Analysis of Interneuron Cell Numbers in the Motor Cortex in Huntington's Disease

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Abstract

Huntington's disease is a neurodegenerative autosomal dominant disease caused by an expansion of a CAG repeat in the huntingtin gene. This results in an expanded polyglutamine repeat in the Huntingtin protein. There is loss of motor control, psychiatric and cognitive deficits in HD. The main brain regions that primarily degenerate are the striatum and cortex, where striatal medium spiny neurons and parvalbumin expressing neurons and cortical projection neurons are affected, respectively. Cortical pyramidal neurons are modulated by cortical interneurons and project to the striatum to modulate the function of medium-spiny neurons to control motor activity. Staining of human HD tissue revealed interneuron cell loss in the motor cortex. However, studies in a mutant huntingtin expressing mouse model showed no decrease in interneuron numbers but a decrease in the marker proteins that define these cell types and a decrease in their cell size in motor cortex. To reconcile these findings, we used the bacterial artificial chromosome full-length human mutant huntingtin expressing mouse model which replicates the loss of motor control, psychiatric issues, and cognitive deficits seen in human patients. The BACHD model also shows a significant decrease in the striatal and cortical volume as the behavioral phenotypes worsen with age. We have analyzed whether there are changes in cell number of somatostatin and parvalbumin interneuron types in the cortex of BACHD mice at 2-3 months and 13-15 months of age. We did not observe significant changes in parvalbumin and somatostatin cell number between BACHD and WT mice at either age.

Striatal Somatostatin Interneurons in BACHD mice

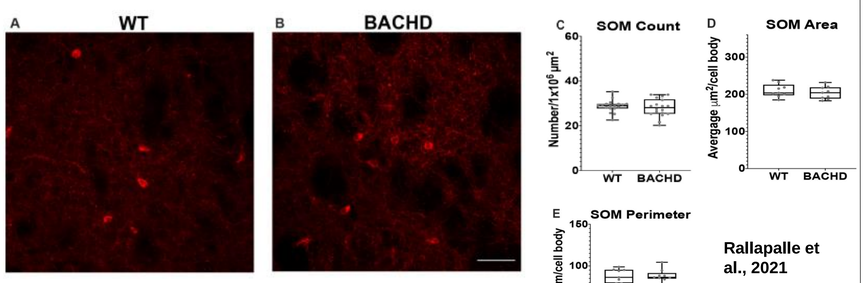

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Figure 1. Striatal SST cells are spared in BACHD mice. IHC confocal images showing SST cells in **(a)** WT mice, and **(b)** BACHD. The two genotypes have similar **(c)** counts, **(d)** area, and **(e)** perimeter.

Striatal Parvalbumin Interneurons in BACHD mice

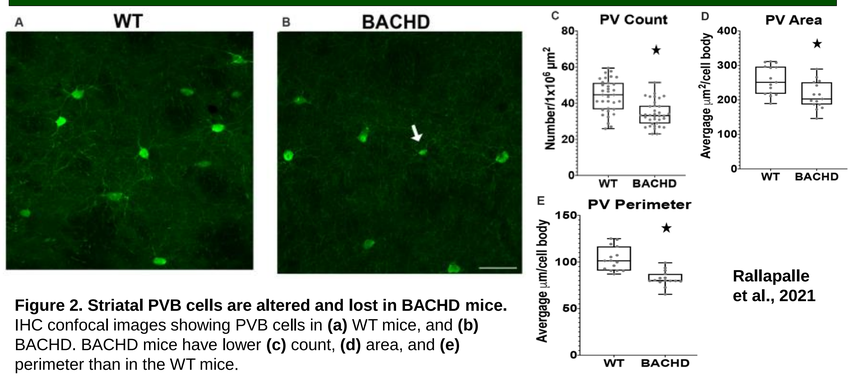

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Figure 2. Striatal PVB cells are altered and lost in BACHD mice. IHC confocal images showing PVB cells in **(a)** WT mice, and **(b)** BACHD. BACHD mice have lower **(c)** count, **(d)** area, and **(e)** perimeter than in the WT mice.

Methods

- **Perfusion –** perfused with 0.01M phosphate buffered saline and 4% paraformaldehyde, and cryoprotected in sucrose
- **Sectioning –** sectioned at 40µm using a microtome
- **Immunofluorescence –** Parvalbumin (Swant PV 235, 1/1500) and Somatostatin (BMA Biomedicals, T-4103, 1/500) Goat anti rabbit cy3 (Jackson Immuno Research 111-165-003, 1/250) Goat anti mouse 488 (Invitrogen, A11029, 1/250)
- **Confocal imaging –** 10x images were taken for analysis
- **ImageJ analysis –** counted using the cell counter plugin

BACHD Mouse Model

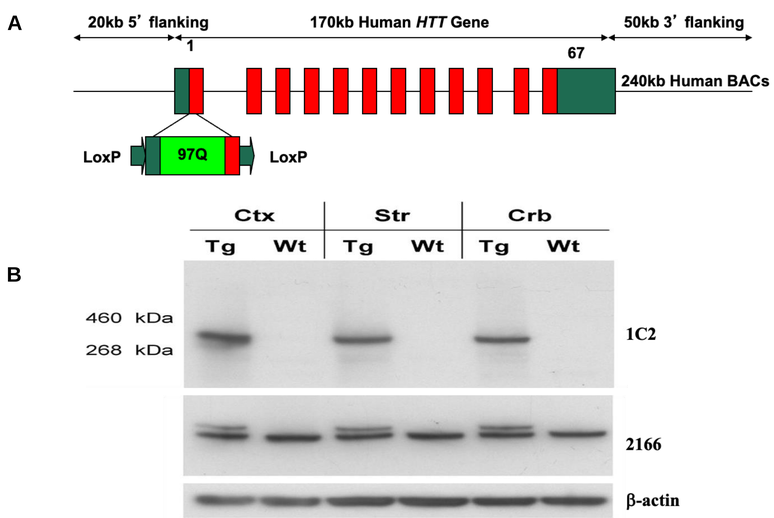

Figure 3. BACHD mice harbor a full-length mutant human huntingtin gene flanked by LoxP sites. (a) BACHD mice genetic construct. The LoxP sites are recognized by cre-recombinase. In the presence of cre-recombinase driven by a cell-type specific promoter, the human huntingtin gene will be floxed out, allowing for cell-type specific knockdown of the human huntingtin gene. **(b)** Western blots showing the presence of expanded polyglutamine in BACHD (Tg) mice, and not present in wildtype mice using the 1C2 antibody. Monoclonal antibody 2166, which recognizes polyglutamine repeats, shows the presence of both wildtype and mutant huntingtin in the BACHD mice, relative to wildtype mice which possess only the endogenous mouse huntingtin.

Interneurons in the Motor Cortex of Young Mice

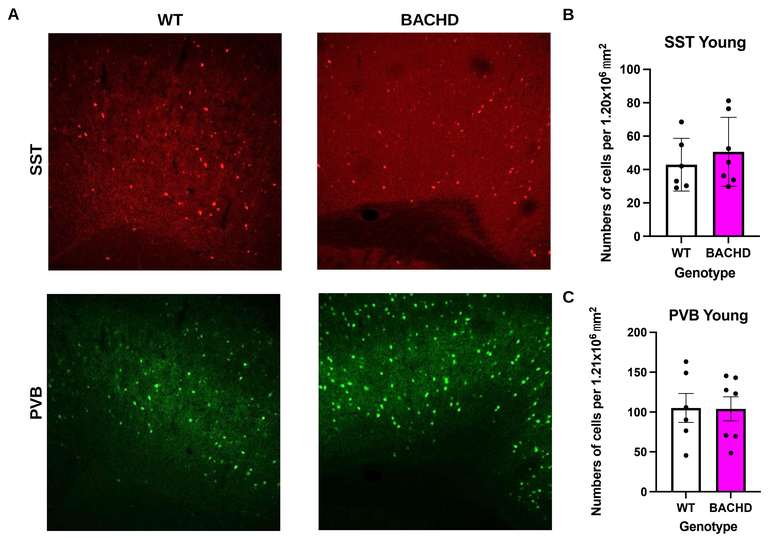

Figure 4. Somatostatin-expressing and parvalbumin-expressing cell numbers are unaltered in 2–3-month-old BACHD mice. (a) Representative 10x confocal images of (top) somatostatin- and (bottom) parvalbumin-expressing interneurons in the motor cortex of both wildtype and BACHD mice. B-C. Quantitation of the number of **(b)** Somatostatin-expressing (SST) interneurons (p=0.4727, t(11)=0.7441, SEM= 7.709 ± 10.36) and **(c)** Parvalbumin-expressing (PVB) interneurons (p=0.9644, t(11)=0.4567, SEM= -1.068 ± 23.38) in the motor cortex of 2–3-month-old BACHD and wildtype mice. n=6-7 per genotype. Data passed normality tests using the Shapiro Wilk tests. Data was analyzed using the unpaired Student's t test.

Interneurons in the Motor Cortex of Aged Mice

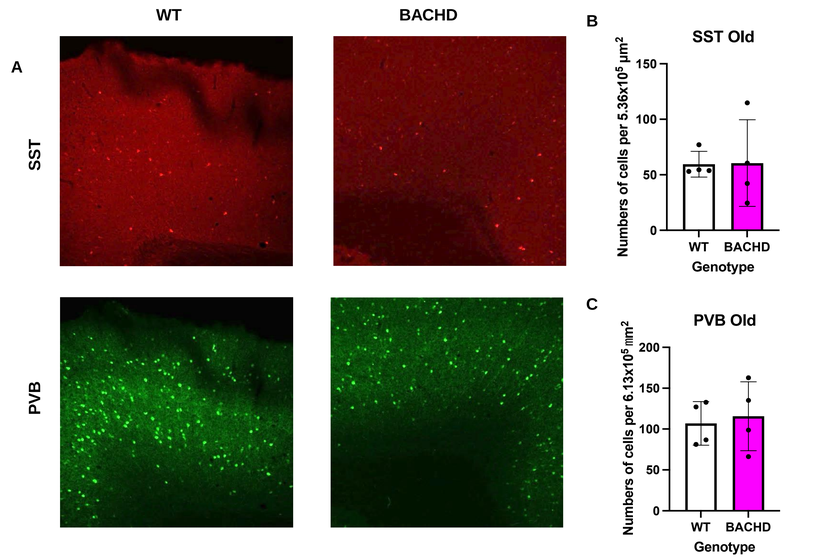

Figure 5. The number of parvalbumin- and somatostatin- expressing cells remain unchanged in the motor cortex of 15-month-old BACHD mice. (a) Representative 10x confocal images of somatostatin- and parvalbumin-expressing interneurons in the motor cortex of both wildtype and BACHD mice. B-C. Quantitation of the number of **(b)** Somatostatin-expressing (SST) interneurons (p=0.9639, t(6)=0.04713, SEM= 0.9600 ± 20.37) and **(c)** Parvalbumin-expressing (PVB) interneurons (p=0.7354, t(6)=0.3541, SEM=8.828 ± 24.93) in the motor cortex of 15-month-old BACHD and wildtype mice. n=4 per genotype. Data passed normality tests using the Shapiro Wilk tests. Data was analyzed using the unpaired Student's t test.

Summary

- **HD is a neurodegenerative disease that affects mainly the striatum in the brain**
- **The BACHD mouse model recapitulates the motor, cognitive, and psychiatric deficits in HD**
- **BACHD mice display atrophy in the striatum (similar to what is seen in HD)**
- **In BACHD mice striatal parvalbumin-expressing cell are lost like what is seen in HD patients**
- **In both BACHD mice and HD patients, somatostatin-expressing cells are not lost**
- **Parvalbumin- and somatostatin-expressing interneurons are not lost in the motor cortex of the BACHD mouse model**

Acknowledgements

This work was supported by NIH/NINDS- 5R01NS089750-08 and the Dixon family foundation.

References

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