Automated phenotyping using intra-home-cage technology

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Similar to other approaches into this direction, the PhenoMaster system (TSE Systems, Germany) is capable to automatically screen rats in a home-cage-like environment for several parameters at a high temporal and spatial resolution. This novel approach generally allows experimenter independent monitoring of laboratory rodents and – a priori – allows avoidance of stress-artifacts, higher throughput, higher sensitivity of measures (online, circadian), and combination of measurements potentially allowing multifactorial analysis and idenification of novel combined behavioral dimensions. Especially, the newly developed PhenoMaster System for rats is conceived as an automated modular high throughput screening system for the assessment of specific gene-associated functions on the physiological and behavioral phenotype of small laboratory animals. It is a modular, multi-purpose tool for investigation of multidimensional physiobehavioral outputs of rats.

Apparatus PhenoMaster

The PhenoMaster system represents a modular set-up that measures indirect calorimetric parameters, activitiy, drinking, feeding, operant wall or wheel and is based on conventional type IV Thermoplast cages, with each cage being equipped with the technical requirements to individually monitor one rat per cage at a time. The present system is set-up to measure 12 animals in parallel with high resolution for activity, calorimetric parameters and food and water consumption. The animals are monitored for the following primary measures: food and water intake, RER, and locomotor activity. Secondary measures are: energy expenditure and other combined readouts. Cumulative feeding and drinking were given and this was used to compare iterative food- and water intake. The calorimetry system is an open-circuit system that determines O_2-consumption, CO_2-production, and RER. To investigate locomotor activity, a photobeam-based activity monitoring system detects and records the number and duration of every total, fine and ambulatory movement, including rearing and climbing movements, in every cage.

This activity detection is achieved using infrared sensor pairs arranged in strips for horizontal (x, y level) and vertical (z level, rearing) activity. Light beams with 32 beams in X, 25 beams in Y dimension and 32 beams in the Z-axis are used. The mean activity per hour (in 20 min intervals) over 72h for each parameter is calculated. Results for locomotor activity are given as number of counts. In addition, depending on the pattern of interruption of the light beams, movements are further subdivided by the PhenoMaster software into fine movements (XF, YF; "grooming") defined as the counting of repeated beam breaks of the same light barrier, ambulatory movement that are the counting breaks of alternate barriers (breaks of Y-axis, XA, YA) and peripheral ambulatory movements (PerT, PerA, PerF). In addition, the total number of interruption of the Z-axis was monitored ("rearing"). Only interruptions of the light beams that are linked with a movement will be recorded, therefore it is possible to define a refractory period so that short-term movements can be ignored and permanently interrupted light beams will not be taken into account as well.

The sensors for detection of movement operate efficiently in both light and dark phases, allowing continuous recording. All measurements are monitored using the PhenoMaster software.

Presently, the system is used to characterize changes in energy-related parameters in rats transgenic for Huntington's disease and Spinocerebellar Atxia type 17 in the course of the European-project RATstream^{TM} (www.ratstream.eu).

Experiments were done in a separate room under standard conditions with control units and computer within the same room. Subsequently, animals were introduced to the system and parameters were continuously and simultaneously measured during 72h. During this period of testing the experimenter only once a day entered the room for controlling proper working of the system and checking health of animals by visual inspection.

Analysis of data

Energy expenditure was measured as VO_2 consumption in ml/(h*kg/BW) and VCO_2 production in ml /(h*kg/BW) and the respiratory quotient (RER) was calculated as the quotient of VCO_2 production divided by VO_2 consumption for each 20 min. Cumulative feeding and drinking was taken as a sum of consumed food- and water intake per 20 minutes iterative. Locomotor activity counts were summed for 20 min intervals from measurements over 1 min intervals for the duration of the experiment. Mean activity counts were calculated for each experimental group and analyzed as activity over time. Raw data derived from automated PhenoMaster system was exported for statistical analysis using R system for statistical computing (version 2.4.1; R Development Core Team, Vienna, Austria; 2006). Imports were examined graphically (Q-Q plots, histograms, boxplots) to determine if normal distribution and equal variances can be assumed for each parameter. For normal distribution, one-way ANOVA with one between-subject factor (genotype) and with repeated measures over time was performed. If data are not normally distributed, non-parametric tests for repeated measures according to the method of O'Brien were applied.