

Characterization of 4L/PS-NA mice for Enzyme Activity, Substrate Concentrations as well as Inflammation to Model Gaucher Disease

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OBJECTIVE

Gaucher disease is the most common lysosomal storage disease. The neuronal disease variant is characterized by aggregated protein accumulations in the brain and associated neurological manifestations. It is autosomal recessively inherited and modeled by 4L/PS-NA mice that express low levels of prosaposin and saposins, as well as a functionally impaired glucocerebrosidase (GCase) with a homozygous point mutation at V394L. To use this model for compound tests against Gaucher disease a detailed characterization of these mice is needed. Thus, we aimed to analyze the 4L/PS-NA mice for GCase activity, glucosylsphingosine (GlcSph) and glucosylceramide (GlcCer) levels as well as inflammation over age.

MATERIALS & METHODS

The GCase activity was analyzed in different tissues as the CBE inhibitable signal in the 4-MUG assay. The levels of GlcSph and GlcCer in the brain extracts were measured by ultrahigh performance liquid chromatography coupled to tandem mass spectrometry. GlcSph and GlcCer were extracted from the brain homogenates using liquid-liquid extraction, deuterium-labeled GlcCer-D5 and GlcSph-D5 were used as internal standards. To explore neuroinflammatory processes, in particular activated microglia and astrocytosis, in this animal model we performed immunofluorescent labeling on brain sections. Furthermore, KC/GRO (CXCL1) cytokine measurement in the CSF was performed by immunosorbent assay. Finally, mouse embryonic fibroblasts (MEFs) of 4L/PS-NA mice were analyzed as *in vitro* screening tool.

Enzyme and Substrate Measurements

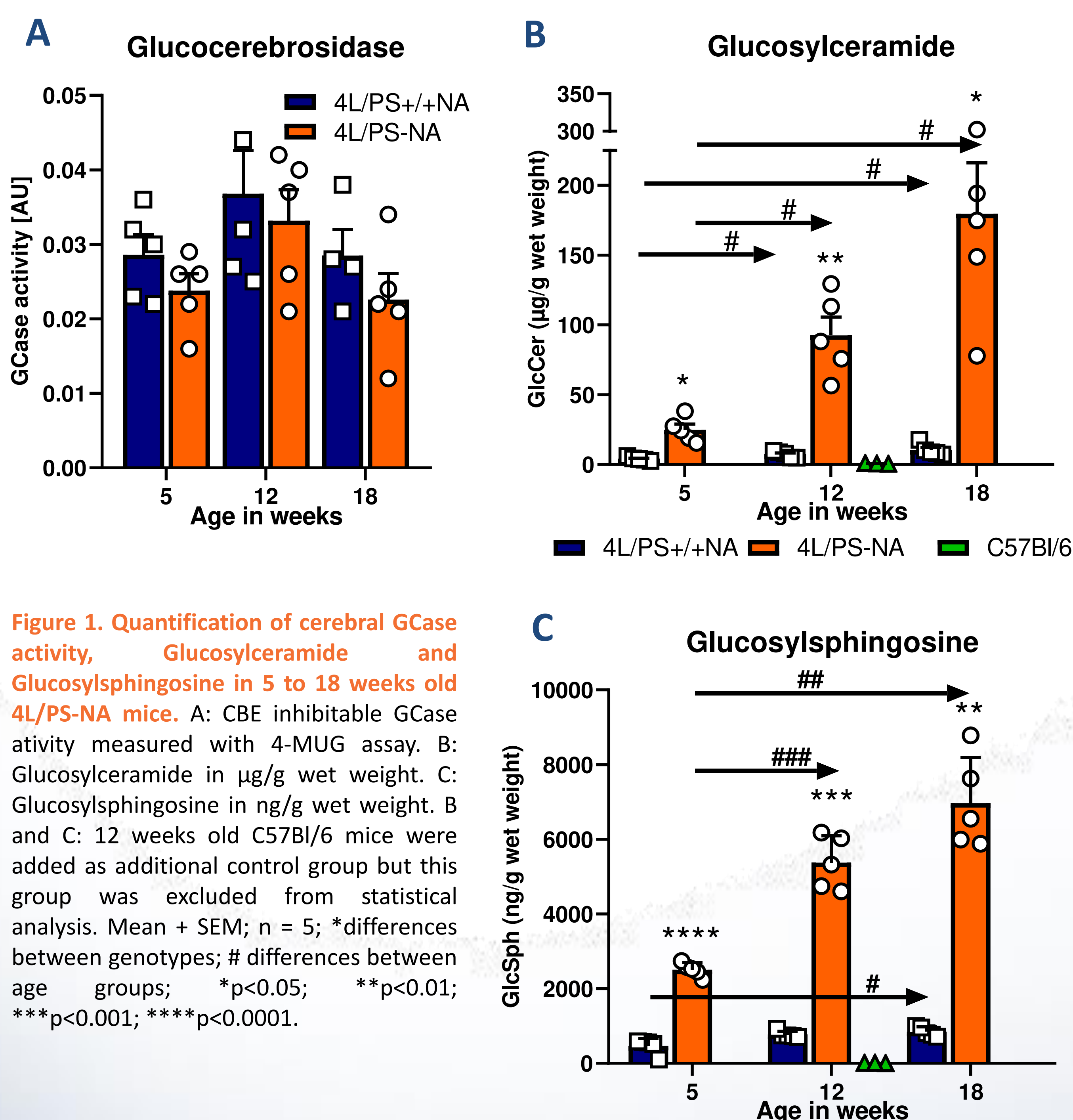


Figure 1. Quantification of cerebral GCase activity, Glucosylceramide and Glucosylsphingosine in 5 to 18 weeks old 4L/PS-NA mice. A: CBE inhibitable GCase activity measured with 4-MUG assay. B: Glucosylceramide in µg/g wet weight. C: Glucosylsphingosine in ng/g wet weight. B and C: 12 weeks old C57Bl/6 mice were added as additional control group but this group was excluded from statistical analysis. Mean + SEM; n = 5; *differences between genotypes; # differences between age groups; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Neuroinflammation

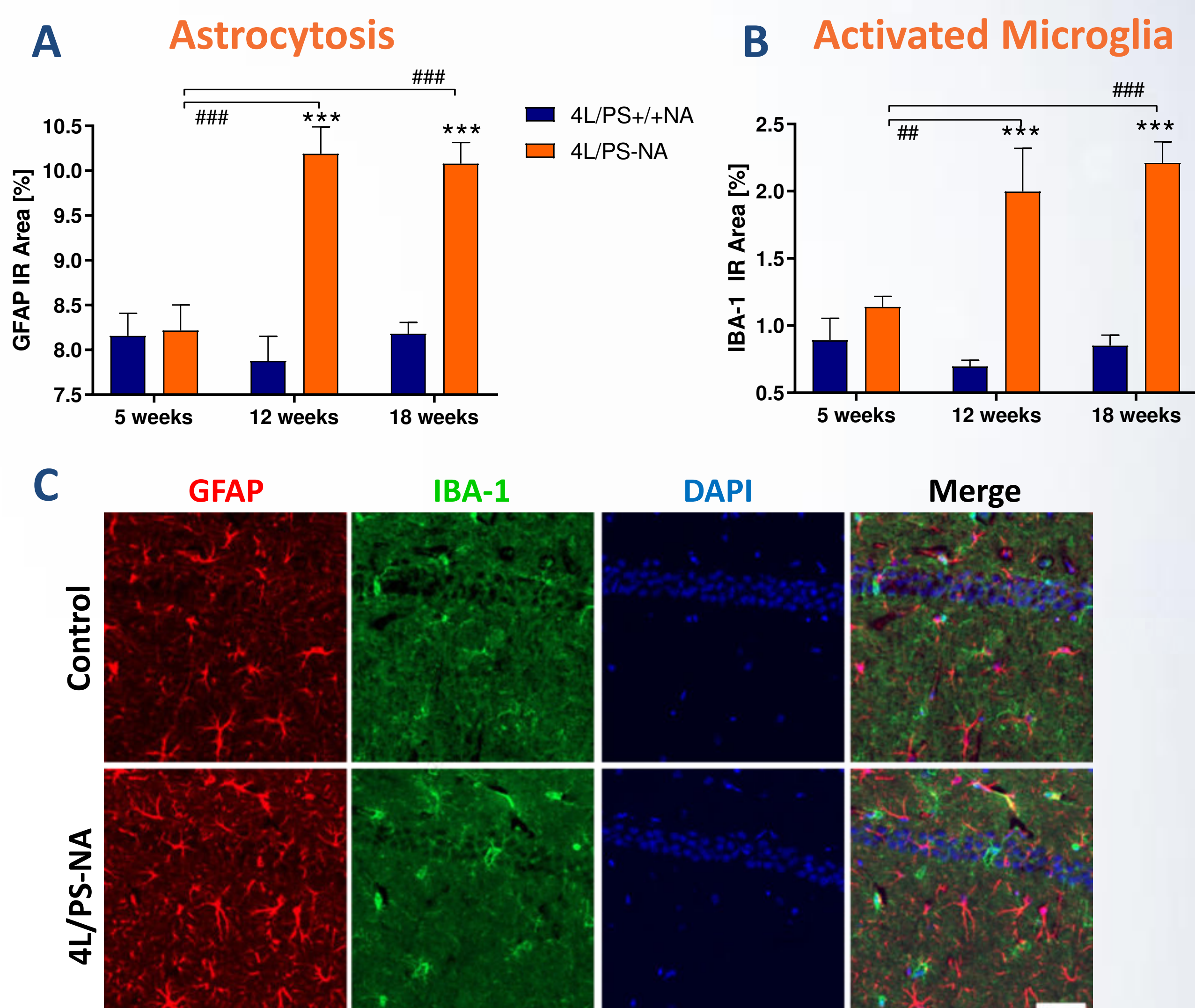


Figure 2. Quantitative analysis of neuroinflammation in 5 to 18 week old 4L/PS-NA mice. GFAP (A) and IBA-1 (B) immunoreactive (IR) area in percent in the hippocampus of 5, 12 and 18 weeks old 4L/PS-NA mice compared with controls. Two-way ANOVA followed by Bonferroni's *posthoc* test. Mean + SEM; n=5 per group; *differences between genotypes; # differences between age groups; **p<0.01; ***p<0.001. C: Representative images of GFAP (red), IBA1 (green) and DAPI (blue) labeling of the hippocampal CA1 region in 18 weeks old 4L/PS-NA and control mice. Scale bar: 50 µm.

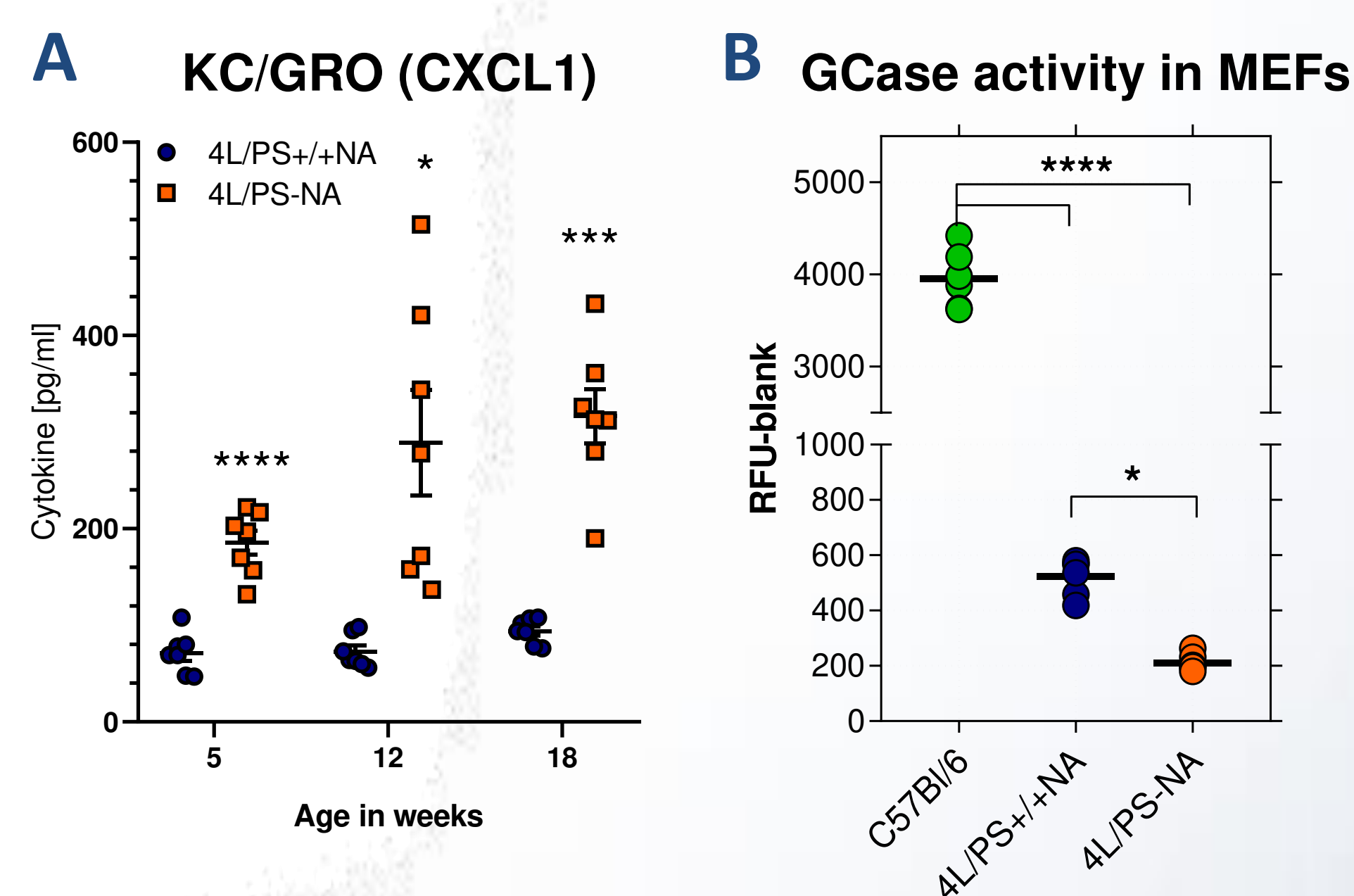


Figure 3A. Quantification of KC/GRO (CXCL1) in CSF of 4L/PS-NA mice over age. Analysis of CSF from 5, 12 and 18 months old animals and age matched controls by immunosorbent assay (MSD). n=7 per group. Two-way ANOVA followed by Bonferroni's *posthoc* test.

Figure 3B. GCase activity in C57Bl/6, 4L/PS+/+NA and 4L/PS-NA mouse embryonic fibroblasts (MEFs) measured with 4-MUG assay. n=6; One-way ANOVA followed by Bonferroni's *posthoc* test. A and B: Mean ± SEM; *p<0.05; ***p<0.001; ****p<0.0001.

RESULTS and CONCLUSION

Analysis of enzyme activity showed a weak decrease of GCase activity in 4L/PS-NA mice but a strong increase of GlcCer and GlcSph substrate concentrations in 4L/PS-NA mice compared to controls. 4L/PS-NA mice exhibited strong neuroinflammation and increased CXCL1 levels. Analysis of MEFs revealed a strongly reduced GCase activity in the cells from 4L/PS-NA mice compared to C57Bl/6 MEFs but only a minor reduction compared to 4L/PS+/+NA mice harboring wildtype prosaposin.

In conclusion, 4L/PS-NA mice mimic the most prominent features of Gaucher disease and are a valuable tool for *in vitro* and *in vivo* drug development.