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Research report

Detection of early behavioral markers of Huntington's disease in R6/2 mice employing an automated social home cage

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ABSTRACT

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder, for which no known cure or effective treatment exists. To facilitate the search for new potential treatments of HD, an automated system for analyzing the behavior of transgenic HD mice is urgently needed. A recently developed behavior screening system, the IntelliCage, allows automated testing of mouse behavior in the home cage employing individual recognition of animals living in social groups. The present study validates the ability of the IntelliCage system to detect behavioral and cognitive dysfunction in R6/2 mice, an established transgenic model of HD. The results indicate that the IntelliCage is a reliable system for recording exploratory activity, drinking behavior, circadian rhythm, spatial preference, and cognition in mice during prolonged periods of assessment. The system detected early dysfunctional behaviors in R6/2 mice, such as decrease in exploratory activity, sleep disturbances, increased drinking, and repetitive behavior. Additionally, the use of various learning tasks, such as spatial avoidance and spatial patrolling, revealed early cognitive changes in R6/2 mice. The simple learning tasks may be used at both early and late stages of the disease.

1. Introduction

Huntington's disease (HD) is a fatal, autosomal dominant, neurodegenerative disorder characterized by a progressive decline in cognitive and motor function [34]. HD is caused by the expansion of trinucleotide CAG repeats (>36) encoding glutamine in the IT15 gene encoding huntingtin (htt) [18]. Wild-type htt is a large, brain-enriched protein involved in intracellular transport, transcriptional regulation, cytoskeletal organization, and metabolism (reviewed by [4,41]). Htt possesses antiapoptotic properties [42] and is required for embryonic development [55] and neurogenesis [52]. Glutamine-expanded htt forms large intranuclear and cytoplasmic inclusion bodies [44]. The precise mechanisms leading to neurodegeneration in HD are not yet fully understood but presumably involve loss-of-function, dominant-negative and gain-of-function mechanisms (reviewed by [6,20,21]).

Currently, HD has no effective treatments, but over the past few years, the search for new HD targets and compounds directed toward these targets has increased considerably [15]. The development of novel therapies critically depends on multiple factors, including appropriate animal models and the development of a simple high-throughput screening platform.

Over the past decade, many rodent transgenic models of HD have been created, with animals expressing either a truncated or full-length form of the mutated htt gene (reviewed by [40]). Among the first HD transgenic models generated, the R6/2 model has been the most extensively studied and used in a number of preclinical therapeutic trials. These mice were generated by expression of exon 1 of human htt with large $(CAG)_{115}$–$(CAG)_{150}$ repeat expansions under the control of the human HD gene promoter [32]. R6/2 mice manifest features similar to patients with HD, including a decline in motor and cognitive performance, weight loss, and premature death [5,29].

A comprehensive behavioral test battery encompassing motor and cognitive function in HD animals has been developed and used in numerous preclinical studies [7,10,16]. However, individual animal testing is an extremely time-consuming and cost-intensive process. Therefore, the development of a simple and fast screening platform that circumvents the need for a complicated, time-consuming battery of separate behavioral tests is highly desirable.

The IntelliCage from NewBehavior (http://www.newbehavior.com) is a newly developed system permitting automated behavioral assessment of spontaneous and complex learning behavior in mice living in social groups [30]. The IntelliCage allows the monitoring of mice in their home cage, thus eliminating the impact of experimental novelty, handling, stress, and anxiety.

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The aim of the present study was to compare the behavioral and cognitive profiles of R6/2 mice and their wild-type littermates during HD progression by means of the IntelliCage system.

2. Materials and methods

2.1. Animals

R6/2 transgenic mice were obtained from Jackson Laboratories (Bar Harbor, ME; strain B6CBA-Tg[HDexon1]62Gpb/3J) and maintained by backcrossing with CBA × C57BL/6 F1 mice at the Department of Experimental Medicine, University of Copenhagen, Denmark.

Genotype was determined by polymerase chain reaction (PCR) of tail-tip DNA using standard procedures and primer sequences provided by Jackson Laboratories. Both primers lie just outside of the CAG repeat region, permitting the monitoring of the number of CAG repeats in the transgene. The amplified ~611 bp fragment corresponds to 130 CAG repeats. To limit the possibility of variation in CAG number, and subsequently in phenotype, only mice with 125–130 CAG repeats were used for breeding and behavioral study.

We used wild-type littermates as controls. Only female mice were used in the study. Mice were housed in mixed-genotype groups with a 12 h light/dark cycle (lights on at 11:15 p.m.) in a temperature- and humidity-controlled vivarium with *ad libitum* access to food and water. Additionally, at 10 weeks of age, animals were provided with a mash food and Solid Drink gel (Triple A Traiding, Tiel, The Netherlands) that may be beneficial for preventing starvation and dehydration in R6/2 mice.

All experiments were performed according to Danish legislation with a license from the Danish Animal Experiments Inspectorate.

2.2. Behavioral characterization of the R6/2 colony

A schematic diagram of the timeline of testing is presented in Supplementary Fig. 1A.

2.2.1. Evaluation of motor skills by rotarod

Starting from 3.5 weeks of age, mice were trained on a TSE RotaRod (TSE, Bad Homburg, Germany) for 3 consecutive days in acceleration mode (4–40 rpm) for 4 min. After establishing stable baselines, the mice were tested once per week. Each animal was given three trials, and the longest latency to fall from the rotarod was recorded and used in the subsequent analysis.

2.2.2. Evaluation of behavior in the open field

Spontaneous activity in the open-field test was measured in a 50 cm × 50 cm × 40 cm white box for 5 min once every 2 weeks starting from 4 weeks of age. The total distance travelled and numbers of rearing during the experiment were automatically recorded using the Ethovision video tracking system (Noldus, Wageningen, The Netherlands). Detection of rearing in the system is based on calculating the change in surface area of the mouse using experimentally predefined thresholds.

2.2.3. Body weight

All mice were weighed weekly.

2.2.4. Determination of survival

Mice were observed twice daily. An animal was euthanized when it was unable to initiate movement after being gently prodded for 2 min. Two independent observers confirmed this criterion, and this time-point of euthanasia was used as the time of death.

2.3. Behavioral assessment in the IntelliCage

2.3.1. Apparatus and subjects

The IntelliCage is a newly developed system for automated recording of home cage activity and learning behavior in mice in social groups (New Behavior AG, Zurich, Switzerland; http://www.newbehavior.com). The system has been described in detail by [12,22]. Briefly, the system includes a cover plate with four operant learning chambers that fit into a standard polycarbonate cage (Techniplast 2000; Supplementary Fig. 2). Each chamber contains two openings, each permitting access to the nozzle of a drinking bottle. The opening can be blocked by a small motorized door. To reach water, animals do not need to rear. Mice are identified by an implanted transponder when entering the chamber via a tubular antenna opening. The chamber is equipped with infrared motion/proximity detectors, lickometers, light-emitting diodes (LEDs) in several colors, and air-puff devices for punishment. The system is controlled by a computer that recognizes visits, nosepokes, and tube lickings of the individual mice. Preprogrammed experimental schedules are used for conditioning in learning corners. Additionally, each cage contained improved housing conditions (e.g., sleeping shelter, plastic tube, and wood sticks). Cages were supplemented with deep bedding material. To reach a corner's opening, an animal does not need to rear.

At 4 weeks of age, mice were injected with sterile transponders (T-IS 8010 FDX-B, Datamars SA, Bedano, Switzerland) under 2% isoflurane inhalation anesthesia and introduced to the IntelliCage 48 h later. The R6/2 mice and wild-type littermates were housed together in the same IntelliCage.

2.3.2. Experimental procedure

A schematic diagram of the timeline of testing is presented in Supplementary Fig. 1B–D.

Adaptation. The experiment started with an adaptation period of 5 days. After being introduced to the IntelliCage, the mice had free access to all water sources (i.e., all doors were open). Twenty-four hours later, a yellow LED was turned on in the chamber when a mouse entered a corner. During the last 2 days of adaptation, all doors were closed, and the mice learned to open the doors with a single nosepoke on the door area.

Home cage activity. The mice were continuously recorded in the IntelliCage for 8 weeks starting from 4.5 weeks of age. During this period, with the exception of days when special tasks were performed, the mice had free access to all water sources.

Place learning and reversal learning. The least preferred corner during the last 12 h session was chosen for each individual mouse. At the start of the experiment, all water access doors were closed, and only the least preferred corner could be opened by a single nosepoke on the door area. No other doors could be opened. A yellow LED was turned on in the chamber when a mouse entered the correct corner. After 72 h, the position of the accessible corner was changed; again, the least preferred corner for each individual mouse was chosen and opened for drinking. The task continued for another 72 h. The total task duration was 144 h.

Place avoidance learning. The most preferred corner during the last 24 h session was chosen for each individual mouse. The mice received a single air puff (0.4 bar, 2 s duration) if they entered this corner. A yellow LED was turned on in the chamber when a mouse entered the other corners. The total task duration was 6 h.

Patrolling behavior. Mice were allowed to drink in one corner (the most preferred corner during the last 24 h for each individual mouse). After a visit to this corner accompanied by at least one nosepoke, the position of the corner that was opened for drinking was changed in a clockwise manner. A yellow LED was turned on when a mouse entered the correct corners. The total task duration was 36 h.

Side alternation task. Preference for right/left side openings was evaluated during a 24 h session for each individual mouse. At the start of the task, water access doors at the most preferred side opening were closed for drinking in all corners. An opposite side door could be opened by a single nosepoke on the door area. The total task duration was 6 h.

2.4. Statistical analysis

Values are expressed as mean ± standard error of the mean (S.E.M.). Statistical analyses were performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA, USA) using Student's *t*-test or two-way analysis of variance (ANOVA) with or without repeated measures, followed by Bonferroni's *post hoc* test. Results were considered statistically significant when $p < 0.05$.

3. Results

3.1. Behavioral characterization of the R6/2 colony

Because of phenotypic variability in age of onset and rate of disease progression in different colonies of R6/2 mice [17], we first characterized the colony used in this investigation by employing a number of well-established behavioral tests. The assessment of phenotype was performed in female hemizygotic R6/2 transgenic mice and female wild-type littermates between 3.5 and 22 weeks of age.

The animals were weighed weekly. As expected, R6/2 transgenic mice exhibited impaired weight gain compared with their wild-type littermates (two-way ANOVA: genotype, $F_{1,366} = 816.5$, $p < 0.0001$; age, $F_{18,366} = 25.1$, $p < 0.0001$; age × genotype interaction, $F_{18,366} = 19.3$, $p < 0.0001$; Fig. 1A). In contrast to the wild-type animals that continued to gain weight throughout the study, the weight of R6/2 mice reached a plateau between 9 and 14 weeks and slowly decreased thereafter. By week 22, R6/2 mice had lost 16.7% of their previously obtained maximal body weight, weighing significantly less than their wild-type littermates.

Motor coordination and balance in the animals were measured using an accelerating rotarod (4–40 rpm) over a 4 min period. The latency to fall off the rotarod within this time period was recorded. The mice were trained on the rotarod for 3 consecutive days before testing began at week 4. At this age, R6/2 mice were indistinguishable from their wild-type littermates. At 8 weeks of age, the R6/2 mice began to display significant motor