

# CBE Treatment of $\alpha$ -Synuclein Overexpressing and Wildtype Mice Models Gaucher Disease Pathology

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## OBJECTIVE

Gaucher disease is the most prevalent lysosomal storage disorder and is caused by autosomal recessive mutations in the glucocerebrosidase gene. Glucocerebrosidase (GCase) hydrolyses the sphingolipid glucosylceramide to glucose and ceramide. Deficiency in GCase activity leads to a multisystemic accumulation of substrate in lysosomes. Additionally,  $\alpha$ -synuclein, tau, ubiquitin, APP and A $\beta$  might build up in affected tissues. Most of these proteins are also accumulated in several other rare diseases and neurodegenerative diseases such as  $\alpha$ -synuclein in dementia with Lewy bodies or Parkinson's disease. In the last years several links between  $\alpha$ -synucleinopathies and lysosomal storage diseases have been reported. We thus combined an inducible mouse Gaucher model with a transgenic Parkinson's disease mouse by treating  $\alpha$ -synuclein transgenic mice and non-transgenic littermates with Conduiritol  $\beta$  Epoxide (CBE) that serves as an irreversible inhibitor of  $\beta$ -glucosidase and is known to cause a Gaucher-like phenotype.

## MATERIALS & METHODS

Six months old PDGF-human  $\alpha$ -synuclein transgenic mice (D-Line; Masliah et al. 2000) on a murine  $\alpha$ -synuclein knockout background (C57BL/6JOLA<sup>Hsd</sup>) and non-transgenic littermates were treated with 100 mg/kg CBE for 15 consecutive days. After CBE treatment, animals were tested for motor impairments in the Beam Walk test and brain tissue was analyzed for  $\alpha$ -synuclein expression, activated microglia and astrocytosis by evaluating  $\alpha$ -synuclein, CD11b and GFAP immunofluorescent labeling.

## RESULTS - Behavior

### Beam Walk Test

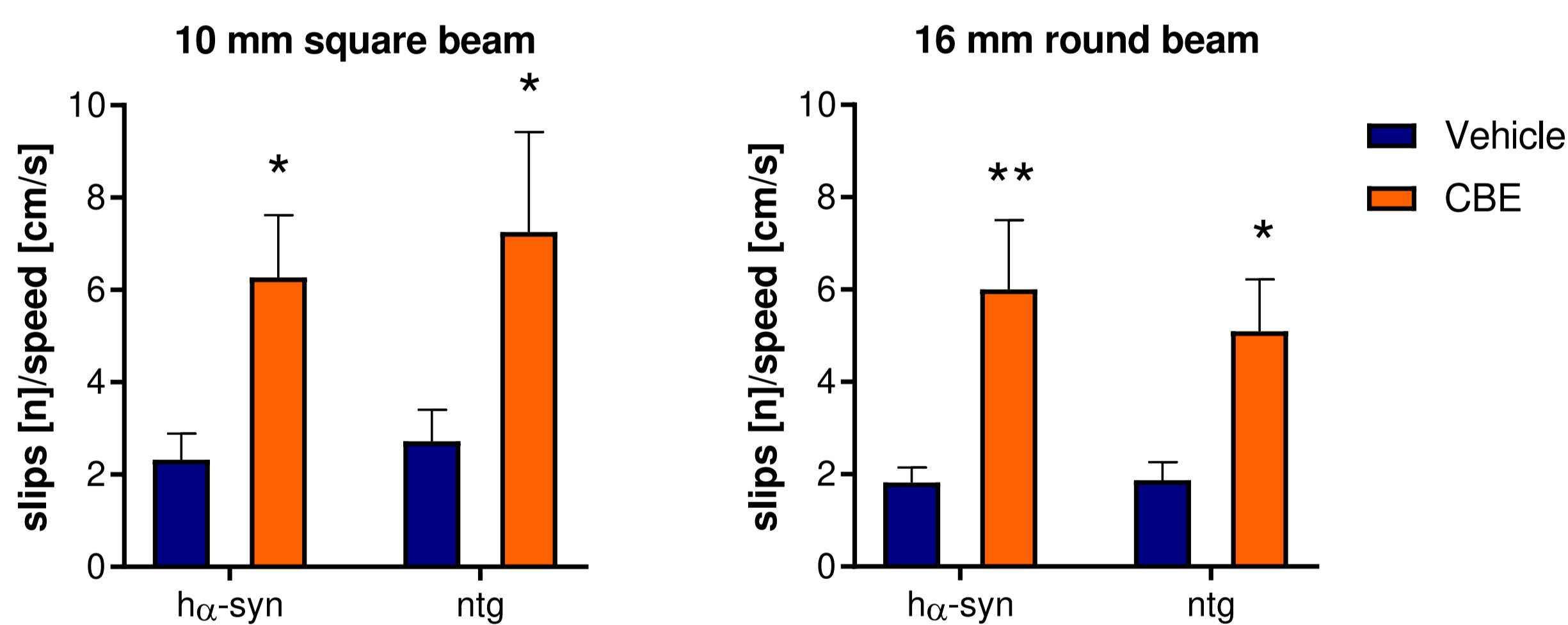


Figure 1: Beam walk test of D-Line (h $\alpha$ -syn) and non-transgenic littermates after CBE treatment. Motor deficits were tested on a 10 mm square beam (left) and a 16 mm round beam (right). Results are shown in slips per speed. Mean + SEM; n = 12 per group; Two way ANOVA followed by Bonferroni's *posthoc* test; \*p<0.05; \*\*p<0.01. All analyzed animals were on a background of murine  $\alpha$ -synuclein knockout.

## RESULTS - Histology

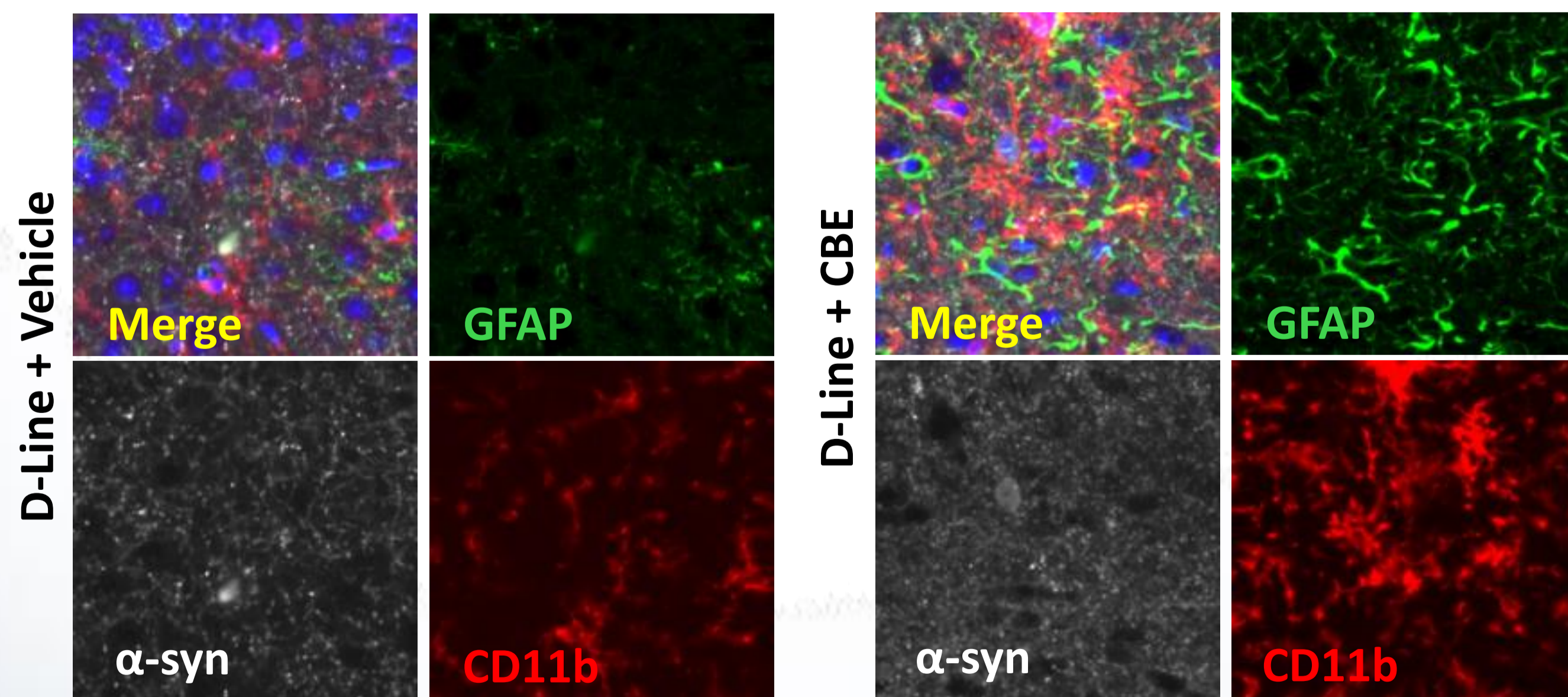


Figure 2: Cortex sections of CBE- and vehicle-treated D-Line mice. CBE treatment causes a dramatic activation and enlargement of microglia (CD11b, red) and induction of astrocytosis (GFAP, green) in D-Line mice.  $\alpha$ -synuclein ( $\alpha$ -syn) immunoreactivity (due to transgene expression) is shown in white.

## $\alpha$ -Synuclein Levels

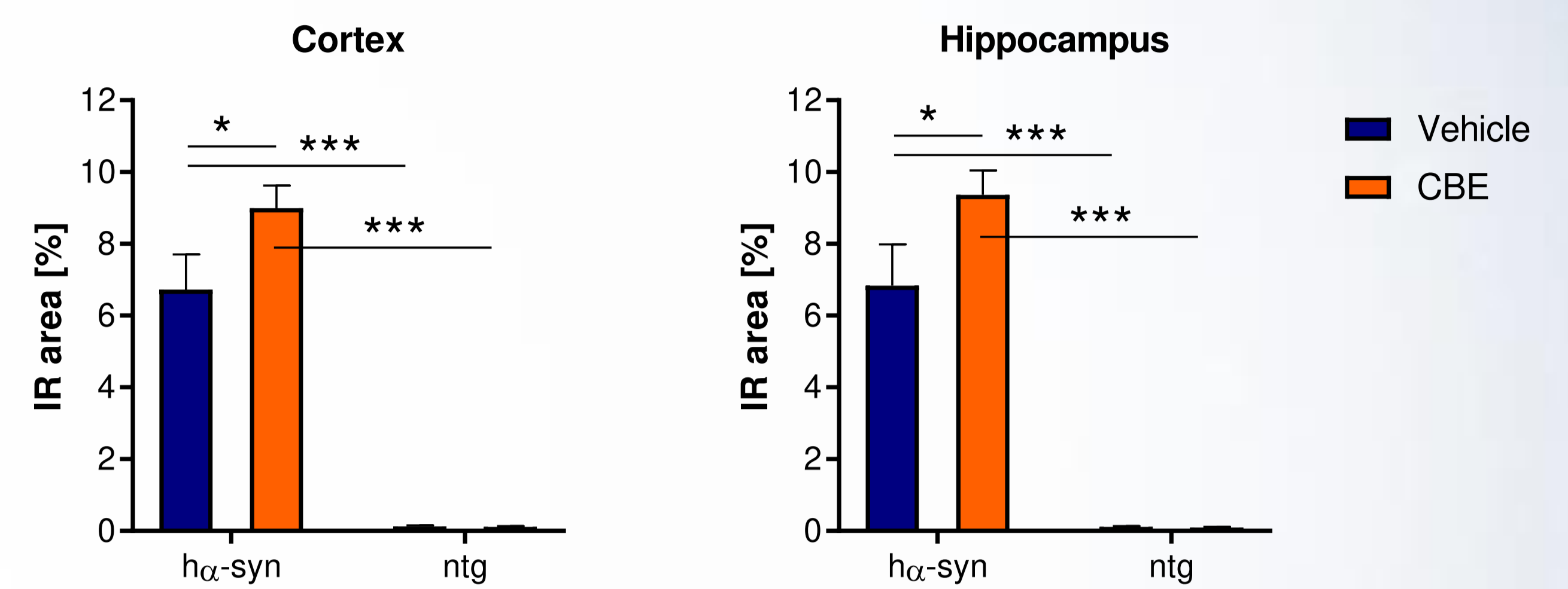


Figure 3:  $\alpha$ -Synuclein levels in the cortex and hippocampus of D-Line mice and littermates after CBE treatment. Immunoreactive (IR) area in percent; mean + SEM; n = 12 per group; Two way ANOVA followed by Bonferroni's *posthoc* test; \*p<0.05; \*\*\*p<0.001.

## Neuroinflammation – Activated Microglia (CD11b)

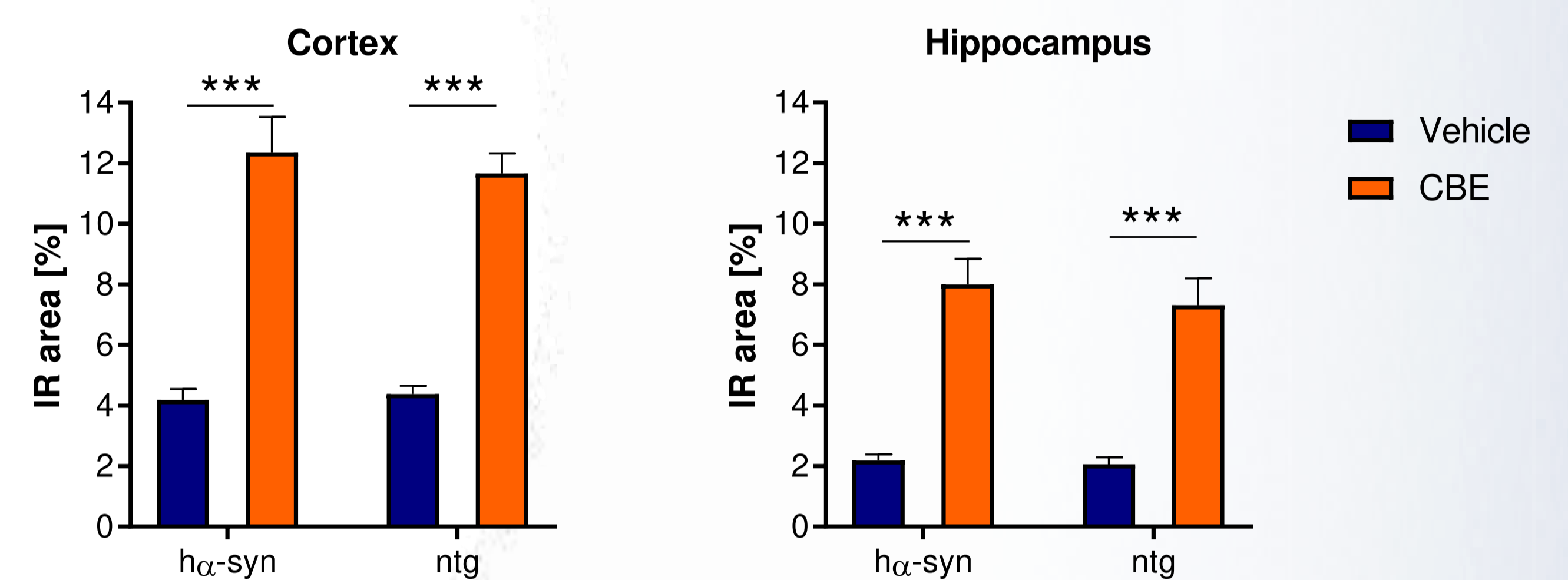


Figure 4: Quantification of activated microglia in the cortex and hippocampus of D-Line and non-transgenic littermates after CBE treatment. Immunoreactive (IR) area in percent; mean + SEM; n = 12 per group; Two way ANOVA followed by Bonferroni's *post hoc* test; \*\*\*p<0.001.

## Neuroinflammation – Astrocytosis (GFAP)

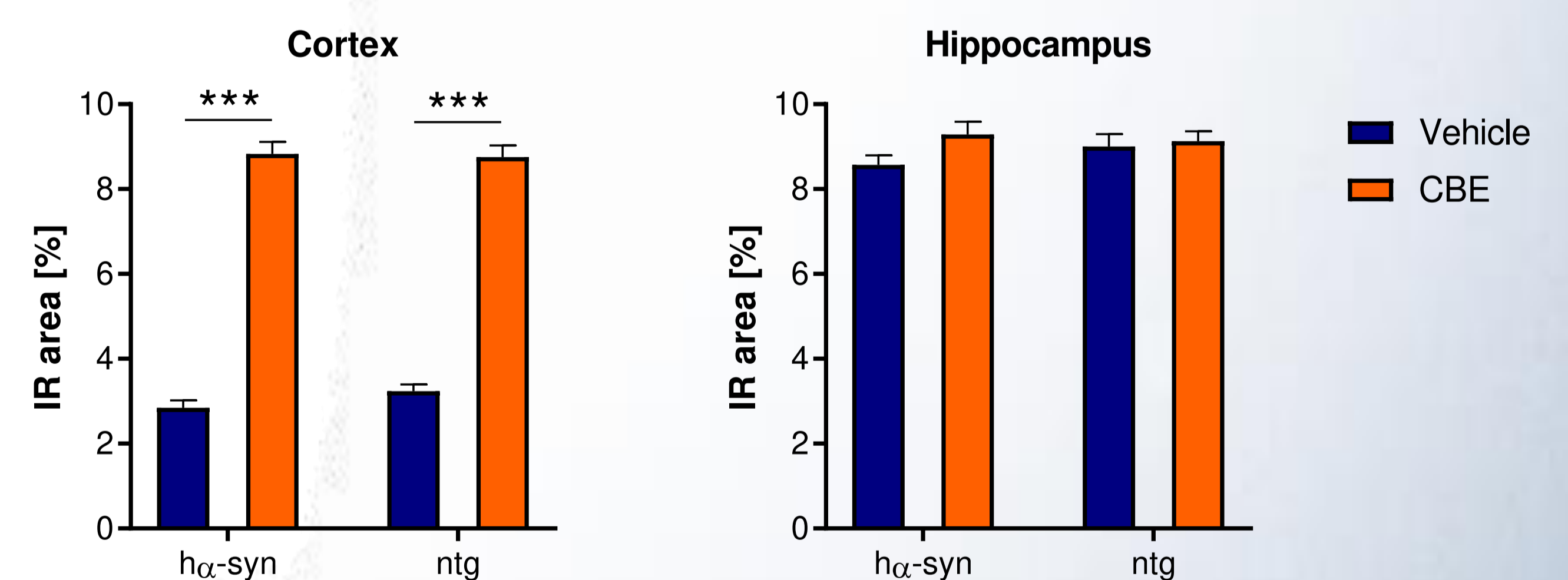


Figure 5: Quantification of astrocytosis in the cortex and hippocampus of D-Line and non-transgenic littermates after CBE treatment. Immunoreactive (IR) area in percent; mean + SEM; n = 12 per group; Two way ANOVA followed by Bonferroni's *post hoc* test; \*\*\*p<0.001.

## SUMMARY

Our results show that CBE treatment caused motor impairments that were not affected by the transgene.  $\alpha$ -synuclein levels were highly increased in transgenic animals compared to non-transgenic littermates and CBE treatment caused a further increase of  $\alpha$ -synuclein levels. Activated microglia and astrocytosis were increased in all CBE treated groups, independent of the transgene.