Pathological hallmarks of a genetic and an induced Gaucher disease mouse model

Magdalena Temmel, Joerg Neddens, Estibaliz Santiago-Mugica, Stefanie Flunkert, Vera Niederkofler, Roland Rabl, David Amschl, Maria Posch, Ewald Auer, Birgit Hutter-Paier

QPS Austria GmbH, Parkring 12, Grambach, Austria

BACKGROUND

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. The disease is autosomal recessively inherited and modeled by 4L/PS-NA mice that express low levels of prosaposin and saposins, as well as β-glucosidase (GCase) with a point mutation at V394L/V394L. Additionally, the disease can be modeled by treating mice with Conduritol-beta-Epoxide (CBE) a specific inhibitor of GCase activity.

To use these models for compound tests against the Gaucher disease a detailed characterization of these mice is needed. We thus analyzed 4L/PS-NA mice for their neurono- and non-neuronopathic features and CBE-treated wildtype mice for their neuronopathic phenotype.

MATERIALS AND METHODS

4L/PS-NA mice were tested behaviorally in the Wire Suspension test at the age of 8, 12, and 18 weeks as well as in the Rota Rod test at 12 and 18 weeks of age.
Wildtype mice lacking endogenous α-synuclein (C57BL/6JOlaHsd) were treated for 15 days daily intraperitoneally with 100 mg/kg CBE starting at 6 months of age. One day after the last treatment, animals were investigated in the Open Field test and on the second day in the Beam Walk test.

Furthermore, brain tissue of both models was analyzed for neuroinflammation by histological methods. The 4L/PS-NA mouse model was additionally examined for visceral symptoms.

RESULTS

4L/PS-NA mouse model

Behavioral Phenotype

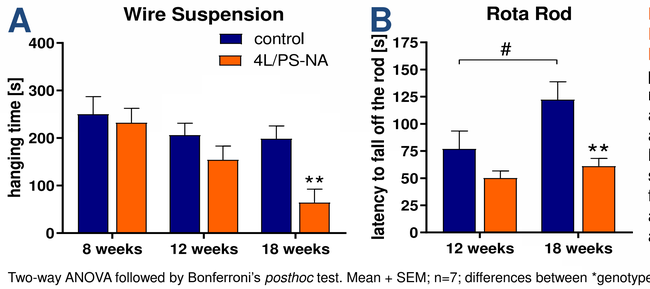


Figure 1. Wire Suspension and Rota Rod impairment in 4L/PS-NA mice. **A:** 4L/PS-NA mice present with a significantly reduced wire hanging time at the age of 18 weeks compared with age-matched controls. **B**: 4L/PS-NA mice present with a significantly reduced latency to fall from the rotating rod at the age of 18 weeks compared with age-matched controls.

Two-way ANOVA followed by Bonferroni's *posthoc* test. Mean + SEM; n=7; differences between *genotypes/ #age groups; *p<0.05, **p<0.01.

Neuroinflammation

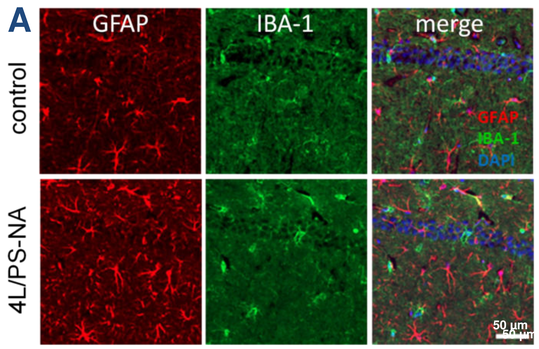
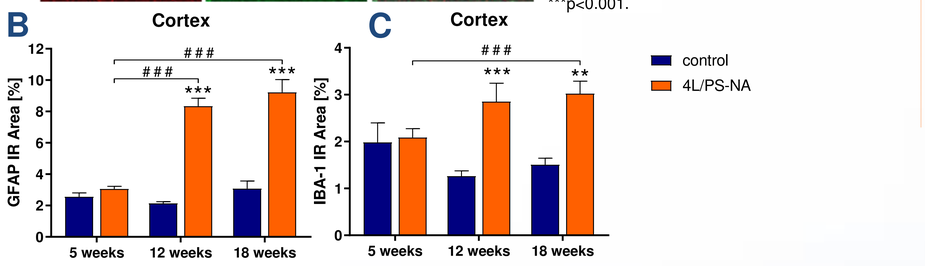


Figure 2. Immunohistological analysis of neuroinflammation in 5 to 18 weeks old 4L/PS-NA mice.
A: Representative images of GFAP (red), IBA1 (green) and DAPI (blue, merge only) labeling of the hippocampal CA1 region in 18 weeks old 4L/PS-NA and control mice.
GFAP (**B**) and IBA-1 (**C**) immunoreactive (IR) area in percent in the cortex 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; *differences between genotypes; #differences between age groups; **p<0.01; ***p<0.001.



Visceral Alterations

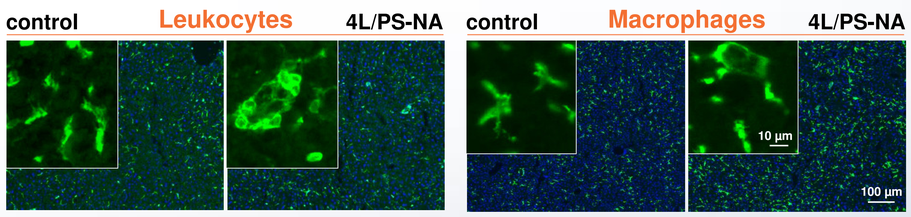


Figure 3. Enlarged leukocytes and macrophages in the liver of 4L/PS-NA mice. The liver of 5 weeks old 4L/PS-NA mice and control littermates was labeled with CD45 antibody for leukocytes (**A**) and F4/80 for macrophages (**B**). CD45 and F4/80 (green); DAPI (blue).

CBE-induced mouse model

Behavioral Phenotype

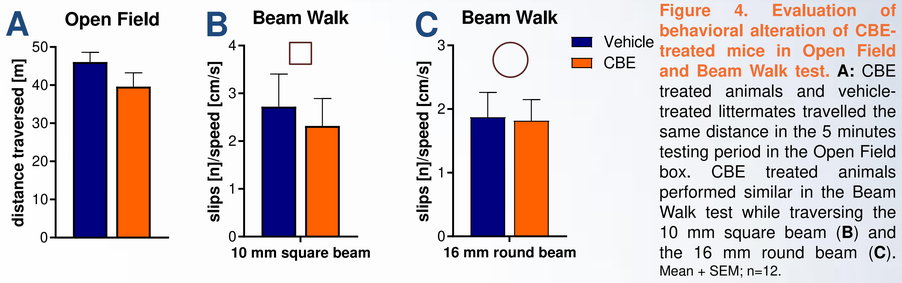


Figure 4. Evaluation of behavioral alteration of CBE-treated mice in Open Field and Beam Walk test. **A:** CBE treated animals and vehicle-treated littermates travelled the same distance in the 5 minutes testing period in the Open Field box. CBE treated animals performed similar in the Beam Walk test while traversing the 10 mm square beam (**B**) and the 16 mm round beam (**C**). Mean + SEM; n=12.

Neuroinflammation

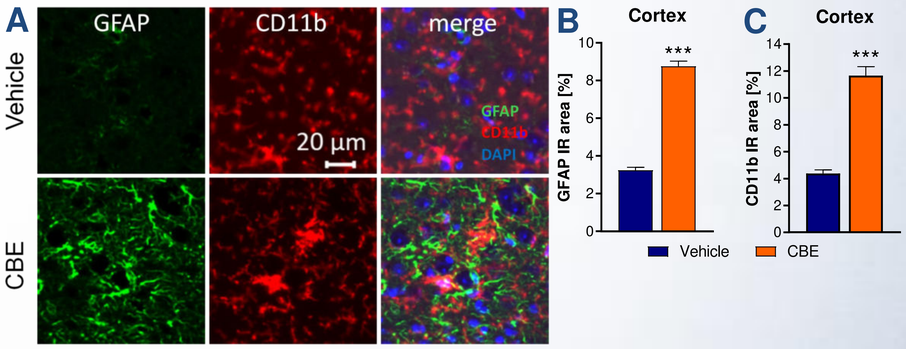


Figure 5. Immunohistological analysis of neuroinflammation after 15 days of CBE or vehicle treatment in wildtype mice. **A**: Representative images of astrocytosis (GFAP; green), activated microglia (CD11b; red) and nuclei (DAPI; blue, merge only) labeling in the cortex. Quantification of GFAP (**B**) and CD11b (**C**) immunoreactive (IR) area in percent in the cortex of CBE treated mice compared to controls. Unpaired t-test. Mean + SEM; n =12; ***p<0.001.

CONCLUSION / SUMMARY

In summary, 4L/PS-NA mice present with highly increased glial activation in the brain that is accompanied by strong motor deficits suggesting that 4L/PS-NA mice are a good model to study chronic neuronopathic type 3 Gaucher disease. Additionally, 4L/PS-NA mice present early alterations in visceral organs and thus further mimicking the non-neuronopathic phenotype of Gaucher disease. Treatment with CBE causes no motor deficits but strong neuroinflammation, suggesting that this induced model has in total a weaker phenotype compared to the 4L/PS-NA model.

Visit us at Booth #3014

Contact: Birgit Hutter-Paier, PhD | Director Neuropharmacology | QPS Austria GmbH | Parkring 12 | 8074 Grambach | Austria | birgit.hutter-paier@qps.com | www.qpsneuro.com