

The power of automated high-resolution behavior analysis revealed by its application to mouse models of Huntington's and prion diseases

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Automated analysis of mouse behavior will be vital for elucidating the genetic determinants of behavior, for comprehensive analysis of human disease models, and for assessing the efficacy of various therapeutic strategies and their unexpected side effects. We describe a video-based behavior-recognition technology to analyze home-cage behaviors and demonstrate its power by discovering previously unrecognized features of two already extensively characterized mouse models of neurodegenerative disease. The severe motor abnormalities in Huntington's disease mice manifested in our analysis by decreased hanging, jumping, stretching, and rearing. Surprisingly, behaviors such as resting and grooming were also affected. Unexpectedly, mice with infectious prion disease showed profound increases in activity at disease onset: rearing increased 2.5-fold, walking 10-fold and jumping 30-fold. Strikingly, distinct behaviors were altered specifically during day or night hours. We devised a systems approach for multiple-parameter phenotypic characterization and applied it to defining disease onset robustly and at early time points.

home cage | neurodegeneration | prion protein | polyQ

The value of mouse models for human diseases has created a keen need for high-throughput behavioral analyses, as has the ambitious goal of systematic characterization of the complete mouse genome (1, 2). Variability in standard behavioral tests hinders comparative studies (3–5) and most sample “snapshots” of behavior. Anxiety caused by being handled by the researcher complicates interpretation and obscures subtle phenotypes. Testing behavior during the daytime may not reflect normal behavior, because mice are nocturnal animals. Finally, manual data collection has inherent investigator bias and high labor costs.

We tested and helped develop a conceptual framework for analysis of mouse behavior by evaluating and improving a beta version of HomeCageScan (HCS), a video-based behavior-recognition platform. The original version of the software functioned only in proof-of-principle experiments: short recordings of a single mouse. It proved unable to give any meaningful phenotypic data in the laboratory setting. By working iteratively with the software designers (Clever Systems Inc., Reston, VA) we overcame numerous video recording and hardware problems, increased the throughput of the system, and refined behavioral definitions. Further details of the modifications to the system are described in [supporting information \(SI\) Methods](#). In the end, the system provided very high-resolution analysis and allowed us to characterize behavior with equally high resolution during the entire light and dark phases, with virtually no intervention by the investigator. We explored the benefits of video-based behavior recognition by conducting high-resolution automated mouse behavior analysis (AMBA) of the home cage (HC) behaviors of two mouse models of neurodegenerative disease, Huntington's disease (HD) and infectious prion disease (PrD).

HD is an autosomal dominant disorder caused by the expression of huntingtin protein with an expanded glutamine repeat (6). Degeneration of the striatum and cortex leads to severe

psychological and motor abnormalities, ending in death (7). The R6/2 mouse is the most widely used HD model, showing an early, severe disease phenotype, with declines in motor performance, cognitive abilities, and weight and premature death (8–10). PrDs have different etiologies and affect distinct regions of the brain. Acquired genetically, spontaneously, or through exposure to infectious material (11), their hallmark features are the misfolding of the prion protein (PrP), dementia, severe ataxia, and death. The most common and robust PrD model involves infecting WT mice with established “strains” of prions. Prion-infected mice exhibit hunched posture, ataxia, tail rigidity, priapism, and death (12). Behavioral abnormalities such as disturbances in food intake and activity vary depending on the mouse strain as well as the prion strain (13, 14). The diversity of PrD symptoms provides excellent candidates for higher-resolution behavioral analysis.

High-resolution AMBA enabled us to characterize models of HD and PrD in unprecedented detail and to discover previously uncharacterized disease phenotypes. Using a systems approach to data analysis, we describe unique behavioral signatures for each disease. Combinatorial behavioral metrics allowed earlier assignment of disease onset. This approach will be extremely useful for phenotypic discovery and as an entry point to more specific behavioral tests.

Results

Experimental Setup. Previously reported behavior acquisition platforms reduce the mouse to a point in space or count the number of breaks of laser beams. Both give very limited information. HCS uses video images collected at a rate of 30 frames per second in the HC and software algorithms to categorize a diverse set of mouse behaviors. The software extracts the image of the mouse as it moves and automated recovery tools adapt to changes in lighting and bedding. Information on the sequence of postures and position of body parts is used to deduce behaviors by comparisons with pretrained data sets. For example, “walking” comprises the mouse being in a particular posture and performing a concerted movement of torso and limbs that changes the position of the mouse along the horizontal axis. For

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Conflict of interest statement: A.D.S., W.S.J., O.D.K., and S.L. initially purchased HCS and received an additional software license in exchange for assistance with further refinement of the program. None of the authors have received remuneration from Clever Systems, Inc. as a stockholder, consultant, or employee.

Abbreviations: AMBA, automated mouse behavior analysis; HC, home cage; HCS, HomeCageScan; HD, Huntington's disease; m.p.i., months postinoculation; PrD, prion disease; Tg, transgenic.

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“eat,” the snout must cross the plane of the food bin with a reared (forelimbs off the ground) posture. For “chew,” which is scored after “eat,” the mouse moves into a vertically huddled position with its paws in front of the snout. Detailed definitions of behaviors are in *SI Methods*.

We recorded control and diseased mice and assessed the accuracy of AMBA by viewing and surveying individual videos for ≈ 20 mice for 3–4 h each. Initially, HCS recorded one mouse at a time. We increased the throughput of the system from one cage to four cages by adding extra cameras. HCS initially also misscored behaviors so frequently that we could detect no differences between WT mice and those suffering from neurodegenerative disease. We worked reiteratively with Clever Systems Inc. to improve ease of use, data transfer capabilities, and, most importantly, the accuracy of scored behaviors through numerous modifications to the algorithms and retests.

The final accuracy assessment was conducted by inspecting ≈ 100 instances of each behavior (according to HCS) in a 24-h video of a single WT C57BL/6 mouse (summary in *SI Table 1*). For 9 of 17 behaviors, $\geq 90\%$ of the instances identified by HCS agreed with manual assessments. For example, 100 of 100 behaviors identified by HCS as “hang upside down” were confirmed, 67 of 70 for “rest,” and 95 of 100 for “walking.” For “hang vertical,” 75 of 100 instances identified by HCS were confirmed. However, most errors were for related activities. The 25 behaviors that HCS misidentified as “hang vertical” were all due to “rearing.” Both require the animal to be in a vertical posture. For the former, the forelimbs touch the wire rack and the hindlimbs lift off the ground; for the latter hindlimbs remain in contact with the ground. A small fraction of behaviors (1–2% of recording time) were unassigned because of failure of the software to recognize the mouse or a behavior (*SI Figs. 5 and 6* for HD and PrD, respectively). The former often occurred immediately after light/dark transitions. The misclassification of behaviors was generally unbiased between WT and diseased mice, except for the later time points for PrD, when the mice move very rapidly and erratically.

Home Cage Behavioral Abnormalities in HD Mice. Standard methods for detecting the HD transgenic (Tg) phenotype are weight loss, clasping, and declining performance in rotarod and grip-strength tests (8, 15). In other laboratories and in our own, these metrics did not reliably detect disease onset until 9–11 weeks (10, 15). Recently earlier detection has been achieved by examining running-wheel activities (16). We recorded the behaviors of seven HD Tg mice and seven gender-matched littermate controls, from a side-view of the HC (*SI Fig. 7*) for two 24-h periods weekly, beginning at 5–6 weeks of age until 11–13 weeks, the terminal phase. High resolution AMBA demonstrated many abnormalities in HD Tg behaviors, many of which were previously unknown. Relative to controls, time spent “resting” progressively declined in HD Tgs (*Fig. 1A*). (The behavior we have defined as “resting” roughly approximates “sleep,” but because we have not validated it with electroencephalogram analysis, we use the more general term “rest.”) Commensurate with less time spent resting, awakening events were dramatically increased in HD Tgs, with Tg mice awakening from rest as much as 2.5-fold more than controls (*Fig. 1B*). “Twitching,” defined as a movement during rest, was elevated in HD mice at the earliest time point tested, and this difference was maintained throughout (*Fig. 1C*). This difference in resting and awakening between HD Tgs and controls was one of the earliest behavioral abnormalities detected and remained significantly different from 6 weeks onwards for awaken and at 6 time points for rest (*Fig. 1A and B*). The early detection of rest abnormalities highlights the remarkable sensitivity of AMBA to clearly and unambiguously detect subtle phenotypic changes before more obvious disease onset at 9–10 weeks of age.

High resolution AMBA also revealed decreased exploratory behaviors in HD Tgs. For example, distance traveled provided a robust metric of overall activity and motor performance. By 9 weeks, there was a 30% decline in the distance traveled by HD Tgs (*Fig. 1D*). AMBA also detected hypergrooming in HD Tg mice. At 5 weeks, HD Tgs groom for $\approx 15\%$ of their time, increasing steadily to 25% at terminal stages of disease (*Fig. 1E*). Control mice showed stable week-to-week grooming behavior, 12–14% of total time (*Fig. 1E*). HD Tgs groomed more frequently than controls rather than grooming for longer bouts (data not shown).

Consistent with previously reported motor abnormalities, high-resolution AMBA also quantified defects in behaviors requiring significant grip strength and coordination. Hanging vertically was reduced in HD Tgs even at the earliest time point tested, 5 weeks, declining ≈ 100 -fold by 10 weeks (*Fig. 1F*). Despite considerable variation in hanging behaviors of diseased and control mice, statistically significant differences between HD Tgs and controls were detected at the early age of 8 weeks. Stretching was similar for HD Tgs and controls until 9 weeks, when stretching severely declined for HD Tgs (*Fig. 1G*). This finding is consistent with their advancing disease “hunched” posture (8), and by 13 weeks HD Tgs rarely stretched ($0.003 \pm 0.001\%$ SEM of total time compared with $0.074 \pm 0.02\%$ SEM in WT controls). Jumping, another complex motor behavior, showed a dramatic decline in HD Tgs (*Fig. 1H*). Despite large intermouse variability of this behavior among controls, a significant difference appeared in HD Tgs by 10 weeks. Alterations in many other behaviors were detected, such as remain low, pause, walk, turn, sniff, rear, eat, chew, drink, and hang upside down (*SI Fig. 5*).

Home Cage Behavioral Abnormalities in PrD Mice. Standard methods for detecting PrD rely on subjective assessments that are not readily quantified: ruffled coat, hunched posture, priapism, and ataxia (12). Consistent with a multitude of reports from other laboratories, when we injected mice with prions harvested from brains of infected animals, these symptoms were easily and reliably detected only 3–4 weeks before the endpoint of disease [5.0–5.5 months postinoculation (m.p.i.)]. For AMBA, we recorded eight similarly infected C57BL/6 males and eight mock-inoculated controls for two 24-hour periods monthly or twice monthly until the terminal phase, 5.5 m.p.i. AMBA detected alterations in PrD mice behavior much earlier than previously reported and detected many previously uncharacterized phenotypes.

By 3.5 m.p.i., prion-infected animals exhibited a significant decrease in resting, and, at the final stages of disease, PrD mice rested only half as much as mock-injected mice (*Fig. 1I*). Awakening from rest showed a significant increase in early stages of PrD at 3, 3.5, and 4 m.p.i. and then decreased in the late stages of disease of 5–5.5 m.p.i. (*Fig. 1J*). Movement during rest, or twitching, was significantly reduced from a very early time point, 3 m.p.i. (*Fig. 1K*). Thus, rest abnormalities were one of the most sensitive metrics of disease onset for PrD and HD.

Unexpectedly, PrD mice showed an enormous increase in activity concomitant with overt disease onset. Beginning at 4.5 m.p.i., the PrD mice traveled 1,378 (± 411 SEM) meters compared with 96 (± 5 SEM) meters in controls (*Fig. 1L* and *SI Movie 1*). PrD mice showed a sharp decline in grooming; by advanced disease (5.5 m.p.i.), they spent half as much time grooming as controls (*Fig. 1M*). In sharp contrast to HD Tg mice, the exploratory activities sniffing and rearing were highly elevated in PrD mice at 3.5 m.p.i., and this increase persisted until the final time point at 5.5 m.p.i. (*Fig. 1N and O*). Despite ataxia and imbalance, PrD mice spent significantly more time jumping than controls from 4.5 to 5.5 m.p.i. (*Fig. 1P*). These behavioral alterations occurred at very early time points, long before classic

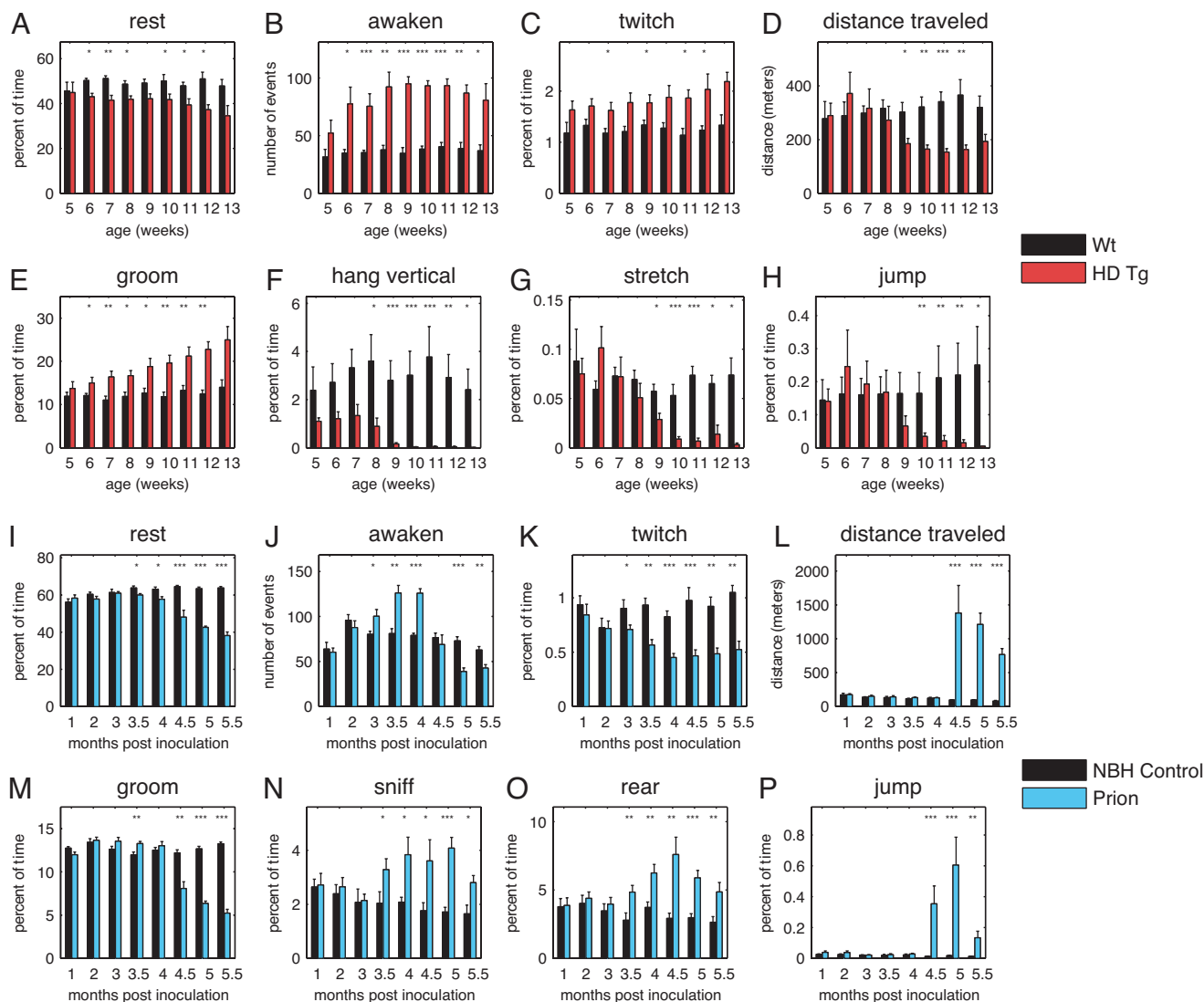


Fig. 1. Behavioral alterations in Huntington's and PrD mice. (A–H) Mean values (\pm SEM) for HD and controls are shown for rest (A), awoken (B), twitch (C), distance traveled (D), groom (E), hang vertical (F), stretch (G), and jump (H). (I–P) Mean values for PrD and controls are shown for rest (I), awoken (J), twitch (K), distance traveled (L), groom (M), sniff (N), rear (O), and jump (P). *P* values were computed by using a two-tailed Wilcoxon rank-sum test (nonparametric) and are indicated as follows *, *P* < 0.05; **, *P* < 0.01; and ***, *P* < 0.001 (sample sizes for HD were *n* = 5 HD Tg and WT control pairs for week 5, *n* = 7 for weeks 6–11, *n* = 6 for week 12, and *n* = 4 for week 13; for PrD, *n* = 8 for every time point except for 5 m.p.i., where *n* = 7 prion and *n* = 8 controls).

symptoms developed. Many other behavioral alterations were detected, such as remain low, pause, walk, turn, eat, chew, drink, stretch, hang upside down, and hang vertical (SI Fig. 6).

Behaviors of HD and PrD Mice in the Light and Dark Phase. Data collection over 24 h allowed us to examine behaviors with respect to the light and dark phase, when mice are normally more active. WT mice were most active during the first half of the dark phase as demonstrated by decreased rest and increased hanging vertical behaviors (Fig. 2*A* and *C*, respectively). In the second half of the dark phase, resting increased, and hanging vertical decreased for WT mice (Fig. 2*A* and *C*).

At 6 weeks, HD Tgs rested a similar amount of time as controls during the dark phase (Fig. 2*A*); however, even at this early stage, HD Tgs rested less during the light phase. This difference in rest during the light phase was observed again at a later stage of disease (Fig. 2*B*). HD Tgs spent slightly less time hanging vertical than did control mice during the dark cycle at 6 weeks, but during the light cycle, the hanging behavior was the same

(Fig. 2*C*). By 11 weeks, the difference in hang vertical behavior with respect to light and dark cycles was much more profound (Fig. 2*D*). The HD Tgs showed a statistically significant decrease in hanging vertical throughout the entire dark phase. Thus, these behavioral alterations observed in HD Tgs were often observed only in light or dark phases.

PrD mice also showed remarkable changes in behaviors, distinct from HD Tgs, with respect to light and dark phases. Resting was similar between PrD and control mice at 2 m.p.i., before disease symptoms (Fig. 2*E*). Resting for both PrD and control mice was fairly constant throughout the light and dark phase, with a slight increase for both in the light phase (Fig. 2*E*). At 5 m.p.i., PrD mice barely rested during the dark phase, in striking contrast to controls, whereas little difference was observed in rest during the light phase (Fig. 2*F*). Upon first entering a new cage, both PrD and control mice spent time walking, indicative of exploring the new cage (Fig. 2*G*). In PrD and control mice at 2 m.p.i., walking was uniformly distributed during the light and dark phases (Fig. 2*G*). However, by 5 m.p.i.,

