

Spatial transcriptomics reveals novel adult neurogenesis niche in cephalopods

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INTRODUCTION

Cephalopods (octopus, squids, cuttlefish, and nautilus) are emerging model organisms due to their independently evolved, large complex brains, and advanced cognitive abilities¹. Despite over 600 million years of evolutionary divergence between humans and cephalopods, commonalities of sophisticated behaviors and centralized brain allows for exploration of potentially universal principles of neural development and regeneration. In contrast, evolutionary pressures also allows for discovering novel cephalopod-specific pathways that provide beneficial neural engineering. In embryonic neurogenesis, a structure known as the “lateral lips” which encompasses the optic lobes have been implicated in neurogenesis due to its association of *proliferating cell nuclear antigen (pcna)* and bHLH group A transcription factors, *achaete-scute (ascl)* and *neurogenin (neurog)*². Cephalopods are capable of post-embryonic neurogenesis throughout their lifespan, generating approximately a thousand times the size of the brain from hatchling (200,000 cells) to adult (2 million cells)¹. However, the neurogenesis mechanism in post-hatchling cephalopods have yet to be determined. Adult neurogenesis has been demonstrated in cephalopods indicated by increased levels of PCNA and poli (ADP-ribose) polymerase 1 in enriched environments³. To understand the mechanism of neurogenesis in post-hatchling cephalopods, we have utilized the cuttlefish, *Euprymna berri* to determine proliferative regions involving the central nervous system to identify neural progenitors via thymine labeling. Proliferative cells are verified as neurogenic by the use of spatial transcriptomics.

METHODS



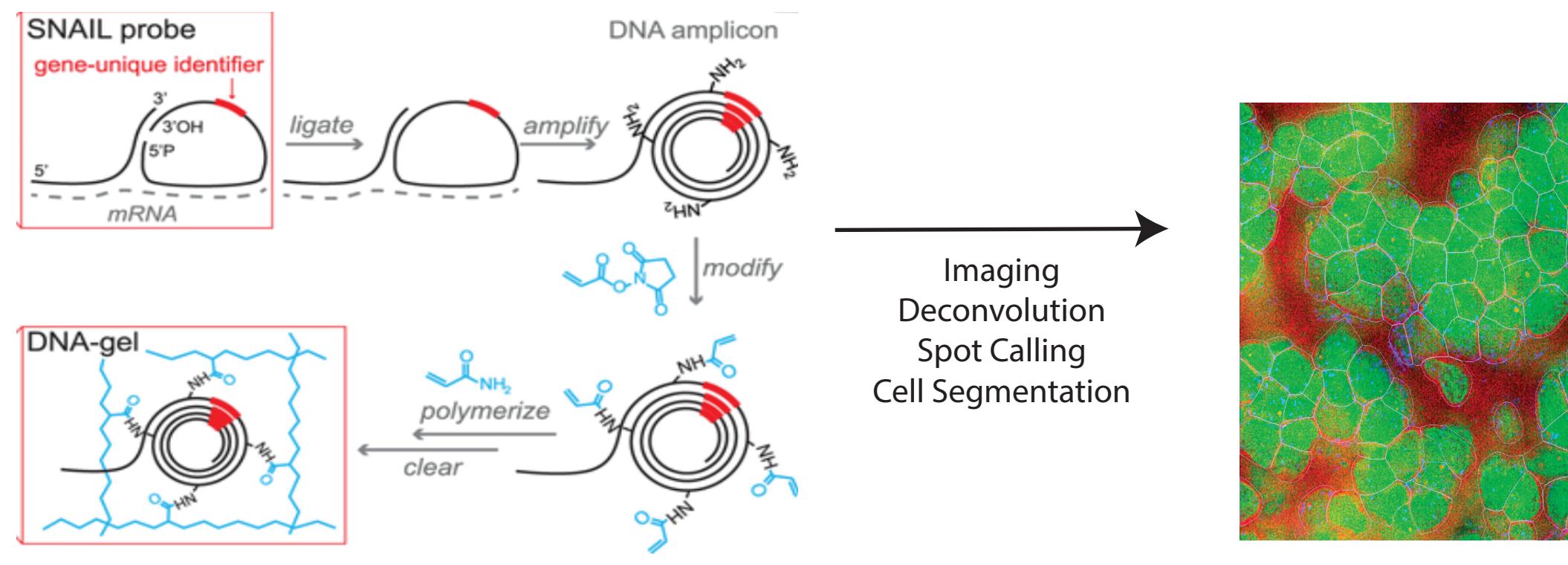
Sample Preparation

- *E. berryi* were submerged in 100 µM F-ara-EdU and subsequently chased in all natural seawater, if required.
 - Specimens were anesthetized using 3.5% MgCl₂, fixed with 4% PFA and prepped for whole brain imaging or OCT embedding.

Specimen Imaging

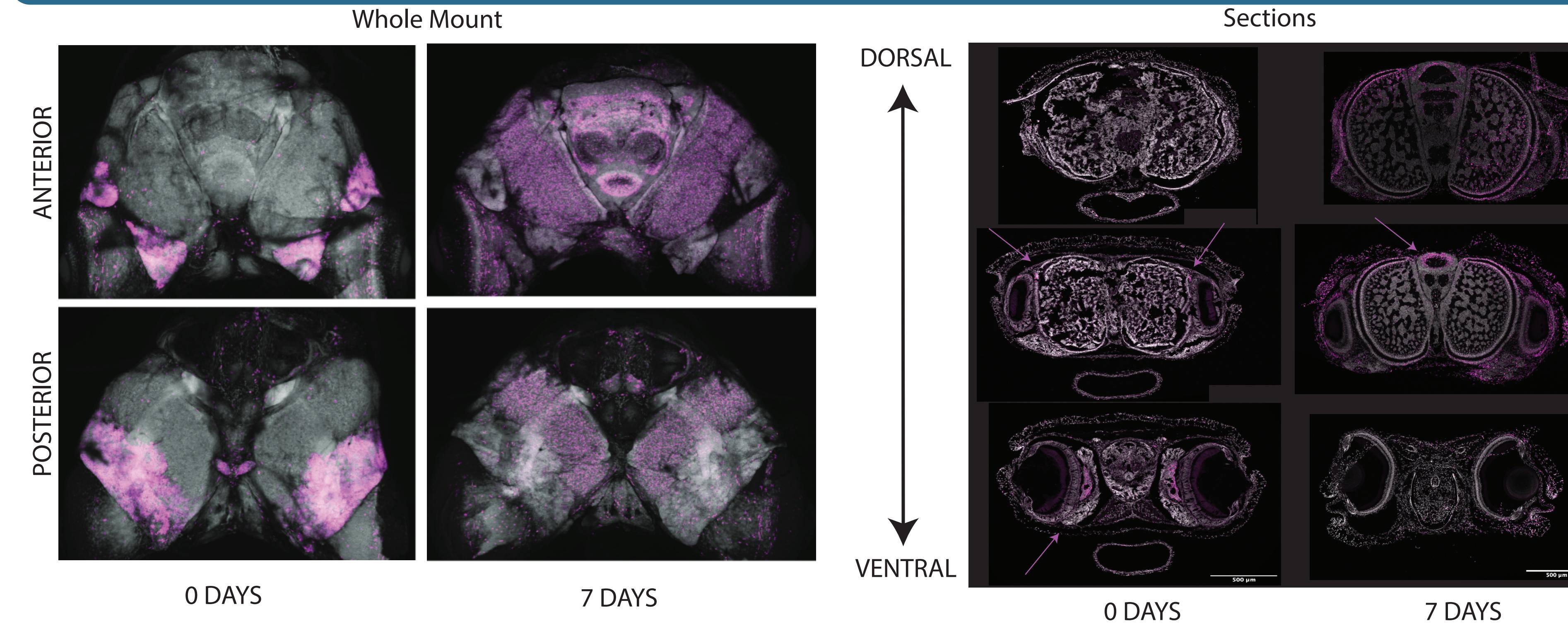
- Samples with F-ara-EdU⁴ underwent copper-catalyzed azide reaction and DAPI staining prior to confocal imaging.

Spatial Transcriptomics



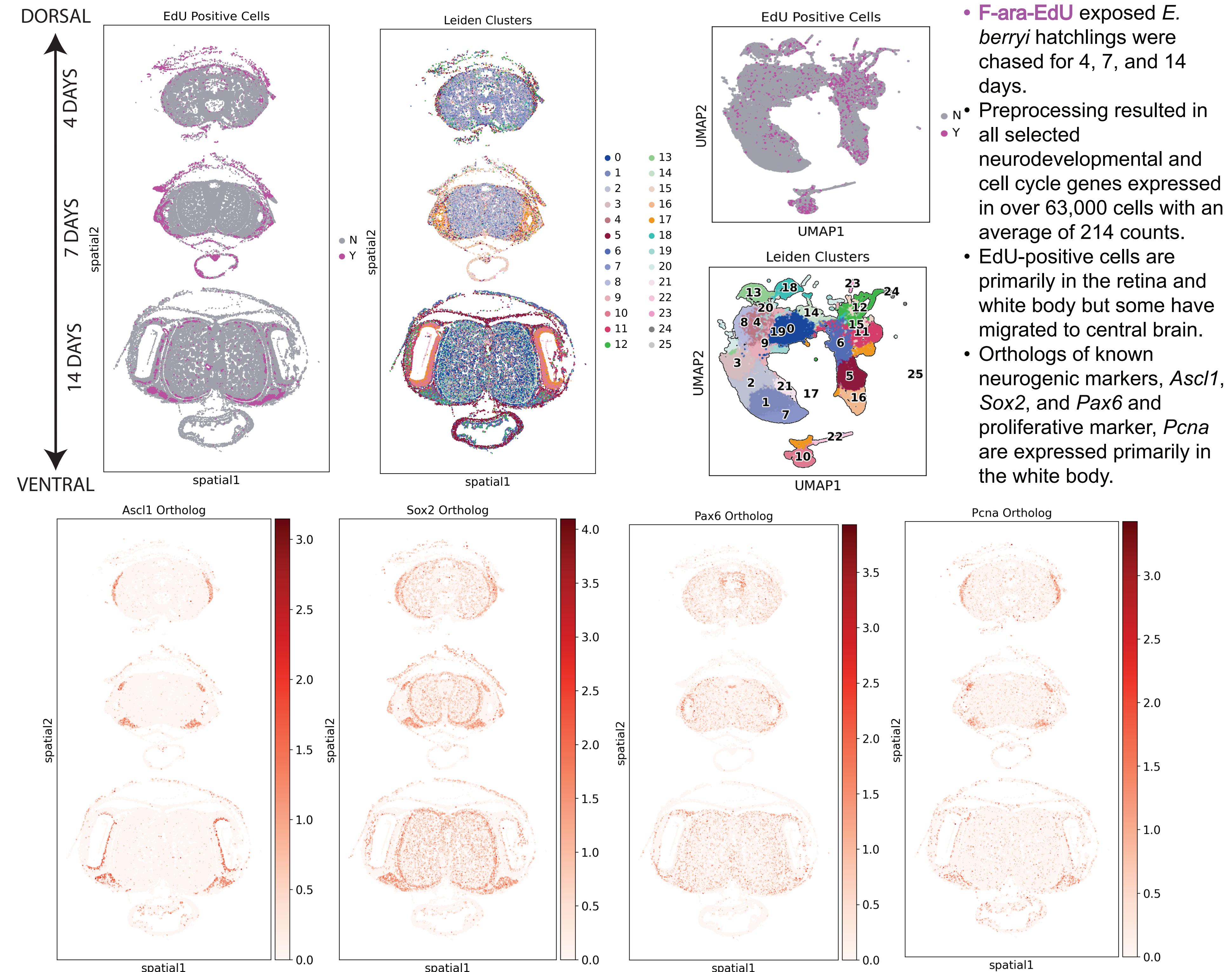
RESULTS

Extended thymidine analog labelling in hatchling squid identifies high proliferation outside central nervous system



- *E. berryi* hatchlings were exposed to thymine analog label, **F-ara-EdU** to identify proliferating cells in contrast with DAPI.
 - Whole mount brains with no chase primarily show the white body, a known hematopoietic organ outside the nervous system as proliferative. Sections verified there were **no significant proliferation within the brain**, but primarily in the white body.
 - Samples with 7 day chase show EdU-positive cells within the brain both in whole mount and sections with less EdU-positive cells in the white body.

The white body is a proliferative neurogenic niche outside the central nervous system in post-hatchling squid



The white body is a heterogenous tissue

CONCLUSION

Cell proliferation occurs outside the central nervous system.

- F-ara-EdU positive cells were shown primarily in the white body, a tissue outside the central nervous system.
 - When thymine analog is extended by a chase, EdU-positive cells are shown dispersed in the central nervous system with less EdU-positive cells in the white body.

The white body is a neurogenic niche outside the central nervous system

- Spatial transcriptomics at various extended thymine analog labeling and regions of the head determined gene-specific cell clusters.
 - Known neurogenic markers, *Ascl1*, *Sox2*, and *Pax6* show association primarily in the white body.

The white body is a heterogenous tissue

FUTURE DIRECTIONS

- Establish a cell type atlas throughout ages and conditions for the white body
 - Verify neural progenitor genes utilizing HCR in whole mount heads and sections
 - Identify neural progenitor trajectories originating from white body to central nervous system location
 - Identify the migration mechanism of neural progenitor cells

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