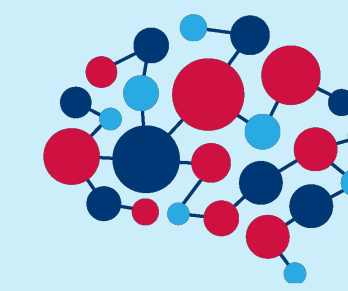


Single-Nuclei Transcriptomics Identifies Estrogen-Dependent Pathway Alterations Relevant To Alzheimer's Disease In Hippocampal Cells

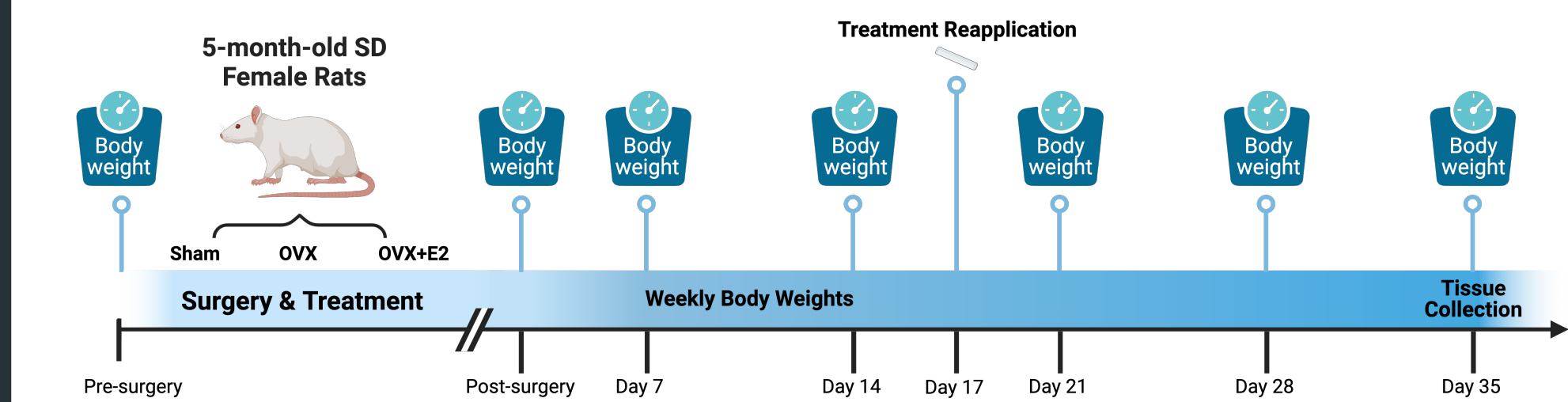
John W. McLean^{1,2}, Tian Wang¹, Yuan Shang¹, and Roberta Diaz Brinton^{1,3,4}¹Center for Innovation in Brain Science, ²Neuroscience Graduate Interdisciplinary Program,³Departments of Pharmacology and ⁴Neurology, University of Arizona, Tucson, AZ

INTRODUCTION

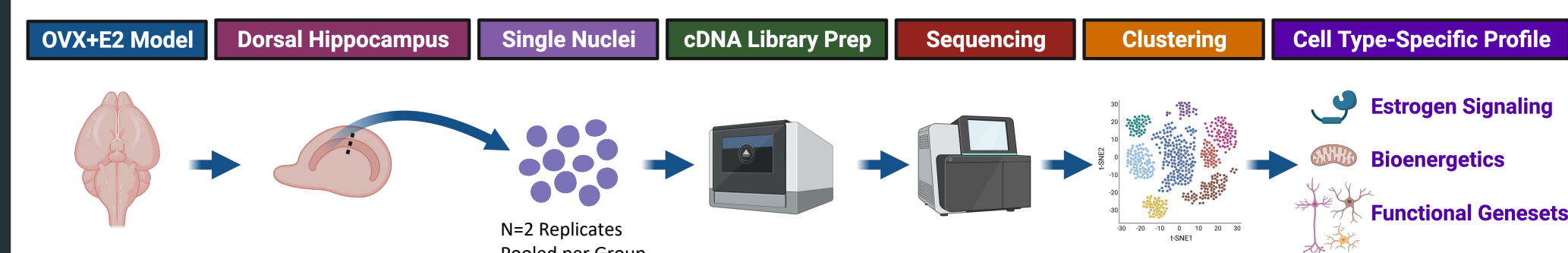
- Estrogen acts as a master regulator of systems biology in the female brain, coordinating metabolic, immune, and neuronal homeostasis. The loss of estrogen during the menopausal transition is associated with a decline in brain glucose metabolism and increased neuroinflammation, which together may contribute to neuronal vulnerability and lead to the doubled lifetime risk of Alzheimer's disease (AD) in postmenopausal women.¹
- Multiple brain cell types, including neurons, astrocytes, and microglia, express estrogen receptors, and the cell-specific response to estrogen deficiency is incompletely understood.²
- To identify brain cell populations and pathways sensitive to estrogen loss, single-nuclei transcriptomics from dorsal hippocampi in an ovariectomized rat model are reported here.

METHODS

- Outlined below, 5-month-old female Sprague Dawley (SD) rats were randomly assigned to one of the following groups: sham ovariectomized (SHAM; n=5), ovariectomized plus vehicle (OVX; n=6), or OVX with 17 β -estradiol (OVX+E2; n=7). Immediately after surgery, silastic capsules containing vehicle (cholesterol powder) or 0.8mg E2 were inserted subcutaneously in the animal's upper back. Animals were weighed before and weekly after surgery; 5 weeks after surgery, animals were sacrificed and collected tissues were flash-frozen.

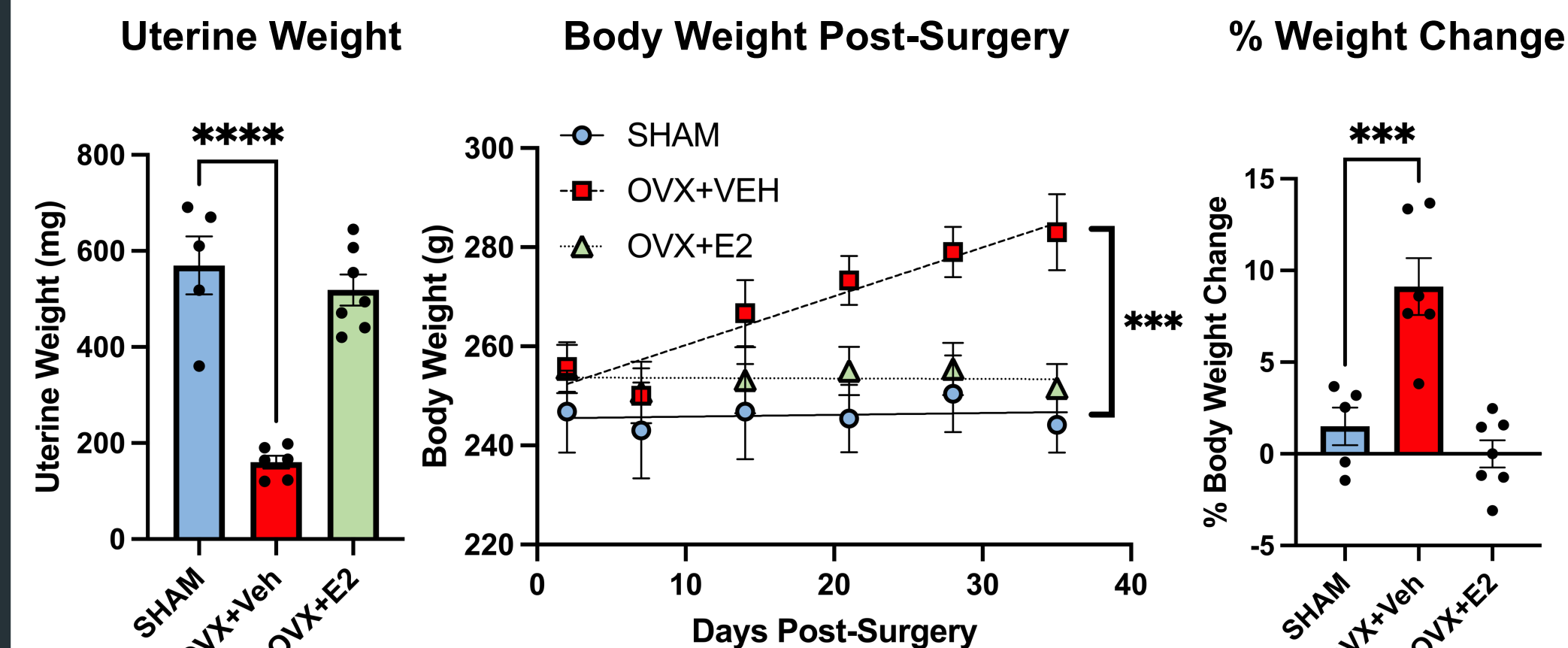


- The following day, nuclei were isolated from dorsal hippocampus using 10X Genomics Chromium Nuclei Isolation kit. 2 biological replicates per treatment group were pooled and a 10X Chromium Controller was used to generate a single-nuclei cDNA library for each condition. Libraries were sequenced on an Illumina NovaSeq6000, processed with Cell Ranger v9.0.1, and analyzed in R/Seurat after QC filtering, including removal of nuclei with >5% mitochondrial reads.

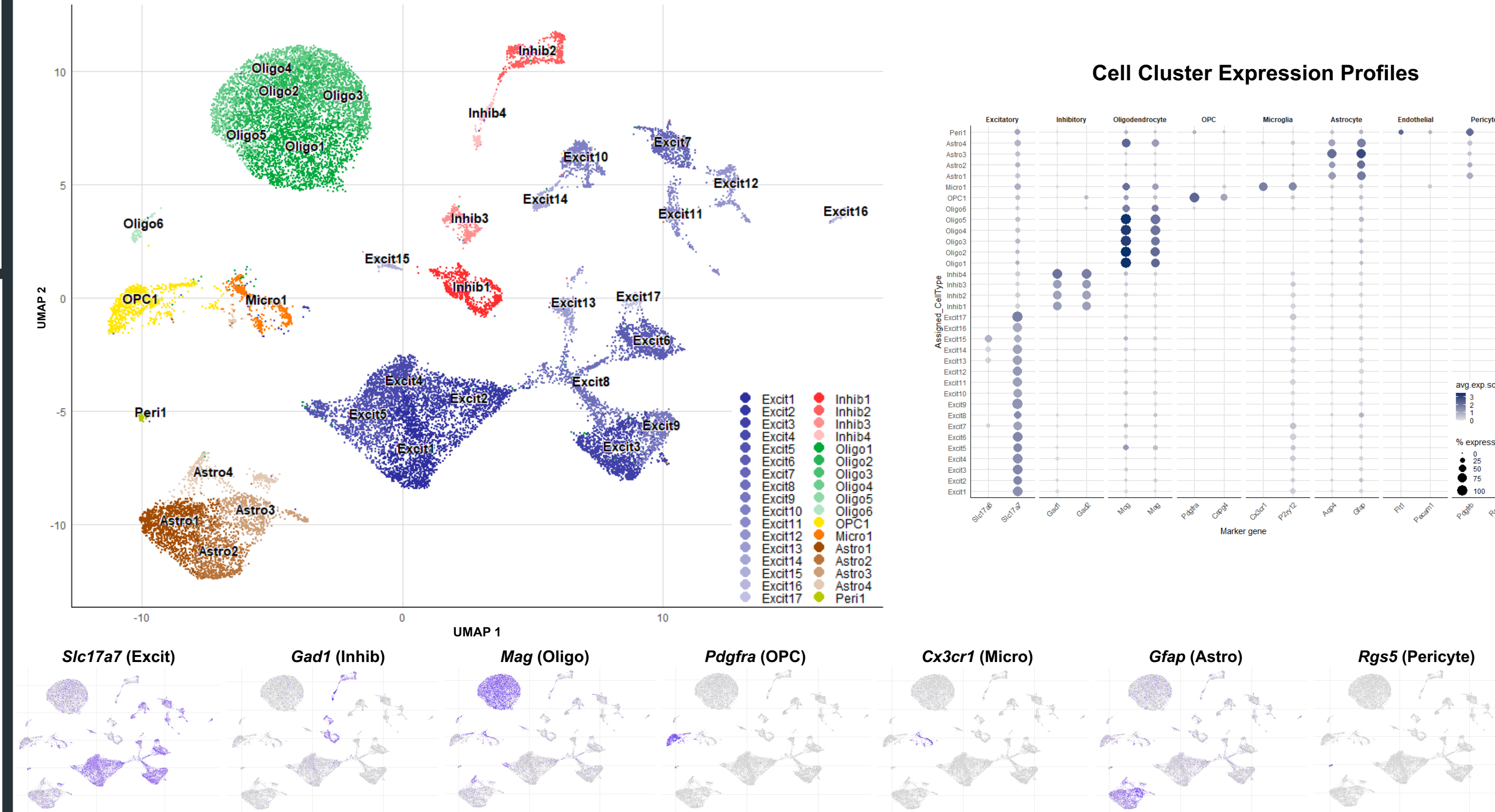


- Uterine and body weight differences were evaluated via *t*-tests Dunnett-corrected for multiple comparisons against SHAM condition. Fisher's exact tests were used to assess proportional shifts in cell type for OVX:SHAM and OVX+E2:OVX, followed by FDR-correction using the Benjamini-Hochberg method.
- Significance is denoted as **p*<0.05, ***p*<0.01, ****p*<0.001, and *****p*<0.0001.

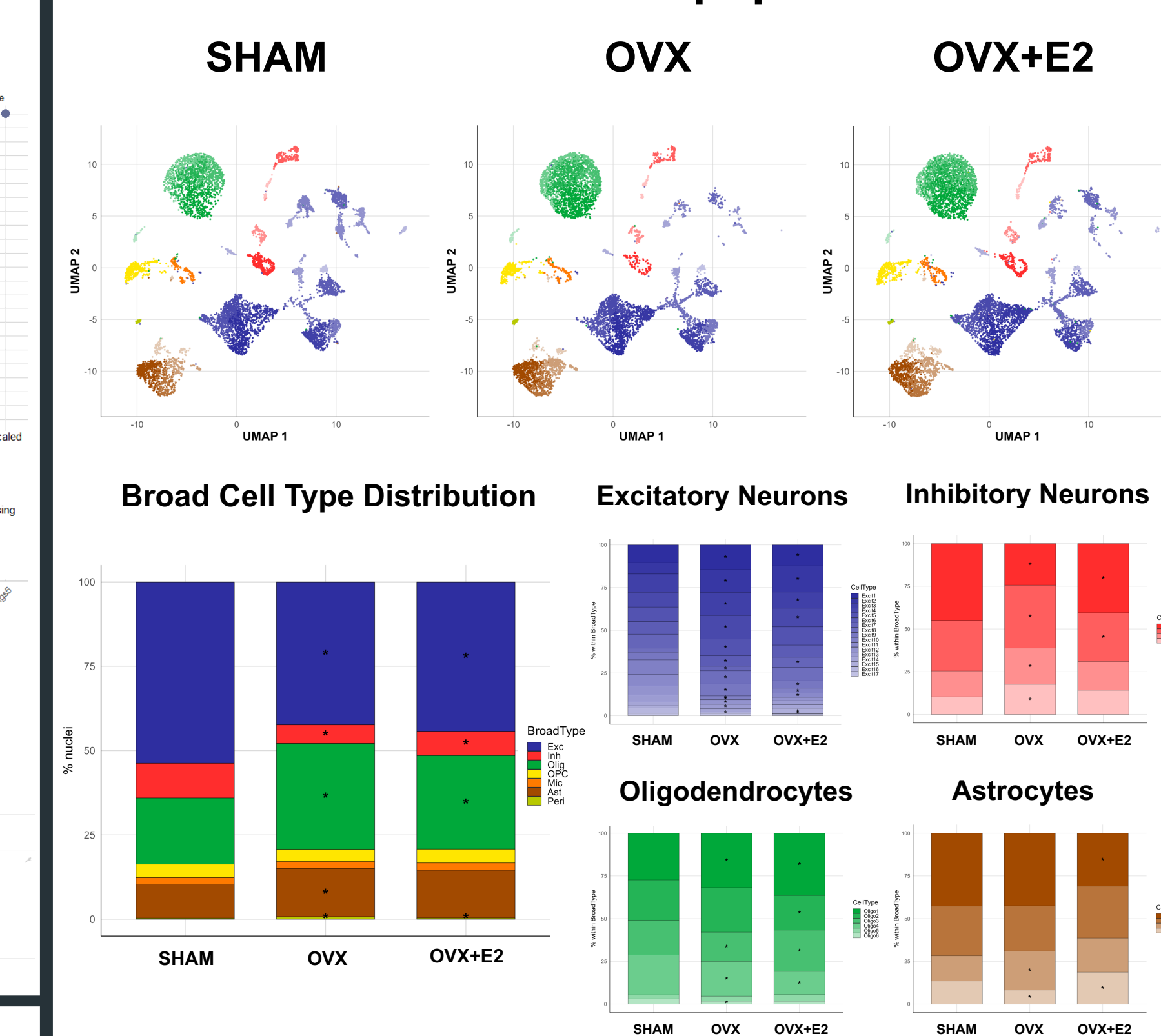
OVX+E2 model recapitulates weight changes associated with menopausal transition



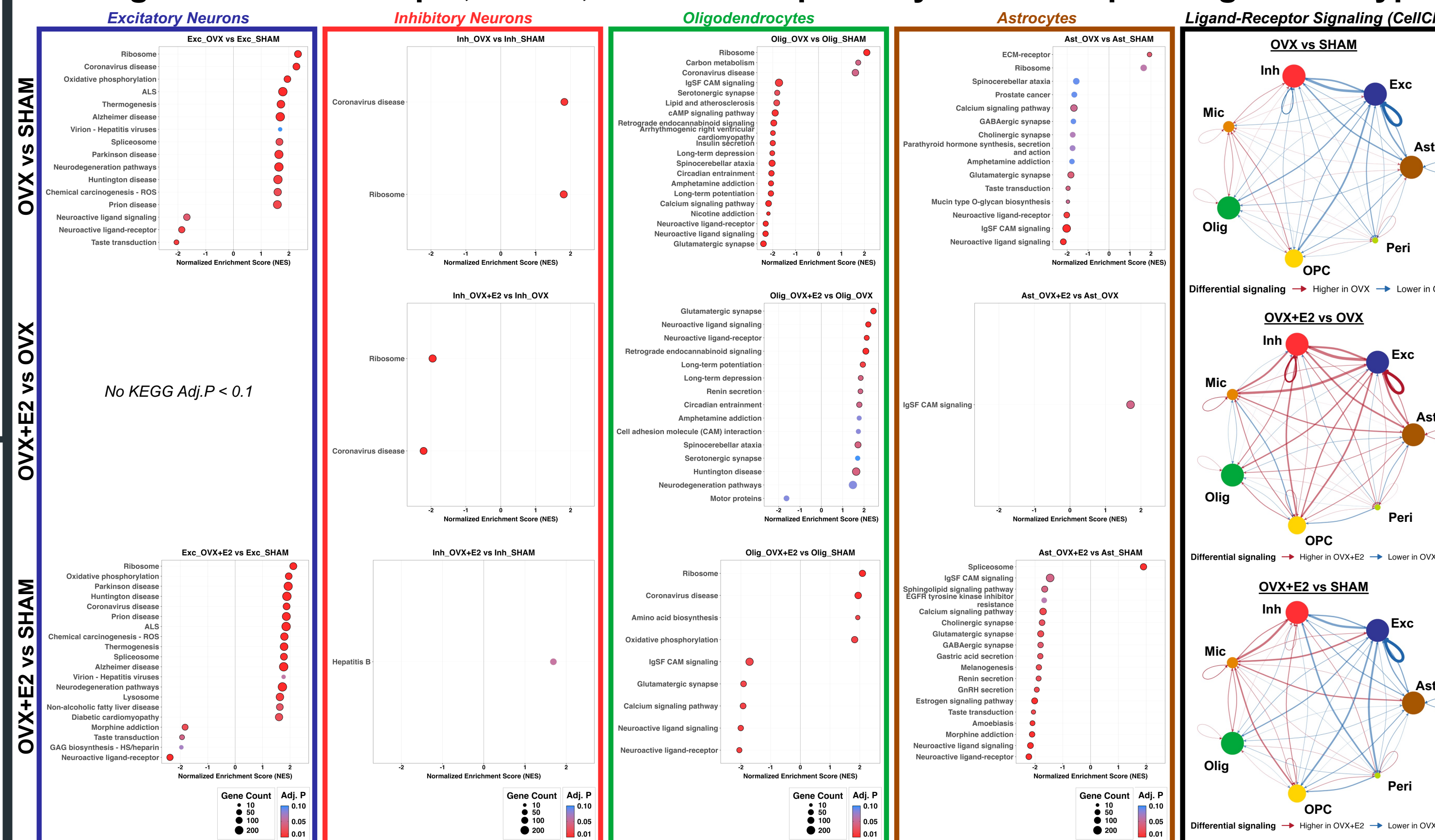
Pooled analysis results in 34 clusters representing 7 hippocampal cell types



Separating by treatment identifies estrogen-sensitive cell sub-populations



Estrogen rescues multiple, not all, AD-relevant pathway deficits depending on cell type



RESULTS & DISCUSSION

- Single-nuclei transcriptomics of dorsal hippocampus identified major brain cell populations across SHAM, OVX, and OVX+E2 animals. OVX altered hippocampal cell composition, reducing excitatory/inhibitory neuronal proportions and increasing oligodendrocyte representation relative to SHAM. OVX+E2 showed lesser broad compositional shift, suggesting partial moderation.
- KEGG GSEA revealed cell-type-specific pathway alterations following OVX, with excitatory neurons showing enrichment of AD-associated pathways, while oligodendrocytes and astrocytes showed altered inflammatory, metabolic, calcium signaling, and neuroactive ligand-receptor pathways. E2 treatment partially mitigated OVX-associated pathway dysregulation.
- CellChat³ ligand-receptor analysis nominated estrogen-sensitive intercellular communication changes, including altered synaptic/contact and neuroactive signaling pathways across hippocampal broad cell types, supporting a network-level effect of estrogen loss beyond cell-intrinsic DEG changes.
- Together, these data suggest that menopause-associated estrogen loss may increase hippocampal vulnerability through both cell-type-specific pathway disruption and altered intercellular signaling networks.
- Ongoing analyses will refine subpopulation-specific drivers of these effects and prioritize estrogen-sensitive pathways for validation as candidate mechanisms linking menopause biology to AD risk.

ACKNOWLEDGEMENTS

Research was supported by NIA grants T32AG061897, P01AG026572, and R37AG053589, and by the Center for Innovation in Brain Science (CIBS), to RDB. We also thank Zisu Mao for their technical expertise and contribution.

REFERENCES

- Rettberg, J. R., Yao, J., & Brinton, R. D. (2014). Estrogen: a master regulator of bioenergetic systems in the brain and body. *Frontiers in neuroendocrinology*, 35(1), 8–30. <https://doi.org/10.1016/j.ynrne.2013.08.001>
- Sato, K., Takayama, K. I., & Inoue, S. (2023). Expression and function of estrogen receptors and estrogen-related receptors in the brain and their association with Alzheimer's disease. *Frontiers in endocrinology*, 14, 1220150. <https://doi.org/10.3389/fendo.2023.1220150>
- Jin, S., Guerrero-Juarez, C. F., Zhang, L., Chang, I., Ramos, R., Kuan, C. H., Myung, P., Plikus, M. V., & Nie, Q. (2021). Inference and analysis of cell-cell communication using CellChat. *Nature communications*, 12(1), 1088. <https://doi.org/10.1038/s41467-021-21246-9>

Center for Innovation
in Brain Science