

Sex And Regional Differences In Microglial Response To Pathological Tau Proteins

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BACKGROUND

- There is no satisfactory explanation for the known sex- and brain region-specific vulnerability to pathological tau forms (fibrils)—neurotoxic protein aggregates that underlie clinical trajectories in aging and various forms of dementia including Alzheimer's disease.
- Microglia, the main brain-resident macrophages, are tasked with the uptake and clearance of tau fibrils. This process is critically modulated by extrinsic factors but poorly elucidated.

RATIONALE

- Astrocytes modulate microglial uptake of amyloid- β (A β) aggregates, a hallmark of Alzheimer's disease (alongside tau fibrils), to mitigate or worsen amyloidosis *in vivo*.
- Perhaps, the propensity of microglia to internalize tau fibrils is similarly modulated by cell-extrinsic factors in brain, which may contribute to sex and regional vulnerability to tau pathology in aging and related neurodegenerative disorders.

¹Lee, Si., Yu, J., Lee, H. et al. Astrocyte priming enhances microglial A β clearance and is compromised by APOE4. Nat Commun 16, 7551 (2025). <https://doi.org/10.1038/s41467-025-62995-1>

HYPOTHESIS

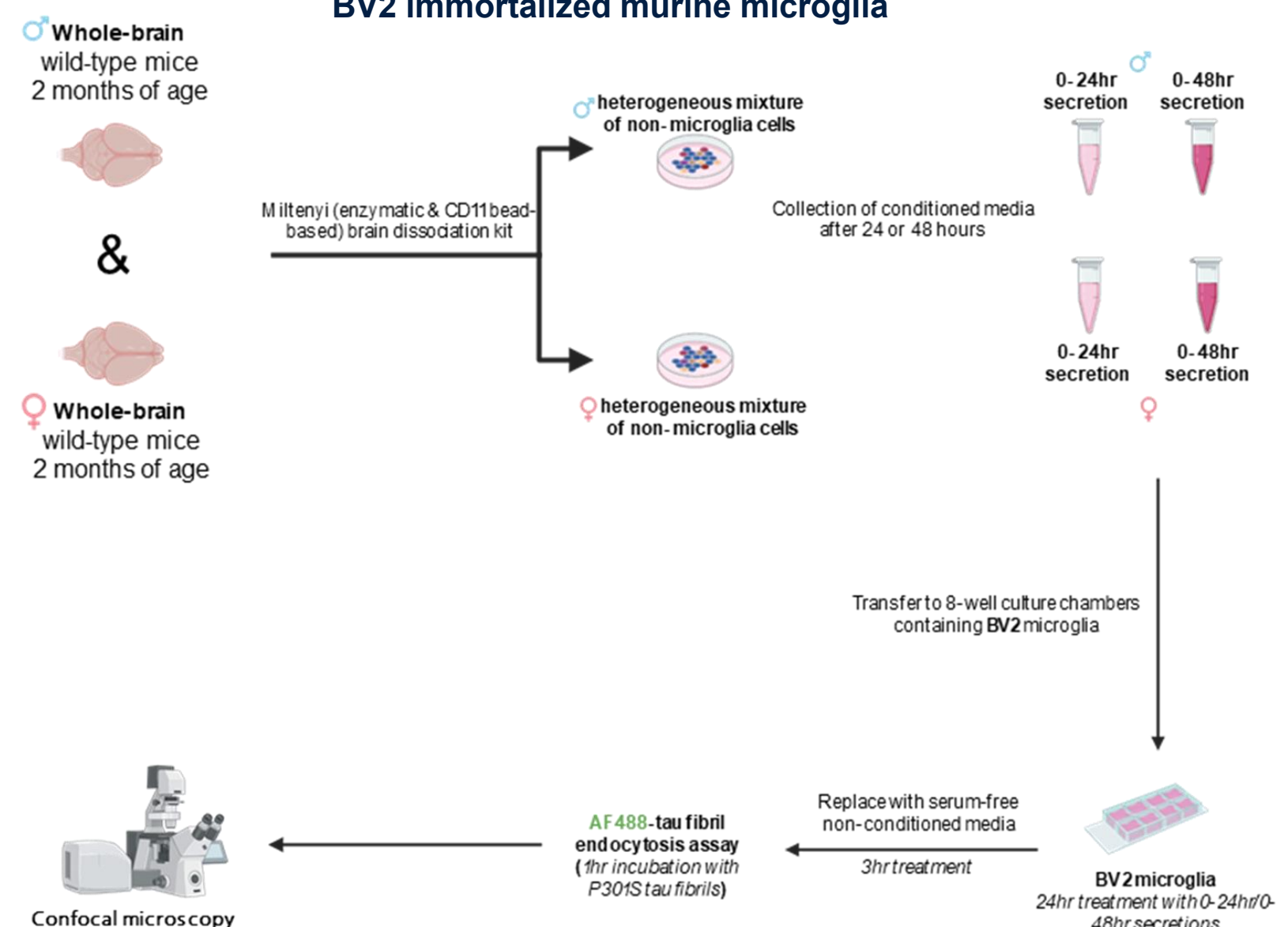
Cues (secreted factors) from mixed cultures of *non-microglia* cells from adult mouse brain will modulate *microglial* propensity for tau uptake, in sex- and region-specific manner.

OBJECTIVE

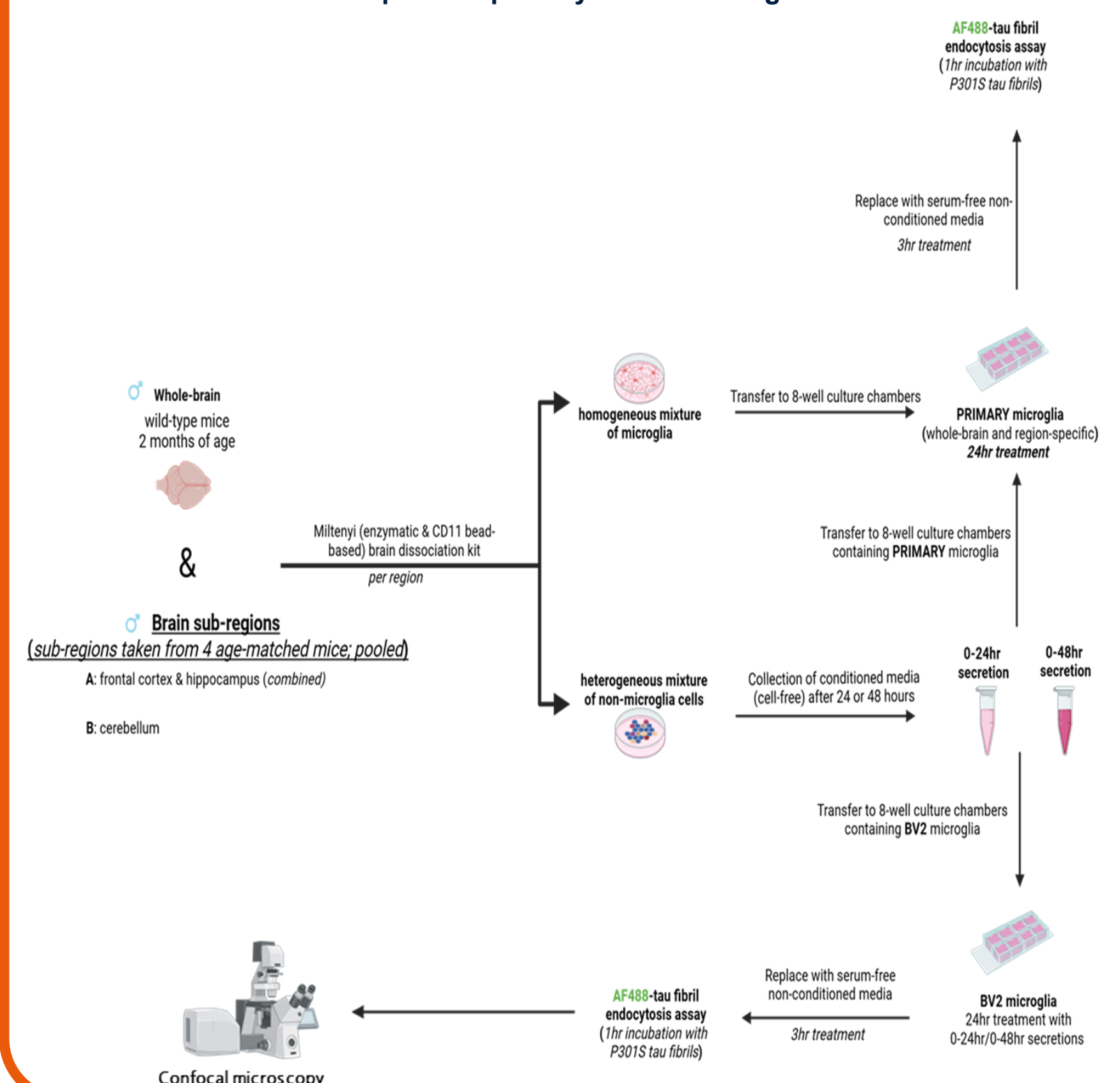
To examine microglial propensity for tau uptake after pre-treatment with conditioned media from mixed cultures of adult mouse non-microglia cells, males or females, collected from whole brains and separate brain regions (cortex+hippocampus, and cerebellum).

METHODOLOGY

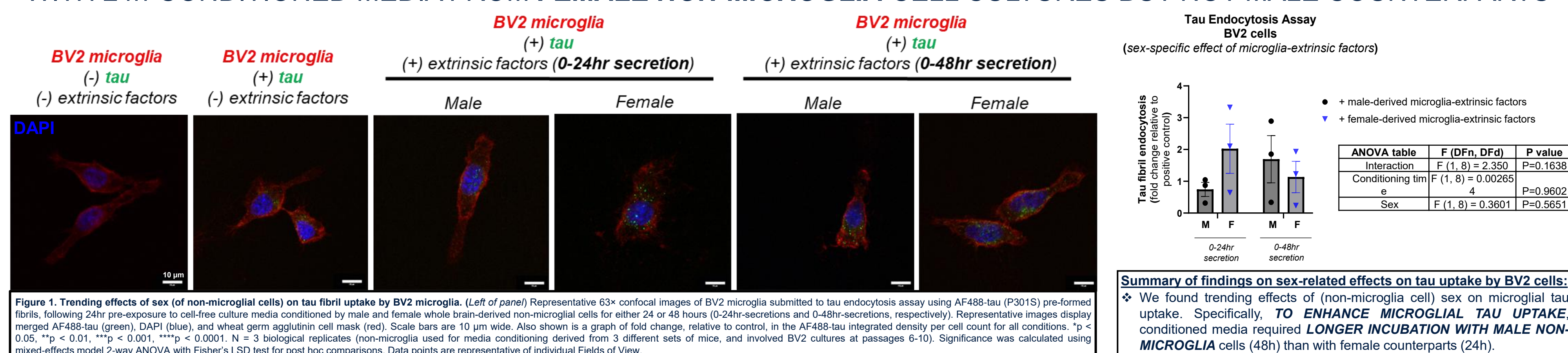
SEX-dependent effect of microglia-extrinsic factors on tau uptake in BV2 immortalized murine microglia



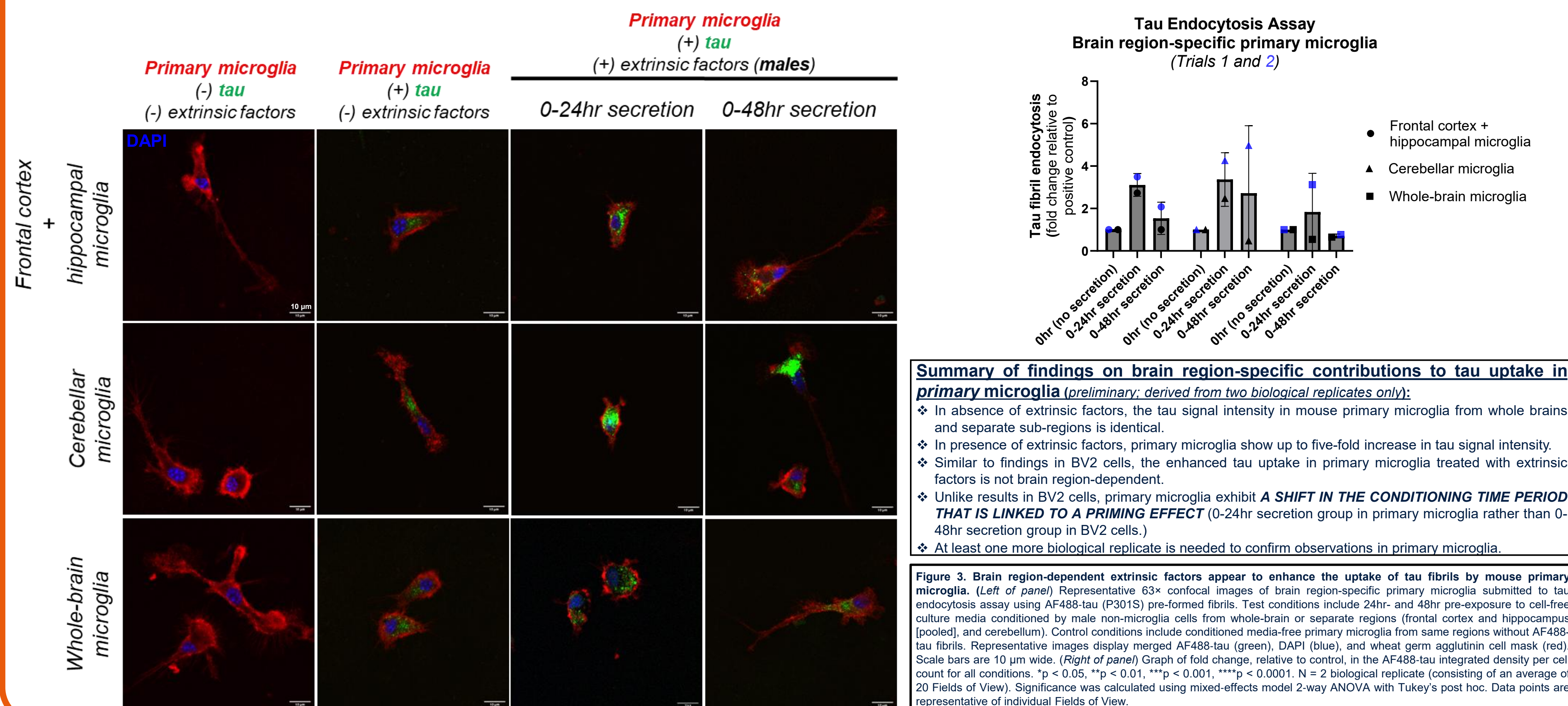
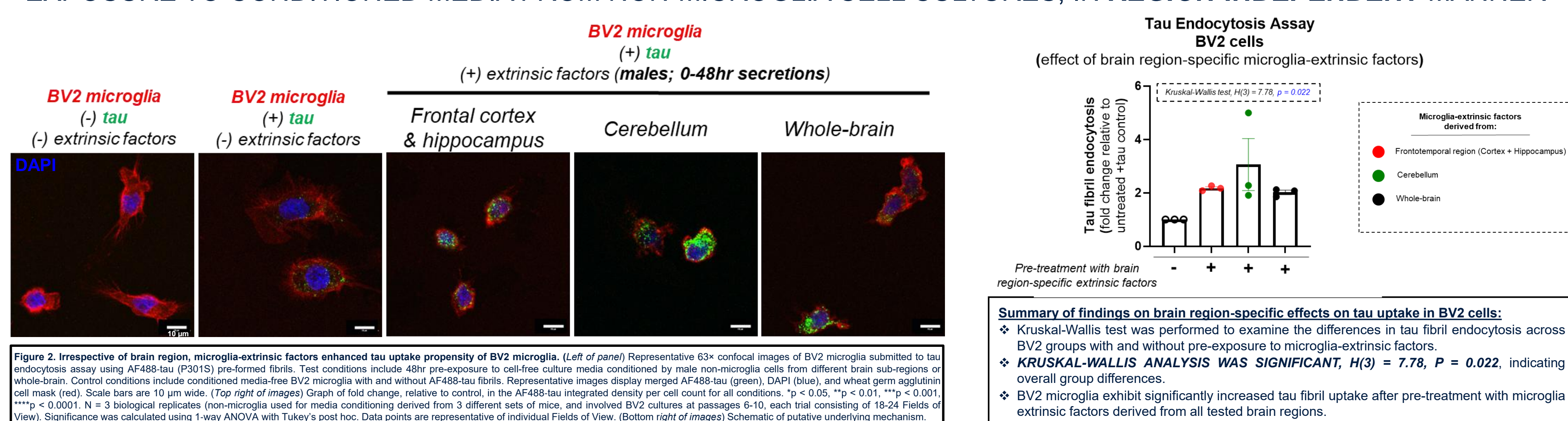
BRAIN REGION-dependent effect of microglia-intrinsic and -extrinsic factors on tau uptake in primary mouse microglia and BV2 cells



TRENDING EFFECT OF SEX: MICROGLIA SHOW ENHANCED UPTAKE OF PATHOLOGICAL TAU AFTER PRE-TREATMENT WITH 24h-CONDITIONED MEDIA FROM FEMALE NON-MICROGLIA CELL CULTURES BUT NOT MALE COUNTERPARTS

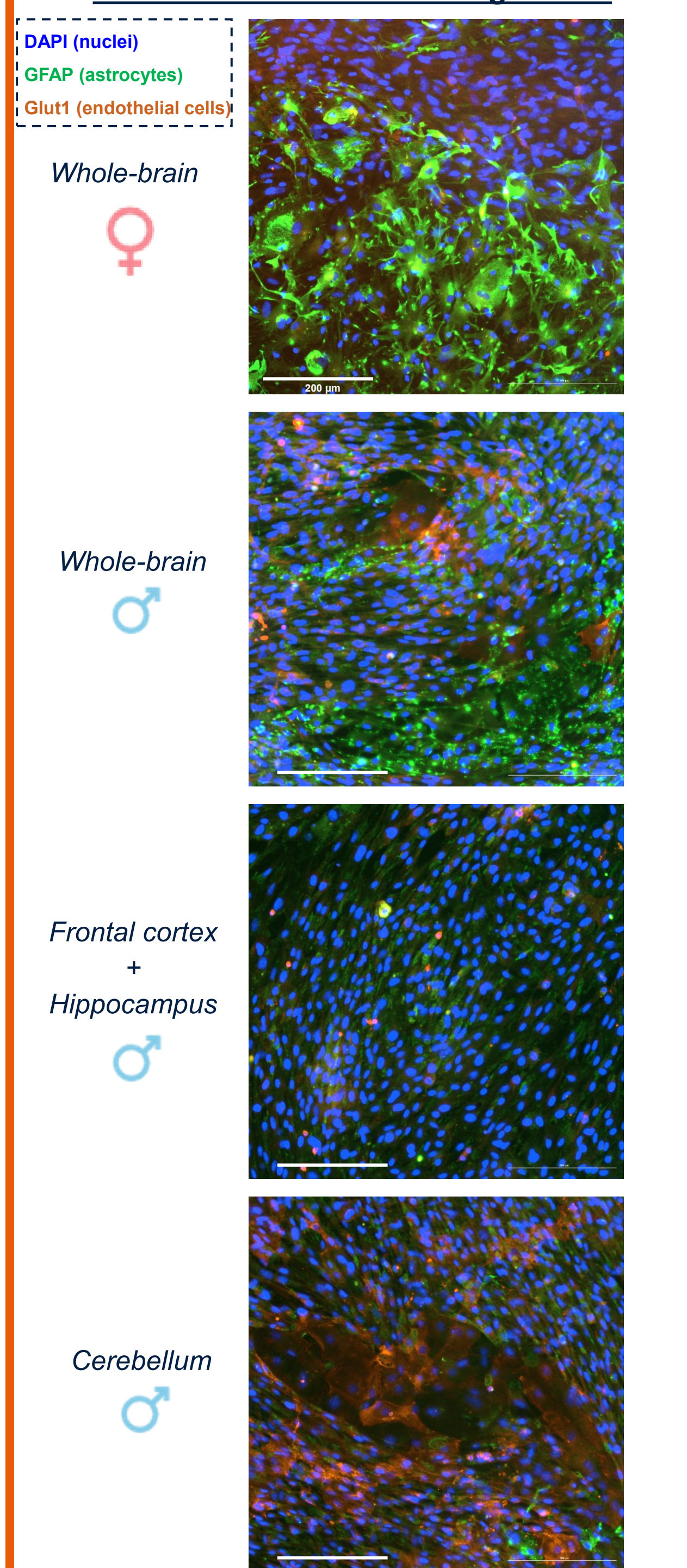


SIGNIFICANT EFFECT OF REGIONAL CUES: MICROGLIA DISPLAY ELEVATED TAU FIBRIL UPTAKE FOLLOWING PRE-EXPOSURE TO CONDITIONED MEDIA FROM NON-MICROGLIA CELL CULTURES, IN REGION-INDEPENDENT MANNER



REACTIVE ASTROCYTES AND BRAIN ENDOTHELIAL CELLS ARE ENRICHED IN NON-MICROGLIA CULTURES

(Preliminary) immunofluorescent staining of mixed cultures of non-microglia cells



Summary of observations in sex- and brain region-specific mixed cultures of non-microglial cells collected from adult mouse brains:

- Mixed cultures are predominantly enriched with astrocytes and endothelial cells.
- Oligodendrocytes and microglia are not evident in mixed cell cultures (based on immunofluorescent staining using, respectively, Olig2 and Iba1 antibodies; not shown).
- Qualitatively, although astrocytes and brain endothelial cells can overlap within cell culture, these also appear to occupy distinct niches.
- Additional experiments are needed to validate findings.

Figure 4. Mixed cultures of sex- and brain region-specific non-microglial cells are chiefly enriched with astrocytes and endothelial cells. At in vitro day 10, non-microglial cells were submitted to immunofluorescence (IF) analysis. Representative images display merged IF staining of non-microglial cell cultures from male and female whole-brains, as well as the pooled frontal cortex and hippocampus regions, and cerebellum from males only, using the astrocyte marker, GFAP (green), and the endothelial cell marker, Glut1 (orange), alongside DAPI (blue). Scale bars represent 200 μ m. Microscopy was performed via BioTek Cytation 5 Cell Imaging Multimode Reader. Scale bars are 200 μ m wide. N = 1 biological replicate. Images starting from the top: female whole-brain, male whole-brain, male (pooled) frontal cortex and hippocampus, and male cerebellum.

DISCUSSION

- Present data **SUPPORT OUR HYPOTHESIS THAT** non-microglial cells modulate microglial uptake of tau fibrils, revealing **TRENDING EFFECTS OF SEX** that are contingent on the duration of secretion (of extrinsic cues by male and female non-microglial cells) to highlight a putative layer of biological complexity.
- Current results also appear to **REJECT OUR HYPOTHESIS THAT** microglia-extrinsic factors will drive **BRAIN REGION-SPECIFIC** differences in microglial uptake of tau fibrils. Thus, suggesting that regional vulnerability to tau pathology seen in dementia types may be driven by other related mechanisms (i.e., immune response).
- Although still preliminary, immunofluorescent staining analysis of non-microglial cell cultures positions **INFLAMMATORY FACTORS AS PUTATIVE MODULATORS** of the conditioning media's sex-related microglia-priming effect. Such observations are consistent with literature on the topic, which our work expands upon via delineation of sex- and region-specific contributions (or lack thereof).

FUTURE DIRECTIONS

- Observed differences in microglial tau signal intensity could be explained by **CHANGES IN TAU ENDOCYTOSIS DRIVERS** and/or **CLEARANCE MECHANISMS**. Thus, the following next steps:
 - Assess levels of tau endocytosis drivers in treated microglia: tau receptors (LRP1, and HSPGs) and regulators of clathrin-mediated endocytosis, the main route of tau uptake in microglia.
 - Assay protein clearance mechanisms in treated microglia: autophagosome lysosome.
 - Quantify cytokines in non-microglia cell cultures, namely, those linked to microglia-priming effects by astrocytes (TNF- α , IL-6, and IL-1 β , among others)

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