Procedural memory-associated transcriptome and epigenome are severely impaired in Huntington's disease mice

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Introduction

Huntington's disease (HD) is a genetic neurodegenerative disease leading to cognitive, motor and psychiatric symptoms, due to primary dysfunction and degeneration of medium spiny neurons (MSN) in the striatum. The molecular mechanism underlying behavioural deficits in HD remains unclear. Epigenetic and transcriptional mechanisms are essential for learning and memory processes, through chromatin dynamic regulation (including histone acetylation and chromatin looping) and transcriptional programs controlling neuronal transmission and synaptic plasticity. Here we investigated whether such mechanisms might be impaired in HD. We showed in previous studies that epigenetic and transcriptional regulations are altered in HD striatum, in basal condition (Achour *et al.* 2015; Le Gras *et al.* 2017; Merienne *et al.* 2019; Alcalá-Vida *et al.* 2021). Specifically, we found that genes defining the identity and function of MSN (e.g. *Drd1*, *Drd2*, *Rgs9*, *Pde10a*…) are selectively down-regulated in HD striatum, and this associates with reduced H3K27 acetylation (H3K27ac). However, it remains to be determined whether altered epigenetic gene regulation contributes to HD behavioural symptoms. To address the question, we have investigated HD epigenome and transcriptome, combining ChIPseq, circular chromatin conformation capture (4C-seq) and RNAseq in the context of learning/memory. We have used a behavioural test assessing striatum-dependent learning/memory based on the double-H maze (e.g. procedural learning/memory, Cassel *et al.* 2012). Our results reveal selective impairment of procedural learning and memory from early symptomatic stage in HD R6/1 mice. In agreement with this, learning/memory-induced transcriptome was severely compromised in the striatum of R6/1 mice. At epigenetic level, we first investigated 3D chromatic architecture using the striatum of R6/1 and control behaving mice (e.g. subject to the double-H task), as the regulation of chromatin looping is a key memory-associated mechanism, notably controlling the induction of immediate-early genes (IEGs), through modulation of enhancer-promoter interactions (Watson and Tsai, 2017). We generated 4C-seq data at basal stage and during learning process for crucial IEGs and control genes. Chromatin looping was specifically regulated by memory process at IEGs, and to similar extent in R6/1 and WT contexts. Additionally, we investigated the effects of striatal learning/memory formation on histone acetylation genome-wide using ChIP-seq. We generated H3K27ac, H3K9ac and RNA Polymerase II (RNAPII) ChIP-seq data for WT and R6/1 animals in basal and behaving conditions. Our data showed robust alterations in R6/1 mouse striatum as compared to WT animals for H3K27ac, H3K9ac and RNAPII, consistent with previously described HD signatures. In addition, H3K9ac was significantly modulated by striatal learning/memory process in WT animals, an effect significantly attenuated in R6/1 mice. To gain insights into mechanisms leading to impaired learning-induced histone acetylation in R6/1 mice, we integrated cell-type specific ChIP-seq data generated by fluorescent-activated nuclear sorting (FANS) using NeuN as a selection marker. We observed that learning/memory-induced increase in H3K9ac was enriched at glial-specific enhancers involved in oligodendrocyte differentiation and myelin formation in WT mice striatum, while this signature was impaired in R6/1 mice. Thus, using genome-wide molecular approaches on behaving animals, we show for the first time that dynamic regulation of striatal epigenome and transcriptome during cognitive task are severely compromised in HD mice. More specifically, our results uncover that H3K9ac is dynamically modulated by striatal-dependent learning/memory, and that this process is impaired in HD mouse striatum, suggesting a critical role of epigenetic alterations in HD cognitive symptoms.

1. Procedural memory is progressively impaired in R6/1 mice

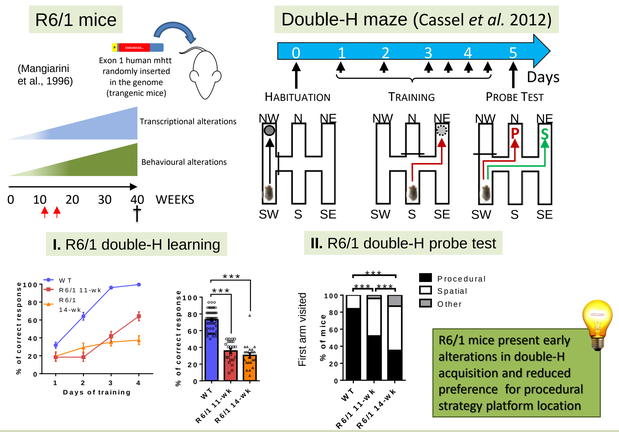


Figure 1. Procedural memory is early impaired in HD R6/1 mice. On top, explanatory scheme of R6/1 mice model and procedural memory task using the double-H maze and time points. **I.** Left panel, graph showing the curves of tendency of the percentage of correct response (mean ± sem) in 11 and 14 wk-old WT and R6/1 mice. Two-ways ANOVA with repeated measures, with Tukey post-hoc test. Right panel, bar graph showing mean percentage of correct response across the 4 days of training (+/- sem). Kruskal-Wallis test, with Dunn post-hoc test, ***p<0,005, R6/1 vs WT. **II.** Bar graphs showing first and second arms visited during the probe test for 11 and 14 wk-old WT and R6/1 mice. Binomial test (Percentage of mice visiting procedural arm vs other arms; Percentage of mice visiting spatial arm vs other arms), *p<0,05, ***p<0,005, R6/1 vs WT.

2. Memory-associated transcriptome is early compromised in R6/1 mouse striatum

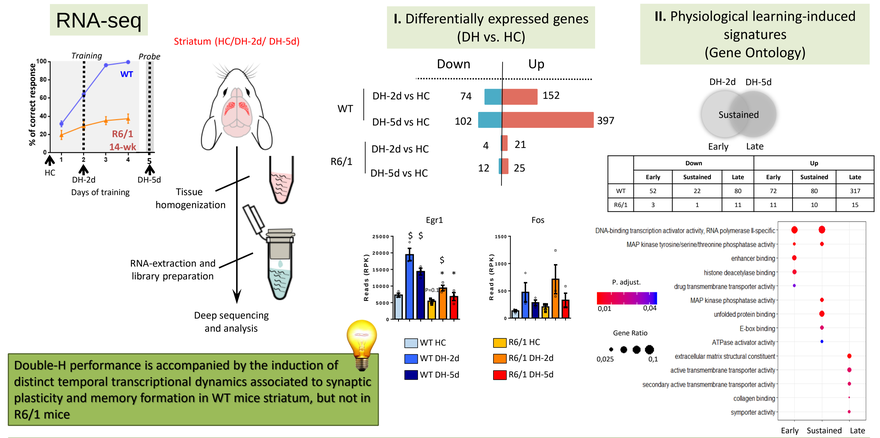


Figure 2. Memory-regulated transcriptome is impaired in HD R6/1 mouse striatum. On the left, explanatory scheme showing the time points used for RNA-seq data generation. **I.** On top, bargraphs showing the numbers of differentially expressed genes (FDR < 0.1) in Double-H day 2 (DH-2d) vs HomeHC and DH-5d vs HC comparisons, in WT and R6/1 striatal samples. On the bottom, bargraphs representing mRNA levels (mean ± sem) of the immediate early genes *Egr1* and *Fos* in striatal samples. Expression values were computed from RNAseq data. RPK, reads per kilobases. The Benjamini and Hochberg method was used for multiple testing correction. **II.** On top, explanatory Venn-diagram illustrating early, sustained and late genes, corresponding to genes only changed at 2 days vs HC, genes changed at both 2 and 5 days vs HC and genes changed only at 5 days vs HC, respectively and summary table of number of genes per category. On the bottom, Gene Ontology analysis of early, sustained and late up-regulated genes in WT. Significant biological processes are shown using dot size proportional to gene ratio and heatmap reflecting adj. pvalue. To note, Early genes were enriched in terms associated to transcriptional regulation (as well as part of Sustained genes); Sustained genes were enriched in processes related to protein folding, ATPase activity and MAP kinase pathways and Late genes were enriched in terms related to extracellular matrix and transmembrane transport activity (synaptic plasticity).

3. Memory process induces dynamic changes in chromatin looping at Immediate early genes, which is not altered in R6/1 striatum

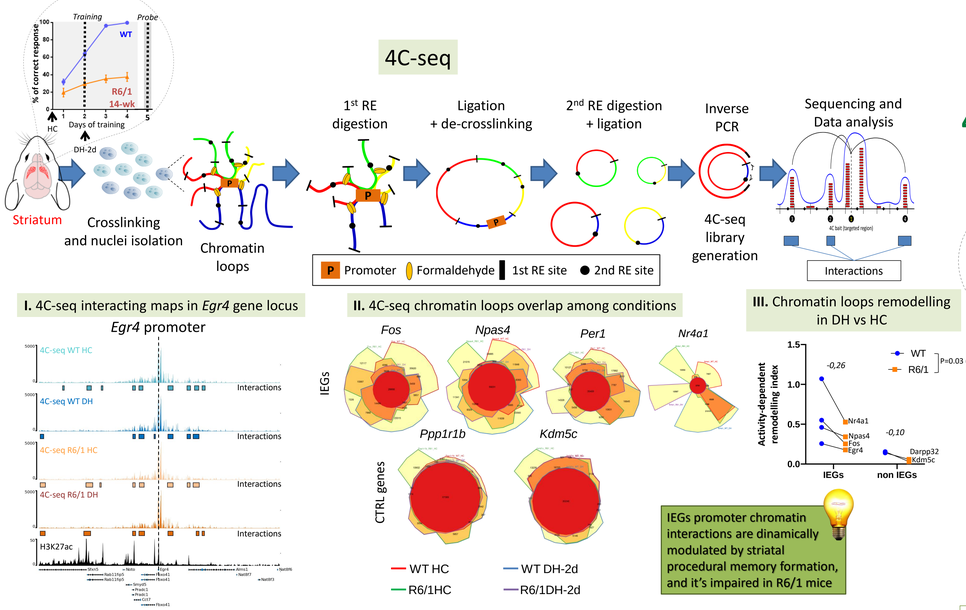
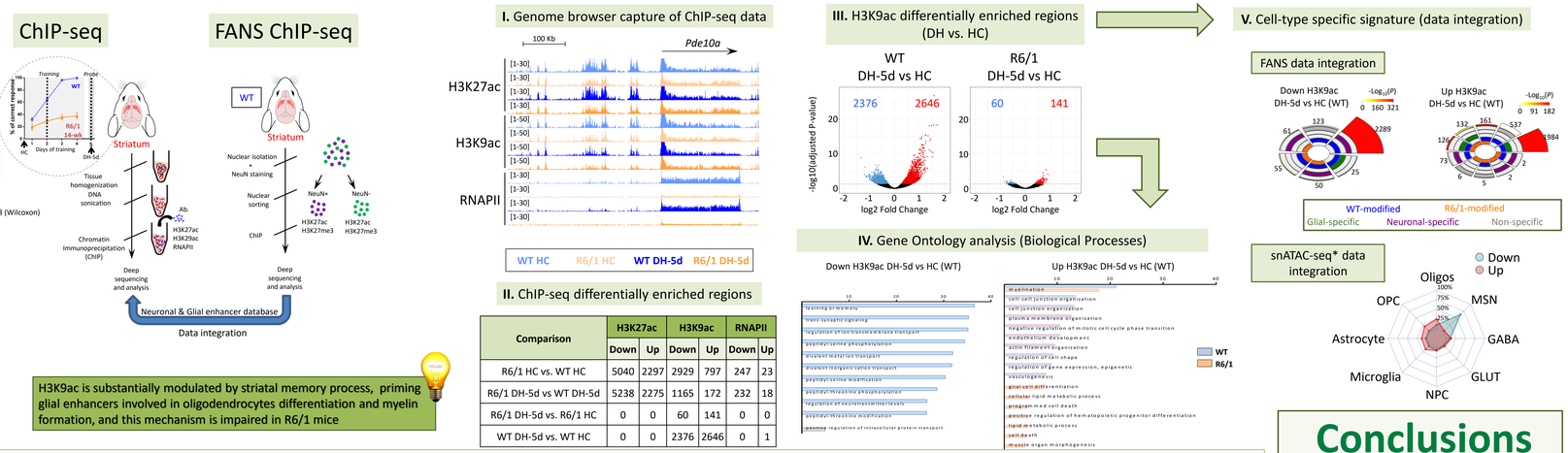


Figure 3. Memory-induced chromatin loop remodelling in WT and R6/1 mouse striatum. On top, scheme showing 4C-seq-major steps using WT and R6/1 mouse striatum at basal and at 2 days of memory process in the double-H maze. In brief, nuclear preparations are generated from frozen striatal tissue and chromatin loops are stabilized by a cross-linking step using formaldehyde. Afterwards, a fist restriction enzyme (RE) is used to fragment the chromatin, preserving the native chromatin interactions and posteriorly de-crosslinked and ligate, generating circular DNA products of inter-spaced interacting DNA regions. These products are digested with a second RE and ligate to generate smaller fragments. Finally, 4C-seq libraries containing all promoter-regulatory regions interactions are generated by inverse PCR targeting specific gene promoters. **I.** Examples of 4C-seq interacting maps at Egr4 gene locus using WT (blue) and R6/1 (orange) mouse striatum in basal (faint) or DH-2d (dark) stages. 4C-seq quartile normalized read counts are plotted as the main lane for each condition and regions interacting significantly with *Egr4* promoter are shown as blocks below each track. H3K27ac coverage is shown in black and gene annotations are included in the bottom track. **II.** Area-proportional Chow-Ruskey plots showing the overlap between significant promoter interacting regions detected in 4C-seq data across the different experimental conditions (WT HC, R6/1 HC, WT DH-2d, R6/1 DH-2d). Data were generated using several immediate early genes (*Fos, Npas4, Egr4 and Nr4a1*) and control genes, which were not transcriptionnally modulated by memory process (*Ppp1r1b* and *Kdm5c*). Of note, the overlap between the different conditions is greater for *Ppp1r1b* and *Kdm5c* genes as compared to *Fos*, *Npas4, Egr4* and *Nr4a1* genes, indicating more dynamic changes in chromatin loop at genes actively participating in memory-related transcriptional programs. **III.** 3D chromatin remodeling index showing doubl-H induced changes at IEGs and Ctrl genes for WT (blue) and R6/1 (orange) mouse striatum, calculated as : (number of chromatin interaction nucleotides specific to double-H) / (total number of chromatin interaction nucleotides obtained for home-cage).

4. Memory-associated histone acetylation is early compromised in R6/1 mouse striatum



Comparison	H3K27ac		H3K9ac		RNAPII	
	Down	Up	Down	Up	Down	Up
R6/1 HC vs. WT HC	5040	2297	2929	797	247	23
R6/1 DH-5d vs WT DH-5d	5238	2275	1165	172	232	18
R6/1 DH-5d vs. R6/1 HC	0	0	60	141	0	0
WT DH-5d vs. WT HC	0	0	2376	2646	0	1

Figure 4 H3K9ac is a procedural memory substrate early impaired in HD R6/1 mouse striatum. On the left, scheme showing major steps of ChIP-seq approach and datasets generated using whole striatal tissue from WT and R6/1 mice in basal (home-cage, HC) or in learning (double-H 5 days, DH-5d) conditions and in WT mouse striatum in NeuN+ and NeuN- populations using Fluorescence-activated Nuclear Sorting (FANS). **I.** UCSC genome browser capture showing representative H3K27ac, H3K9ac and RNA Polymerase II (RNAPII) signals in the striatum of WT and R6/1 mouse striatum at basal (home-cage, HC) or in learning (double-H 5 days, DH-5d) conditions at *Pde10a* gene, a striatal identity genes, in the adult striatum. **II.** Table summarizing the number of differentially enriched regions for H3K27ac, H3K9ac and RNAPII ChIP-seq data for the different biological conditions analysed using deseq2 (N=2). **III.** Volcano plots representation of H3K9ac differential enriched regions in WT (left) and R6/1 (right) mouse striatum between basal (home-cage, HC) and learning (double-H 5 days, DH-5d) conditions (FDR<0.05) for WT (blue) or R6/1 (orange) mouse striatum ChIP-seq data. **V.** On top, circular plots illustrating all possible intersections between different sets of genomic regions and the corresponding statistics. The five tracks in the middle represent the five genomic regions sets (H3K9ac WT-modified, H3K9ac R6/1-modified, H3K27ac glial-specific, H3K27ac neuronal-specific and H3K27ac non-specific regions), with individual blocks for each colour indicating the presence or absence of the genomic regions sets in the particular intersection. The height of the bars in the external layer is proportional to the intersection sizes (number of intersecting regions indicated by the numbers on the top of the bars) and the color intensity of the bars represents the P value significance of the intersections. On the bottom, radar plot showing the cell-type specific distribution of H3K9ac regions significantly (adj. P value <0.05) decreased (DOWN, blue) or increased (UP, red) in WT mice striatum between basal basal (home-cage, HC) and learning/memory (double-H 5d, DH-5d) conditions when intersected with top cell-specific striatal chromatin accessible regions from single nuclei ATAC-seq data (Zhong et al, 2020*). Values represent the percentage from total number of intersecting regions per category (DOWN or UP).

Conclusions

- **Procedural learning and memory alterations in R6/1 mouse striatum establish at early disease stage**
- **Memory-associated transcriptional programs are impaired in R6/1 mouse striatum**
- **Chromatin looping at immediate early genes is dynamically regulated upon striatal memory process, and is substantially altered in R6/1 mouse striatum**
- **H3K9ac is a striatal memory substrate implicated in epigenetic priming of myelin genes, and this mechanism is impaired in R6/1 mice**

Bibliography

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