization testing revealed a late-stage sexual disinterest in R6/1 HD mice compared to WT littermates. We show that essential, species-relevant functions are disrupted by the HD mutation. The development of integrative behavioral assays which more closely model 'activities of daily living' (ADL) will facilitate the testing of novel therapeutic interventions in animal models as well as their clinical translation.

1. Introduction

Huntington's disease (HD) is a neurodegenerative disorder caused by an abnormal expansion of a trinucleotide repeat in the HD gene [39]. HD patients show decline in a characteristic triad of cognitive, affective and motor symptoms as well as circadian, metabolic and peripheral dysfunctions [1,73]. This culminates in the impairment of activities of daily living (ADL) [33], basic activities necessary for self-care such as dressing, personal hygiene and mobility [43]. Assessments which incorporate ADL measures such as the Unified Huntington's Disease Rating Scale (UHDRS) are sensitive to impairments in gene carriers prior to disease diagnosis [6].

Animal models have been essential in the advancement of therapies and understanding etiology in HD [23]. Transgenic mouse models of HD show excellent face and construct validity and can recapitulate well the symptoms of HD using the standard test battery for behaviors [13,67]. However, impaired ADL such as the early decline in occupational performance and safe driving [6] cannot be directly modeled in animals. Patient-based outcomes such as ADL assessments are instrumental in the evaluation of pharmacological agents in disease [85]. Assessment of behaviors relevant to the model species (ethological behaviors) may reveal how essential functions are impacted by the HD mutation. This ethological approach may offer an improved approach to pre-clinical testing of therapeutics.

Three behaviors relevant to daily function in a mouse are olfactory detection, building a nest and sexual communication. Olfaction

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is a primary sensory modality in rodents [12,20]. In a HD mouse model, Hölter recently reported olfactory discrimination and learning deficits during late-stages of the disease [37]. However, the protocols employed to test olfactory sensitivity in HD mice have not shown any deficits in the ability to detect an odor [37,68]. HD aggregates have been found in olfactory regions [32,44] however, even prior to diagnosis, HD gene carriers show impairments in olfactory detection and discrimination [2,10,17,46,48,54,58]. We believe this important ethological function in mice can be further exploited with improved behavioral testing. A protocol focused on assessing the sensitivity to odors could reveal early, clinically-relevant deficits in HD mouse models.

Nest-building is important for mice during pregnancy and lactation but also for shelter, protection from predators and thermoregulation in the absence of offspring [47,87]. For a laboratory mouse, building a nest is also a source of stimulation and play [4]. The quality of a nest is a behavioral reflection of general health [27], lesions [16], pharmacological treatments [49] and genetic mutations [3]. The impact of the HD mutation on this important activity in mice has not yet been investigated.

In addition to olfaction, acoustic signals are instrumental to mouse communication and social functions [12]. Ultrasonic vocalizations (USVs) are uttered during heterosexual encounters, the majority of which reflect sexual arousal from the male [15,60]. Sexually experienced males vocalize to cues from females such as urine, saliva and vaginal fluids [9,84]. A behavioral test for urine-induced vocalizations has been developed for male mice during which the vocal responses may indicate sexual reward [50]. Sexual behavior is aberrant in HD patients [18,40,74] but the contributions of social, cognitive and hormonal dysfunctions have not been established.

In the present study, we describe the protocols and assessment of three behaviors relevant for daily function for a mouse—olfactory detection, nest-building and sexual communication. The impact of the HD mutation was assessed in the clinically relevant R6/1 transgenic mouse model and WT littermates. R6/1 mice develop affective and cognitive impairments by 8 weeks of age [53,69], followed by further cognitive decline and motor deficits which are clearly manifest by 14 weeks of age [31,52,57,80]. We hypothesized that tests of olfactory and nest-building behaviors would be sensitive to early dysfunctions in R6/1 HD mice. We therefore tested over 6–14 weeks of age. Sexual behavior was tested from sexual maturity (8 weeks of age). These behaviors should also decline with disease progression.

2. Methods

2.1. Mice

R6/1 hemizygote males originally from the Jackson Laboratory (Bar Harbour, ME, USA) were bred with CBA × C57BL/6 (CBB6) F1 females to establish the R6/1 HD colony at the Florey Institute for Neuroscience and Mental Health. R6/1 offspring and their wild-type (WT) littermates were genotyped by polymerase chain reaction (PCR) from toe and tail biopsies. CAG repeat length sequencing showed a mean length of 136.4 ± 3.3 repeats. At 3–4 weeks of age, mice were randomly allocated to single-sex groups ($n = 3$–5) of mixed genotype. Standard housing consisted of laboratory cages (15 × 30 × 12 cm) with bedding and two facial tissues. Mice were maintained under a 12-h light/dark cycle (7.00am light on) with access to food and water ad libitum. Behavioral testing was performed during the light cycle on mice at 6–14 weeks of age. All experiments were approved and conducted in accordance with the guidelines of the Florey Institute Animal Ethics Committee and the National Health and Medical Research Council (NHMRC).

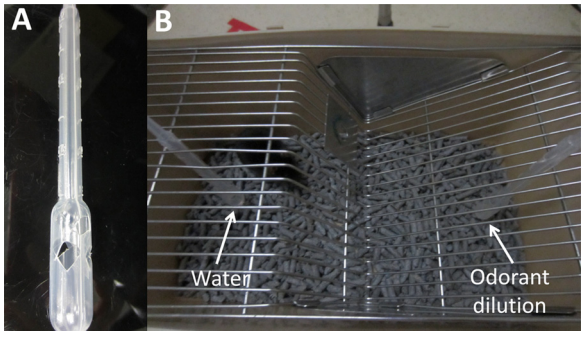

Fig. 1. Set up of the olfactory sensitivity test.
The dilution of peanut butter or water was presented in an odorant vessel made from 3 ml pipettes (A). Two vessels were inserted bulb-side down into the cage and a preference for sniffing the dilution of peanut butter (10^{-1}, 10^{-2}, 10^{-3}) compared to water indicated correct detection of the odorant (B). Left-right positioning of water and order of dilution presentation were randomized across trials.

2.2. Experimental design

Three behaviors were tested over time in male and female R6/1 HD mice and their WT littermates. Olfactory sensitivity was assessed at 8 weeks and 12 weeks of age. Nest-building was assessed at 6, 8, 10, 12 and 14 weeks of age. Social and sexual vocalizations were assessed at 8, 12 and 14 weeks of age. This vocalization protocol is only suitable for male mice [50]. A separate cohort was used for each behavioral test. Mice were handled for 3 days prior to the first assessment. Mice were acclimatized to the testing room for 1 h unless otherwise indicated.

2.3. Olfactory sensitivity test

We modified protocols used by others [29,86] to determine the threshold for detecting a non-social food odorant (peanut butter, PB) that was attractive to our strain of mouse. Mice were single-housed in standard cages with normal bedding and two facial tissues but food and water were absent. Mice were acclimatized to the test room for 2–3 h under low light setting (4 lx). Two odorant vessels (see below) were also present in each cage during acclimatization. For each testing trial, whipped peanut butter (Kraft Foods Australia Ltd.) diluted 10^{-1}, 10^{-2} or 10^{-3} in water (Milli-Q, Merck Pty. Ltd., Australia) was paired with water alone. To ensure that only the odor vapor was accessible, we created odorant vessels from plastic graduated pipettes (3 ml, Copan, USA). Five holes (5 mm × 8 mm) were cut into each pipette bulb (3 half-way up, 2 at the top of the bulb) (Fig. 1A). Two pipettes were inserted bulb-side down into each cage lid (Fig. 1B) from the beginning of the acclimatization phase. During testing, 500 μl of water was pipetted into one bulb and 500 μl of a dilution of PB in the other. The left–right positioning of water was randomized for each trial. Each mouse received one test trial per day (3-min) for a total of three trials. After testing was completed for each trial, mice were returned to group housing. The sequence of dilutions (10^{-1}, 10^{-2} or 10^{-3}) presented to each mouse was also randomized. The peanut butter was diluted fresh every day prior to testing.

A video camera was mounted from above and the time spent sniffing (nose touching the bulb) was later scored blind to genotype and treatment. A preference for sniffing peanut butter (time spent sniffing [PB/(PB + water)] × 100) compared to water was regarded as a correct detection of the odorant.

2.4. Nest-building test

Nest quality can represent a wide variety of behaviors including a measure of home-cage activity and general health [27,55].