Altered basal ganglion activity in the R6/1 model of Huntington's disease

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INTRODUCTION

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by polyglutamine expansion of the CAG trinucleotide in HD gene coding for Huntingtin protein. This expansion causes selective alteration of medium spiny projection neurons (MSN) in striatum, while fast-spiking Interneurons seem spared, at least, at early stage of the disease.

We have recently performed single-unit recording of striatal activity in freely behaving R6/1 mice (Cayzac et al, PNAS, 108, 9280, 2011). This study has shown that MSNs are scarcely recruited during a procedural learning task which requires the integrity of striatum and basal ganglia circuit.

Here, we report a quantitative analysis of striatal activity using c-fos immunostaining, approach being impossible with single-unit recording, to confirm our previous finding. Fos imaging also has a considerable advantage because the analysis could be extended to different relays of basal ganglia loop and their input and output structures.

To map disease-associated changes in c-fos activity patterns in R6/1 mice, we investigated early (2 months), pre motor-symptomatic (4 months) and motor-symptomatic (6 months) ages .

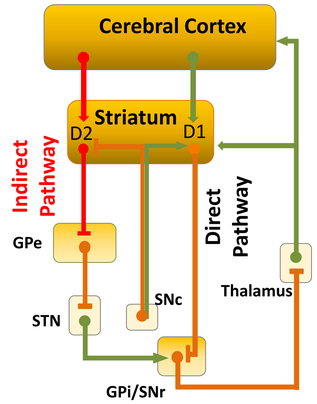

Figure 1. Oversimplified basal ganglion loop with **weakened circuits in HD**. **Excitatory** and **Inhibitory** pathways.

MATERIALS AND METHODS

Male R6/1 (125 CAGs) and wild-type littermates of 3 age (2, 4 and 6 months) groups of mice.

Behavioral task used an operant conditioning in a Skinner operant box as in Cayzac et al (2011).

Active group consisted in testing mice in behavioral task for 30 min one hour before brain perfusion and extraction.

Negative control group remained in home cage.

Fos data (fos positive nuclei/mm²) are expressed in terms of **differences between active and control groups** for the same age and genotype.

Experimental plan	R6/1		Wild-type		
Age	Active	Control	Active	Control	Total
2 months (early age)	9 mice	9	9	7	34
4 months (presymptomatic)	8	6	8	5	27
6 months (symptomatic)	9	5	7	5	26
Total	**26**	**20**	**24**	**17**	**87**

Results: Behaviors

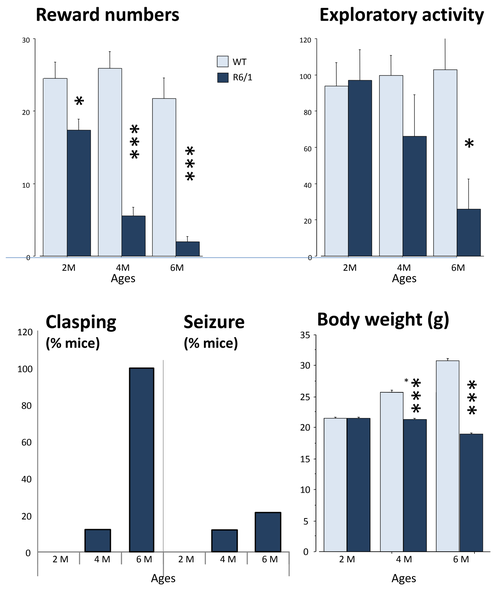

Figure 2. Behavioral results: age-dependent deficits in R6/1 mice. Rewards received and exploratory activity (number of nose poke holes explored), clasping and seizure phenotypes.

Results: Immunocytochemistry

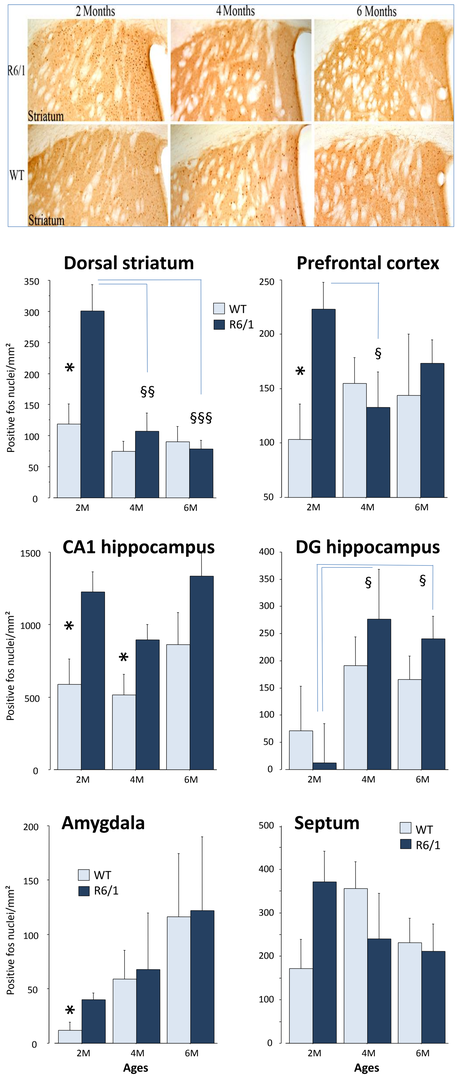

Figure 3. Positive fos nuclei in R6/1 of 2, 4 and 6 months of age. Operant conditioning -related c-fos activity was unexpectedly increased in the striatum, prefrontal cortex and CA1 field of the hippocampus of 2-month-old R6/1 mice as compared to age-matched WT littermates. Then, fos levels dropped to WT levels in the striatum and prefrontal cortex but not in CA1 at older ages. * significant difference between age-matched genotypes, § different from 2 month-old R6/1 mice.

Results: Summary and synthesis

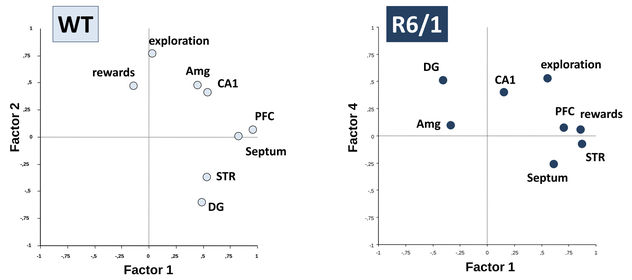

Figure 5. Factorial analysis for fos in different neural regions and behavioral data (rewards, exploratory nose poke activity) in both WT and R6/1 mice. In WT mice, behavioral parameters remained independent of neural activity of a large cluster that includes all regions studied. To the contrary, in R6/1 mice, behavioral accuracy measure appeared in a closed cluster that contains only prefrontal cortex, striatum and septum. Amg: Amgdala, PFC: prefrontal cortex, STR: striatum.

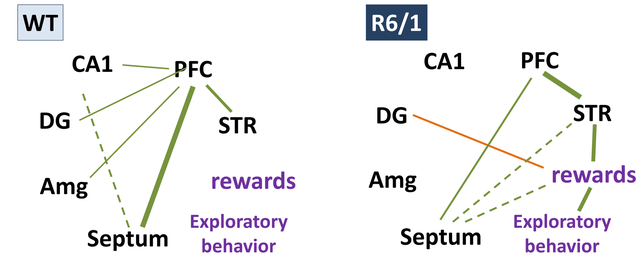

Figure 4. Inter-relations (correlations) of activity in different regions and behaviors in both WT and R6/1 mice (2-6 month s combined). **Positive** and **negative** correlations. Thickness of lines is proportional to statistical significance (0.05>p>0.0001), and dotted lines represent 0.5<p<0.08.

CONCLUSION

1. We found dramatic and unexpected increased levels of c-fos in prefrontal cortex – striatum in early asymptomatic R6/1 mice of 2 months of age, and this aberrant activity faded away with age.
2. In R6/1 mice, a closed and limited cortico-striatal circuit activity is highly correlated with striatal-dependent behavioral performance, which is impaired. To the contrary, in WT mice, a broader and extended functional network connectivity is not correlated with behavioral performance. at least at early learning stage.
3. This remarkable difference may point to altered functional connectivity of neural networks underlying cognitive impairments in R6/1 mice, this presumably due to their deficient synaptic plasticity and physiology.

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