Metabolic Characteristics of Primary Neuron Cultures from BACHD Rats Compared to Induced Lesion Models

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Background

The BACHD rat is by now a well-characterized animal model of Huntington's disease (HD), presenting several disease relevant symptoms and pathologies. The BACHD rat represents one of the few animal models that overexpresses the full length human mutant huntingtin (mHTT) and is thus of great value for HD research. The aim of this study was to compare the metabolic properties of primary striatal, hypothalamic and cortical neurons of BACHD rats with the L-glutamate or MPP+ induced rat striatal lesion models to establish BACHD primary cells as valuable *in vitro* HD model.

Materials and Methods

Hemizygous BACHD and wildtype rat pups were dissected at embryonic day 19 and primary neurons of the striatum, hypothalamus and cortex were cultivated. Cells were analyzed after 1, 7 and 14 days *in vitro* (DIV). For the lesion models, primary striatal embryonic day 19 wildtype rat neurons were cultivated for 15 days and lesioned with L-glutamate or MPP+ for 24 hours. All samples were analyzed with the LDH- and MTT-assay.

Results

Our data show that primary neurons (PNs) of embryonic BACHD rats have a significantly decreased metabolic activity in the striatum and hypothalamus (Fig. 1, 2). These results are comparable with data obtained by L-glutamate or MPP+ lesions in primary striatal neurons of wildtype rats (Fig. 3). Our most recent method development shows that polyQ HTT can be quantified by sandwich immunosorbent assay with a high specificity (Fig. 4).

Quantification of polyQ HTT

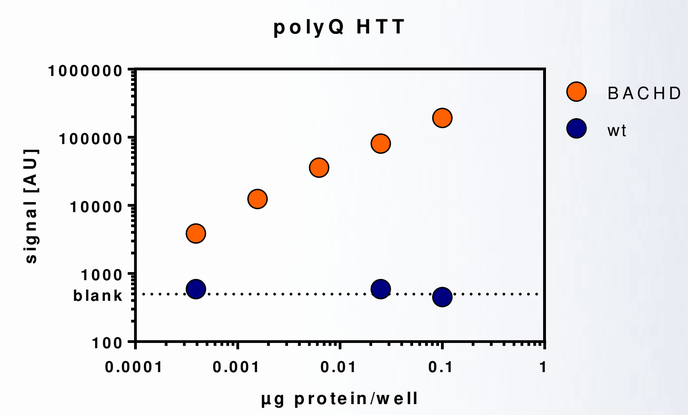

Fig. 4: Quantification of polyQ HTT by sandwich immunosorbent assay using the MesoScale Discovery platform. Whole brain lysate of a 6 months old hemizygous rat and a non-transgenic littermate were used in a dilution series of 1:10, 1: 40; 1:160; 1:640 and 1:2560.

Primary Neuron System of BACHD Rats

LDH measurement

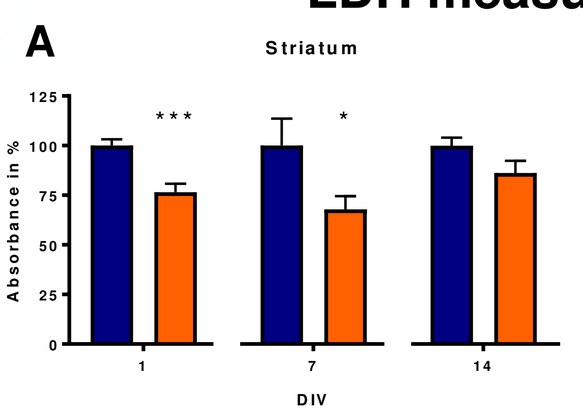

Fig. 1: LDH levels in BACHD primary neurons. DIV 1 cultures from BACHD animals showed a significant decrease of lactate dehydrogenase (LDH) activity in the cell culture media of all regions compared to wt PNs. The level of LDH activity in the cell culture media is a indicator of the cell viability, where high activity points to an increased rate of cell death and lysis. At DIV 7 only striatal BACHD PNs showed a significantly decreased LDH level, whereas after 14 DIV no significant difference of LDH levels could be observed (Unpaired t-test & Mann-Whitney test).

MTT measurement

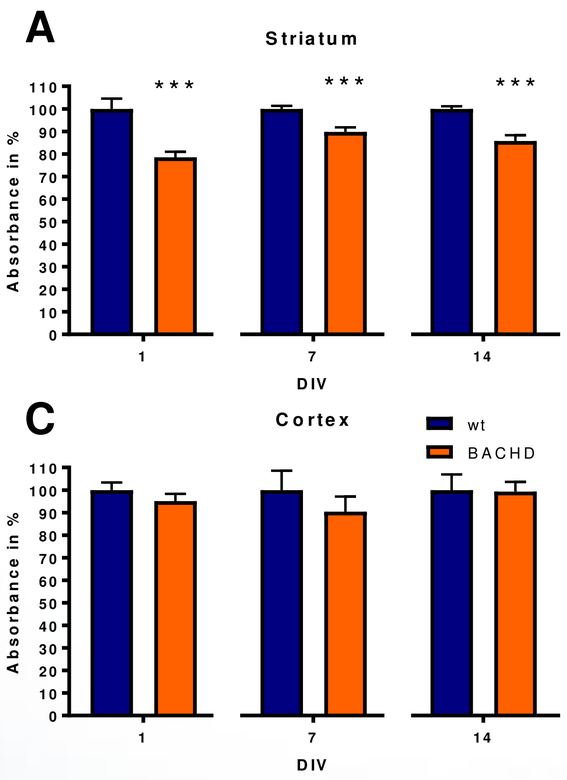

Fig. 2: MTT levels in BACHD primary neurons. Striatal and hypothalamic PNs of BACHD rats show a significantly decreased metabolic activity at DIV 1 & 7, as less of the tetrazolium dye MTT was reduced to its detectable form formazan compared to PNs of wt rats. This could also be shown in striatal PNs at DIV 14 but not in hypothalamic PNs. No difference could be observed in cortical PNs at all time points. (Unpaired t-test & Mann-Whitney test).

Lesion Models of Wildtype Striatal Neurons

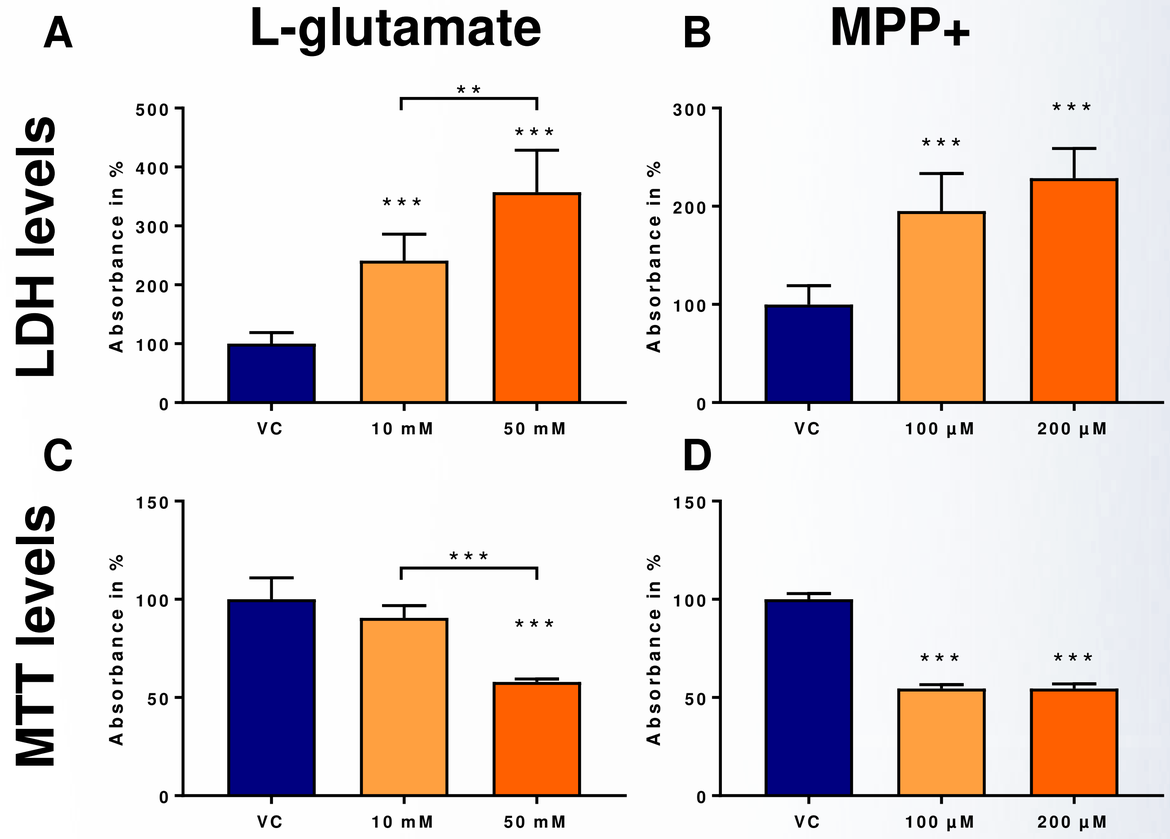

Fig. 3: LDH and MTT levels in lesioned primary striatal neurons. Striatal PNs of wt rats lesioned with L-glutamate (A) or MPP+ (B) show a concentration dependend increase in LDH levels while MTT levels decrease (C,D). PN were cultured for 15 days and lesioned for 24 h. One Way ANOVA followed by Tukey's multiple comparison test.
All figures: *p<0.05; **p<0.01; ***p<0.001.

Summary and Conclusion

We conclude that the BACHD rat model is a valuable tool for the *in vitro* evaluation of HD-related metabolic properties. Future experiments can further be analyzed by polyQ HTT immunosorbent assay.

We look forward to support your research

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