

# Early spontaneous behavioural signs of Huntington's disease in R6/2 mice detected in home cage situation

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## Introduction

Behavioural characterization of the earliest signs of Huntington's disease (HD) in animal models is crucial for future testing of drugs aimed at slowing or stopping the progression of this disease.

Traditionally, behavioural studies on these models are conducted by using test batteries (clasping test, rotarod, grip strength test) in order to investigate specific evoked responses separately. Moreover these tests usually do not lead to any differences before 8 weeks of age. Alternatively, we use an integrated system based on video-tracking in a home cage situation in which several aspects of the disease and its spontaneous behavioural changes can be simultaneously investigated. The advantages are: reduction of the confounding effects of stress, transportation, novelty, handling caused by human interference and the possibility of investigating longitudinal time course of changes.

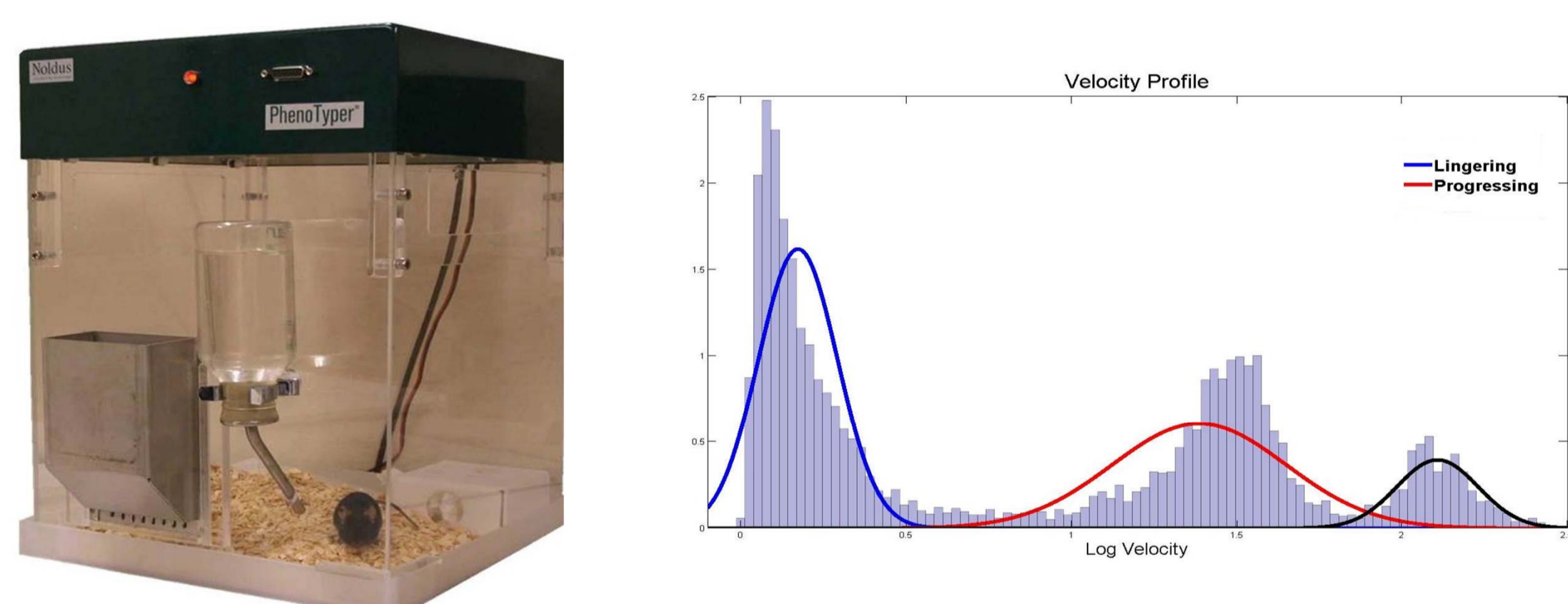
Mice (R6/2) were generated that are transgenic for the 5' end of the human HD gene carrying (CAG)<sub>188</sub>-(CAG)<sub>222</sub> repeat expansions (Mangiarini et al. 1996). R6/2 mice as well as the wild type littermates are singly housed in the home cage system for several weeks. The spontaneous locomotor activity is continuously monitored as well as the behavioural responses to the presence of shelter, food, water and an aversive bright light for conditioning avoidance responses.

## Aim

We propose to use an integrated approach, based on a video tracking system, in which several aspects of the disease can be investigated without the confounding effects of stress, transportation, novelty and handling caused by human interference. The main objective of this work is to detect early onset of behavioural signs of HD in the locomotor activity of the R6/2 mouse model by using a fully automated home cage environment.

## Methods

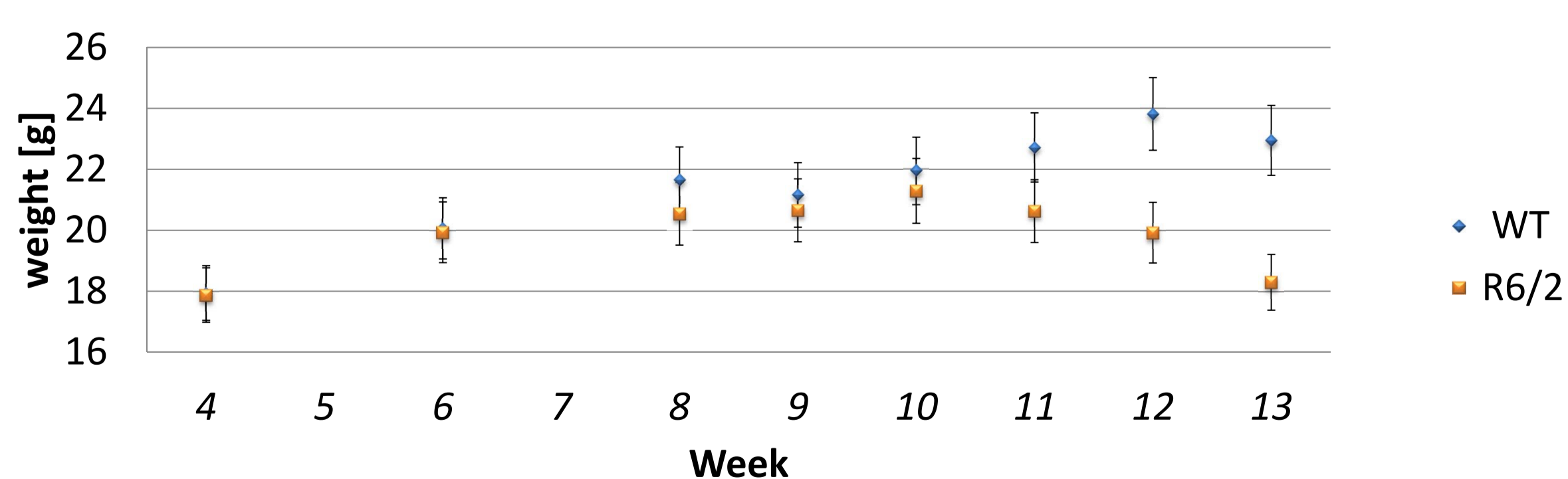
15 R6/2 mice as well as 13 wild type littermates were part of this study. All the mice were singly housed into the home cage system (PhenoTyper®, Noldus Information Technology, Wageningen, The Netherlands) for several weeks and tested at 4 and 8 weeks of age. The spontaneous locomotor activity in their home cage was monitored using EthoVision® XT (Noldus Information Technology, Wageningen, The Netherlands). Several parameters related to the motor activity of the mice as well as the spontaneous behaviour of the mice (time spent in the shelter, feeding, drinking) were extracted and further analyzed using MATLAB (MathWorks). A full velocity profile (Figure 1, right) of the animals have been calculated to better characterize the locomotor behaviour distinguishing lingering from progressing periods (Drai et al, 2001)



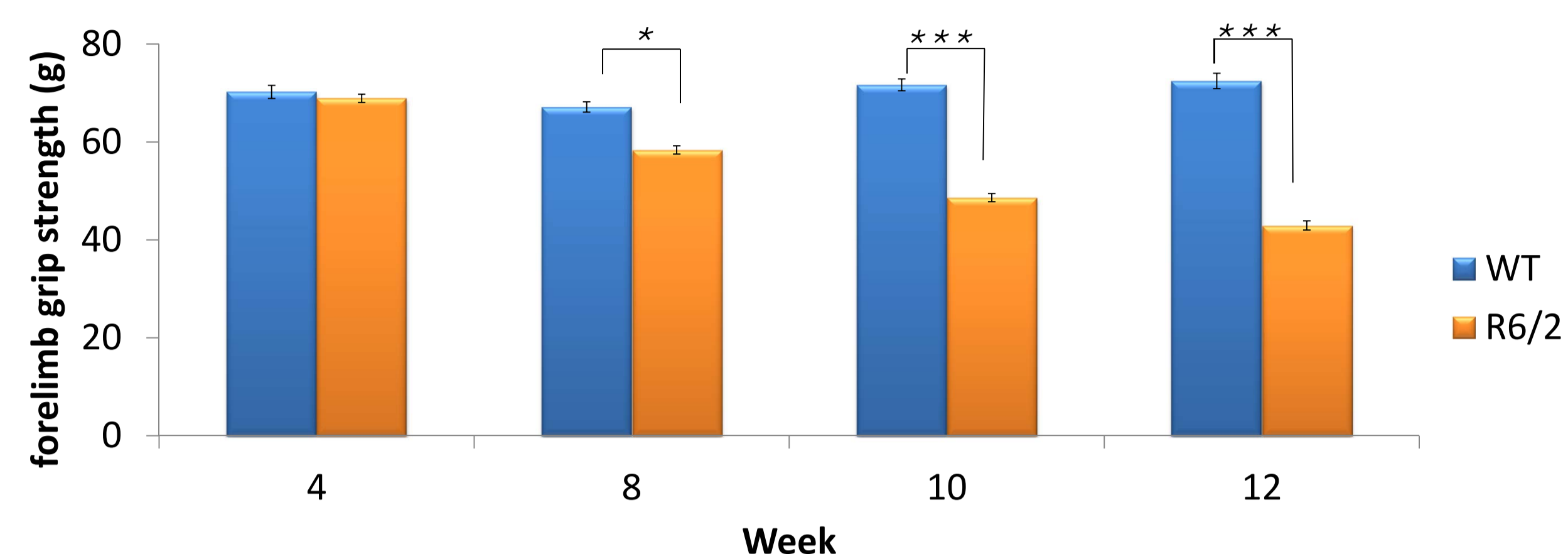
**Figure 1.** On the left the PhenoTyper cage, on the right an example of the velocity profile of a mouse. Lingering, Progressing and a third curve of very high velocities are clearly separated

## Results

The R6/2 mice showed a significant weight loss at 11 weeks of age (Figure 2). However using the grip strength test we could see a clear difference between the R6/2 and the littermates at week 8 (Figure 3)

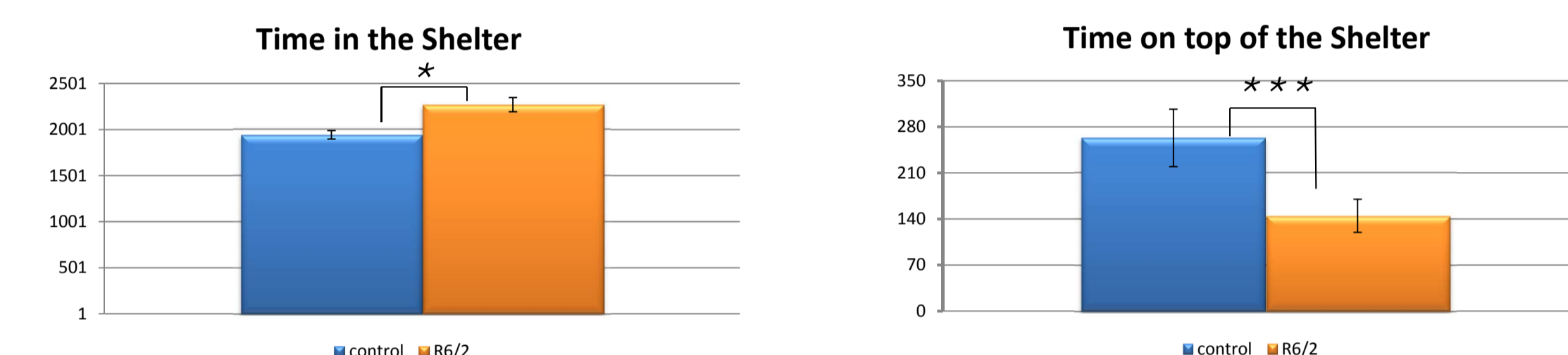


**Figure 2.** All the animals have initially a comparable weight. As expected the R6/2 start losing weight and the difference becomes significant at week 11 of age. Data are represented as mean ± SEM



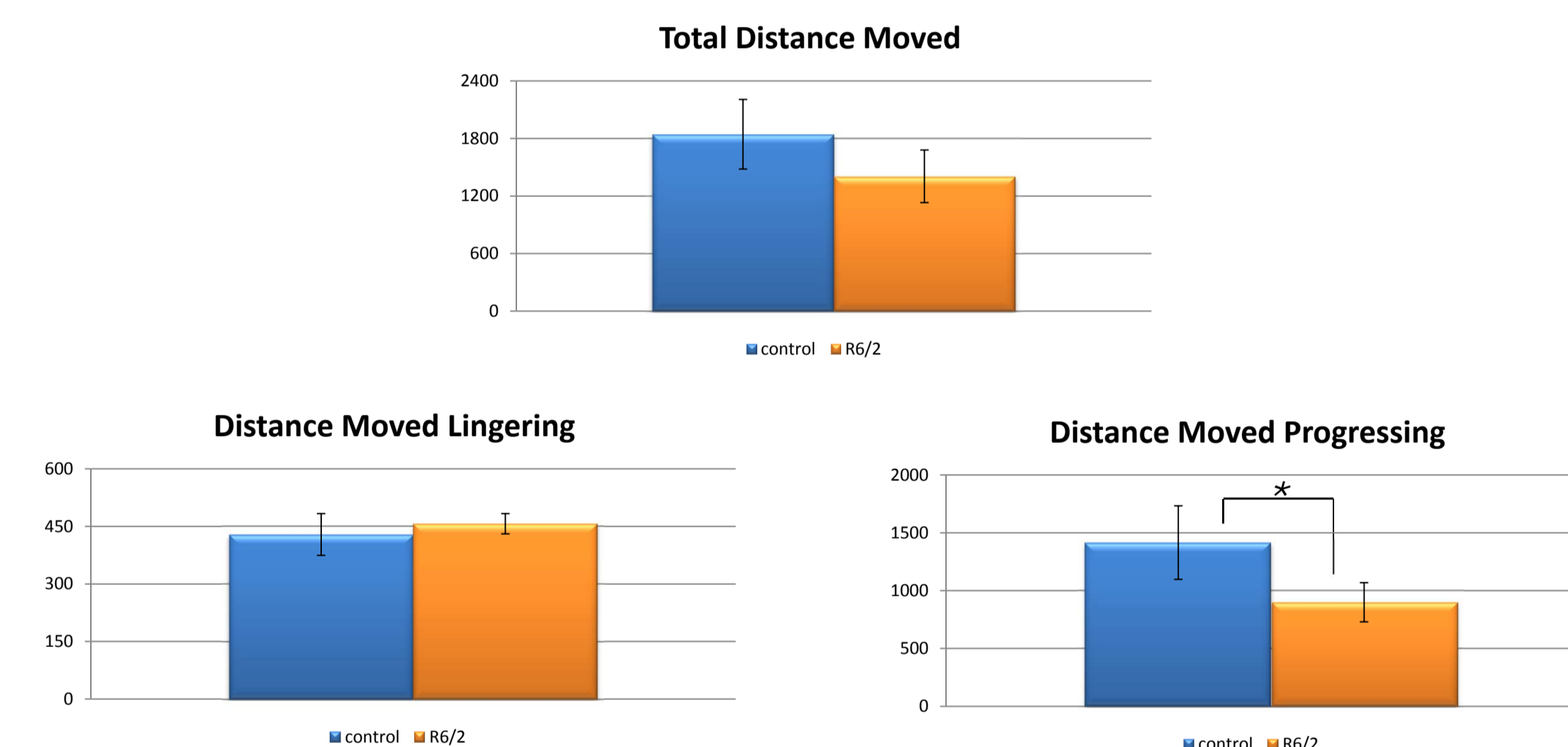
**Figure 3.** R6/2 mice show the first symptoms of muscular loss at 8 weeks of age. Asterisks indicate significant differences between wild-type control and R6/2 transgenic mice (\* $p < 0.05$ , \*\*\* $p < 0.0001$ ). Data are represented as mean ± SEM

However, the earliest behavioural signs in these mice are seen during the home cage testing in the PhenoTyper cages. The R6/2 showed a reduced activity level already at the age of 4 weeks. This is seen both in the time they spend inside the shelter (Figure 4, left) and on top of the shelter (Figure 4, right)



**Figure 4.** Both the duration in the shelter and on top of the shelter show a early clear difference in the behaviour of the R6/2 when compared to their wild type littermates. Asterisks indicate significant differences between wild-type control R6/2 transgenic mice (\* $p < 0.05$ , \*\*\* $p < 0.0001$ ). Data are represented as mean ± SEM

Moreover, the analysis of the activity level using parameters such as distance moved or velocity lead to the same results, showing symptoms of hypoactivity at the earliest age of 4 weeks. However, as shown in figure 5, only by differentiating lingering from progressing it is possible to see a difference in the spontaneous locomotor behaviour of these mice (Figure 5, bottom).



**Figure 5.** A significant difference in the distance moved by the mice is only visible while the mice are progressing. Asterisks indicate significant differences between wild-type control and R6/2 transgenic mice (\* $p < 0.05$ ). Data are represented as mean ± SEM

## Conclusion and future work

The presented approach reveals that behavioural signs of HD are evident already at the age of 4 weeks. The traditional measurements for phenotyping the R6/2 as a mouse model of HD such as weight loss and muscular strength decline are compared with our automated approach in which several other parameters can be extracted and analyzed.

Our latest development in describing the velocity parameter by making a velocity profile has improved the capabilities of the current system and has allowed us to better characterize our animal models.

Furthermore, the current home cage system allows automated cognitive testing which is now being explored. Gait analysis will be also part of future investigations, as it is an important feature of HD.

These early characteristics can be used for testing the efficacy of therapeutic treatments in an early phase of the disease when neuroprotective strategies may lead to slow or even stop the progression of the neurodegeneration.

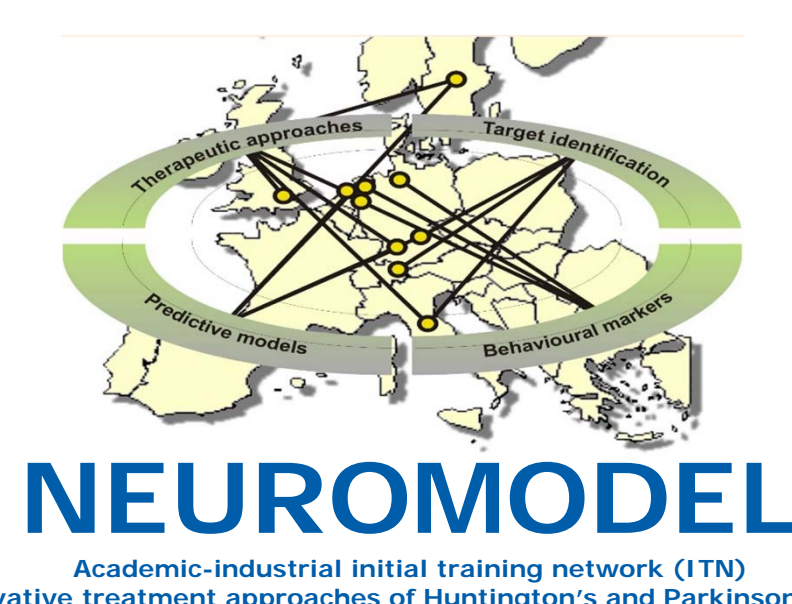
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Academic-industrial initial training network (ITN)  
on innovative treatment approaches of Huntington's and Parkinson's disease

