Mitigating Stress-Driven Potentiation of Amyloid Pathology via Frequency-Specific Audiovisual Flicker Stimulation

Fall 2025 Research Report

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Background.
Psychological stress can more than double the risk of neurodegenerative disease.[1] Current mitigation approaches for undesirable stress effects either involve pharmacology and/or other interventions associated with poor efficacy or adherence. Our lab recently showed that audiovisual (AV) flicker, or rhythmic light and sound stimulation at specific frequencies, ameliorates pathology under conditions of stress[2] or neurodegeneration[3] alone. However, the effects of flicker have not yet been examined at the intersection of both stress and neurodegeneration. In order to address this gap, we use a model of chronic psychological stress in the context of Alzheimer's disease pathology to test whether our non-invasive, drug free flicker intervention approach can mitigate stress-induced exacerbation of neurodegenerative diseases. We hypothesize that stress is a potentiator of neurodegenerative vulnerability and that 40 Hz flicker is a promising noninvasive strategy to counteract these effects.

Experimental Approach.
Male and female adult 5xFAD mice were exposed to chronic unpredictable stress (2 stressors/day) for a total of 30 consecutive days with concomitant flicker stimulation at either 0Hz (no stimulation, constant dark), 10 Hz, or 40 Hz for 1 hour/day (**Figure 1**). All stress groups (0-40Hz flicker intervention) were compared to stress naïve control that receive 0Hz no stimulation. These animals showed stress-induced physiological changes, including weight gain (**Figure 2**), that is mitigated by frequency specific flicker intervention.

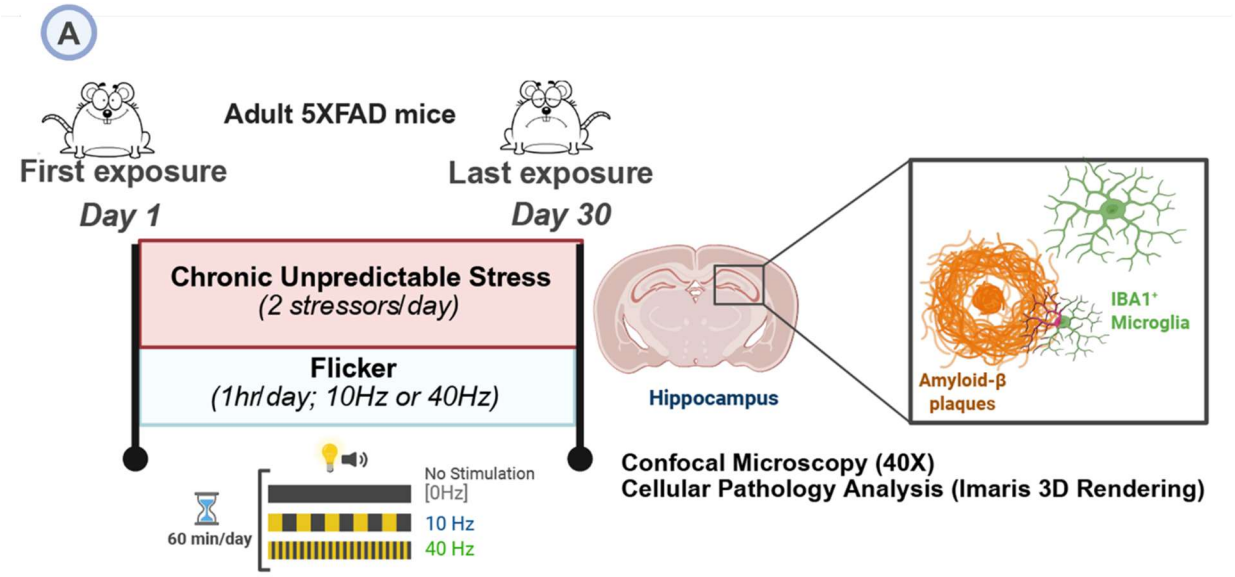

Figure 1. Experimental paradigm. 5xFAD mice exposed to both chronic unpredictable stress (0 or 2 stressors/day) and flicker stimulation (0Hz, 10Hz, or 40Hz) for 1hr/day. Stressors included unpredictable periods of food or water restrictions, cage shaking, white noise, aversive odors and reverse light exposures.

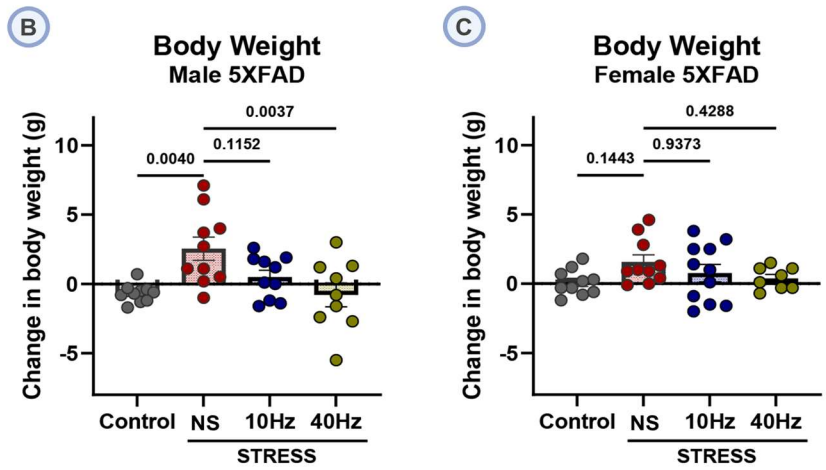

Figure 2. Frequency specific flicker mitigates stress-induced weight gain in 5XFAD mice. One-way-Anova for flicker found a significant difference ($F = 5.831$, $p = 0.0024$). Bonferonni's multiple comparison test found a significant difference between Stress-No stim and Control ($p = 0.004$) and Stress-No stim and Stress-40 Hz ($p = 0.0037$). C. There was no significant difference found in females.

Accurate 3D rendering and reconstruction of both microglia and amyloid plaques is critical for data analysis, which was largely the focus of my work this semester. This approach enables precise 3D quantification of plaque burden and glial morphology, which is essential because microglia are the brain's primary neuroimmune cell, and their state can help track neurodegenerative progression and resolution. To accomplish this, coronal brain sections were used for immunohistochemistry labeling of glia populations (IBA1 Synaptic Systems 234 308, GFAP Abcam ab4674) and amyloid debris (Cell Signaling D54D2). Amyloid plaques and microglia cells within the CA1 and CA3 subregions of the hippocampus were then imaged using Zeiss 900 series confocal microscopy, creating 3D datasets which were then processed using Imaris, an image analysis software that enables 3D reconstruction of plaque and microglial surfaces. I rendered all the surfaces used in this project using a machine-learning segmentation model for microglia and amyloid beta plaques. However, in order to get an accurate read-out, these surfaces had to undergo additional filtering and editing.

Protocol Improvement

The rendered amyloid plaques underwent a first pass of filtering based on volume. To avoid oversampling of the signal, inclusion of plaques was limited to amyloid signal with a volume 6 μm^3 or above. This filtering approach eliminated signal that was too small in size to be considered a "plaque" or was fluorescence noise that might have been incorrectly picked up in the staining process. We then found that the surface rendering was oversampling the actual amyloid staining, and thus a second pass of filtering was implemented. We determined that using an intensity mean threshold was the most effective at capturing the true staining, and thus we used this as a filter to remove any surface oversampling.

Exclusion criteria

Microglia were edited to only include whole and partial cells, following the criteria that we determined in the table below:

Inclusion	**Exclusion**
Soma + process	Loner soma: a soma (cell body) with no attached processes.
	Loner processes: a process without an affiliated soma (cell body).

Table 1. Inclusion and exclusion criteria for microglia editing.

Microglia surface unification

A “quickpass” of the unedited microglia surface (**Figure 3**, left) selected any cells that were potentially paired soma and processes, and excluded any puncta (noisy signal), loner processes, or loner soma. This selection was duplicated into a new surface, which is where the editing primarily took place. Cells were unified to correctly join processes and soma together, and any extraneous surfaces were deleted. The final result was an edited surface, named L2 Microglia (**Figure 3**, right), that only included unified soma + process pairs.

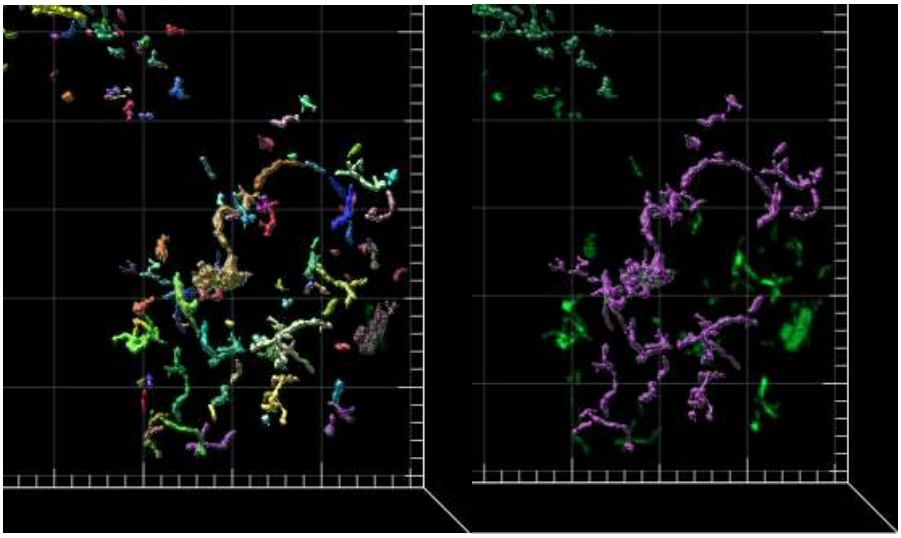

Figure 3. Side-by-side comparison of original quickpass selection (left) and final edited microglia surface with any extraneous surface deleted (right). Disconnected segments are labeled in various rendered colors. Unified cells are represented in a single rendered color.

Microglia infiltration into amyloid plaque

The filtered amyloid surface was used to find microglia infiltration within amyloid plaques, an analysis necessary to determine the effect of flicker on microglia response to the compounding effect of amyloid presence and chronic stress. This surface was masked with the unedited microglia channel to create a new colocalization channel, and a model, Microglia-Amyloid Infiltration Model A (**Figure 4**, *merged*), was used to render a surface for this channel. Data from

the colocalization surface along with data from the microglia surface were output for further statistical analysis.

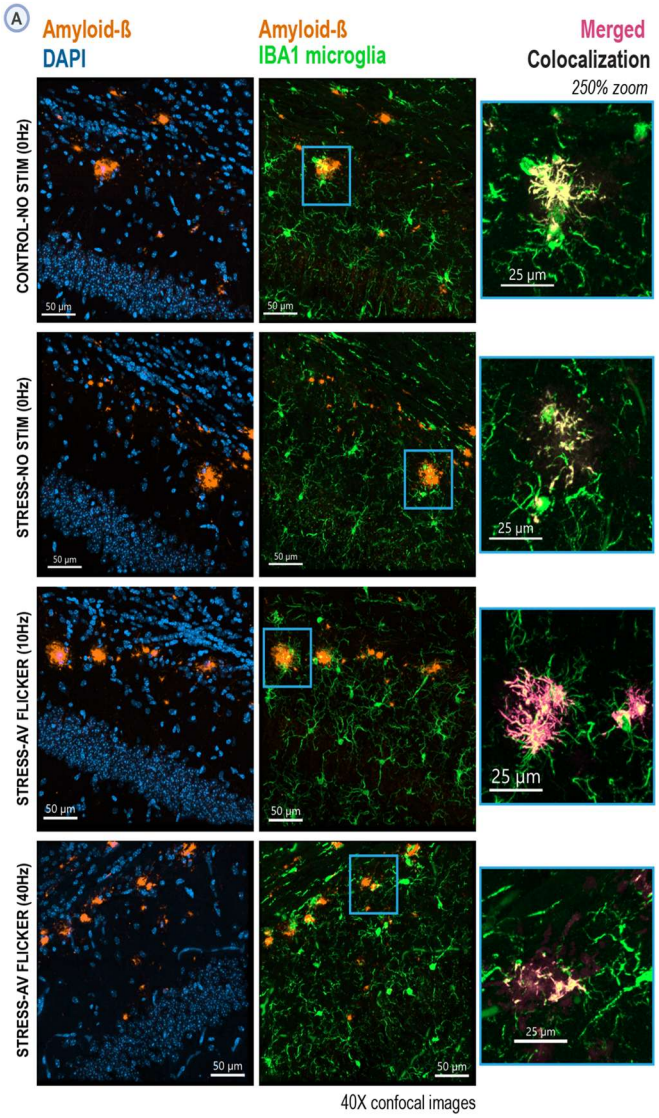

Figure 4. 10Hz flicker increases microglia colocalization with plaques and reduces overall plaque number in males. Representative images of each flicker condition (Control-NS, Stress-NS, Stress-10Hz, Stress-40Hz) of amyloid burden (left), microglia signal (middle), and colocalization (right).

Results.

Based on both the cellular assessment and the Imaris data outputs (colocalization of microglia and amyloid, total plaque volume, and large plaque volume), the following results were obtained. Stressed male 5XFAD mice show decreased microglia contact with amyloid plaques (**Figure 5A,** *NS vs Control*). This deficiency prevented with frequency specific flicker and even enhances microglia contact with amyloid at 10Hz (*10Hz vs Control*). Moreover, stressed male 5XFAD mice show increased vulnerability to stress-induced amyloid accumulation in the CA1 subregion of the hippocampus (**Figure B**). Flicker mitigated this potentiation of amyloid accumulation in male 5XFAD mice in a frequency specific manner. Specifically, 10Hz is protective against stress-

induced accumulation of large plaques in CA1, but this protective effect appears to be absent at 40Hz (**Figure 5B**).

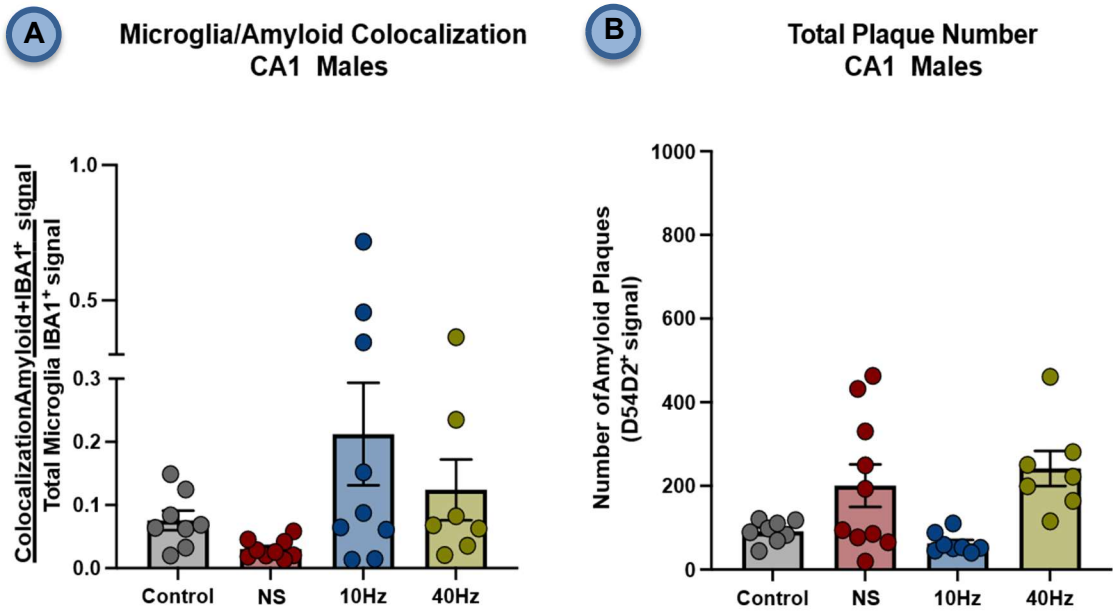

Figure 5. (A) Colocalization is measured as the ratio of IBA1 signal overlapping with amyloid signal / total IBA1 signal and (B) large plaque size of >10um3 in CA1 subregion of male 5XFAD mice. One-Way-Anova for flicker found a significant overall difference among the four groups ($F = 5.98$, $p= 0.0032$). Post hoc pairwise comparisons (Dunn's test) found a significant difference between Stress-40Hz and Stress-10Hz ($p= 0.030$), Stress-NS and Stress-10Hz ($p= 0.002$), Stress-10Hz and Ctrl-NS ($p= 0.0414$). C. Total Number of Plaques is measured as all amyloid signal. No significance found.

Conclusions.

Our results show that psychological stress exacerbates pathological and physiological outcomes in an Alzheimer's disease mouse model, with males showing greater vulnerability. Additionally, frequency-specific chronic AV flicker mitigates several stress-induced effects, notably weight gain and hippocampal amyloid-beta accumulation. Specifically, 10 Hz AV flicker in males enhances microglial association with hippocampal plaques, suggesting a sex-dependent, frequency-specific microglial response that may underlie protection. Our future directions include characterizing effective engulfment of plaque material in microglia and other phagocytic cells like astrocytes that may lead to protective cellular effects seen at 10 Hz in males. Although there was no significant effect of stress or flicker on female mice, it is possible that flicker is helping recruit another neuroimmune cell, such as astrocytes, which is why this is important to look at.

Reflections.

My work in the lab this semester has helped obtain important data for the lab's research goals and has enabled me to better train more undergraduates who will also contribute to the data analysis pipeline. This semester, I transitioned from just executing my portion of the data-analysis pipeline to gaining a deeper understanding of the lab's scientific goals. I presented at the Fall Undergraduate Neuroscience Symposium, forcing me to articulate not just what I was working on

at the lab, but also why it mattered, which enabled me to connect my day-to-day work to the larger questions that were driving our research. I also took more ownership over our methods by drafting, refining, and finalizing protocols which required me to develop skills in clarity and attention to detail. Furthermore, I was heavily involved in peer mentoring which included interviewing and screening new undergraduates, as well as training them on Imaris which made me translate my own understanding of my tasks into guidance for others. This helped me build both my communication skills and further developed my understanding of my work at the lab.

References.

1. Justice, N. J. (2018). The relationship between stress and Alzheimer's disease. *Neurobiology of stress, 8,* 127-133.
2. Franklin, T. C., Bitarafan, S., King, A. T., Goodson, M., Rutledge, C., Gajelli, T., ... & Singer, A. C. (2025). Sensory neurostimulation promotes stress resilience with frequency-specificity. *bioRxiv*.
3. Singer, A. C., Martorell, A. J., Douglas, J. M., Abdurrob, F., Attokaren, M. K., Tipton, J., ... & Tsai, L. H. (2018). Noninvasive 40-Hz light flicker to recruit microglia and reduce amyloid beta load. Nature protocols, 13(8), 1850-1868.