INVESTIGATING THE NEUROPROTECTIVE EFFECTS OF FINGOLIMOD ON VISUAL SYSTEM DEFICITS IN ALZHEIMER'S DISEASE

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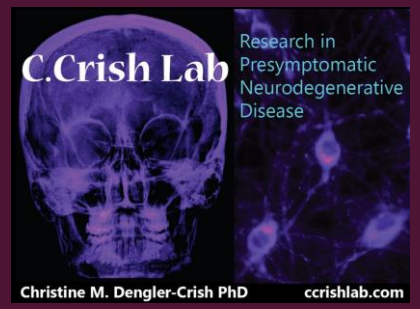
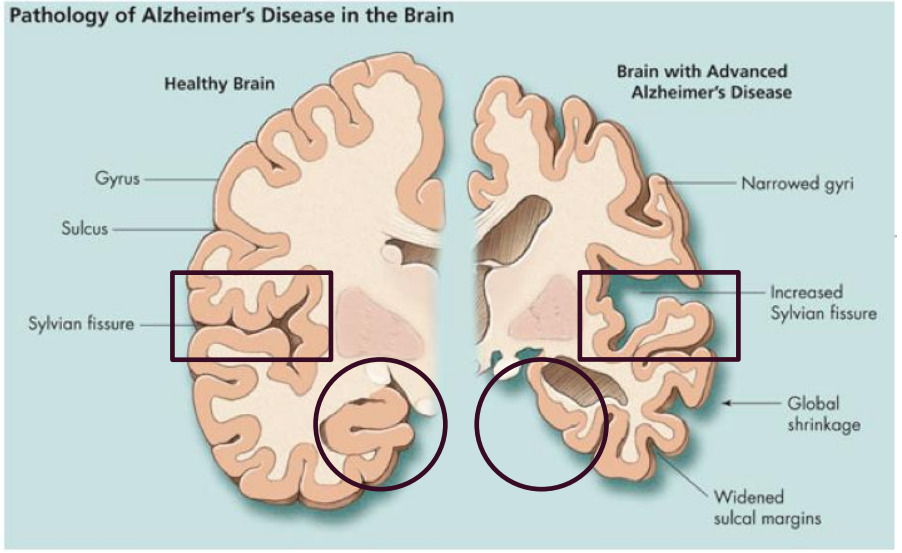


ALZHEIMER'S DISEASE

- Progressive neurodegenerative disease
- Hallmark pathologies
  - Amyloid beta plaques
  - Hyperphosphorylated tau (ptau)
  - Neuroinflammation
- Deficits in memory, cognition, emotional regulation, and speech



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ALZHEIMER'S DISEASE AND VISION

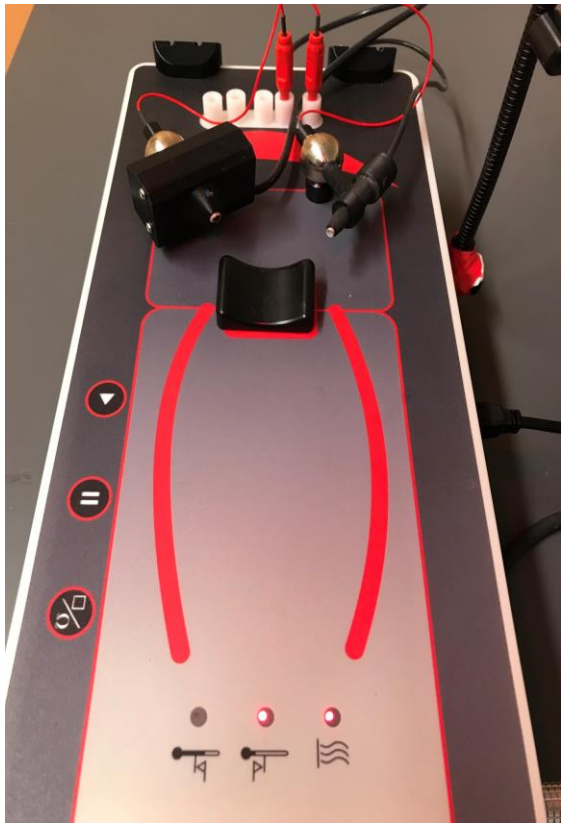
- Alzheimer's disease pathology has been shown to occur in the retina and precedes accumulation in the brain and associated cognitive deficits
- Patients with Alzheimer's disease often report visual deficits prior to AD diagnosis, however, these symptoms are usually attributed to general aging
  - Decreased visual acuity and deficits in contrast sensitivity
- The visual system is an attractive target for early detection of AD given the ability to non-invasively visualize and monitor it over time

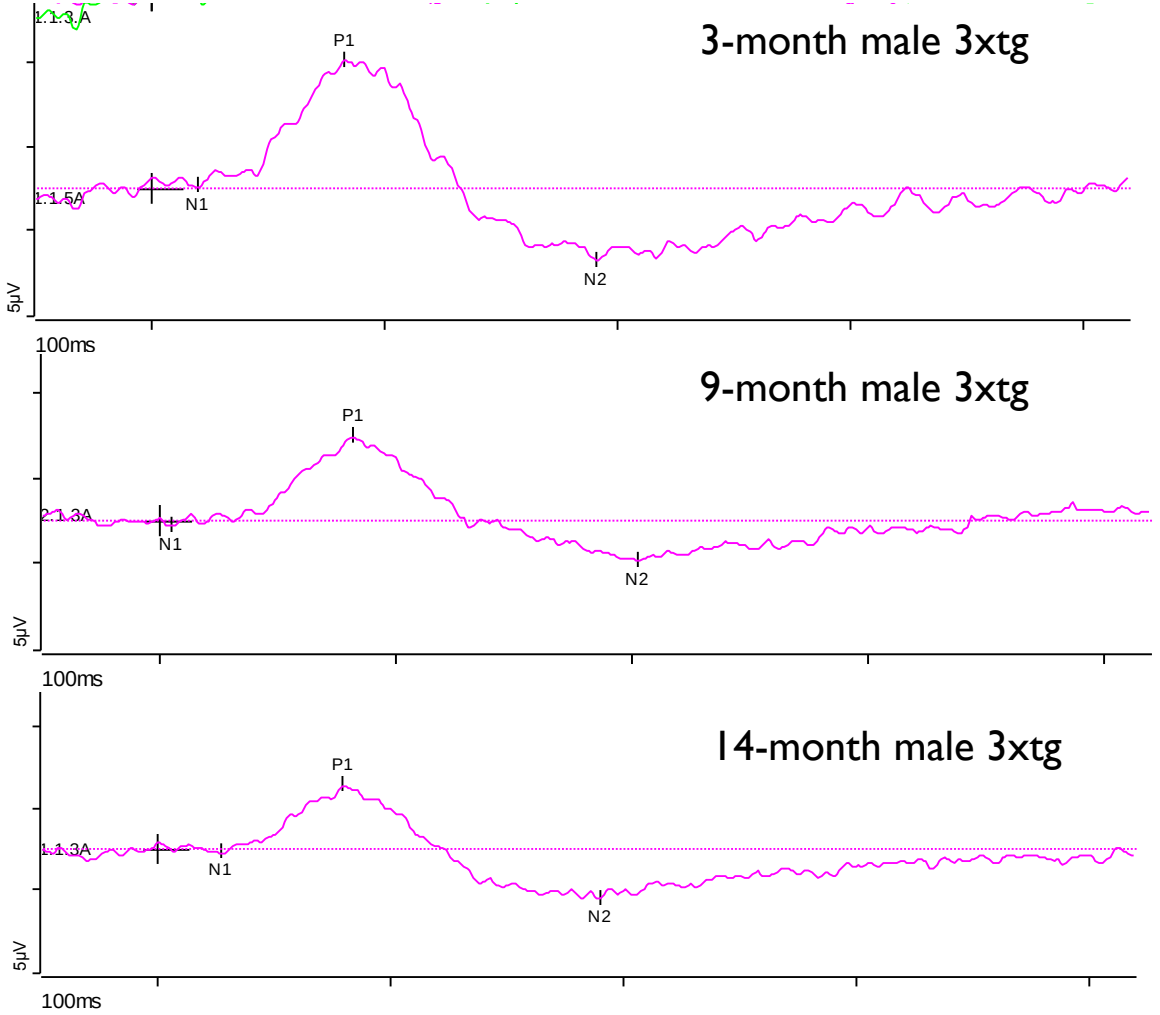
WHY SHOULD WE CARE?

- Currently, no disease-modifying treatments are available, with research focusing on methods for early detection and disease management
  - ~6 million Americans currently affected
  - 6th leading cause of death in the US
  - Only top 10 cause of death in the US without intervention available
- Alzheimer's diseases robs people of their independence and drastically reduces their quality of life as the disease progresses
  - Visual deficits only further exacerbate these effects, but also prevents otherwise healthy individuals from completing essential day to day tasks
  - In patients with both Alzheimer's disease and visual deficits, symptomology of Alzheimer's disease may be exacerbated as patients struggle to recognize faces and navigate the world around them

AIM 1: OPTIMIZATION OF METHODS FOR IN-VIVO DISEASE DETECTION AND MONITORING

PATTERN ELECTRORETINOGRAM





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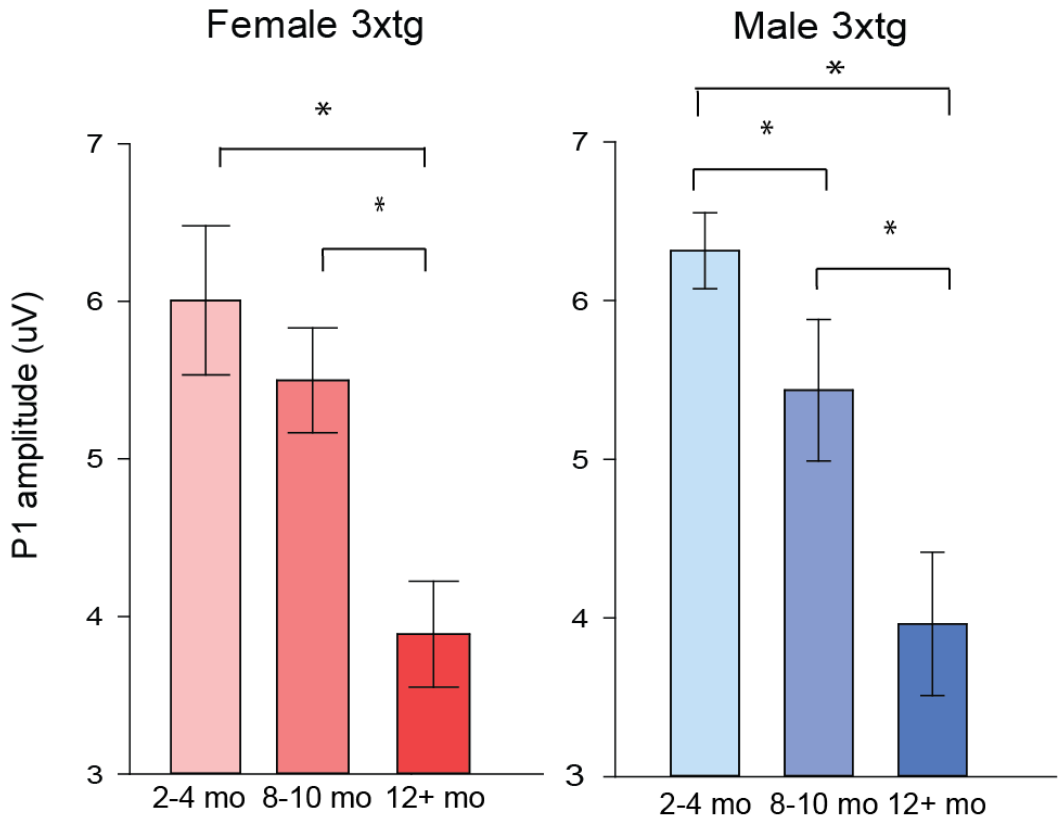
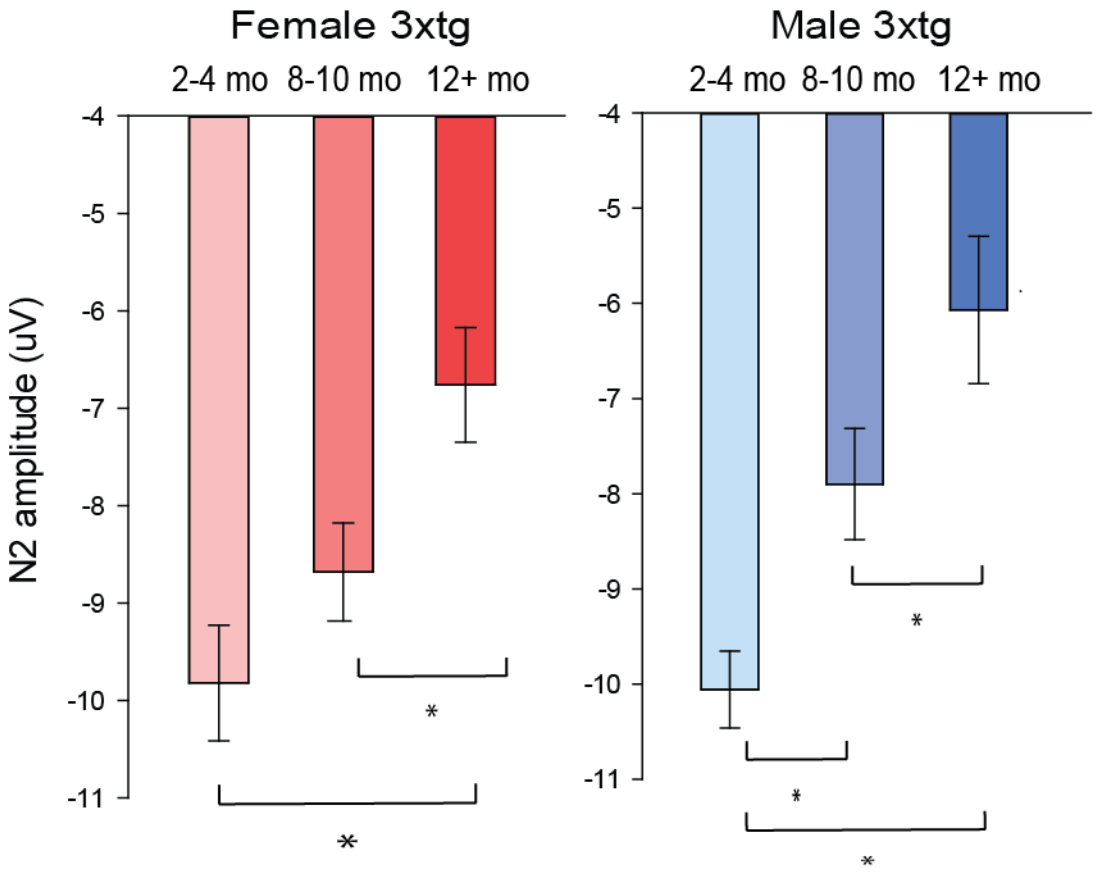
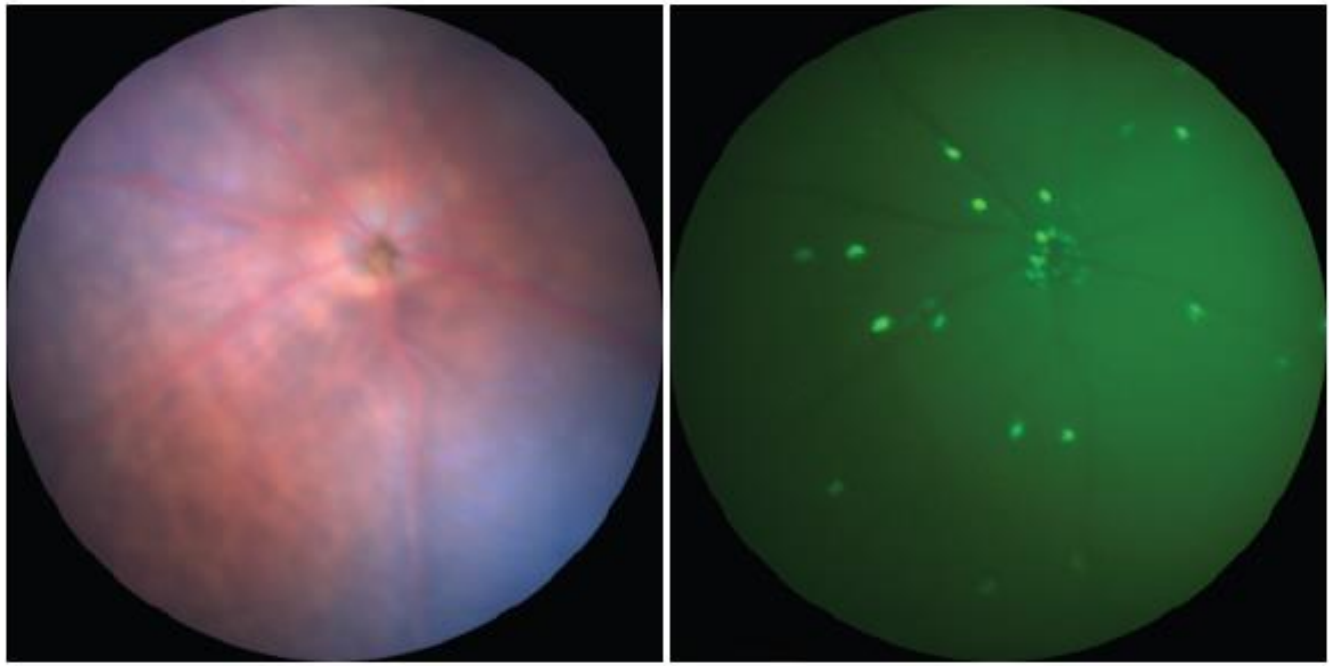




IN VIVO RETINAL IMAGING

APP/PS1 (female, 4 months) Left retinae



Bright field

Alexafluor488-tagged anti-Aß

- Injected 1.5 µL of anti-amyloid beta conjugated to Alexa Fluor 488 (Santa Cruz, sc28365 AF488)
- Imaged using Micron IV from Phoenix Technology Group

AIM 2: DETERMINING FINGOLIMOD'S EFFICACY AS A NEUROPROTECTIVE AGENT

THERAPEUTIC STRATEGIES FOR ALZHEIMER'S DISEASE

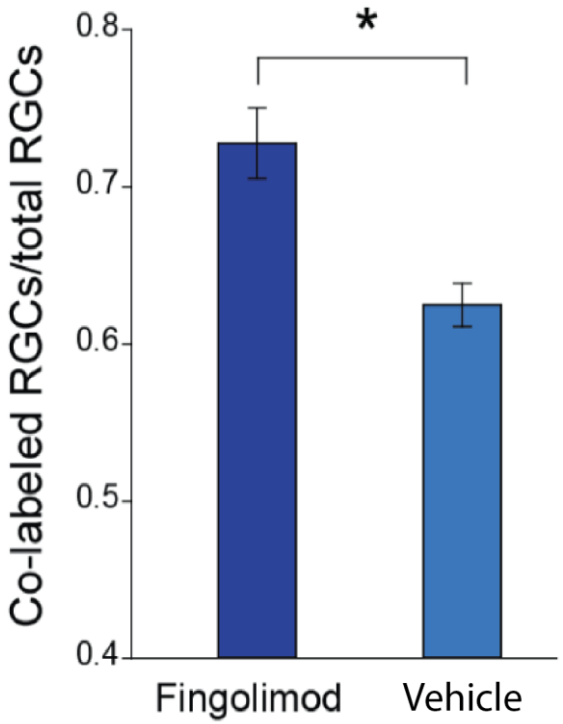
Current therapies (non-disease modifying)

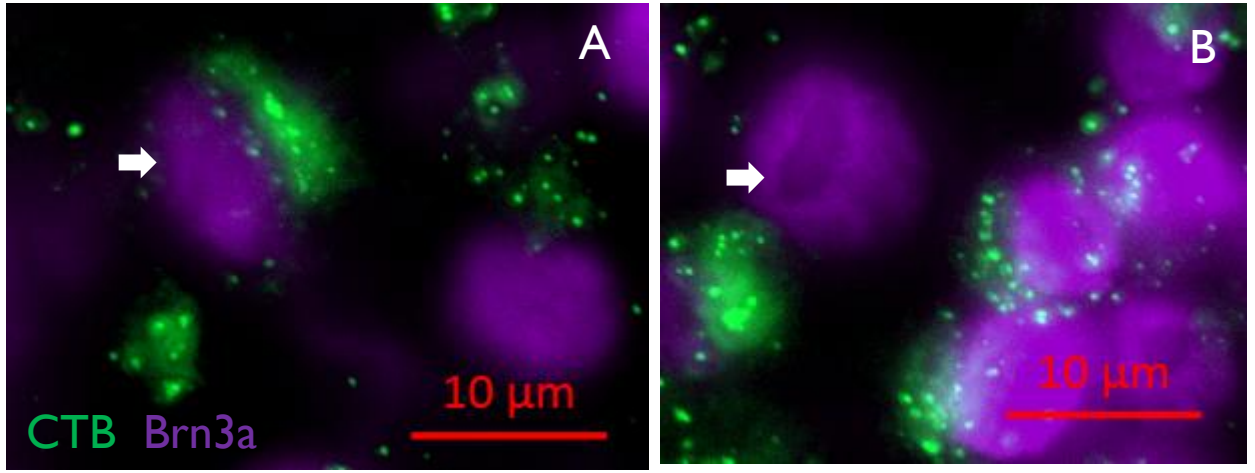
- Acetylcholinesterase Inhibitors (AChEIs)
  - Prolong action of acetylcholine at the synapse
- NMDA receptor antagonist
  - Reduces calcium influx; protective against glutamate toxicity
- Mood stabilizers

Proposed therapy (disease-modifying)

- Immunomodulator
  - Fingolimod
    - Currently used for treatment of multiple sclerosis
    - Reduces inflammatory responses
    - Activator of protein phosphatase 2A (PP2A)

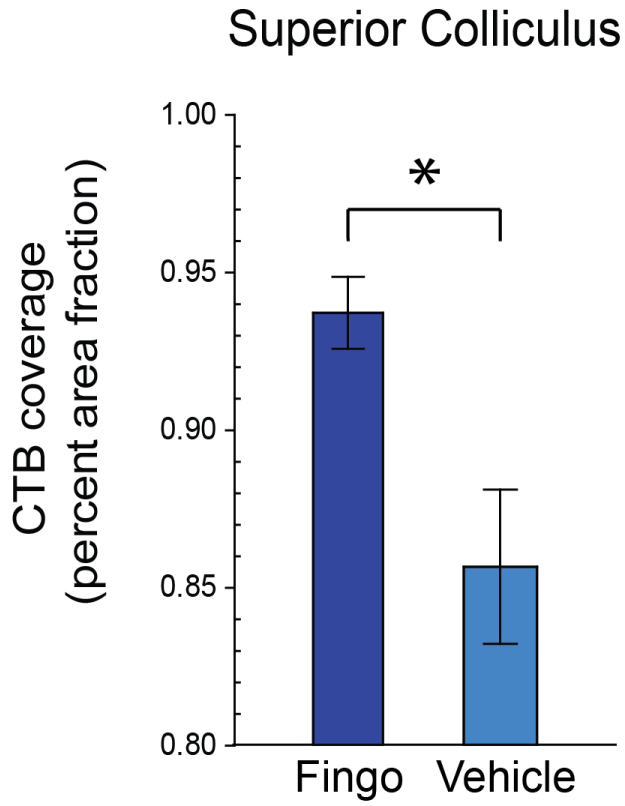
RETINAL GANGLION CELL FUNCTION AFTER FINGOLIMOD TREATMENT

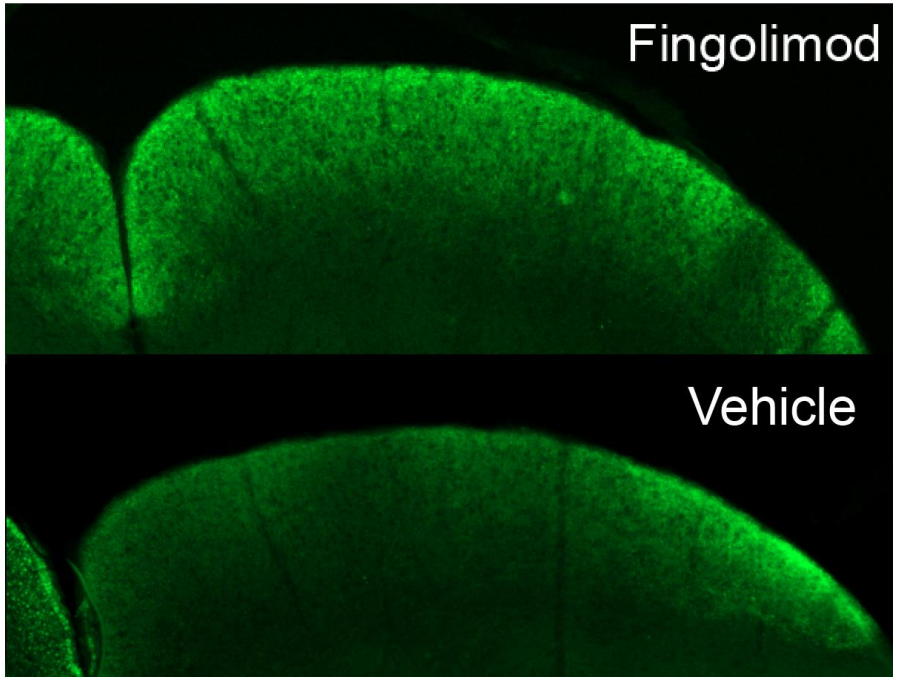




High magnification images of a 6-month-old 3xtg female mouse retina showing (A) an example of colocalization of CTB and Brn3a-positive cells and (B) and Brn3a-positive cell with no colocalization of CTB.

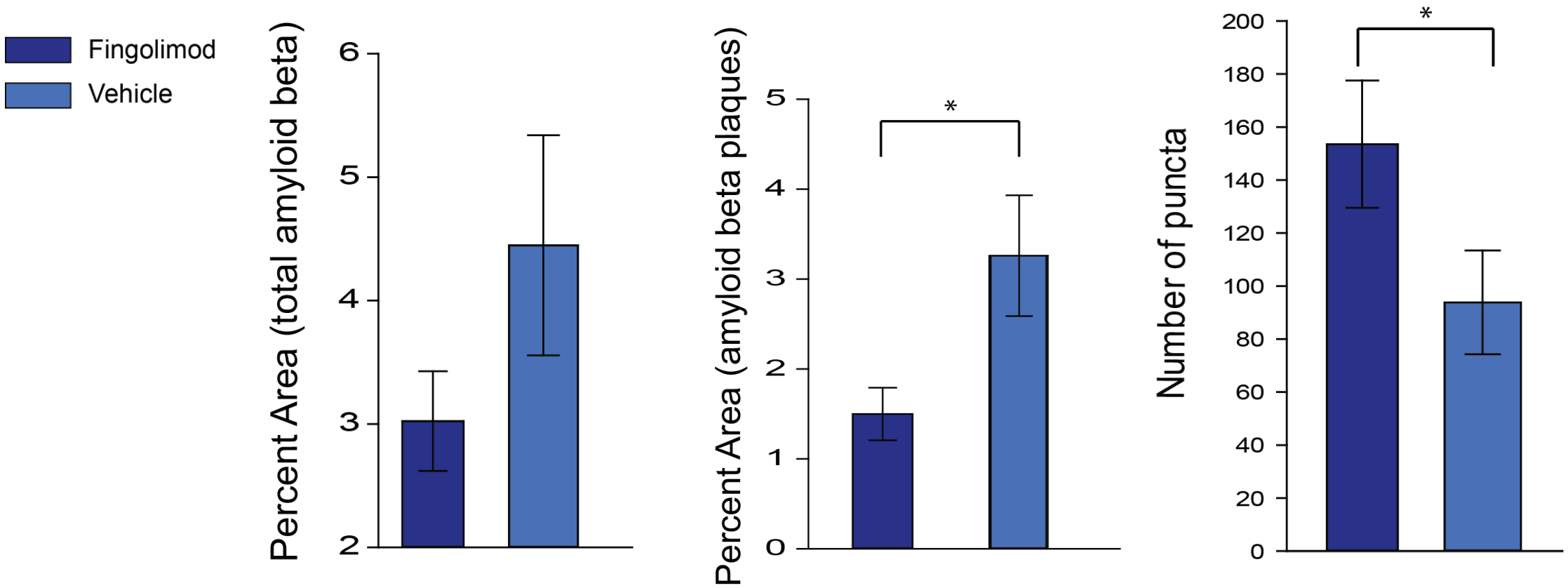
AXONAL TRANSPORT AFTER FINGOLIMOD TREATMENT





Representative images CTB coverage in the superior colliculus of fingolimod and vehicle treated 3xtg mice (female, 6 months).

CORTICAL AMYLOID PATHOLOGY AFTER FINGOLIMOD TREATMENT



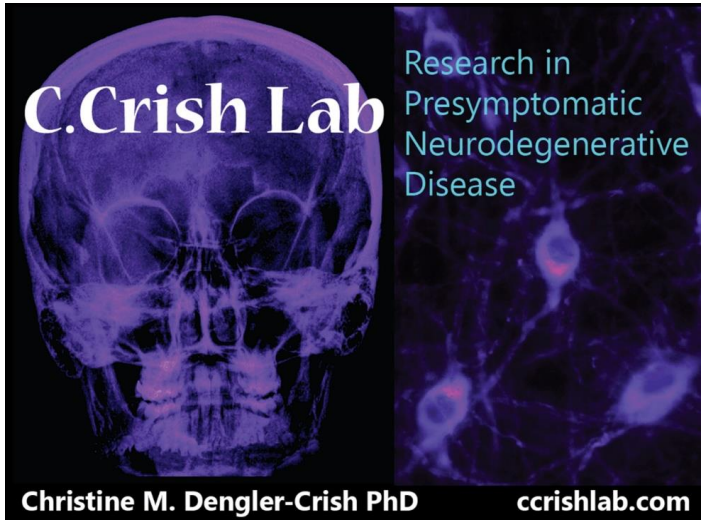
CONCLUSIONS

- Pattern electroretinogram recordings displayed a significant decrease in amplitude throughout disease progression, showing promise as a method for monitoring visual dysfunction associated with Alzheimer's disease progression
- Demonstrated proof-of-concept of visualizing retinal amyloid beta in vivo
- Preliminary evidence suggests that fingolimod:
  - may preserve retinal ganglion cell functionality after disease pathology onset in the visual system
  - may be effective as a neuroprotectant against cortical amyloid pathology

FUTURE DIRECTIONS

- Chronic administration of fingolimod to an amyloid dominant Alzheimer's mouse model
- Characterize various components of immune response (microglia, inflammatory cytokines) following fingolimod administration
- Behavioral and cognitive testing of fingolimod-treated Alzheimer's mice
- Further optimization of PERG recordings for various Alzheimer's mouse models

ACKNOWLEDGEMENTS



- Dr. Christine Crish
- Dr. Matthew Smith
- Katie Bretland
- Emily Simons
- Li Lin

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QUESTIONS?

METHODOLOGY

PATTERN ELECTRORETINOGRAM

- **Preparation of mice**
- Dark-adapted for 1-hour prior to recording
- Anesthetized with ketamine/xylazine (i.p.)
- Pupils dilated with 1% tropicamide

- **PERG response measurement using Diagnosys Celeris system**
- Mice were placed on the apparatus
- Electrodes for stimuli and reference were oriented to each eye as described in Figure 1
- Black and white bar pattern stimuli presented at spatial frequency of 0.155 cycles/degree and a luminance of 50 cd/m^2
- 600 sweeps were recorded for each eye; then averaged values for amplitude and latency of P1 and N2 were computed

IN VIVO RETINAL IMAGING

- Intravitreal injections of fluorescence-tagged amyloid antibody
  - Mice anesthetized with 2.5% isoflurane
  - 1.5 µL of anti-amyloid beta conjugated to Alexa Fluor 488 (Santa Cruz, sc-28365 AF488) was injected into vitreal chamber of eye via a Hamilton syringe using 33G needle
  - Mice were allowed to recover for 4-48 hours prior to retinal imaging

- Retinal imaging with Micron-IV ophthalmoscope (Phoenix Technology Group)
  - Mice anesthetized with ketamine/xylazine (i.p.)
  - Pupils dilated with 1% tropicamide
  - Mice placed into holding frame and ophthalmoscope was positioned on eye
  - Images were focused around the optic disc for consistency between animals
  - Both antibody-injected eyes and un-injected eyes were imaged under white light and fluorescent 488 channel.

CTB INJECTIONS AND TISSUE COLLECTION

- Intravitreal injections of 1.5 µL 0.1% cholera toxin B conjugated to Alexa Fluor 488 (CTB) were administered to each eye
- After 48 hours, mice were transcardially perfused with PBS and 4% paraformaldehyde
- Brain, retina, and ON were dissected and post-fixed
- Coronal sections (50 µm) of brain tissue were taken through midbrain on a freezing microtome for assays

MICROSCOPY AND ANALYSIS

- Superior colliculus (SC) sections were imaged for CTB on a Zeiss Axio Imager M2 microscope
- Percent area fraction of label across SC area from each image was quantified for each label using a custom-written macro on ImageJ (Dengler-Crish et al., 2014)
- Analysis of Brn3a/CTB colocalization involved generating a z-stack of images throughout a section of tissue which was then compressed into a single image
  - Images were analyzed by looking for colocalization of signal from the two channels used
  - z-stacked images were utilized if there was a question whether there was true colocalization of CTB and Brn3a or if the colocalization seen was due to compression of the image