RETINAL AND STRIATAL PROFILING OF THE R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

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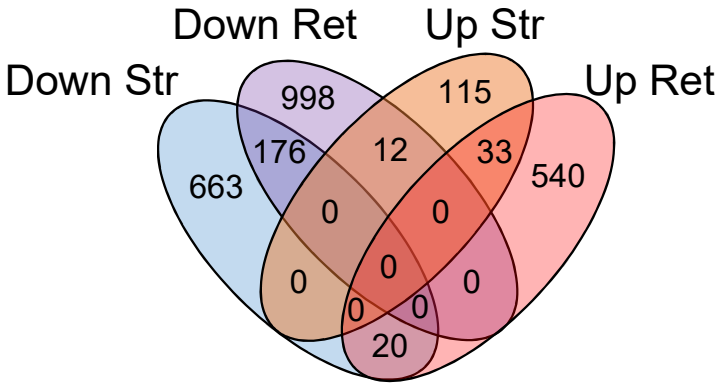
INTRODUCTION

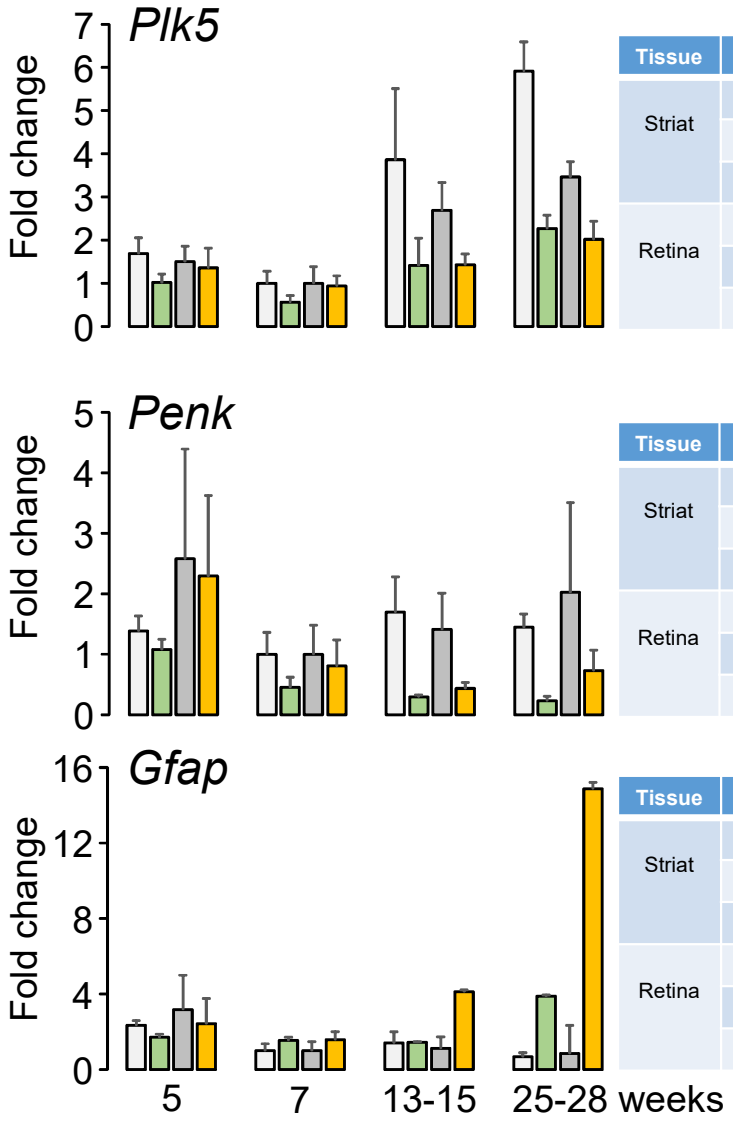
Increasing evidence indicates compromised structural and functional integrity of visual pathways in Huntington's disease (HD) mouse models and patients. The retina can be an accessible window of the central nervous system through non-invasive methods to monitor the health status and response to potential treatments in patients. However, no correlation has been firmly established between the molecular events occuring in the retina and in the most affected brain area by the HTT polyQ expansion, the striatum.
The aim of this study is to explore the molecular and cellular alterations linked to the disease in the retina of the R6/1 strain in order to define its suitability for biomarker screening.

1. R6/1 RETINAS SHOW EXTENSIVE TRANSCRIPTIONAL DYSREGULATION



Tissue	Change	Top Tissue Expression / Biological Process term
Striatum	Down	UP_TISSUE: Brain
		GO_BP: Intracellular signal transduction
	Up	UP_TISSUE: Brain
		GO_BP: Cell adhesion-related
Retina	Down	UP_TISSUE: Retina
		GO_BP: Visual perception
	Up	UP_TISSUE: Bone marrow
		GO_BP: Immune system process

RNA-seq analysis:
- Differentially expressed genes (DEG) between 15 weeks-old R6/1 and wt littermates in striatum (Str) and retina (Ret), adj *P*-value < 0.05
- Top terms from DAVID Gene Enrichment tool



Tissue	Variable	F, d.f.	*P*-value
Striat	Genotype	7.91, 1	0.007
	Age	5.14, 3	0.004
	Interact	1.51, 3	0.226
Retina	Genotype	4.96, 1	0.032
	Age	5.89, 3	0.002
	Interact	1.58, 3	0.218

Tissue	Variable	F, d.f.	*P*-value
Striat	Genotype	14.48, 1	4×10^{-4}
	Age	1.09, 3	0.362
	Interact	1.52, 3	0.223
Retina	Genotype	0.92, 1	0.344
	Age	1.20, 3	0.324
	Interact	0.13, 3	0.944

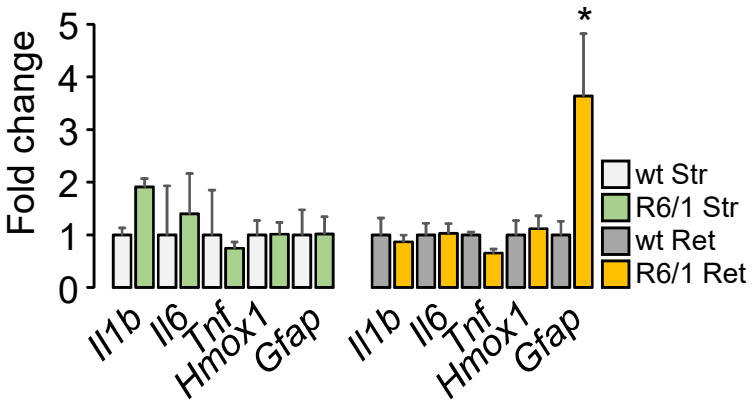
Tissue	Variable	F, d.f.	*P*-value
Striat	Genotype	0.57, 1	0.455
	Age	0.73, 3	0.538
	Interact	1.70, 3	0.183
Retina	Genotype	9.37, 1	0.004
	Age	8.57, 3	2×10^{-3}
	Interact	10.88, 3	3×10^{-5}

Time-course analysis of selected DEG in RT-qPCR assays using independent samples. Two-way ANOVA results in the tables: significant *P*-values < 0.05

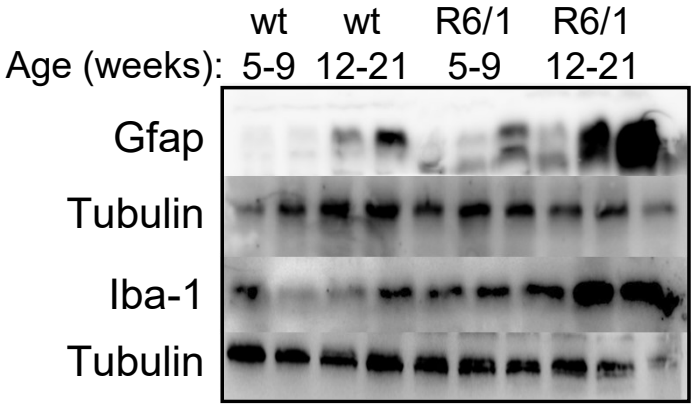
wt Str
R6/1 Str
wt Ret
R6/1 Ret

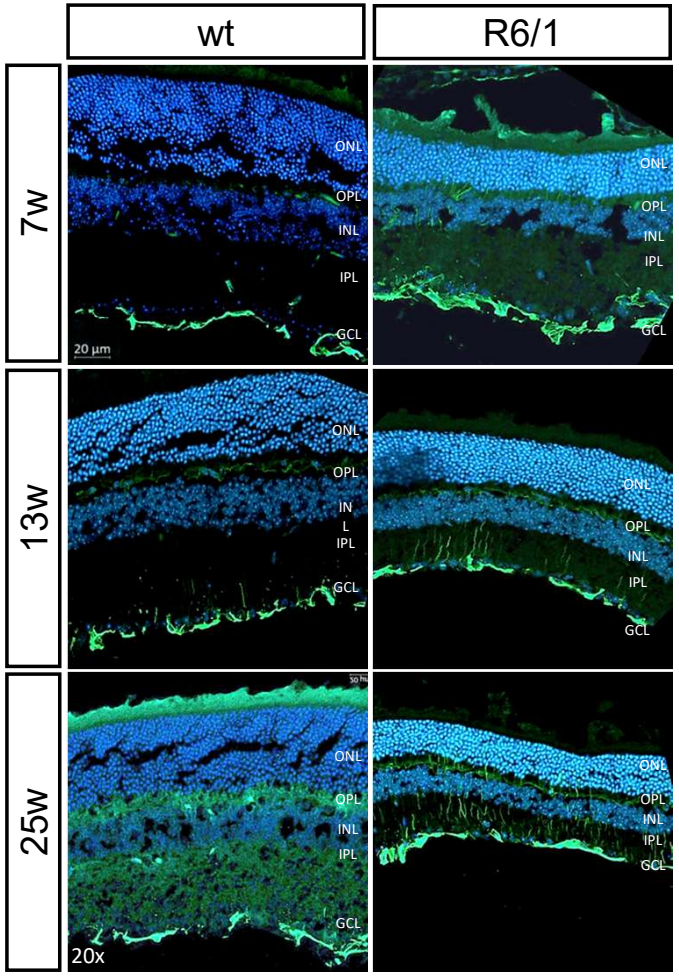
2. GFAP AND IBA-1 ARE INDUCED IN R6/1 RETINAS

RT-qPCR assays in 13-15 weeks-old R6/1 and wt littermates: *, *P*<0.05, Mann-Whitney U-test. In agreement with RNA-seq results

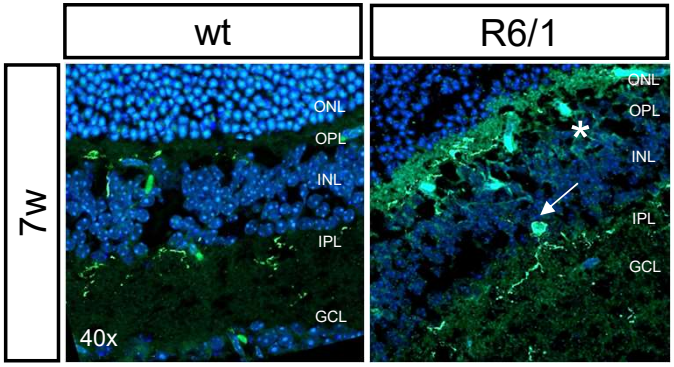


Western blotting assays in R6/1 and wt retinas

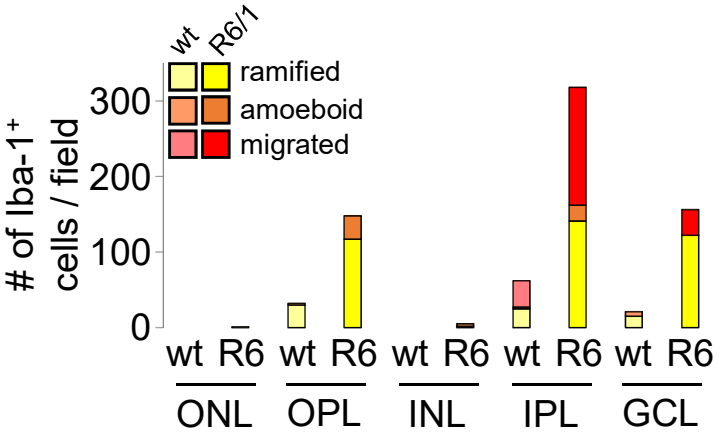




Gfap / DAPI: increasing staining of GCL and OPL, later comprising INL and IPL

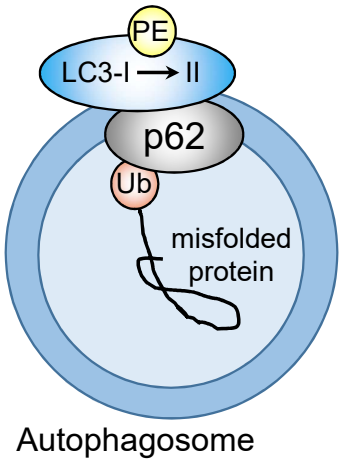


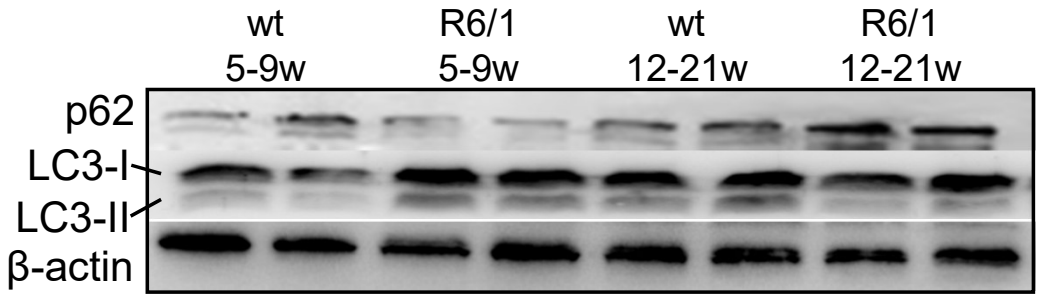
Iba-1 / DAPI: arrow, ramified resting microglia; *, ameboid microglia

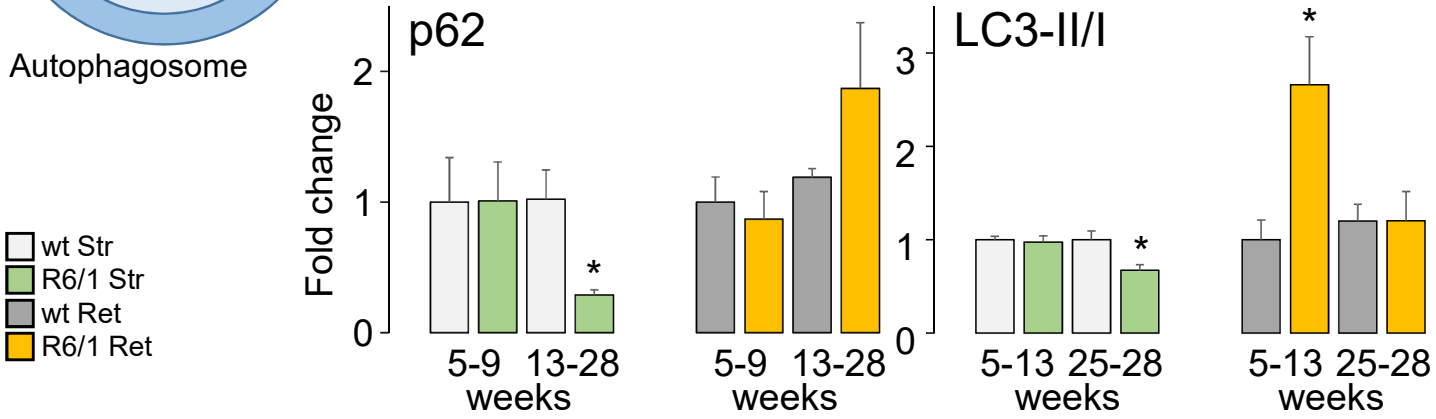


Morphology analysis indicates microglia activation. O, outer; I, inner; N, nuclear; P, plexiform; GCL, ganglion cells layer

3. LC3 AND p62 PROFILES ARE DIFFERENT IN THE RETINA AND STRIATUM OF R6/1 MICE

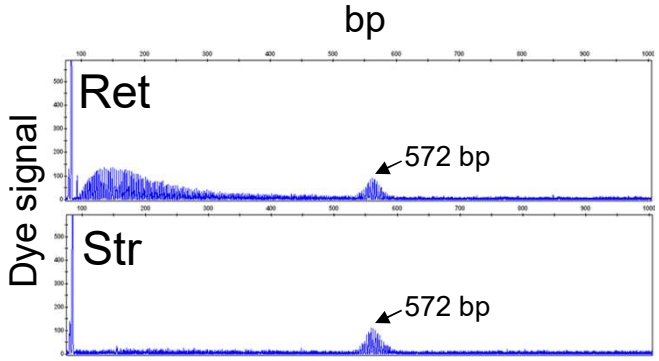


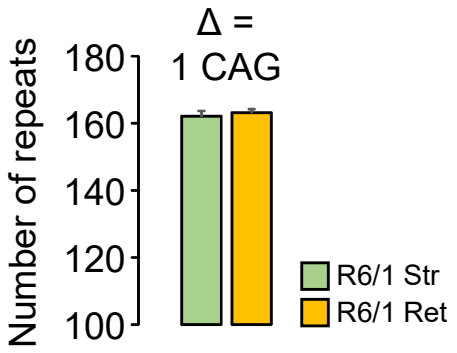




Western blotting analysis of R6/1 and wt. *, *P*-value < 0.05 Mann-Whitney U-test. Representative blots from retina are shown. Autophagy may be altered in the retina (autophagic induction in early stages) and in the striatum (autophagic flux impairment in advance stages). p62 and LC3 may be compensated in retina by upregulation of *SQSTM1* and *MAP1LC3B* genes, respectively (RNA-seq results).

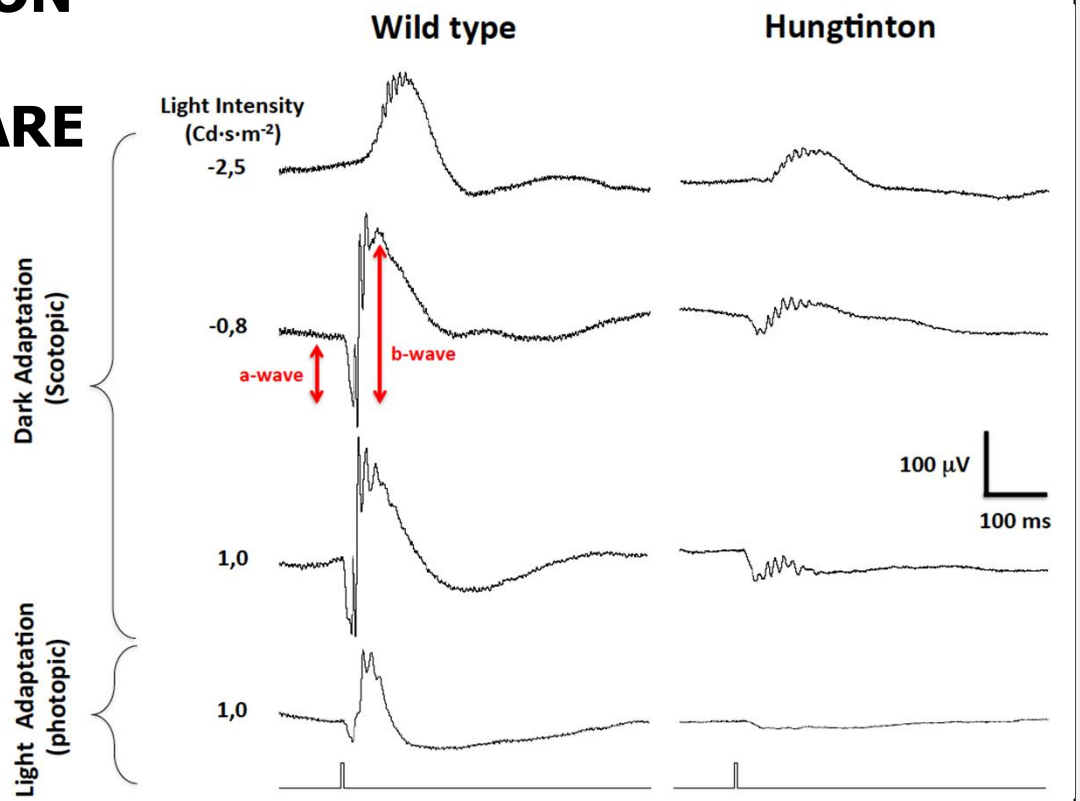
4. INSTABILITY OF CAG REPEATS DO NOT EXPLAIN TISSUE DIFFERENCES





CAG repeat analysis in retina and striatum of the same R6/1 animals

5. FUNCTION OF R6/1 RETINAS ARE IMPAIRED



Electroretinogram of 24 weeks-old R6/1 and wt littermates

CONCLUSIONS

* R6/1 retinas show a profound transcriptional dysregulation containing an important retinal-specific component.
* There is a delimited inflammatory response characterized by gliosis and microglia activation but without the induction of other markers. The presence of degeneration-related markers in R6/1 retina justifies further exploration in other models. This is not clearly evident in the striatum.
* Currently, we are exploring in further detail autophagic processes as a potential explanation for retina malfunctioning in the R6/1 mice.

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