MAPPING THE MOUSE BRAIN BEFORE IT BECOMES ONE: THE SPATIOTEMPORAL LOGIC OF NEURAL DEVELOPMENT

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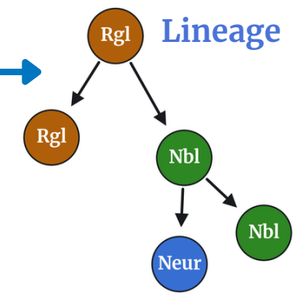
Introduction

The embryonic mouse brain develops over a ~19-21-day pregnancy as 19 segments called neuromeres. Within each, stem cells differentiate into **RADIAL GLIA to NEUROBLASTS to NEURONS**, following distinct trajectories and speeds. Yet which specific cell population gives rise to which remains unclear, because each developmental stage is measured in a different embryo, so the same cells cannot be directly followed over time. We analysed more than 4 million cells across six stages (embryonic day E9.5 to E15.5) to develop a pipeline for reconstructing lineage connections and uncovering how and when distinct neural populations develop. Previous studies have identified a limited number of lineage relationships, providing benchmarks for this study.



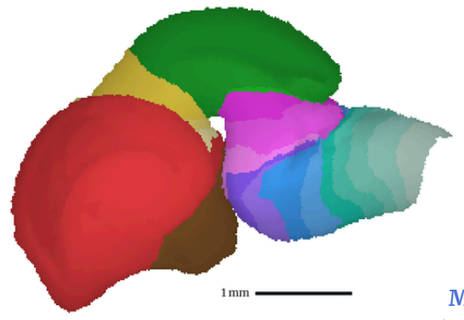
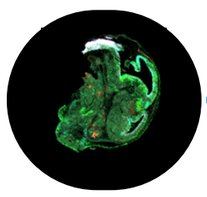


Figure 1. Mouse brain anatomical hierarchy by neuromere (E13.5)

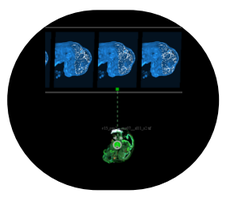
Methodology

A) Database creation using ABBA

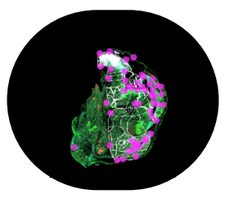
Embryonic tissue sections are processed for RNA imaging.



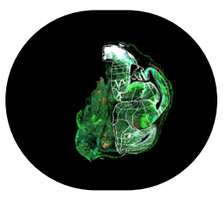
HybISS* section of E15.5 embryonic mouse brain



Aligning the section to the reference atlas of the E15.5 time point

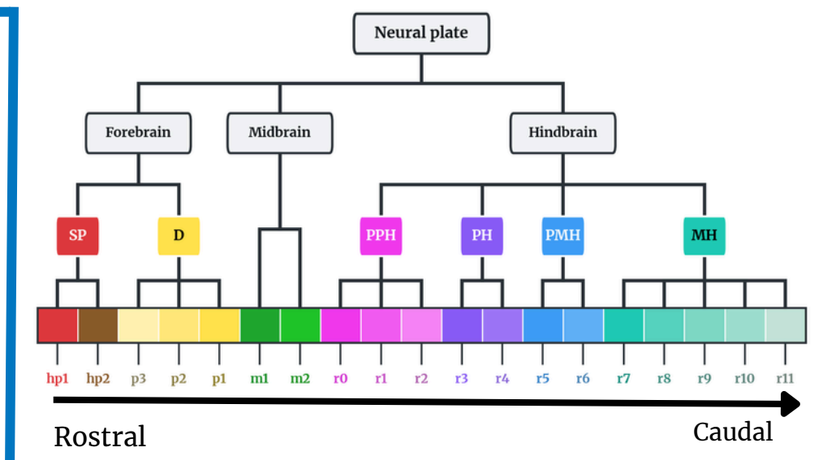


Placing anchors and expanding the section to match the borders of the atlas



Aligned section : each cell inherits the anatomical region it falls in

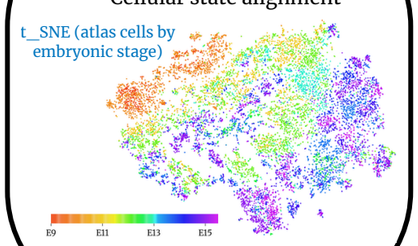
14 embryos
121 aligned sections



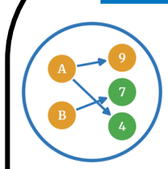
B) Established process by this study for candidate lineage construction and selection

1. PROJECTION

Cellular state alignment



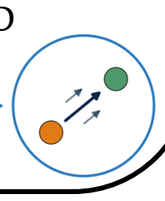
2. MATCH



Candidate population-to-population transitions
Moscot Optimal Transport[2]

AND

Direction of transitions and cell fates



RNA Velocity + CellRank[3]

3. FILTER

Statistically supported transitions

Enrichment : how strong the tansition is

$$E_{ij} = \frac{f_{ij}}{c_j}$$

i, j a population, and one at the next stage
f_{ij} the share of what i sends out that lands on j
c_j the share of all mass arriving that lands on j
$E = 1$ chance level; $E = 3$, three times the expected traffic

Empirical p : how likely that strenght is by chance

$$p_{ij} = \frac{1 + \#\{\, b : E^*_{ij} \geq E_{ij} \,\}}{B + 1}$$

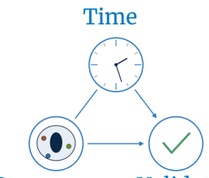
E^* enrichment of a null coupling: parent and child independent, at the observed margins and cell numbers
b of B the 200 null draws per transport problem
$\#\{\cdot\}$ how many draws match or beat the observed E; the added 1 holds p above 1/201

Best candidates : low p, high enrichment, False Discovery Rate < 0.05 and transition mass > 2% mass of parent

4. CHECK

Biologically coherent transitions
- tests whether transitions follow anatomical and developmental organization

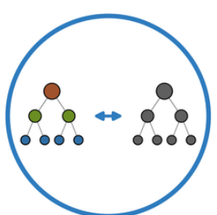
Time



Space Validation

5. COMPARE

Validation against established literature



*HybISS : hybridization-based in situ sequencing, a technique for detecting where specific genes are expressed within intact tissue

Results

Figure 2. Cell-type distribution within different neuromeres across the 6 developmental stages

Figure 2A. obtained from RNA-aligned data using Napari

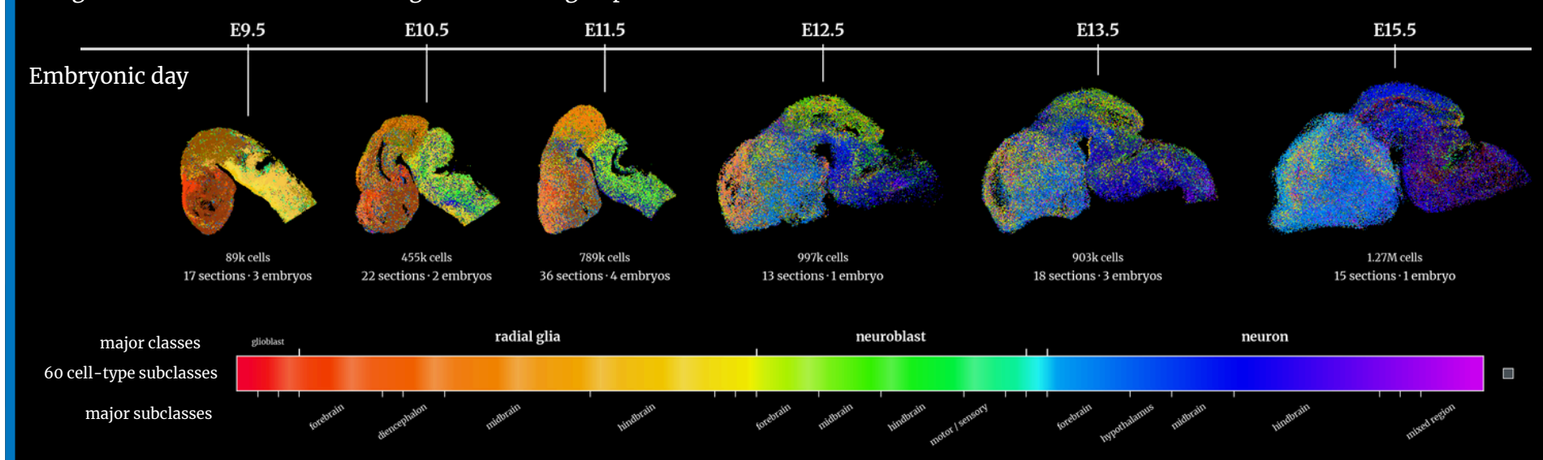
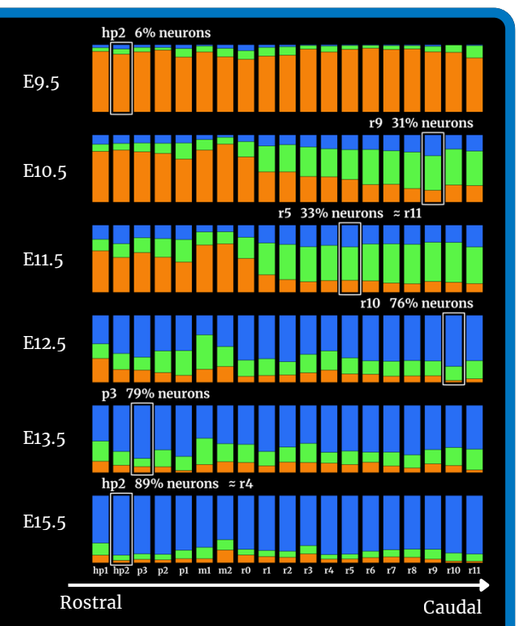


Figure 2B. Differentiation across neuromeres and developmental stages

- Neuromeres with the most advanced differentiation (highest percentage of neurons) are circled.



Observations and analysis

Together, these two panels let us follow how differentiation progresses within each neuromere. They show in particular which neuromeres differentiate first, and allow a chronological order to be established:
- E9.5 : Development has just begun, with most cells still stem cells and little difference between segments.
- E10.5 : r9 in the posterior brain, located next to the developing spine and muscles, receives early differentiation signals from surrounding tissue, promoting the transition from stem cells to neurons.
- E11.5 : r5 establishes its neural identity early and rapidly produces neurons involved in eye movement, an essential early function.
- E12.5 : The posterior brain is still leading. r10, at the junction with the spinal cord, is the furthest through the transition, with around 76% of its cells already neurons.
- E13.5 : p3 begins differentiating later because its local developmental control centre only becomes active around E10.5, approximately two days after the posterior regions.
- E15.5 : The anterior brain finishes differentiation last. hp2 leads at 89% of cells becoming neurons, while hp1 lags behind because it contains the telencephalon, which continues stem-cell production for the future cerebral cortex.

Figure 3. Output of the established pipeline

Figure 3A. Parent-child connections called by both methods (step 2)

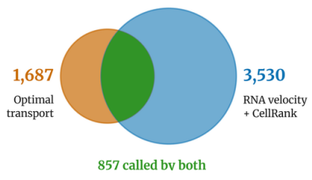
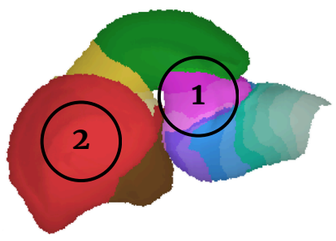
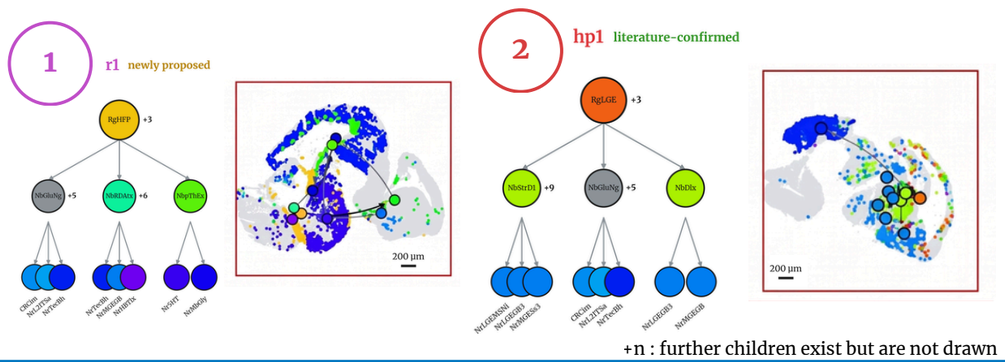


Figure 3B. A few examples of lineage trees with zoomed section (cells from different stages registered in the same section)





+n : further children exist but are not drawn

Limits and challenges

- 55% of possible connections: populations could be linked, but the direction could not be determined
- only ~4 genes detected per cell which limits the ability to distinguish closely related populations
- Lineage is mainly inferred statistically, never observed. Parent-child links come from matching snapshots; no cell is followed since the same cell cannot be followed across stages once each embryo has been sampled.

Conclusion

- **4,360 candidate parent-daughter** relationships of which only half are confirmed by a second method and just 60 have an unambiguous direction
- **11 of 11** literature relations recovered; **7 of 17** trees match preivous PhD work[1] in this same lab ; **8** new lineage trees
- Different differentiation profiles across neuromeres reflecting anatomical organization and regional identity

new spatial transcriptomics-based pseudolineage analysis, validated against established literature

Outlook

- Improving the pipeline by adding more embryos, more data, and more transcripts per cell to increase the resolution and reliability of the resulting lineage trees.
- Understand how brain region and cell position shape how cells develop within lineage trees across neuromeres.
- Compare proliferation and differentiation across stages to determine when different cell populations develop within each lineage.
  - **Broader applications** : Use lineage trees to identify where and when proliferation or differentiation is disrupted in neurodevelopmental disorders, and determine which cell populations and developmental stages are affected. This could help identify where abnormalities arise along specific developmental trajectories.

References
1. Khven, I. (2025) Fine-grained mapping of neural progenitors subtypes in the embryonic brain, PhD thesis, EPFL
2. Klein, D. et al. (2025) "Mapping cells through time and space with moscot", Nature, 638, pp. 1065–1075
3. La Manno, G. et al. (2018) "RNA velocity of single cells", Nature, 560, pp. 494–498.