

Adenylate kinase 1 (Ak1) knockout mice may be protected from alpha-synuclein pathology



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INTRODUCTION

- α-synucleinopathy is characterized by α-synuclein aggregation, leading to neurodegeneration in Parkinson’s disease (PD) and Dementia with Lewy Bodies (DLB).
- Despite its prevalence, disease-modifying therapies for PD and DLB remain lacking.
- Our prior genetic screen in a *Drosophila* α-synucleinopathy model identified adenylate kinase1 (AK1) as a glial gene that influences neurodegeneration
- Glial knockdown of Ak1 reduced neuronal α-synuclein toxicity and increased extracellular adenosine in α-synuclein expressing flies as compared to control flies.
- We hypothesize that glial Ak1 knockdown elevates adenosine, reducing neuronal excitotoxicity, neuroinflammation and oxidative stress.
- The current study investigates whether AK1 knockout (Ak1^{-/-}) mice exhibit protection in a mammalian α-synucleinopathy model.
- This work aims to validate Ak1 as a novel therapeutic target for α-synucleinopathies.

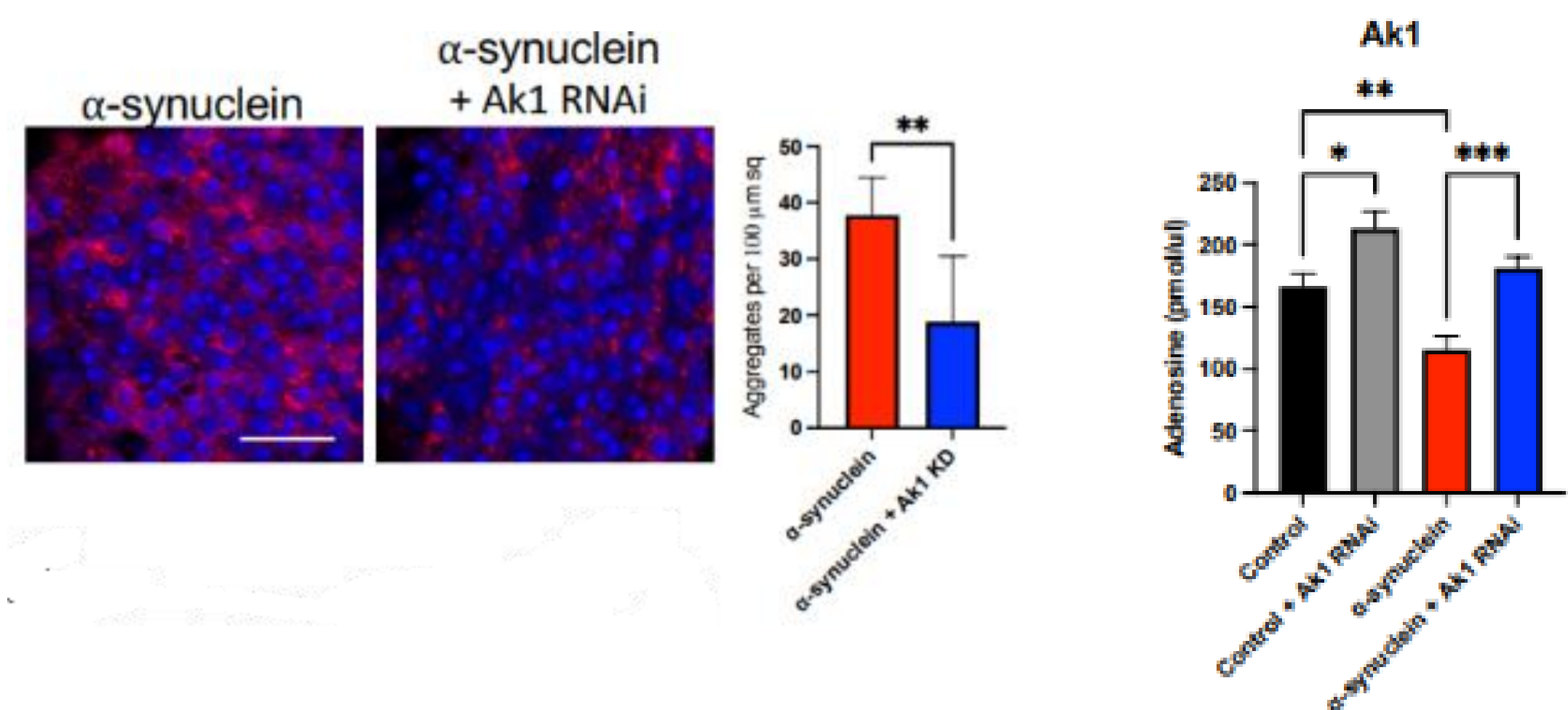
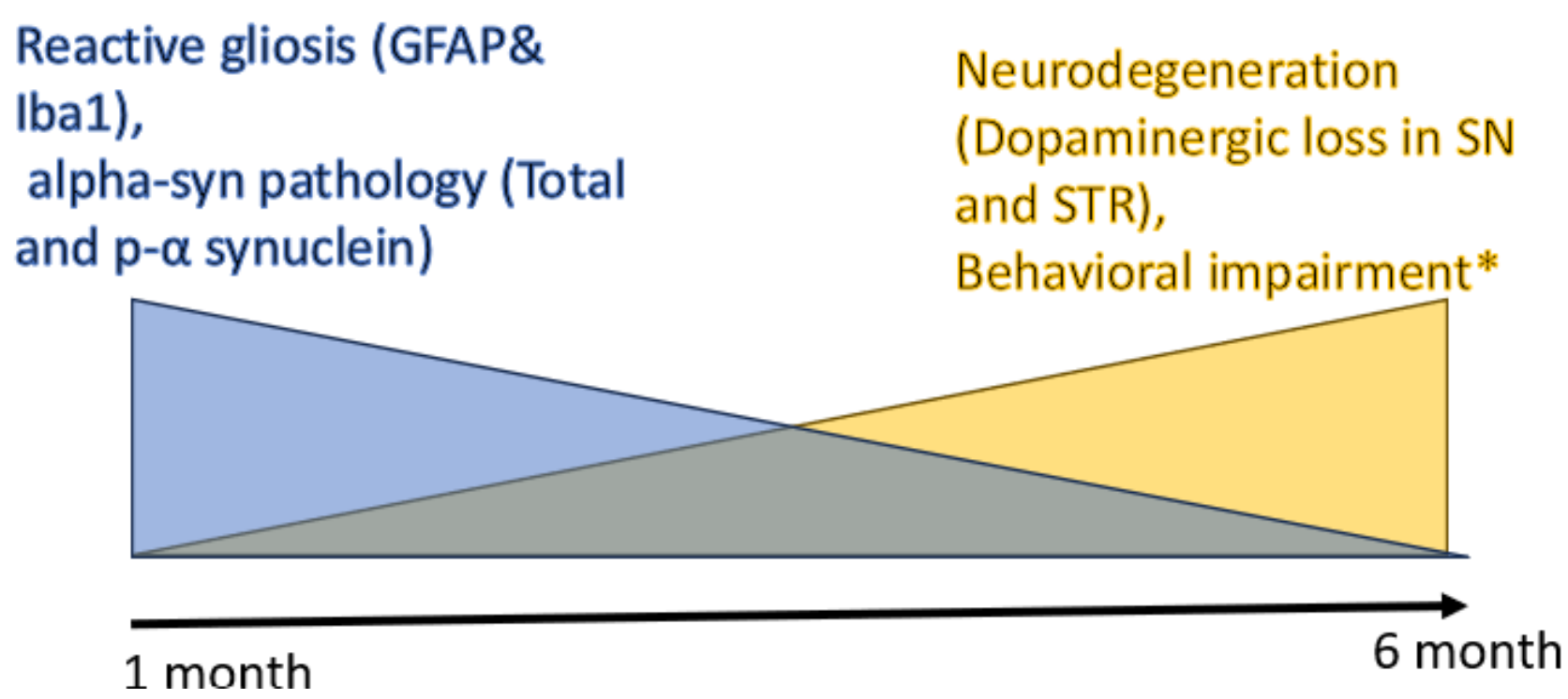


Figure 1 Prior work demonstrates that knockdown of Ak1 in glia rescues α-synuclein aggregation and increases adenosine levels.

METHODS

- We assessed the involvement of Ak1 in α-synucleinopathy using unilateral stereotaxic injection of AAV–α-synuclein-GFP or AAV–GFP into three-month-old C57BL/6 wild-type (Ak1^{+/+}) and Ak1 knockout (Ak1^{-/-}) mice.
- Pathological changes were assessed at one- and six-month post-injection.
- Gfap, Iba1, S129 phospho α-synuclein and total human α-synuclein were assessed by immunofluorescence at one-month post-injection.
- TH(+) cells in substantia nigra were counted by stereology and TH+ nerve terminals were assessed by immunofluorescent at six-months post-injection.
- Behavior was assessed at six-months post-injection using the open field test and rotarod test.
- Statistical analysis was done using ANOVA with post hoc test (p < 0.05).



RESULTS

Ak1^{-/-} mice may have fewer reactive microglia and less α-synuclein than wild type littermates at one month post-injection

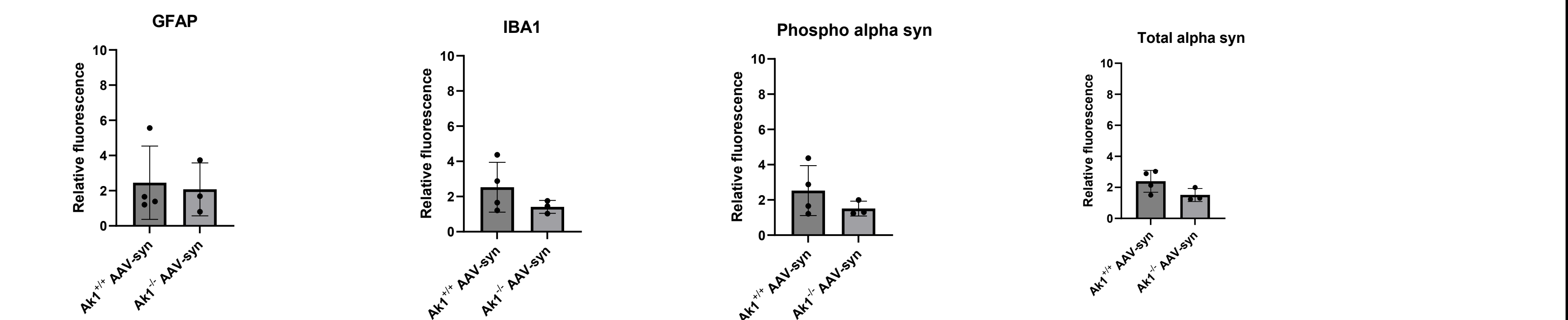


Figure 2 Relative fluorecence intensity of Iba1, Gfap, total α-synuclein, and phospho-α-synuclein was measured in anterior, middle, and posterior brain sections (ipsilateral). Background-subtracted (contralateral) values and AAV-GFP controls were used for normalization. At the one-month time point, preliminary data indicated a decrease in Iba1, phosphorylated α-synuclein and total human α-synuclein expression levels in Ak1^{-/-} mice compared to wild type (Ak1^{+/+}). More animals are needed to determine statistical significance.

Ak1^{-/-} mice may have more TH+ nerve terminals in the striatum than wild type mice at six-month post-injection

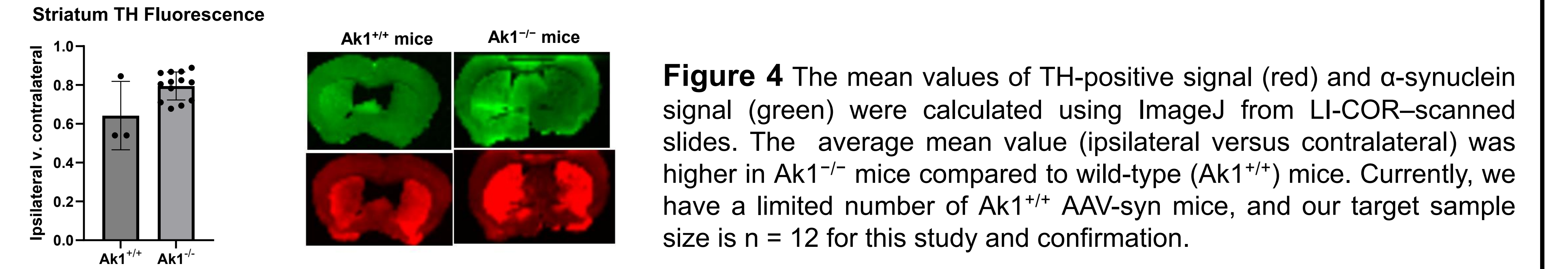


Figure 4 The mean values of TH-positive signal (red) and α-synuclein signal (green) were calculated using ImageJ from LI-COR–scanned slides. The average mean value (ipsilateral versus contralateral) was higher in Ak1^{-/-} mice compared to wild-type (Ak1^{+/+}) mice. Currently, we have a limited number of Ak1^{+/+} AAV-syn mice, and our target sample size is n = 12 for this study and confirmation.

Ak1^{-/-} mice may have preserved TH+ neurons in the substantia nigra at six-months

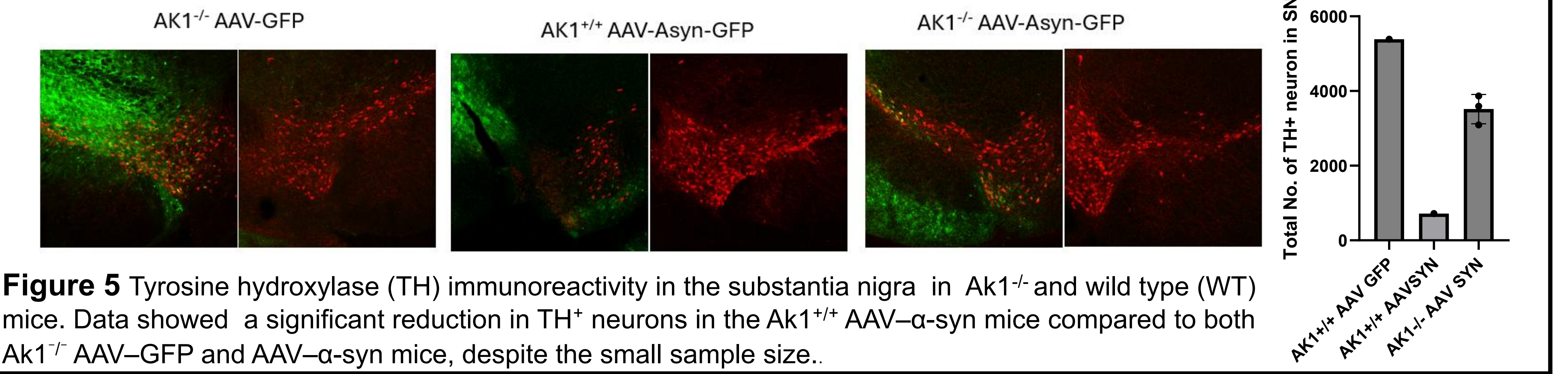


Figure 5 Tyrosine hydroxylase (TH) immunoreactivity in the substantia nigra in Ak1^{-/-} and wild type (WT) mice. Data showed a significant reduction in TH⁺ neurons in the Ak1^{+/+} AAV–α-syn mice compared to both Ak1^{-/-} AAV–GFP and AAV–α-syn mice, despite the small sample size..

Ak1^{-/-} mice have worse motor performance than wild type mice, independent of AAV-α-synuclein injection, at six-months post-injection

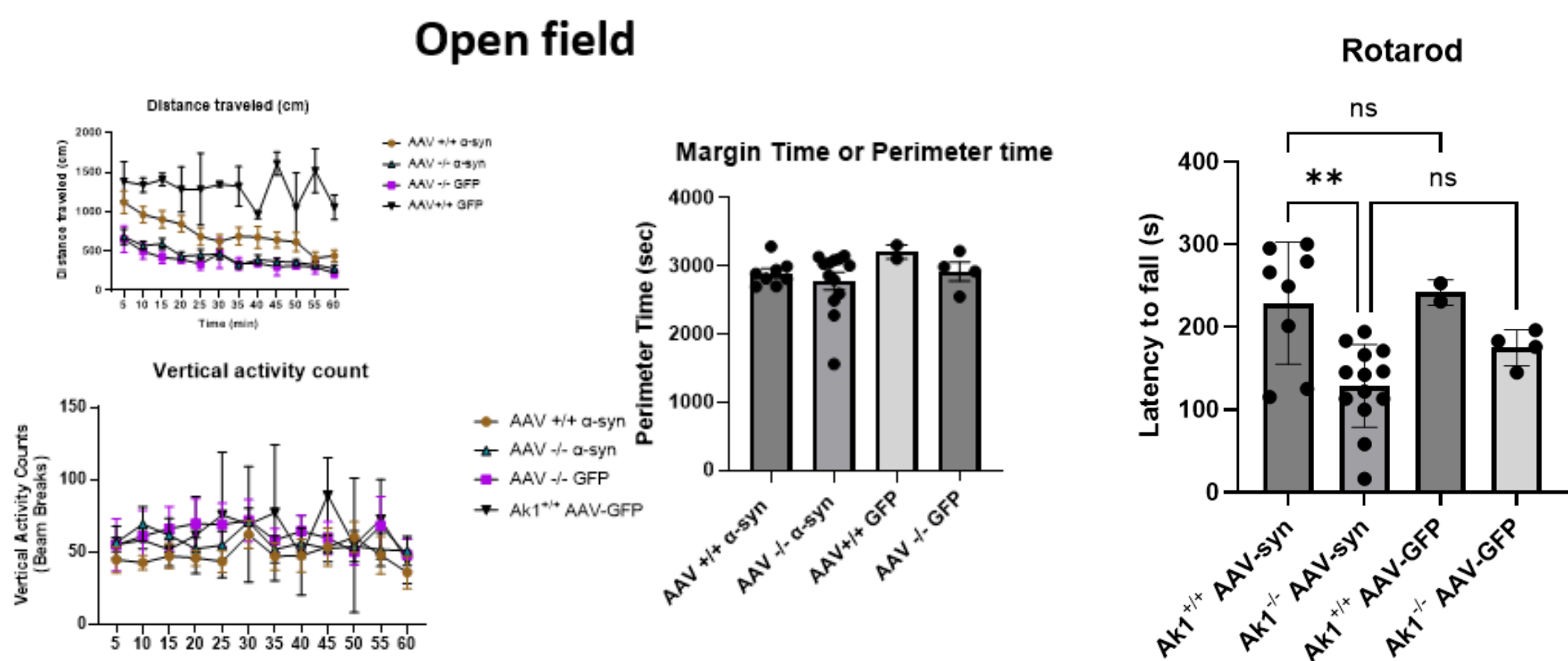


Figure 3 At six months, wild type mice injected with AAV-α-synuclein have lowered distance traveled in the open field test compared to wild type mice injected with AAV-GFP. They perform equally well in the rotarod test, however. Surprisingly, Ak1^{-/-} mice have lower distance traveled and worse performance on the the rotarod than wild type mice, independent of the AAV-α-synuclein or AAV-GFP injection.

Acknowledgements

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Conclusions and Future Directions

- We have successfully implemented a mouse α-synucleinopathy model involving stereotaxic injection of AAV2-α-synuclein virus or AAV2-GFP into C57BL/6 wild-type and Ak1^{-/-} mice.
- The data presented here represent preliminary results from a small number of mice and will require a larger sample size before firm conclusions can be reached.
- Preliminary results indicate that Ak1^{-/-} mice may have a reduction in indicators of α-synuclein pathology compared to wild type.
- There are mixed reports in the literature as to whether this model causes enough loss of dopaminergic neurons to induce behavioural deficits. We find that wild type mice injected with AAV2-α-synuclein virus have impairments in distance traveled in the open field test, and, surprisingly, Ak1^{-/-} mice have impairments in both rotarod and open field compared to wild type littermates, independent of the virus.
- Future directions include measuring adenosine after Ak1 knockdown and expanding our cohort to include 12 mice per genotype, per sex, per virus, per timepoint.