CORTICAL INHIBITORY NEURONS EXHIBIT CELL-TYPE SPECIFIC MATURATION PROGRAMME

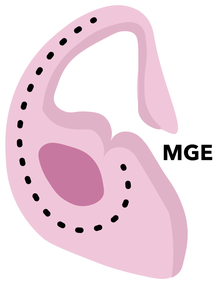
INTRODUCTION

The full diversity of somatostatin-expressing interneurons is not well understood, particularly timings of differentiation and laminar allocation. To illuminate upon these points, we take a combined approach to characterise the range of neuronal cell identities across embryonic timepoints in the mouse cortex, both by their transcriptomic profile and by spatial position.

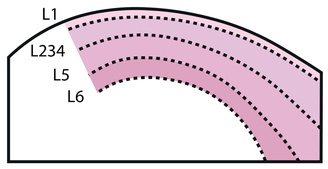
During cortical development progenitor cells originate in the embryonic subpallium. These cells undertake a tangential migration, then travel radially to reach a final position.[1] Several potential models of this process exist. A dominant model proposes late diversification as a result of neural input, before which cells follow the same maturation program. Alternatively, cells may have cell-type specific unique trajectories detectable early in development alongside shared maturation modules.

A further model[2] proposes three stages in which

1. Major types are specified early in development.
2. Subtypes generate during a refinement period before cells settle in the cortex.
3. Functional roles and circuitry are established.



Reproduction of Fig. 1b Lim et al.



Schematic representation of cortical layers.

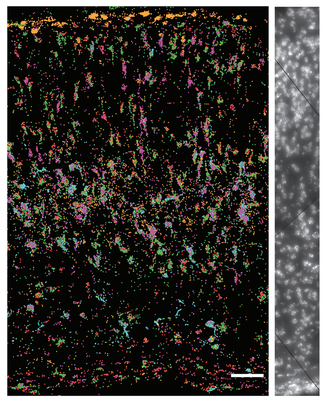
METHODS

SINGLE CELL RNA SEQUENCING

Developmental timing of cortical interneurons.

To explore a comprehensive range of cortical development we sample the mouse cortex at three stages. The earliest, embryonic day 16, is the stage at which interneurons are expected to be tangentially migrating. At postnatal day 1, they are radially migrating and establishing laminar allocation. Postnatal day 5 is just prior to programmed cell death. We generate single cell profiles by 10x Chromium sequencing. After QC we analyse ~9,000 RNA profiles across the three developmental stages.

By bioinformatic analysis cells are clustered and annotated as one of three classes: Martinotti (MC), non-Martinotti (NMC) and long-range projecting (LRP). Cell pseudotime as an approximation of maturation is computed by diffusion map. We performed spatial transcriptomics on mouse cortex samples from P5 using Resolve Bioscience's Molecular Cartography platform - a method based on single molecule fluorescence in situ hybridization with a proprietary probe labelling.

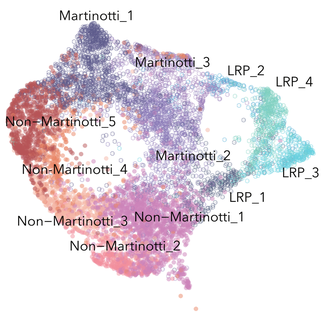


Sample single cell flourescence outcome showing discrete identified trascripts

RESULTS

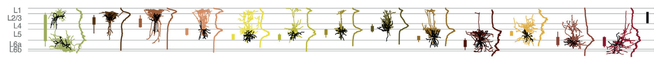
INTERNEURON DIVERSITY

By application of an iterative clustering algorithm to scRNA-seq data we identify four LRP subtypes, three MC subtypes and five NMC subtypes annotated by differentially expressed markers. We find several LRP subtypes, suggesting potential diversity of function within this sparse population. To verify functional properties associated with these transcriptomic types we use a reference dataset of adult mouse cells from Gouwens et al.[3] which includes cell classification based on morphogenic and electrophysiological features (MET-types).

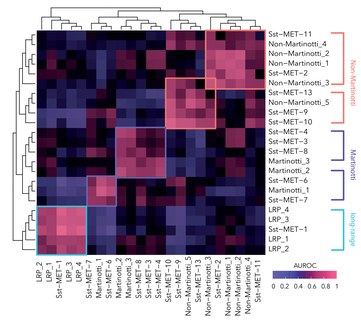


UMAP embedding of scRNA-seq cell profiles labelled by cell type annotation.

Using correlation networks, we generate an AUROC measure for each cluster pair between our data and the reference. This works as a similarity metric, and we find our clusters align to MET-types with the expected projections and properties. This alignment allows us to estimate expected soma depth for each cluster

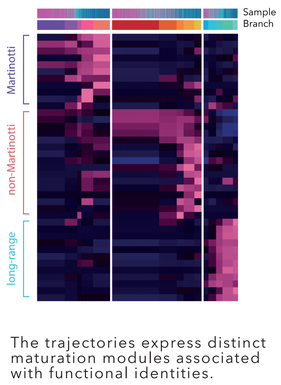


Morpho-electric types described by Gouwens et al. [3]



Transcriptomic identity Metaneighbor correspondences to MET types.

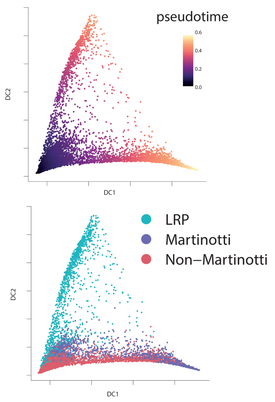
EARLY DIVERGENT MATURATION



The trajectories express distinct maturation modules associated with functional identities.

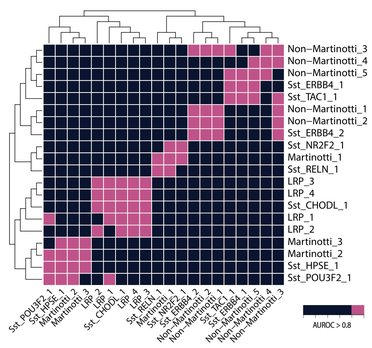
We define a module of developmental genes which are used to order cells by maturation. We find each cell type expresses a unique gene module along the gradient of maturation pseudotime, revealing their distinct maturation programmes.

Diffusion map embedding shows a clear divergence in the transcriptomic profile of LRP cells as early as E16. This implies a decision point at which molecular differences define cell fate with implications for difference in migration route and localisation.



LRP cells diverge from MC/NMC trajectory.

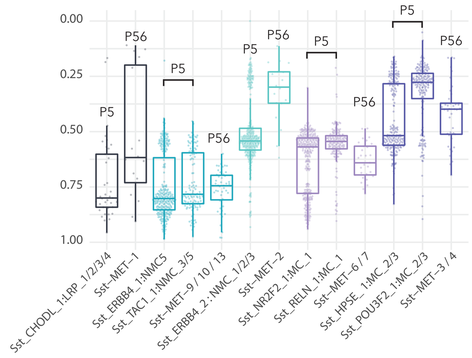
LAMINAR DISTRIBUTION



Correlation between spatial and scRNAseq data.

We isolate ~2,300 SST+ interneurons in the spatial data, and annotate clusters by predominant markers. The observed in soma depth for LRP, MC, and NMCs generally match expectations. Within each class there is some variation in subtype soma depth, suggesting transcriptomic differences reflect differences in localisation. Further, we compute AUROC between spatial groups and transcriptomic types (left). This allows us to find correspondence between targeted-panel spatial subtypes and the more carefully defined identities derived from whole-transcriptome scRNAseq. From these we refer to our early plot to find associated MET-types, and so form a chain of correspondence between the three data sets.

We extract soma depth approximations from MET-type data and compare with the corresponding developmental cell types. In some cases P5 cells are differently located to adult cells, while in others cells from both stages are at a similar depth. This is exemplified in the bimodality of non-Martinotti P5 cells compared to MET types 9,10, and 13. Difference in location suggests that cells reach their final position with different timings. In conjunction with the above pseudotime results, we conclude that early-expressed developmental programmes may have the potential to influence cell type specification, migratory timings and establishment of functionally diverse circuitry.



Estimated soma depth compared between spatial data from P5 and corresponding MET-type cells from P56. Boxes colour-coded by high-correspondence cell class.

REFERENCES

1. Lim L, Mi D, Llorca A, Marín O. Development and Functional Diversification of Cortical Interneurons. Neuron. 2018;100(2):294-313. doi:10.1016/-j.neuron.2018.10.009

2. Fishell G, Kepecs A. Interneuron Types as Attractors and Controllers. Annu Rev Neurosci. 2020 Jul 8;43:1-30. doi: 10.1146/annurev-neuro-070918-050421. Epub 2019 Jul 12. PMID: 31299170; PMCID: PMC7064158.

3. Gouwens, N. W. et al. Integrated Morphoelectric and Transcriptomic Classification of Cortical GABAergic Cells. Cell 183, 935-953.e19 (2020).

Authors: Josephine Fisher, Marieke Verhagen, Rani Moors, Monika Moissidis, Da Mi, Oscar Marín, Lynette Lim