Cell type diversity in a developing octopus brain

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Introduction

Cephalopods have developed **intricate neural systems** that are comparable with vertebrates in terms of cell number, complexity and size.

At hatching, the brain possesses all the structural lobes and main connections of an adult brain, but it remains elusive which **cell types** are present in this alien brain.
An adult octopus brain consists of **200 million cells**, but at hatching this is only 200 000. Exactly how an octopus increases its cell number so dramatically, and how it makes **specific synaptic connections** to enable higher cognitive function and complex behavior is still unknown.

Recent genome sequencing of several cephalopods has revealed expansions of a specific transmembrane protein family, **the protocadherins**, which are essential for mammalian neural development and might play a role in wiring in the octopus' brain.

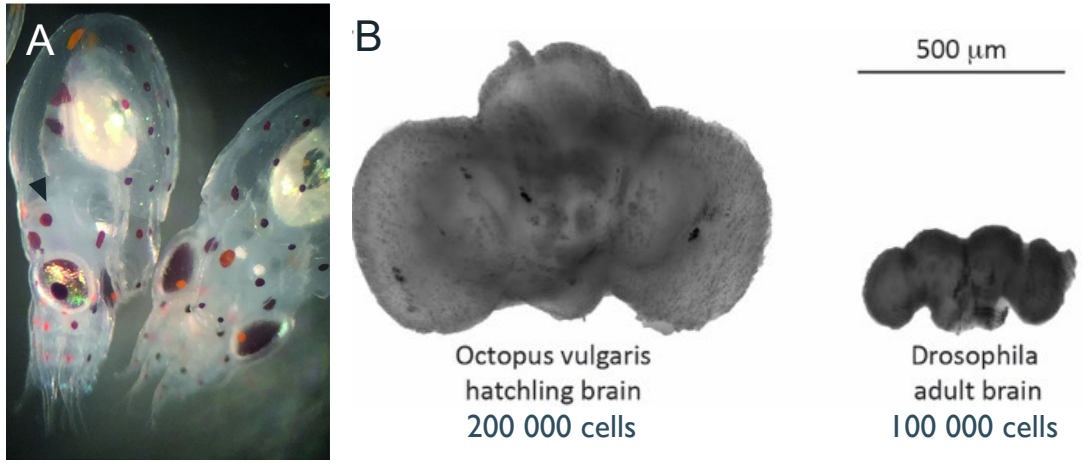

Figure 1: A. *Octopus vulgaris* paralarvae (one day post-hatching). The brain is localised between the eyes and is annotated with an arrow. **B.** At hatching the brain is estimated to possess about 200 000 cells.

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Methods

Experimental design

To profile the cell diversity in the octopus brain we applied 10x Genomics' single-cell/nuclei RNA sequencing technology.

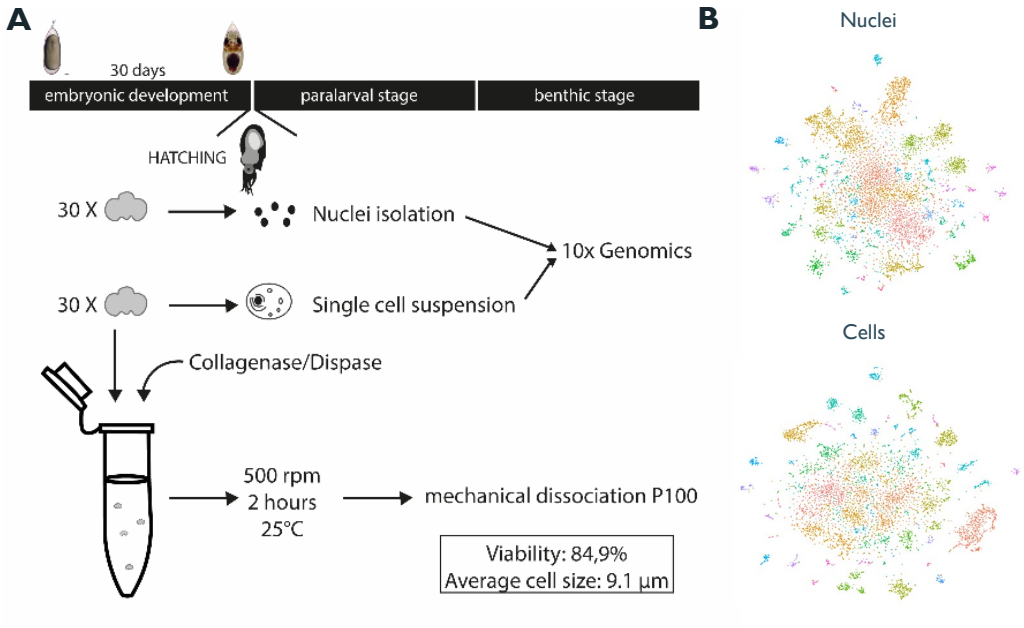

Figure 2: A. We dissected 30 brains of one-day old paralarvae. One sample was used for single-nuclei RNAseq and one for single-cell RNAseq. We obtained 247 457 191 reads for the cells and 202 402 758 reads for the nuclei. The sequencing saturation was between 34,5%-20,5%. **B.** tSNE plots of the two datasets are shown.

Improving the genome annotation

Since the final 10x libraries are 3' biased and the existing gene models often did not include the 3' ends we took several steps to improve the gene annotation (see Figure 3).

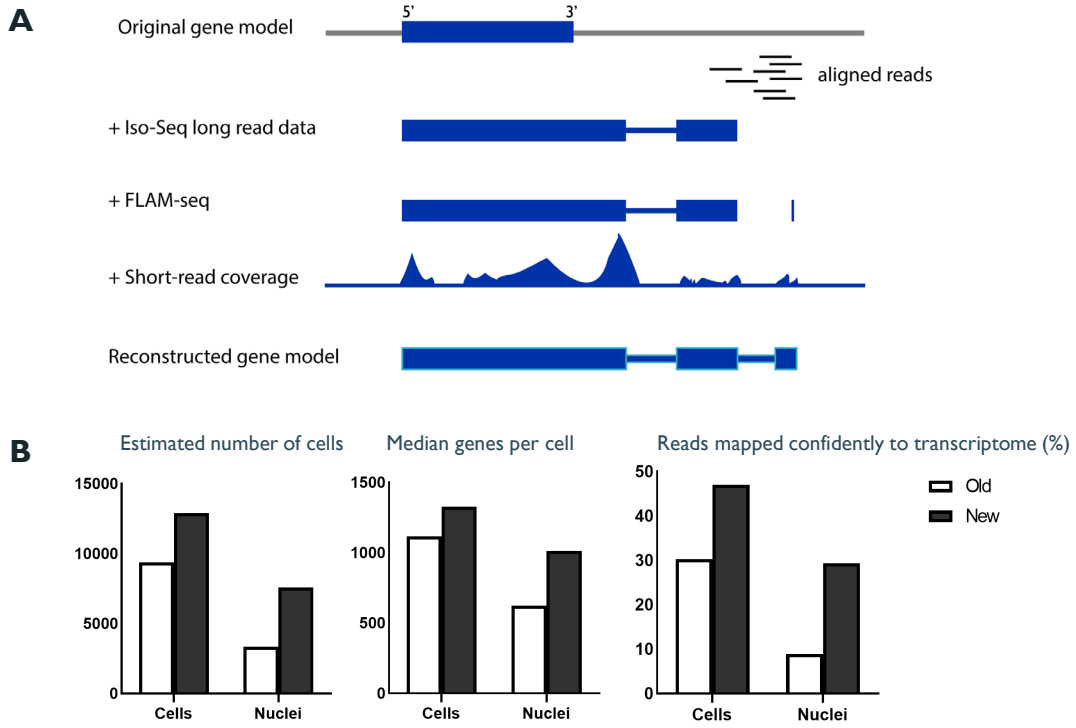

Figure 3: A. Starting from the Gnomon NCBI gene annotation and the published EVM models (Li et al., 2020), we included long-read RNAseq data (Iso-Seq TAMA models + SQANTI2). By including full-length mRNA sequencing (FLAM-seq), we were able to accurately predict gene ends. Lastly, we also extended the 3' ends based on short-read coverage. **B.** Paired comparisons for several important parameters to assess the mapping of the reads to the genome reference are shown. The new genome annotation based on the reconstructed gene models performs better than the old annotation.

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Results

Stringent filtering and integration of both datasets resulted in **56 clusters** where we can **identify major cell types** based on the expression of known mammalian and invertebrate markers.

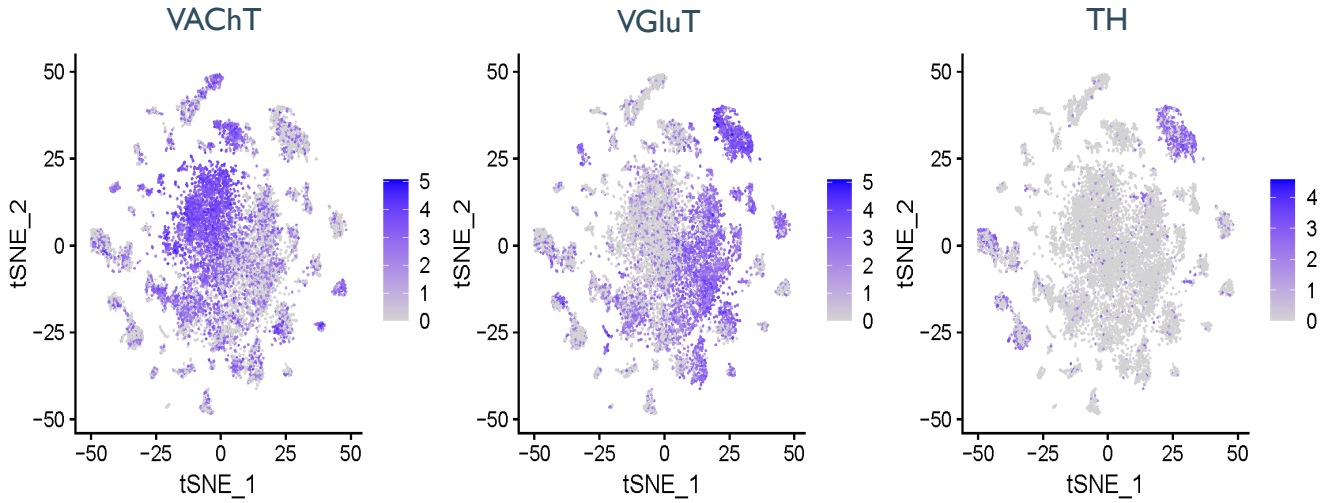

Figure 4: t-SNE plots of the integrated dataset. Cells are colored by expression of VAChT, VGluT, TH and demonstrate the main neuronal types in the paralarval brain.

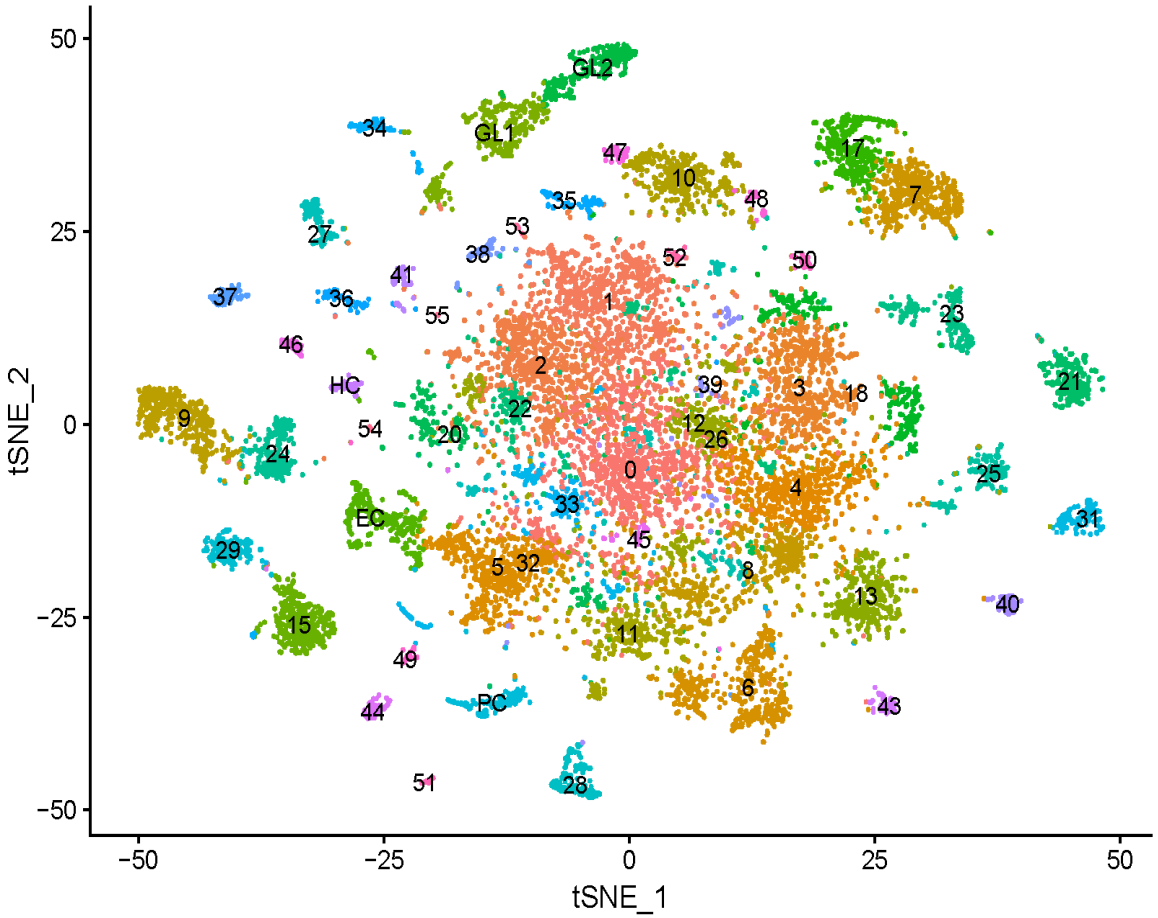

Figure 5: t-SNE plot of the integrated dataset with the major cell types annotated. Progenitor cells (PC), Glial subtype I (GI1) and Glial subtype II (GI2), Hemocytes (HC) and Endothelial cells (EC).

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The majority of **octopus protocadherins (PCDHs)** are differentially expressed between different putative cell types and they might play a role specifying cell type identity. Moreover, we investigated whether certain PCDHs are always co-expressed and form 'co-expression modules'. We were able to identify several of these modules and found that these co-expressed PCDHs are also cluster-specific.

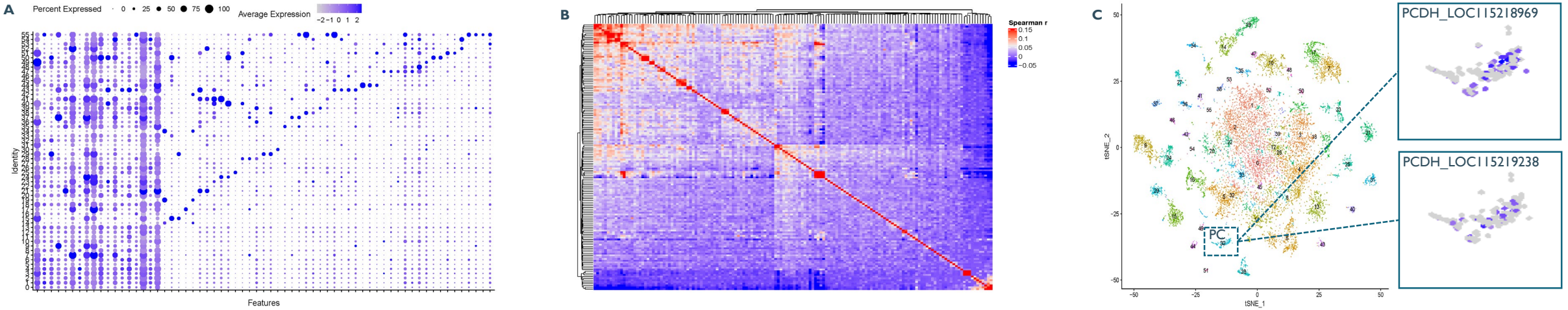

Figure 4: A. Dotplot of all HVG Ov-PCDHs in the integrated dataset. **B.** Correlation matrix of all Ov-PCDHs. **C.** t-SNE plot of the integrated dataset. Cluster 30 represents the progenitor cells (PC) and exhibits co-expression of two Ov-PCDHs.

Conclusion

For non-model organisms **improving the gene models** can be very beneficial for analysing 3'-biased scRNAseq data.
We found that the developing brain of *Octopus vulgaris* possesses **a wide variety of cell types** and have identified marker genes for glial cells, hemocytes and different neuronal populations.
Moreover, it seems that some protocadherins might play a role in defining cell types, while others are more ubiquitously expressed.

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