Subcellular Localisation of Histone Deacetylase (HDAC) 4 and HDAC5 at the Synapse in R6/2 Mice

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ABSTRACT

The disruption of synaptic connections in patients with Huntington's disease (HD) in part is attributed to early cognitive decline and neuronal connection loss. Therefore, understanding how mutant Huntingtin (HTT) affects synaptic function is critical for unravelling the selective cortico-striatal neuropathology seen in HD. Histone deacetylase (HDAC) 4 and HDAC5 are transcriptional repressors which contain glutamine rich domains. Recently, HDAC4 was shown to associate with HTT in a polyglutamine-length dependent manner and co-localise with cytoplasmic inclusions. Furthermore, upon the genetic reduction of *Hdac*4, HD phenotypes were ameliorated by delaying cytoplasmic aggregate formation and rescuing neuronal and cortico-striatal synaptic function in HD mouse models. This was accompanied by an improvement in motor co-ordination; neurological phenotypes and lifespan. Given this crucial role for HDAC4 in the cytoplasmic aggregation process in the molecular pathology of HD, here, we performed cell fractionations to further ascertain the subcellular localisation of HDAC4 and HDAC5. Using sucrose gradient centrifugation methodologies, either cytoplasmic versus nuclear fractionations, or synaptosomes enriched with pre- and post-synaptic proteins were isolated from fresh R6/2^{Q200} transgenic brain tissues, to study cellular localisation and/or synaptic changes. Our results showed that both HDAC4 and HDAC5 steady state levels are predominately cytoplasmic and reduce with age, regardless of genotype. Interestingly, both HDAC4 and HDAC5 (along with HDAC3) were also present at the mammalian synapse. Given this observation, future work will now address what role(s) HDACs play at the synapse in the aggregation process, given that historically HDACs have been typically classified as *bona fide* "nuclear" histone deacetylases.

Fig.1: HDAC4 / HDAC5 are predominantly cytoplasmic in the mouse brain

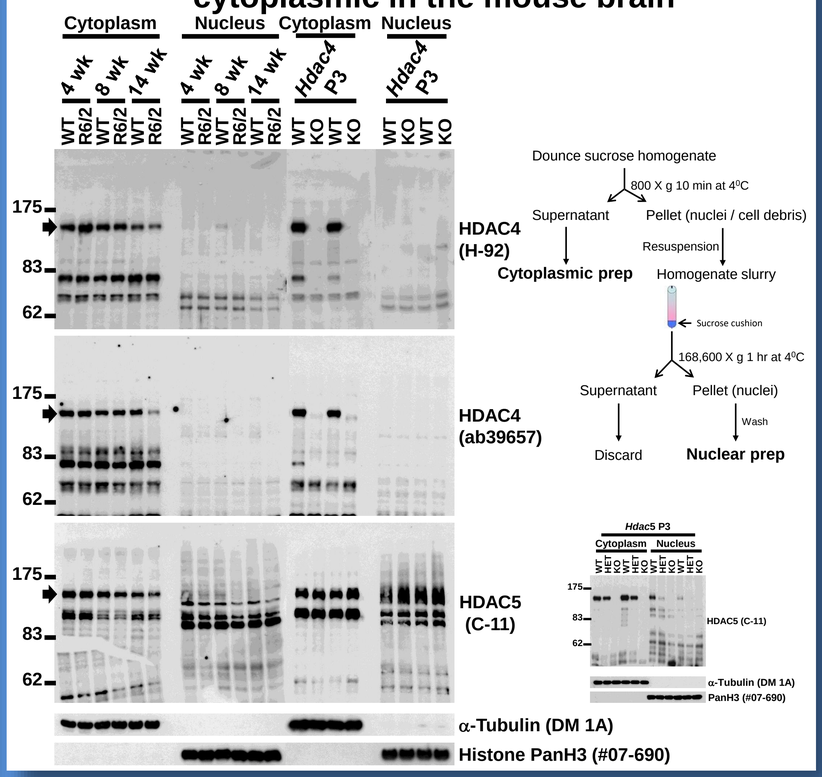


Fig.2: Sucrose gradient centrifugation of synaptosomes

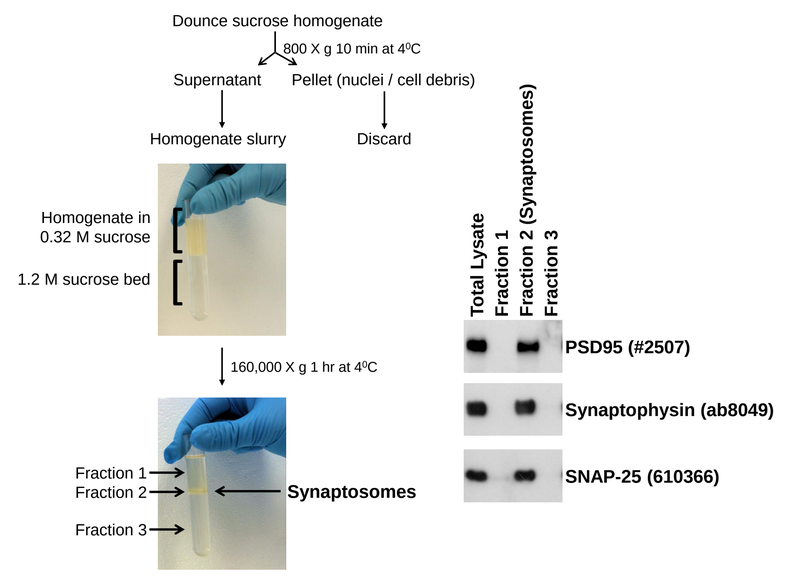


Fig.3: Western blotting analysis of synaptosomes from R6/2 brain

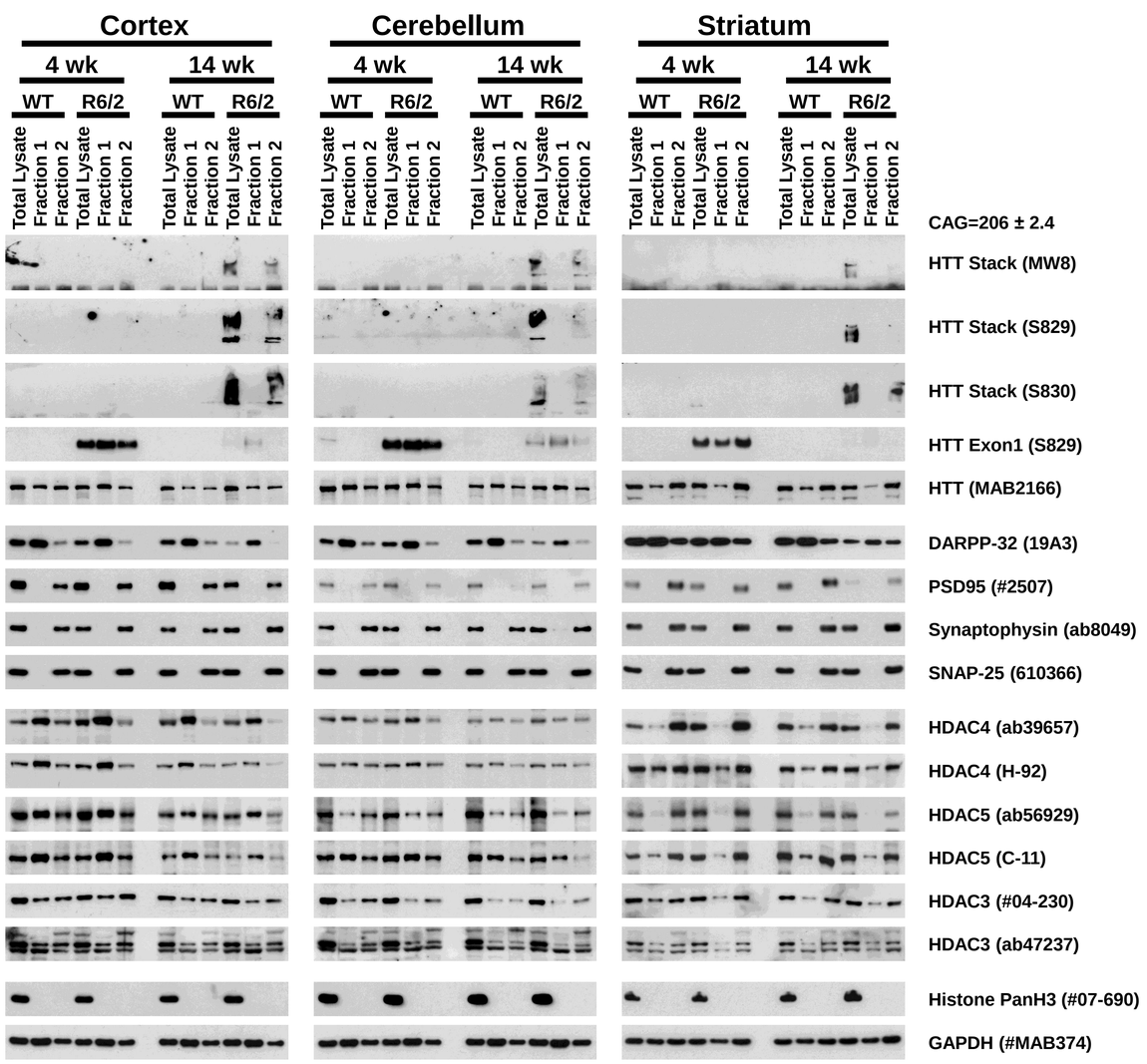
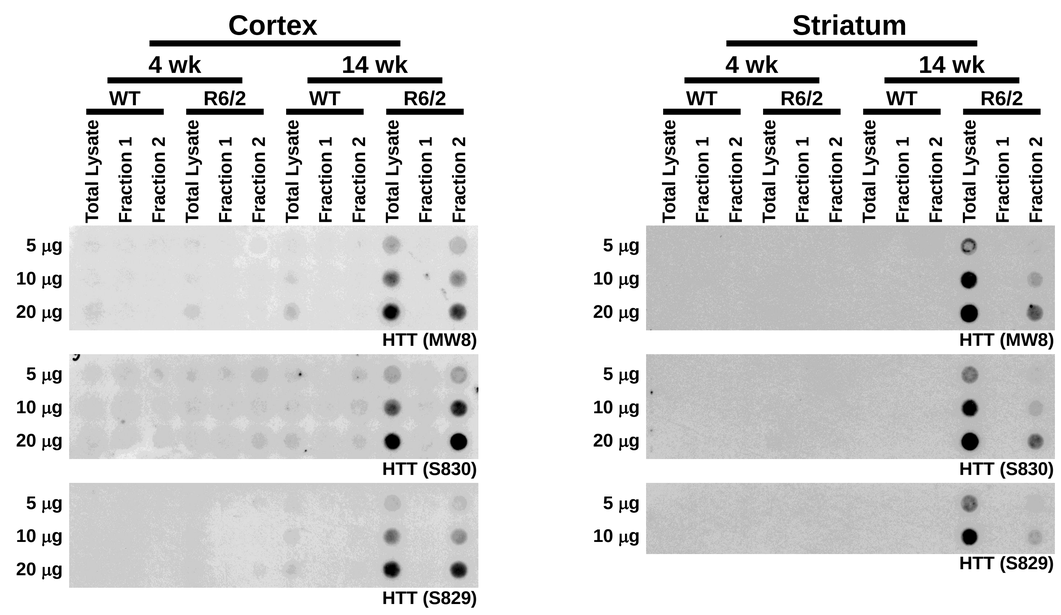


Fig.4: Filter-trap analysis of synaptosomes from R6/2 brain



SUMMARY

• By cytoplasmic and nuclear fractionation in brain, HDAC4 and HDAC5 are localised predominantly in the cytoplasm, and steady state levels were reduced with ageing (4 wk to 14 wk). Trace amounts were detected in the nucleus.

• By synaptosome fractionation of brain tissues, HDAC4, HDAC5 (and HDAC3) were detected in the synaptosome purified fraction. In the cortex and cerebellum, both HDAC4 and HDAC5 were localised predominately in cytosolic compartment (Fraction 1), whereas in the striatum both HDAC4 and HDAC5 were localised predominately in the synaptic compartment (Fraction 2).

• In R6/2 mice, both HDAC4 and HDAC5 levels were reduced at the synapse with disease severity, whereas HDAC3 levels remained unchanged.