Age-Related Neurodegeneration in the Visual Thalamus of a Leigh Syndrome Mouse Model

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Introduction

Leigh syndrome is a fatal pediatric mitochondrial disorder that causes progressive neurodegeneration and vision loss. It commonly results from loss of Ndufs4, a subunit required for complex I assembly and energy production (Figure 1). Ndufs4$^{-/-}$ mice survive only to about postnatal day 50 and show accelerating retinal degeneration between weeks 5 and 7.[1] The dorsal lateral geniculate nucleus (dLGN), the thalamic relay receiving retinal input, is composed of energy-demanding neurons and may be especially vulnerable, yet it has not been examined. We compared synaptic integrity and neuronal density in the dLGN of wild-type and Ndufs4$^{-/-}$ mice at P22 and P50, hypothesizing age-dependent neuronal loss.

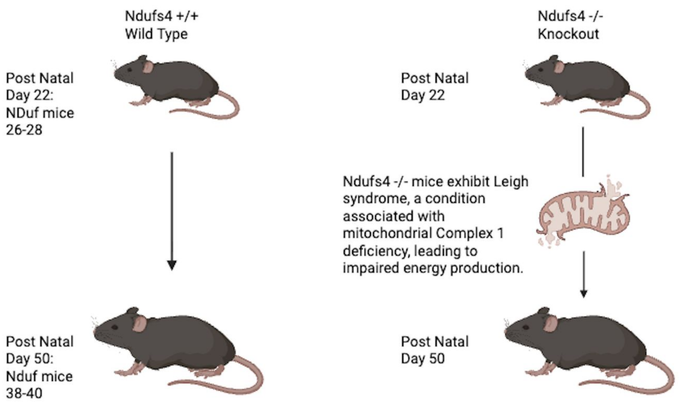

Figure 1. Experimental design. Control (Ndufs4+/+, n = 3) and knockout (Ndufs4 -/-, n = 3) mice were examined at postnatal day (P) 22 and P50. All analyses were performed blind to genotype and age.

Methods

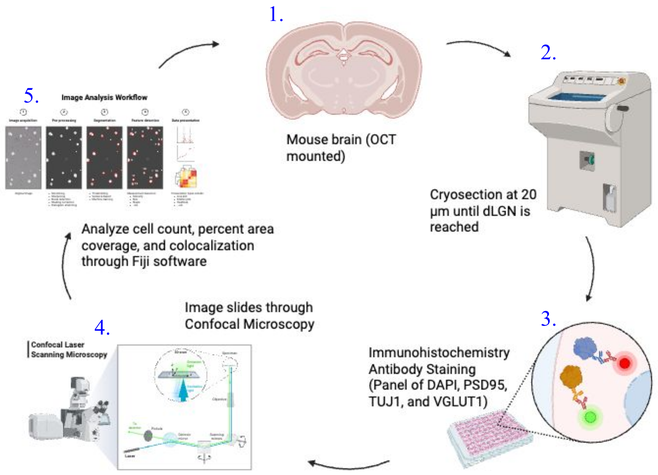

Figure 2. Experimental workflow. The same procedure was applied to all six mice: cryosectioning, staining with consistent antibodies and ratios , imaging, and analysis.

Brains from wild-type and Ndufs4$^{-/-}$ mice were cryosectioned coronally at 20 µm through the dLGN.

Following antibody panel optimization, sections were stained for DAPI (highlighting cell nuclei), VGLUT1 (highlighting presynaptic terminals), PSD95 (highlighting postsynaptic terminals), and Tuj1 (highlighting class III beta-tubulin, found in neurons) and imaged by confocal microscopy as stitched z-stack montages (Figure 2).

Synaptic integrity was quantified by VGLUT1–PSD95 colocalization in Fiji/ImageJ. Neuronal density was quantified from DAPI-labeled nuclei in a single mid-stack optical section to avoid projection artifacts. A size and circularity filter excluded elongated endothelial nuclei, and identical parameters were applied across all samples.

References

1. Avrutsky MI, Lawson JM, Smart JE, Chen CW, Troy CM. Noninvasive ophthalmic imaging measures retinal degeneration and vision deficits in *Ndufs4*$^{-/-}$ mouse model of mitochondrial complex I deficiency. *Transl Vis Sci Technol.* 2022;11(8):5. doi:10.1167/tvst.11.8.5
2. Lake NJ, Bird MJ, Isohanni P, Paetau A. Leigh syndrome: neuropathology and pathogenesis. *J Neuropathol Exp Neurol.* 2015;74(6):482–492. doi:10.1097/NEN.0000000000000195

Results

While VGLUT1–PSD95 colocalization showed no interpretable results, nuclear density was reduced in Ndufs4$^{-/-}$ mice compared with wild-type littermates (338 versus 585 nuclei/mm²). This reduction appears to be age-dependent: at P22, knockout density fell within the wild-type range, whereas at P50 both knockout animals showed less than half of wild-type density. Mean nuclear size was comparable between groups, indicating a loss of cells rather than altered nuclear morphology.

Figure 3. Finalized antibody panel. Representative confocal images of dLGN immunostained for (A) PSD-95, a postsynaptic marker (green); (B) TUJ1 (βIII-tubulin), a neuronal marker (red); and (C) VGLUT1, a presynaptic marker (magenta).

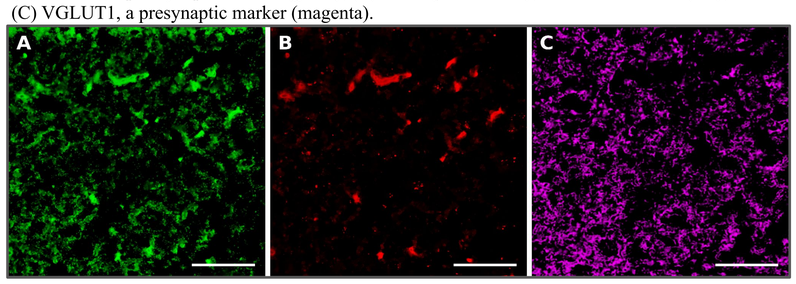

Table 1. dLGN nuclear density by animal

Animal	Age	Genotype	Nuclear density (nuclei/mm²)
ND26	P22	WT	510
ND27	P22	WT	656
ND28	P22	*Ndufs4*$^{-/-}$	487
ND38	P50	WT	588
ND39	P50	*Ndufs4*$^{-/-}$	268
ND40	P50	*Ndufs4*$^{-/-}$	259

Table 2. Group summary

Genotype	Age	n	Mean density (nuclei/mm²)	KO as % of age-matched WT
WT	All ages	3	585 ± 73	—
Ndufs4$^{-/-}$	All ages	3	338 ± 129	58%
WT	P22	2	583	—
Ndufs4$^{-/-}$	P22	1	487	84%
WT	P50	1	588	—
Ndufs4$^{-/-}$	P50	2	264	45%

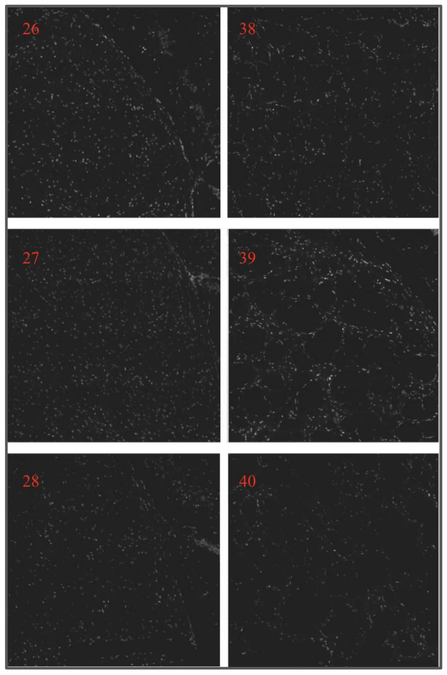

Figure 4. DAPI nuclear staining in dLGN of all six mice. Representative confocal images of DAPI-stained sections from control (Ndufs4 +/+) and knockout (Ndufs4−/−) mice at P22 and P50.

Conclusion

VGLUT1–PSD95 colocalization did not yield interpretable differences between genotypes. Nuclear density, however, was reduced in Ndufs4$^{-/-}$ mice compared with wild-type littermates (338 versus 585 nuclei/mm²). This reduction appeared to be age-dependent: at P22, knockout density fell within the wild-type range, whereas at P50 both knockout animals showed less than half of wild-type density. Mean nuclear size was comparable between groups, indicating a loss of cells rather than altered nuclear morphology. Knockout tissue also showed enlarged vasculature within the dLGN, most prominently at P50. This suggests that vascular changes accompany neuronal loss as the disease progresses, perhaps indicating that the dLGN develops Leigh-type lesion pathology as the disease progresses.

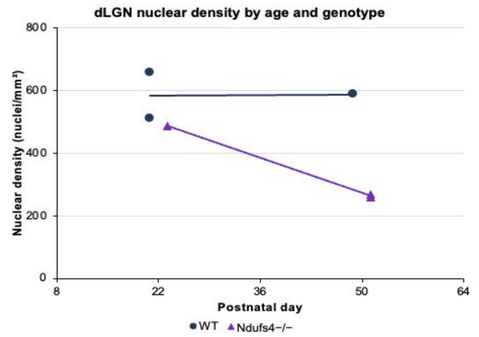

Figure 5. Nuclear density in the dLGN declined with age in Ndufs4 −/− mice. Knockout mice showed lower nuclear density at P50 than at P22; control mice did not.

Future Directions

Larger, age-matched cohorts, including intermediate timepoints, will allow statistical testing and define when neuronal loss begins. NeuN labeling will confirm that the lost cells are neurons. Quantifying vessel diameter will test whether vascular enlargement tracks with neuronal loss. This baseline will support the lab's upcoming study of a potential therapeutic intervention in the Leigh Syndrome model mice.

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