Longitudinal assessment of the effect of the removal of the neomycin resistance cassette on behavioral phenotype in z_Q175KI mice

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Introduction

Huntington's disease is caused by an expansion of a polyglutamine tract in exon 1 of the huntingtin (HTT gene). Of the many mouse models available for pre-clinical testing, heterozygous knock-in mice most closely resemble the genetic mutation responsible for Huntington's disease. Knock-in mouse lines have expanded polyglutamine (CAG) tracts in exon 1 of HTT (of either mouse or human origin) which are targeted to the mouse HTT allele. The z_Q175KI mouse line (C57BL/6J; CHDI-81003003; z_Q175) is a well characterized mouse model that arose from a spontaneous germ line expansion of the pure CAG tract in the CAG140KI line. This line expansion to an approximate repeat size of 175 was first identified at PsychoGenics. Through judicious breeding strategies this line has been stabilized to a CAG repeat number of approximately 190. In recent years, a new knock-in mouse line denoted z_Q175KI(neo-) (C57BL/6J; CHDI-81003019; z_Q175DN) with a comparable CAG repeat length has been generated by the genetic excision of the neomycin resistance cassette that was present in the 5' sequence flanking the HTT locus of the z_Q175 line. The excision of the neomycin resistance cassette results in a modest but significant increase in mutant HTT mRNA levels (Figure 1) and a trend towards an increase in soluble mutant Htt protein and inclusions.

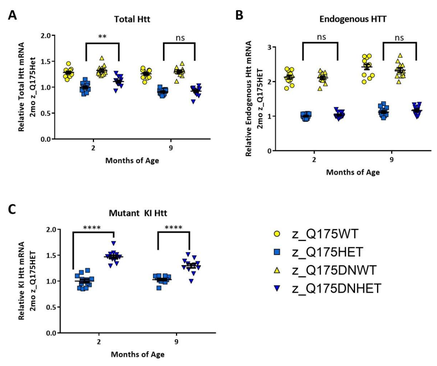


Figure 1: Excision of the neomycin resistance cassette results in increased mutant HTT mRNA levels in the striatum of z_Q175DN mice compared to the z_Q175 line at both young (2M) and old ages (9M). A) Total HTT mRNA levels, B) Endogenous HTT mRNA levels and C) Mutant KI HTT mRNA levels. QPCR data is normalized to 2M z_Q175HET and presented as mean ± SEM.

The effect, if any, of these changes in steady state mutant HTT mRNA on the behavioral phenotype has not previously been evaluated. We set out to compare the behavioral phenotypes of the z_Q175 and z_Q175DN lines (back-crossed onto a pure C57BL/6J background) utilizing PsychoGenics proprietary cubes platform technology and standard behavioral testing paradigms at 2, 4, 6 and 10 months of age.

Methods

Animals:

Male and female heterozygous and wild-type mice from the twolines z_Q175KI (C57BL/6J; CHDI-81003003; z_Q175) and z_Q175KI (neo-) (C57BL/6J; CHDI81003019; z_Q175DN) were generated at Jackson Laboratories and shipped to PsychoGenics for behavioral evaluation.

Animals were housed in homogenous genotype and line groups in OptiRat cages (n=10 per cage) for the duration of the study. Behavioral assessments were performed at 2, 4, 6 and 10 months of age.

Behavioral Analysis:

Phenocube®

PhenoCube® is a high-throughput platform that assesses circadian, cognitive, social and motor behavior exhibited by group-housed mice.

Smartcube®

SmartCube® is a platform that employs computer vision to detect changes in body geometry, posture and behavior both spontaneous and in response to particular challenges.

Neurocube®

The Neurocube® system is a platform that employs computer vision to detect changes in gait geometry and gait dynamics. Mice were tested for 5minutes in a rectangular Neurocube® chamber where mice were allowed move freely back and forth through the rectangular walkway. Complex bioinformatics algorithms are employed to subtle phenotypes related to gait.

Open Field

Locomotor activity was measured over a 30 minute interval in a plexiglas square chamber (27.3 x 27.3 x 20.3cm with 16 x 16 x 16 infrared photobeam sources (Med Associates Inc., St Albans, VT). Horizontal activity (distance traveled) and Vertical activity (rearing) were measured by consecutive beam breaks.

Tapered Balance Beam

The tapered balance beam test consisted of a training session (five trials) followed 24hrs later by a testing session (3 trials with an inter-trial interval of 2-3minutes). The tapered balance beam consisted of a tapered angled beam elevated from the floor with a goal box located at the steepest end. Video recordings of each mouses' three test session traversals were later manually scored for foot-slips.

Data Analysis:

Cubes analysis: data was only included from animals who completed all three cubes testing paradigms at 2, 4 and 6mths of age (10mth) animals were not analyzed due to testing attrition).

Open Field and Tapered balance beam: data was only included from animals who completed testing at 2,4,6 and 10mths of age.

Results

Early phenotypic differences are detectable in both lines, but disease progression differs using a Three Cubes Analysis

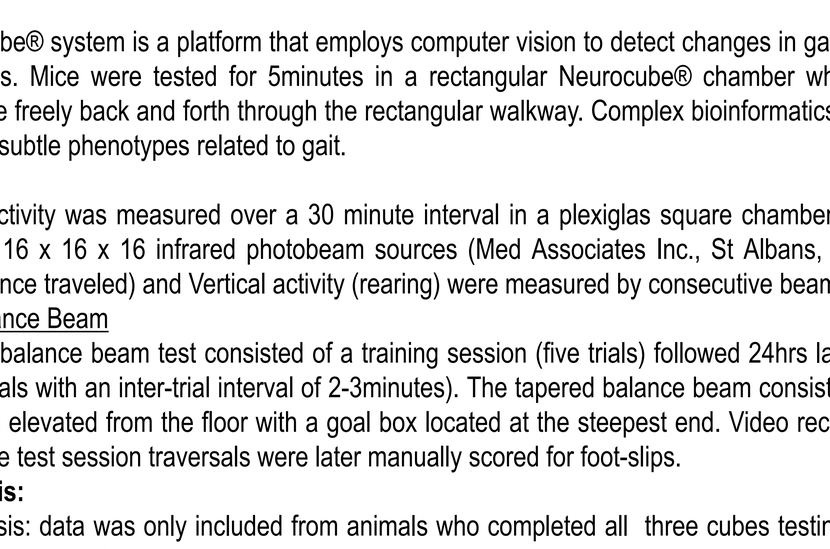


Figure 2: Cloud analysis and discrimination values in combined data assessment of SmartCube®, PhenoCube® and NeuroCube® of the two lines at 2, 4 and 6 months of age: z_Q175Het vs WT littermates; z_Q175DNHet vs WT littermates.

PhenoCube® can discriminate between z_Q175 and z_Q175DN mice by 6mths of age

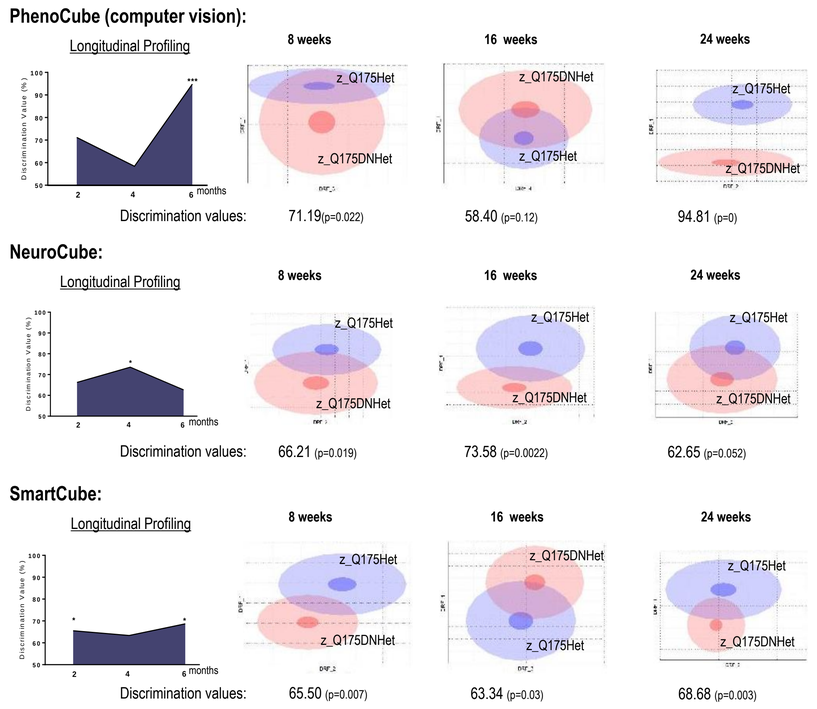
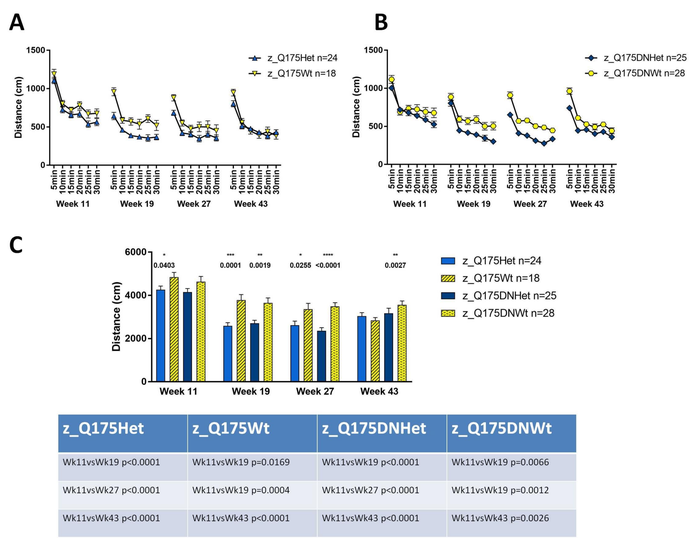


Figure 3: Cloud analysis and discrimination values in data assessment of PhenoCube®, NeuroCube® and SmartCube® individually by the two knock-in lines at 2, 4 and 6 months of age: z_Q175Het vs z_Q175DNHet.

Comparable age related decline in activity is detected in both z_Q175 and z_Q175DN lines



z_Q175Het	z_Q175Wt	z_Q175DNHet	z_Q175DNWt
Wk11vsWk19 p<0.0001	Wk11vsWk19 p=0.0169	Wk11vsWk19 p<0.0001	Wk11vsWk19 p=0.0066
Wk11vsWk27 p<0.0001	Wk11vsWk19 p=0.0004	Wk11vsWk27 p<0.0001	Wk11vsWk19 p=0.0012
Wk11vsWk43 p<0.0001	Wk11vsWk43 p<0.0001	Wk11vsWk43 p<0.0001	Wk11vsWk43 p=0.0026

Figure 4: Total distance travelled (cm) in 30 minutes of open field testing by z_Q175 and z_Q175DN lines at 2, 4, 6 and 10mths of age A) z_Q175Het vs z_Q175Wt B) z_Q175DNHet vs z_Q175DNWt C) z_Q175Het vs z_Q175DNHet. Data is presented as mean ± SEM of distance travelled in 5 minute bins. D) Sum of total distance travelled for heterozygous z_Q175 and z_Q175DN mice at 2, 4, 6 and 10mths of age. Data is presented as mean ± SEM.

❑Age related declines in activity (as measured by distance travelled) are detected in heterozygous mice from both lines. There is no detectable difference in activity between heterozygous mice from both lines at any age tested.

❑ Apparent differences in disease progression between the lines (as measured by comparison to their wild-type) is due to age related declines in performance of wild type mice.

Coordination Deficits are detected in z_Q175 at 10 months of age

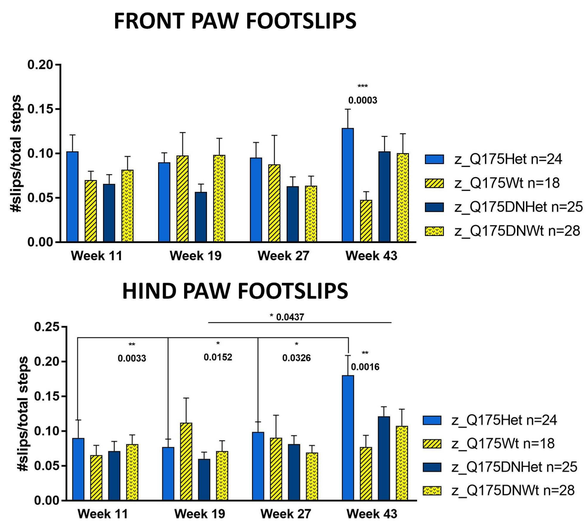


Figure 5: Number of front and hind paw footslips (normalized to total number of steps) at 2, 4, 6 and 10 months of age. Data is presented as mean ± SEM. No statistically significant differences were detected in traverse time between the three lines or their wild-type counterparts at any age tested.

❑ Coordination deficits are detectable at older ages in z_Q175 line but not the z_Q175DN line when comparing heterozygotes to their wild-type littermates.

❑ The apparent difference in disease progression between the lines (as measured by comparison to their wild-type) is due to age related declines in performance of wild type mice from the z_Q175DN line.

Conclusions

❑ Standard behavioral testing paradigms including open-field and tapered balance beam do not reveal phenotypic differences between heterozygous mice from the two lines even at older ages (10mths).

❑ However, Phenocube® is able to robustly discriminate between the z_Q175 and z_Q175DN lines at 6mths of age.

❑ Apparent differences in disease progression between the z_Q175 and z_Q175DN lines observed in Cubes and standard behavioral testing paradigms is most likely due to reduced performance of their wild-type littermates with age rather than an effect of the excision of the neomycin resistance cassette.