Body weight gain is impaired in rats and mice during automated homecage system observations

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Introduction

Detailed behavioral characterization is an important part of establishing novel animal models in a variety of research fields. Automated homecage observation has been promoted to provide better measurements of behavioral parameters than classical tests [1], and several systems for computer-based acquisition of homecage activities of rats and mice as well as analysis software are currently in use [2–7]. These systems offer a standardized testing environment, while also allowing standardized customization to run specific protocols. As the measurements are highly computerized, behavioral data is gathered objectively. Moreover, the systems are able to measure a broad spectrum of behaviors including activity, food and water intake as well as cognitive aspects and thus a well-functioning automated homecage system could in theory be used for the complete behavioral characterization of an animal model.

A further aspect of the promotion by both, companies selling the systems [8, 9] and researchers using them [5, 10–11], concerns the increase in research quality by improving animal welfare. As the tests involve less handling than classical tests, they are believed to be less stressful for the animals, and the results are thought to better represent the animals' natural behavior. The latter is further improved by the opportunity of assessing behavioral parameters over longer periods of time, so that novelty-induced and baseline behavior can be properly separated [1, 5]. However, most systems are designed to measure the behavior of individual animals, and thus require social isolation for the time of observation. Individual housing of rats and mice is usually avoided though, as it is considered stressful and alters an animal's behavioral response [12–13]. From this perspective, both welfare and scientific benefits of using automated homecages might be lost.

Behavioral experiments

It should be noted that data discussed here, were collected in multiple studies as part of the behavioral characterization of different mouse and rat models and as such, the studies were not specifically designed for extracting potential stress effects of the automated homecage environment.

All experiments were approved by the commission for animal experiments at the Regierungspraesidium Tuebingen in accordance with the guidelines of the German animal welfare act. The behavioral experiments were carried out by experimenters trained and experienced in laboratory animal research.

Male, wild type rats and mice were kept under controlled environmental conditions (21–23 °C ambient temperature, 55 +/- 10 % humidity and a 12/12 h light/dark cycle) in two different animal facilities. They were housed in social groups of 3–4 animals in cages with wooden bedding and nesting material. Standard chow and tap water were delivered *ad libitum*.

Automated behavioral phenotyping was performed with the three automated homecage systems listed in Table 1. For this purpose, the animals were transferred to an experimental room and placed individually in the testing cages. The behavior of the animals was investigated over a period of 70 h, during which they had *ad libitum* access to food and water, and were left undisturbed except for a short daily visit. Body weight was measured before and after the test. For some cohorts, body weight was additionally recorded on a weekly basis over a period of several months, enabling the comparison of body weight gain before, during and after behavioral testing.

Table 1. Automated homecage systems used for the behavioral observation of rats and mice. The PhenoMaster systems for mice and rats were provided by TSE Systems, Germany, the PhenoTyper for rats by Noldus Information Technolgy, The Netherlands. Their customized setup of the systems' test cages differed considerably from standard home cages.

System	Provider	Species	Cage dimensions length - width - height	Peculiarities compared to the home cage
PhenoMaster	TSE	Rat	48 - 37.5 - 20 cm	Air-tight lid, small free-hanging water bottle and food basket, little amount of bedding
PhenoTyper	Noldus	Rat	45 - 45 - 65 cm	Shelter, two water bottles, large feeding area, non-standard bedding
Home cage		*Rat*	*55 - 38 - 24.5 cm*	
PhenoMaster	TSE	Mouse	20.5 - 36.5 - 14 cm	Small free-hanging water bottle and food basket, little amount of bedding
Home cage		*Mouse*	20.5 - 36.5 - 14 cm	

Results

In our laboratory, automated homecage observation is used for the characterization of animal models of various neurodegenerative diseases [e.g., 14-18]. When analyzing data from these studies, we noticed that the body weight gain of test animals dropped during the test sessions in automated homecages in a similar manner as when running classical behavioral tests (see Figure 1). Comparing the weekly body weight gain of wild type Sprague Dawley rats subjected to behavioral testing to rats that were not tested, revealed a significantly altered development characterized by higher fluctuations in weight gain in the tested rats (see Figure 1a). The effect was even more pronounced when the body weight gain before, during and after behavioral testing was calculated for each rat individually (see Figure 1b), as the exact time of behavioral testing varied between individual rats by up to 12 days.

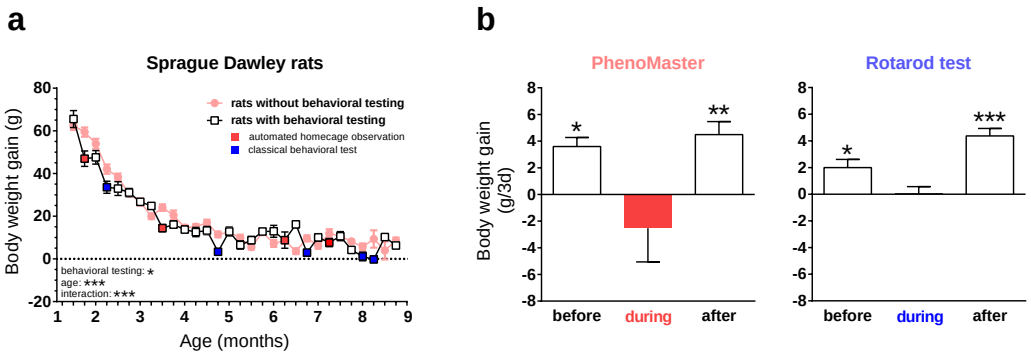

Figure 1. Influence of behavioral testing on body weight gain in Sprague Dawley rats. (a) Body weight gain over 3 days was calculated from weekly measurements for a behavioral test group and a group of rats not subjected to behavioral testing (n = 15 per group). Behavioral test events included observations in the automated PhenoMaster system and classical Rotarod and swim tests. Effects of behavioral testing are likely weakened, since the exact time of behavioral testing varied between individual rats by up to 12 days. Data sets derive from two spatially and temporally separate cohorts of rats. Statistical test results displayed in the graph derive from repeated measurements two-way ANOVA. (b) Body weight gain during the 3 days of PhenoMaster observation and during Rotarod test at the indicated ages are compared to the average body weight gain over 3 days during the week prior and post testing. Data derive from the behavioral test group displayed in Figure 1a and was calculated for each rat individually in order to reveal a more exact measurement. Statistical significance was determined using paired one-tailed t-tests. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

As reported before, the reduced body weight gain was likely to be the result of reduced food intake, since we found relative food intake of rats with impaired weight gain during automated homecage observation to be obscured [14]. This further influenced other parameters obtained during testing, specifically locomotor activity and metabolic rate, in a similar manner [14].

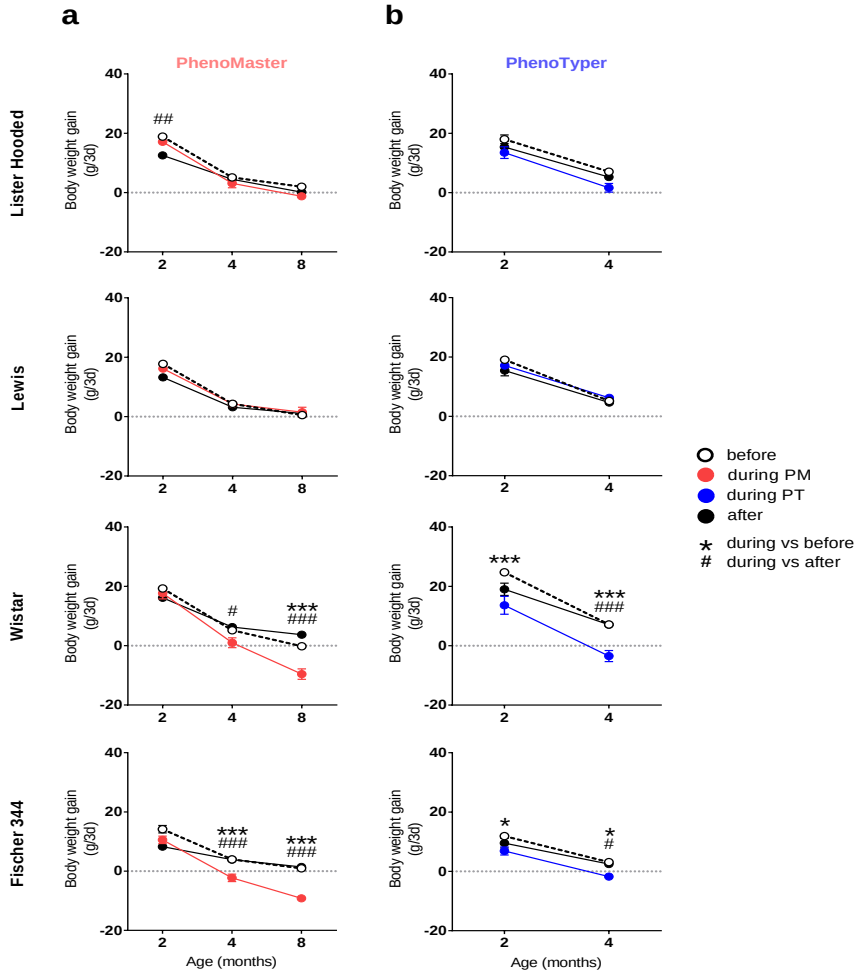

Figure 2. Effect of automated homecage observation on body weight gain in different rat strains. Rats of the four strains Lister Hooded, Lewis, Wistar and Fischer 344 were subjected to automated homecage observation in the PhenoMaster system at 2, 4 and 6 months of age (a) and the PhenoTyper system at 2 and 4 months of age (b). Body weight gain during the 3 days of observation was compared to the average body weight gain over 3 days during the week prior and post testing. Statistical significance was determined using two-way repeated measurements ANOVA and Fisher LSD post test for the indicated comparisons; */# = $P < 0.05$, **/## = $P < 0.01$, ***/### = $P < 0.001$.

The effect of automated behavioral observation on body weight gain increased with age and/or re-exposure to the test cages (see Figure 2a and Figure 3). Furthermore, while younger rats showed a reduced body weight gain during automated homecage observation, older rats experienced explicit weight loss (Figure 2a, Figure 3a). This body weight loss was found as well in aged mice exposed to a mouse PhenoMaster system (Figure 3b).

Discussion

Data presented here, reveal a common impairment of body weight gain during automated homecage observation. This indicates that animals experience some form of stress while being housed in the homecage systems, which is supported by the finding that rat strains regarded as less anxious (i.e. Lister hooded and Lewis rats), did not show the impaired weight gain. The phenomenon, however, seems to be of general importance, as it was found in three out five rat strains investigated, as well as in mice.

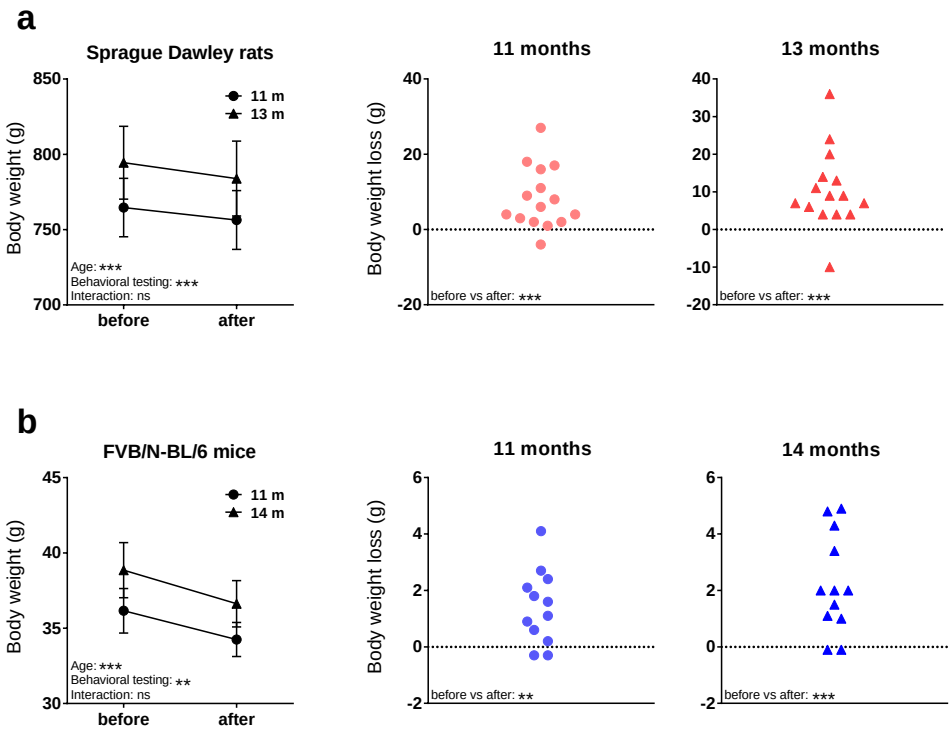

Figure 3. Body weight loss in aged rats and mice during automated home cage observation. Sprague Dawley rats were subjected to automated homecage observation in the rat PhenoMaster at 11 and 13 months of age (a), and FVB/N-BL/6 cross-bred mice were tested in the mouse PhenoMaster at 11 and 14 months of age (b). Body weight was measured before and after 3 days of observation. Statistical test results displayed in the graph on the left side derive from repeated measurements two-way ANOVA. Statistical significance for body weight loss at the two ages (graphs on the right side) was analyzed using paired one-tailed t-tests; * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Since the impairment occurred in systems from two different manufacturers and in three considerably different setups, it appears unlikely that changes in cage properties have caused the anxiogenic effect. The impaired body weight gain is more likely the result of isolating the animals during the time of observation, as this was the common requirement for behavioral observations in these systems. Similar issues have been discussed for metabolic cages [20–21], and should be addressed as well for automated homecage systems.

It is important to note that the changes in body weight were apparently accompanied by changes in other parameters [14]. Thus, such results need to be interpreted carefully. A more thorough investigation of the anxiogenic factors of automated homecage systems is needed in order to elucidate optimal protocols.

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