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Microglia plasticity in a mouse model of Fabry disease

-Hippocampal alterations and behavioural deficits-

Luna-Choconta J, Kalpachidou T, Langeslag M, Kummer KK, Kress M

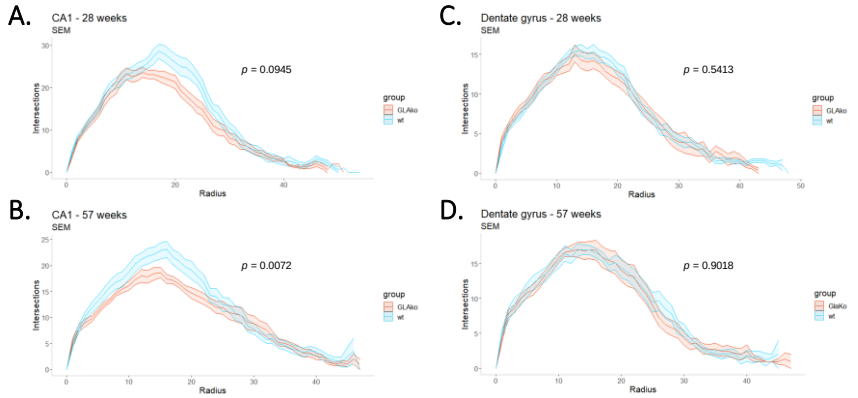
Institute of Physiology, Medical University Innsbruck, Innsbruck, Austria

Introduction and Summary

Fabry disease (FD) is a lysosomal storage disorders caused by deficiency in the x-chromosomal α-galactosidase A (αGal) gene, leading to the accumulation of neutral glycosphingolipids, mainly globotriaosylceramide (Gb3) in internal organs and the nervous systems. While dysfunction of the peripheral and autonomic nervous system already occurs early in childhood, other neurological symptoms such as cognitive deficits are developing with older age. A possible reason for these symptoms might be Gb3 accumulations in diverse regions of the central nervous system. Lysosomal storage disorders with neuronal dysfunction induce an activation of microglia and alteration of their protective phenotype.

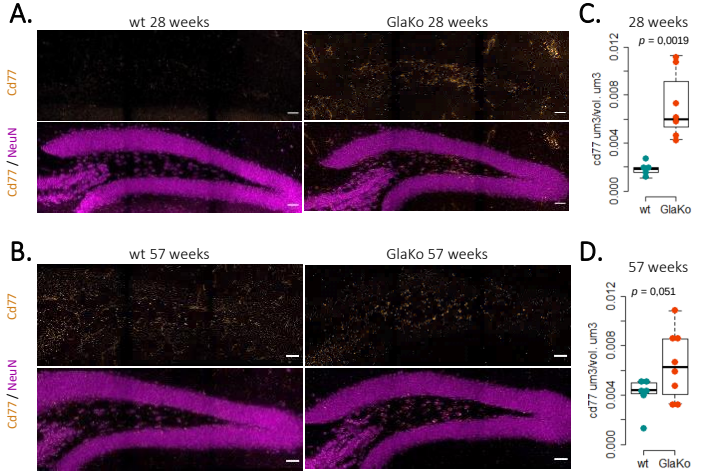
We investigated here Gb3 effects during the progression of FD in a murine transgenic α-Gal A-/0 (Gla-KO) model of FD. We found Gb3 accumulation in the hippocampal dentate gyrus, reduced diameter of blood vessels and altered microglia morphology in aged Gla-KO mice. In addition, Gb3 accumulation in the hippocampal dentate gyrus of Gla-KO mice was associated with a deficit in exploration of new environments but not motor coordination.

1. Microglia morphology of the CA1 hippocampal region is affected in old Fabry mice



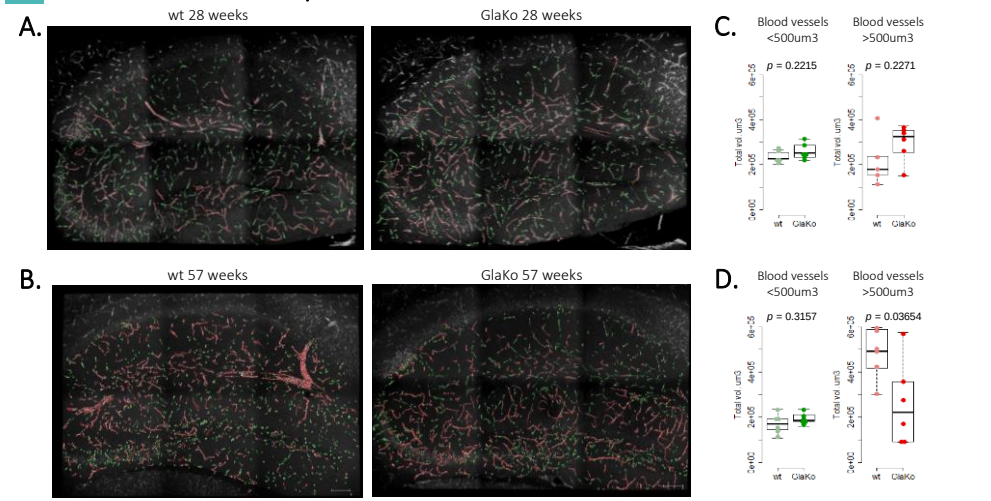
Since the role of microglia activity in FD progression is still unknown, we addressed morphological changes of microglia in GlaKo mice. FD mice displayed a slight morphological alteration in CA1 at 28 weeks (A), however the morphological changes were more pronounced in aged mice (B). Microglia from FD and wt mice in the dentate gyrus were comparable (C and D). Statistics analysis, linear mixed-effects model.

2. Gb3 accumulation in polymorphic layer of the dentate gyrus in Fabry mice



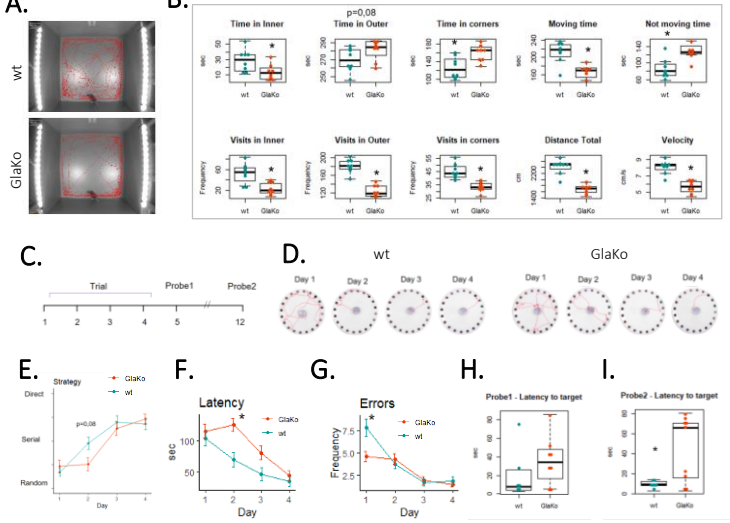
Gb3 deposition in the polymorphic layer of the dentate gyrus in GlaKo mice. Samples were collected from intracardially perfused mice and stained with anti-Gb3 (Cd77) and anti-NeuN. Multiphoton images were analyzed by Imaris software 9.7. Gb3 accumulation in the dentate gyrus was identified at 28 and 57 weeks (A,B). Quantification of Gb3 both 28 and 57 weeks yielded significant differences between FD and wt mice (C and D). Cohort sizes: 28 weeks wt mice (n=6), 28 weeks GlaKo mice (n=7) 57 weeks wt mice (n=6) and 57 weeks GlaKo mice (n=8). Scale bar 50um. Shapiro-Wilk test p>0,05. t-test.

3. Blood vessels in old Fabry mice



Decreased thickness of blood vessels in GlaKo old mice (57 weeks). Cd31 was used to quantified the blood vessels. Multiphoton images were analyzed by Imaris software 9.7. Surface reconstruction of blood vessels from 28 (A) and 57 (B) weeks old mice. Total surface volume of blood vessels from 28 (C) and 57 (D) weeks old mice. n=6. Scale bar 50um. Shapiro-Wilk test p>0,05. T-test.

4. Behavioural deficits in Fabry mice



LPS-induced inflammation reduces total distance and centre time in the open field test, however blocking of microglia activation improves the behavior task (Yang, et al, 2020). We found an abnormal behavior of GlaKo mice (A) with preference to spend more time in the corners and travel less distance (B). Barnes maze was used to evaluate spatial memory in GlaKo mice. Four consecutive days for trial sessions, followed by short (probe1) and long (probe2) term memory assay (C). The strategy of Fabry mice was randomly during first and second day (D and E). GlaKo mice showed increased latency (F) but less errors (G) during trial days. FD mice completed the behavior test both probe1 (H) and probe2(I), however they needed more time to find the target hole. Wt mice n=8 and GlaKo mice n=8. Scale bar 50um. Shapiro-Wilk test. t-test, *p<0.05.

Conclusion

Late stages of FD were associated with phenotypical changes with less branches in microglia from hippocampal CA1 region and Gb3 accumulation specifically in the polymorphic layer of the dentate gyrus. In addition, our results suggest dysfunction of blood vessel walls due to the inability of age-related thickening. Overall our data suggest that these structural alterations might be causally involved in the deficits in explorative performance, where the explorative behaviour was decreased and more time needed to complete a memory retrieval task.

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