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Automated phenotyping and advanced data mining exemplified in rats transgenic for Huntington's disease

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ABSTRACT

Background: The need for improving throughput, validity, and reliability in the behavioral characterization of rodents may benefit from integrating automated intra-home-cage-screening systems allowing the simultaneous detection of multiple behavioral and physiological parameters in parallel.
New method: To test this hypothesis, transgenic Huntington's disease (tgHD) rats were repeatedly screened within phenotyping home-cages (PhenoMaster and IntelliCage for rats), where spontaneous activity, feeding, drinking, temperature, and metabolic performance were continuously measured. Cognition and emotionality were evaluated within the same environment by means of operant learning procedures and refined analysis of the behavioral display under conditions of novelty. This investigator-independent approach was further correlated with behavioral display of the animals in classical behavioral assays. Multivariate analysis (MVA) including Principle Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) was used to explore correlation patterns of variables within and across the two genotypes.
Results: The automated systems traced previously undetected aspects in the phenotype of tgHD rats (circadian activity, energy metabolism, rearing), and out of those spontaneous free rearing correlated with individual performance in the accelerod test. PCA revealed a segregation by genotype in juvenile tgHD rats that differed from adult animals, being further resolved by PLS-DA detecting "temperature" (juvenile) and "rearing" (adult) as phenotypic key variables in the tgHD model.
Conclusions: Intra-home-cage phenotyping in combination with MVA, is capable of characterizing a complex phenotype by detecting novel physiological and behavioral markers with high sensitivity and standardization using fewer human resources. A broader application of automated systems for large-scale screening is encouraged.

1. Introduction

The anticipated increase in the number of transgenic rat models displaying pathological traits of neurological diseases available for research calls for an implementation of the conventional behavioral tests in use, which oblige scientists to face the limitations of resources, validity, reliability, throughput, and data mining offered with such 'classical' approach (Urbach et al., 2010). In our own previous projects, phenotyping of a single mutant rat line requires 24–36 months for initial behavioral characterization and much longer for in depth analysis of subtle behavioral traits (Frerker et al., 2009; Karl et al., 2003a,b,c; von Horsten et al., 2003).

Incomplete phenotyping due to a reduction in the numbers of tests applied, a limited observation period, limited complex statistics on large data sets, limited sensitivity of established testing procedures, and a lack of proper controls (littermates, backcrosses) often cause false negative, or positive, results as has been observed in several transgenic mouse models (Crabbe et al., 1999; Crawley

Abbreviations: tgHD, transgenic Huntington's disease rats; wt, wild-type rats; PM, PhenoMaster; IC, IntelliCage; MVA, multivariate analysis; PCA, Principal Component Analysis; PLS-DA, Partial Least Squares Discriminant Analysis; PPI, prepulse inhibition; TWAA, two-way active avoidance; RER, respiratory exchange rate.

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et al., 1997). The resources (space, personnel), equipment, and expertise (know-how, standardization, validation) necessary for comprehensive phenotyping are not always available, and highly standardized procedures for many "classical" behavioral tests have not been established yet. However, based on first data from mouse studies (Balci et al., 2013; de Visser et al., 2006; Jhuang et al., 2010; Krackow et al., 2010), it can be expected that automated phenotyping technology will also allow the investigation of rat models in a more familiar environment.

In particular, the transgenic rat model of Huntington's disease (von Horsten et al., 2003) used in this study has been systematically characterized by means of classical behavioral and physiological phenotyping (Bode et al., 2008; Casaca Carreira et al., 2013; Nguyen et al., 2006; von Horsten et al., 2003) and is about to be used in translational experimental therapeutical approaches.

In this study we used the tgHD rat model as the positive control for the validation of newly developed automated, intra-home-cage phenotyping systems for rats (PhenoMaster, TSE Systems GmbH, and IntelliCage, NewBehavior AG), as this transgenic rat model shows several behavioral differences and as the phenotype described so far was found by using classical behavioral assays only. We compared the individual performances of tgHD within classical behavioral assays previously identified as suitable to characterize onset and progression of the phenotype in this model with ethological behavior detected within the automated systems. Data mining and classical statistical analysis were supplemented by multivariate statistics, Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) in order to take advantage of the intra-individual longitudinal multidimensional quality of parallel data acquisition within the automated home-cage systems.

The results presented in fact demonstrate how such an approach is able to detect interesting phenotypic traits, which might be age-/gender-/genotype-specific and only clearly identified by an integrated and comprehensive analysis. The behavioral and physiological quality of our study was mostly possible thanks to the home-cage nature of the testing, which virtually introduces no artifacts induced by handling and novelty-related stress. For this reason, large-scale screening of novel as well as existing animal models will benefit from this technology, regarding further validation and application in translational studies.

2. Materials and methods

2.1. Animals

A detailed description of the tgHD rat model has been published (von Horsten et al., 2003). Briefly, these animals are derived from the outbred Sprague-Dawley strain and express 727 amino acids of the HD gene with 51 CAG repeats under the control of 886 bp of the rat huntingtin promoter. A colony of transgenic HD rats was established at the preclinical research center of the Universitätsklinikum Erlangen, and the line was maintained by backcrossing. For genotyping, tail tips were collected at the age of 3 weeks. Rats were housed in gender- and genotype-matched groups of four, according to FELASA recommendations (Rehbinder et al., 1996). All rats were kept under a 12:12 h light:dark cycle (lights on at 6.00, off at 18.00), with food (Sniff lab chow pellets; Germany) and tap water available ad libitum. All research and animal care procedures were approved by the district government of Frankonia, Ansbach, Bavaria, Germany.

2.2. Experimental design

The present study used strictly age-matched male homozygous (hom) and wild-type (wt) littermates. Two sets of animals were used: Set 1 consisted of 12 wt and 12 hom rats, monthly tested in the PhenoMaster and on classical behavioral paradigms; Set 2 consisted also of 12 wt and 12 hom rats, which underwent cognitive assessment in the PhenoMaster operant behavior system (OBS) at the age of 2 and 6 months. It should be noted here that it will be important to consider also females as well as classical inbred strains of rats.

Social interaction and accelerod tests were conducted during the dark phase, whereas prepulse inhibition (PPI), OBS and two-way-active-avoidance (TWAA) were measured during the light phase of the light:dark cycle. The PhenoMaster experiments lasted 72 h, comprising three full light:dark cycles.

2.3. Automated phenotyping using intra-home-cage technology

2.3.1. PhenoMaster

2.3.1.1. Theoretical construct. The PhenoMaster system for rats (TSE Systems GmbH, Bad Homburg, Germany) is capable of automatically screening rats in a home-cage-like environment with a high temporal and spatial resolution. The newly developed PhenoMaster for rats is conceived as an automated modular high throughput screening system, where data relative to physiological and behavioral parameters are continuously collected from the single experimental animals under circumstances that maximize the likelihood of natural behavior to take place, thus representing a refinement of the use of laboratory animals for research.

2.3.1.2. Apparatus. The PhenoMaster measures indirect calorimetric parameters, similar to the older LabMaster (Bode et al., 2008), but is based on conventional type IV Thermoplast cages, equipped to individually monitor one rat per cage. Food and water intake are measured by highly reliable sensors and cumulative feeding and drinking iteratively calculated by the system. Calorimetric parameters are assessed by continuously measuring O_2 and CO_2 concentration in the cages, through an open-circuit. Locomotor activity is measured by a photobeam-based device, in which infrared sensor pairs are arranged in strips for horizontal ($x = 32$ beams and $y = 25$ beams) and vertical ($z = 32$ beams) detection. Light beam breaks are counted in a user-defined time interval over the experimentally defined period of time (e.g. 72 h) and data are stored in a separate binary file. Analysis of these data is achieved by further sub-dividing beam-break counts into: (A) fine movements, or grooming (XF, YF) resulting from the repeated break of the same light beam, (B) ambulatory movements (XA, YA) that are counted as the breaks of consecutive beams, and (C) peripheral ambulatory movements (PerT, PerA, PerF). In addition, the total number of *Z*-axis breaks is also monitored and classified as rearing (*Z*). Only interruptions of the light beams classified as one of the above mentioned behaviors are analyzed so that minimal movements (such as breathing) can be ignored. As with other infra-red beam systems, such devices cannot distinguish between e.g. fine movements and grooming All measurements were monitored using the PhenoMaster software, Version V1.9.20-06/2007, supplied by TSE.

The setup installed allowed the monitoring of 12 animals in parallel, with high resolution for activity, calorimetry, food and water consumption. Telemetric measures of animals' core temperature were integrated using the MiniMitter transponder technology (implanted E-Mitter-transponders; www.minimitter.com) providing a ±0.1 °C accuracy within a range of 33–41 °C body temperature. As modular add-ons, a running wheel and an operant conditioning wall were available for advanced locomotor and cognitive testing.

The operant conditioning units (OBS) used in this study consist of two retractable levers positioned on either side of a central food crib, covered with a clear Perspex panel hinged at the top, so that the animal could push it open to retrieve the food pellets (dustless precision pellets 45 mg, Bio-Serv, Frenchtown, NJ, USA) delivered upon completion of a successful trial. The walls are also equipped