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Astrocyte–Neuron Communication Associated with Ex5 Resilience Across Brain Regions and AD Pathology Stages

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Abstract

Selective neuronal vulnerability is a central feature of Alzheimer's disease (AD), yet some neuronal populations remain relatively preserved despite increasing pathology. A recent single-nucleus RNA-sequencing study identified Ex5 layer 4 excitatory neurons as a relatively resilient population, particularly enriched in the late-affected primary visual cortex (BA17). This project investigated whether astrocyte–neuron communication may be associated with this resilience. Processed snRNA-seq data from GSE263468 were analyzed across BA9, BA7, and BA17 and across low, intermediate, and high AD pathology. Astrocytes, vulnerable Ex2 neurons, and resilient Ex5 neurons were selected using the published annotations. CellChat was used to infer bidirectional ligand–receptor communication, and pathway-level communication probabilities were compared between Ex5 and Ex2 to identify Ex5-biased signaling. Ex5-biased communication varied across regions and pathology stages, with recurrent candidates including NRG, NGL, retinoic-acid-related signaling, and CADM. Functional enrichment of ligand–receptor genes converged on synaptic organization, axonal development, neuronal signaling, and cell–cell adhesion. These findings support a possible contribution of the cellular microenvironment to molecular programs associated with Ex5 resilience, while remaining exploratory because the inferred interactions do not establish causality.

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1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, but neuronal loss and molecular disruption are not spatially uniform. Different cortical regions and neuronal populations show distinct vulnerability to AD pathology, providing a natural framework for investigating mechanisms of neuronal resilience.

Dharshini *et al.* compared three cortical regions that occupy different positions along neocortical AD progression: BA9 (prefrontal cortex) and BA7 (precuneus), which are affected relatively early, and BA17 (primary visual cortex), which is affected later [1]. Using single-nucleus RNA sequencing and spatial transcriptomics, the study resolved excitatory neuronal subtypes including Ex2 and Ex5. Ex2 was used as a prototype vulnerable excitatory population, whereas Ex5, corresponding predominantly to layer 4 intratelencephalic excitatory neurons, was relatively preserved and particularly enriched in BA17 [1].

The original study identified intrinsic molecular programs associated with Ex5 preservation, including genes related to synaptic maintenance, synaptic plasticity, calcium homeostasis, and neuroprotection [1]. However, neuronal resilience may also reflect the local cellular environment. Astrocytes are well positioned to influence neuronal homeostasis and synaptic function through metabolic support, extracellular regulation, and intercellular signaling.

Key Insight

Research Question Is astrocyte–neuron communication preferentially associated with resilient Ex5 neurons, and do these signaling patterns vary across brain regions and AD pathology stages?

The aim of this project was therefore to compare inferred astrocyte communication with vulnerable Ex2 and resilient Ex5 neurons across BA9, BA7, and BA17 and across low, intermediate, and high AD pathology. The analysis was designed to identify signaling pathways preferentially associated with Ex5 and to determine whether these pathways converge on biologically coherent functions.

2 Related Work

2.1 Molecular signatures of neuronal resilience in AD

The primary dataset and biological framework were established by Dharshini *et al.*, who used single-nucleus and spatial transcriptomics to investigate selective vulnerability across neocortical regions [1]. Their analysis showed that Ex5/layer 4 neurons are relatively preserved, particularly in BA17, and identified early resilience-associated genes involved in synaptic maintenance and neuroprotection. Notably, NRG3 and several synaptic genes were among the programs elevated in resilient neurons [1]. The present project extends this work from an intracellular perspective to an intercellular one by asking whether communication with astrocytes also differs between Ex5 and the vulnerable Ex2 reference population.

2.2 Inference of intercellular communication

CellChat infers potential intercellular communication from expression of known ligands, receptors, and cofactors and summarizes ligand–receptor interactions into signaling pathways [2]. This approach enables systematic comparison of signaling inputs and outputs among cell populations,

but the resulting communication probabilities are model-based inferences rather than direct measurements of physical signaling.

2.3 Biological context for selected pathways

Retinoic acid (RA) signaling contributes to synaptic homeostasis in the adult brain. Experimental AD models have shown that amyloid-β can suppress RA synthesis and that pharmacological activation of RARα signaling can attenuate several AD-related pathological features [3]. CADM1 is a cell-adhesion molecule with evidence for glial intercellular interactions in other CNS settings; increased CADM1–CADM1 interactions have been reported in a single-cell study of neuropathic injury, although that work was not performed in AD [4]. These observations provide biological context for interpreting, rather than validating, the pathway candidates identified here.

3 Methodology

3.1 Dataset

Processed single-nucleus RNA-seq data from GEO accession **GSE263468** were used. The processed H5AD file contains published metadata, author-defined cell annotations, dimensionality-reduction coordinates, and a normalized expression layer. The analysis focused on three regions: frontal cortex BA9, precuneus BA7, and primary visual cortex BA17, each represented across low, intermediate, and high AD pathology groups [1].

The dataset can be accessed through GEO at `https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE263468`.

3.2 Cell population selection

Only nuclei labeled `All Nuclei` in the processed dataset were retained. Published author annotations were then used to select three populations: astrocytes, Ex2 vulnerable excitatory neurons, and Ex5 resilient excitatory neurons. No re-clustering or re-annotation was performed.

Across the three regions, 36,230 nuclei were included in the focused analysis. BA9 contained 20,092 nuclei (10,175 astrocytes, 7,275 Ex2, and 2,642 Ex5); BA7 contained 5,398 nuclei (1,890 astrocytes, 2,346 Ex2, and 1,162 Ex5); and BA17 contained 10,740 nuclei (1,655 astrocytes, 2,871 Ex2, and 6,214 Ex5). These counts emphasize the strong representation of Ex5 in BA17.

Table 1 Cell populations retained for communication analysis.

Region	Astrocytes	Ex2	Ex5	Total
BA9	10,175	7,275	2,642	20,092
BA7	1,890	2,346	1,162	5,398
BA17	1,655	2,871	6,214	10,740
Total	**13,720**	**12,492**	**10,018**	**36,230**

3.3 Expression matrix

The authors' processed `layers/normalized` matrix was used directly. A second normalization step was deliberately avoided so that the downstream analysis used the expression representation supplied with the published processed dataset. Region-specific matrices were extracted by matching cell identifiers in the expression matrix to metadata rows, and ordering was explicitly checked before CellChat analysis.

3.4 CellChat analysis

Communication analysis was performed independently for each region and each pathology stage to avoid pooling distinct regional or disease contexts. For every region, low, intermediate, and high pathology subsets were analyzed separately with CellChat [2]. Donor identifiers were retained in the metadata.

For each subset, the human CellChat database was assigned and signaling genes and candidate ligand–receptor interactions were identified. Communication probabilities were calculated using `computeCommunProb(type = "triMean")`. Interactions involving cell groups with fewer than 10 cells were filtered using `filterCommunication(min.cells = 10)`. Significant ligand–receptor interactions were then aggregated to pathway-level communication using `computeCommunProbPathway()` and `aggregateNet()`.

Only the two directions relevant to the research question were retained:

- Astrocytes → Ex2 or Ex5 neurons.
- Ex2 or Ex5 neurons → astrocytes.

3.5 Definition of Ex5-biased signaling

Within each region, stage, direction, and signaling pathway, CellChat pathway probabilities for Ex5 and Ex2 were compared. A pathway was classified as **Ex5-biased** when its inferred communication probability was greater for Ex5 than for Ex2. Importantly, Ex5-biased does not mean that signaling was absent in Ex2; it indicates a relative difference in inferred pathway strength.

The comparison can be written as

$$\Delta P = P_{\mathrm{Ex5}} - P_{\mathrm{Ex2}},$$

where $\Delta P > 0$ denotes Ex5-biased signaling.

3.6 Ligand–receptor extraction and functional enrichment

Ligand and receptor genes underlying Ex5-biased pathway contexts were extracted from the CellChat interaction tables. Gene symbols were mapped to Entrez identifiers with `org.Hs.eg.db`, and Gene Ontology Biological Process enrichment was performed with `clusterProfiler::enrichGO`. Benjamini–Hochberg adjustment was used for multiple testing. Enriched terms were linked back to the CellChat pathways through their contributing ligand–receptor genes, allowing pathway-level communication to be interpreted in terms of broader biological functions.

3.7 Software

The workflow was implemented in R using `rhdf5`, `BPCells`, `Matrix`, `dplyr`, `tidyr`, `CellChat`, `clusterProfiler`, `org.Hs.eg.db`, `ggplot2`, and `ComplexHeatmap`. A reproducible source-code package accompanies the final submission.

4 Results

4.1 Focused neuronal and astrocyte populations

The selected population contained astrocytes together with the vulnerable Ex2 and resilient Ex5 excitatory neuronal subtypes. Ex5 was disproportionately represented in BA17 relative to BA9 and BA7 (Table 1), consistent with the original report identifying layer 4 Ex5 neurons as

a prominent resilient population in the late-affected visual cortex [1]. This regional imbalance motivated analysis within each region rather than pooling all nuclei.

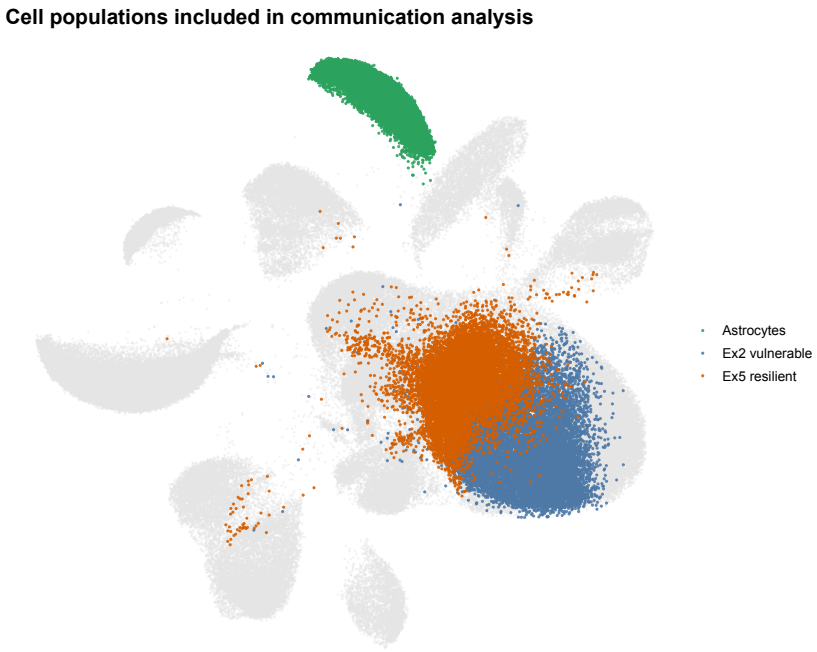


Figure 1 Cell populations included in the communication analysis. Astrocytes, vulnerable Ex2 neurons, and resilient Ex5 neurons are highlighted on the UMAP representation.

4.2 Ex5-biased communication is context dependent

CellChat identified multiple pathways for which inferred astrocyte–neuron communication was stronger for Ex5 than for Ex2. These Ex5-biased pathways were not uniformly distributed across the nine region-by-stage contexts. Instead, the pattern varied according to both brain region and pathology stage and differed between astrocyte-to-neuron and neuron-to-astrocyte directions (Figure 2).

Among the recurrent or biologically notable candidates were NRG, NGL, RA, PSAP, PTN, NCAM, CADM, APP, PTPR/PTPRM, TENASCIN, and UNC5. NRG signaling was recurrent across several contexts, while RA signaling showed a notable astrocyte-to-Ex5 pattern in BA17. CADM signaling included a bidirectional Ex5-biased context in BA17 low pathology. Overall, no single pathway accounted for Ex5-associated communication in every region and stage.

4.3 Functional convergence of Ex5-biased signaling

GO Biological Process enrichment of ligand–receptor genes underlying Ex5-biased pathways revealed a coherent set of neuronal and synaptic functions. Representative enriched terms included ERBB4 signaling, axon development and axonogenesis, modulation of chemical synaptic transmission, synaptic membrane adhesion, synapse assembly, response to axon injury, regeneration-related processes, postsynaptic organization, and cellular response to retinoic acid (Figure 3).

The functional enrichment therefore suggested that apparently distinct CellChat pathways converge on processes related to synaptic and axonal maintenance rather than representing isolated signaling events.

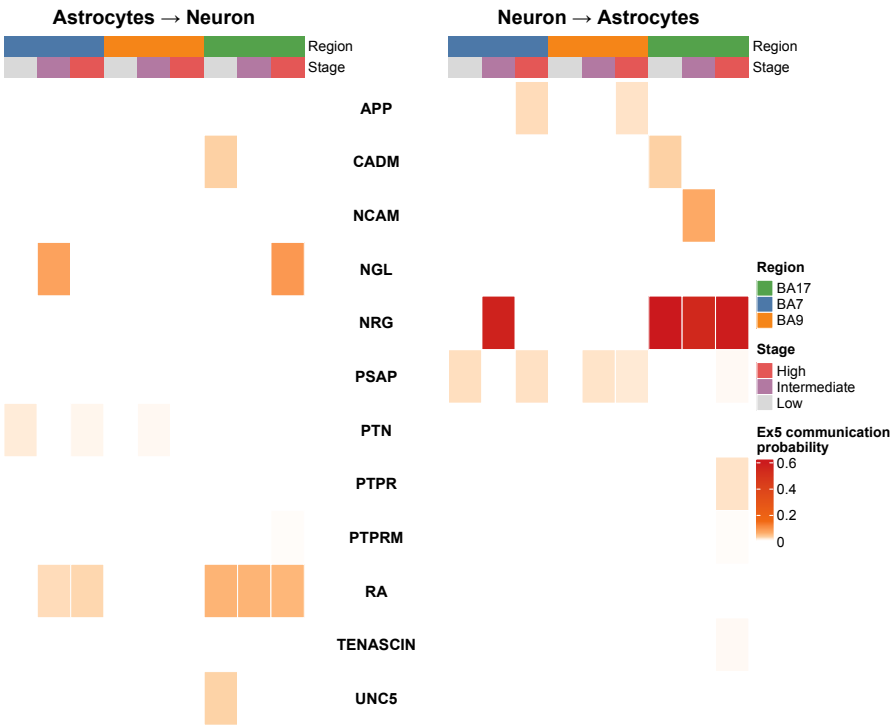


Figure 2 Ex5-biased astrocyte–neuron signaling across brain regions and AD pathology stages. The two panels represent astrocyte-to-neuron and neuron-to-astrocyte directions. Color intensity represents Ex5 communication probability.

Takeaway

Main Result Ex5-biased astrocyte–neuron communication was strongly context dependent, but the genes underlying these pathways converged on synaptic organization, neuronal signaling, axonal biology, and cell–cell interaction processes.

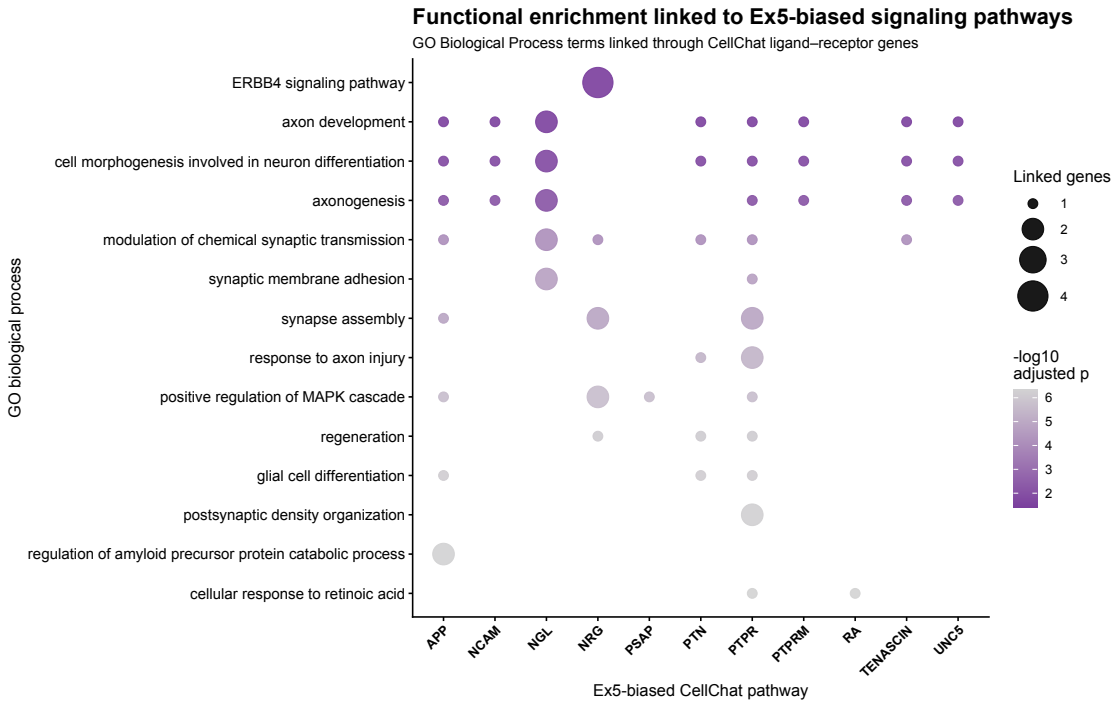


Figure 3 Functional enrichment linked to Ex5-biased signaling pathways. GO Biological Process terms are linked to Ex5-biased CellChat pathways through their ligand–receptor genes. Dot size represents the number of linked genes and color represents adjusted significance.

5 Discussion

This project asked whether the relative resilience of Ex5 neurons might be associated not only with intrinsic molecular programs but also with differences in their communication with astrocytes. The results support this possibility at an exploratory level: Ex5-biased signaling was detected across multiple pathways, but its regional and stage dependence indicates that resilience-associated communication is unlikely to be explained by a single constitutive pathway.

5.1 NRG–ERBB4 signaling

NRG was among the most recurrent Ex5-biased pathway candidates, and the functional analysis recovered ERBB4 signaling. This aligns with the original study, which identified *NRG1* and *NRG3* among genes upregulated in early BA17 and highlighted *NRG3* among genes associated with resilient Ex5 neurons [1]. The agreement between an intrinsic neuronal transcriptional signature and an inferred intercellular NRG pathway is notable because it suggests that the same biological system may appear at both intracellular and microenvironmental levels. It does not, however, establish that NRG signaling causes resilience.

5.2 NGL signaling and synaptic maintenance

NGL signaling showed broad links to axon development, synaptic adhesion, and modulation of synaptic transmission. This functional profile is consistent with the wider synaptic-maintenance signature reported for resilient neurons in the original study [1]. The convergence is more informative than any single ligand–receptor pair because it suggests that Ex5-biased communication may preferentially involve pathways supporting neuronal connectivity and synaptic organization.

5.3 Retinoic-acid-related signaling

RA-related astrocyte-to-Ex5 signaling remained Ex5-biased across BA17 pathology stages in the analysis. This candidate is biologically relevant because RA contributes to synaptic regulation in the adult brain. In experimental AD models, amyloid-β suppresses RA synthesis, while pharmacological activation of RARα signaling attenuates several AD-related pathological features [3]. The enrichment of cellular response to retinoic acid among Ex5-biased ligand–receptor genes provides an additional functional link. These data support RA-related signaling as a candidate for follow-up rather than a demonstrated protective mechanism.

5.4 CADM1–CADM1

Bidirectional Ex5-biased CADM signaling was observed in BA17 low pathology. CADM1 was not among the strongest significant GO terms in the final enrichment summary, so it should be interpreted separately and cautiously. Independent CNS evidence supports a role for CADM1 in cell adhesion and intercellular communication; for example, enhanced CADM1–CADM1 signaling was identified and experimentally examined in glial cells in a neuropathic injury model [4]. Because that evidence comes from a non-AD context and a different experimental system, CADM1 is best regarded here as an exploratory candidate.

5.5 Relationship to the original study

The major conceptual finding is the functional convergence between the inferred microenvironmental signals in this project and the intrinsic resilience-associated programs reported by Dharshini *et al.* [1]. Both analyses highlight synaptic organization, neuronal signaling, cell adhesion, and maintenance-related processes. This does not distinguish whether Ex5 resilience originates from cell-intrinsic properties, the regional environment, or both. Instead, it suggests a model in which intrinsic neuronal programs and local astrocyte-associated signaling may operate together and vary according to cortical region and pathological context.

6 Limitations and Future Work

- **CellChat provides inference, not direct signaling measurements.** Communication probabilities are calculated from transcript abundance and known ligand–receptor relationships. Future work should validate prioritized interactions using spatial colocalization, protein-level assays, or perturbation experiments.
- **Association does not establish a mechanism of resilience.** Ex5-biased signaling may accompany, rather than cause, neuronal preservation. Experimental perturbation of candidates such as NRG–ERBB4 or RA-related signaling would be needed to test causal effects on neuronal survival or function.
- **Regional composition differs substantially.** Ex5 is highly represented in BA17, while the relative abundance of Ex2, Ex5, and astrocytes differs across regions. The analysis addressed this partly by running CellChat independently within each region and pathology stage, but compositional differences may still influence inferred network strength.
- **Pathology groups are cross-sectional.** Low, intermediate, and high pathology represent different donors rather than longitudinal measurements of the same individuals. Therefore, stage-associated differences should not be interpreted as directly observed temporal transitions.
- **The project focused specifically on astrocytes.** Other microenvironmental populations, including microglia, oligodendrocytes, OPCs, vascular cells, and inhibitory neurons,

may also contribute to selective vulnerability or resilience. A systematic multi-cell-type comparison is a natural extension.

- **Functional enrichment depends on the selected ligand–receptor genes.** GO terms provide a useful functional summary but can be sensitive to gene-set size and annotation structure. Independent pathway resources and gene-set approaches could be used to assess robustness.

Future work should therefore prioritize a small number of recurrent candidates, test their donor-level and spatial consistency, integrate ligand and receptor protein expression, and determine whether perturbing these pathways modifies vulnerable or resilient neuronal phenotypes.

7 Conclusion

Astrocyte–neuron communication associated with resilient Ex5 neurons varied across cortical regions and AD pathology stages. Comparison with vulnerable Ex2 neurons identified Ex5-biased signaling candidates including NRG, NGL, RA-related, and CADM pathways, while functional enrichment showed convergence on synaptic organization, neuronal signaling, axonal biology, and cell–cell interaction processes. Several of these functions overlap with intrinsic resilience-associated programs reported in the original study. Together, the findings support a potential contribution of the cellular microenvironment to Ex5 resilience in AD and provide a focused set of signaling candidates for experimental validation.

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