STUDYING NEUROGENESIS IN CEPHALOPODS: WHY AND HOW ?

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INTRODUCTION

The nervous system of cephalopods exhibits numerous sensorial and structural innovations among molluscs. Their developed central nervous system (ganglia fused into a brain) has been used as a comparative model to vertebrates (Young, 1971, 1974, 1976; Messenger, 1979; Hochner *et al.*, 2003) and giant axons have long been an important material for neurocytology, electrophysiology and biophysics.

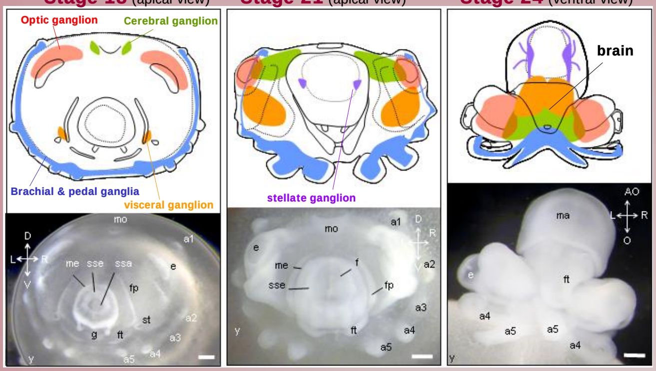
Intense efforts have been conducted to understand physiological function of the brain and giant axons but comparatively nothing is known about the molecular pathways underlying their development. Similarly, the diversity of cephalopod nervous systems indicates a high flexibility and adaptability, which makes them a relevant biological material for evolutionary studies. Nevertheless, neither their development nor the mechanisms that could have led to the emergence of these derived traits have been studied. For example, the process of neural bilaterality establisment remains unknown in these species without neural cord.

DEVELOPMENT & NERVOUS SYSTEM in *Sepia officinalis*

Cephalopods present a **direct development**, there is no veliger stage and no metamorphosis as in other molluscs.

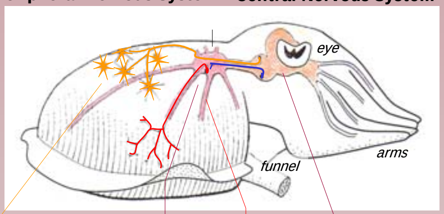
The nervous system is established **before hatching** but changes occur from juvenile to "adult", mostly regarding the relative proportions of brain parts.

Neurogenesis has to be described in *S. officinalis* and this first requires the description of embryonic neural territories ►



Embryonic neural territories in *S. officinalis* are deduced here from that of *Octopus vulgaris* and *Sepioteuthis lessoniana* (squid) embryos. In *S. officinalis*, fecundation is stage 0 and hatching occurs at stage 30. Main organogenetic changes occur between stage 15 and 24. a: arm; e: eye; fp: funnel pouch; ft: funnel tube; g: gill; ma: mantle; me: mantle edge; mo: mouth; ssa: shell sac aperture; sse: shell sac edge; st: statocyste. Scale bar: 500µm.

Adult



the **2 stellate ganglia** (s.g.) are peripheral relays of the brain

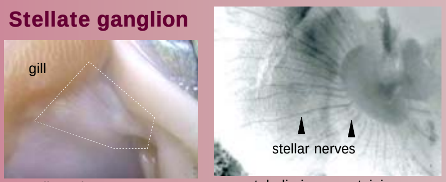
the **brain** is made of fused ganglia (visceral, cerebral, pedal and optic)

chromatophore fibres pass through the s.g. without any connection

in stellar nerves, **giant fibers** are made of fused axons of 3rd-order neurons. Synapses with 2nd order neurons from the brain are located in the s.g.

NEURAL SPECIFICITIES RAISE NEUROGENETIC and EVOLUTIONNARY QUESTIONS

Stellate ganglion

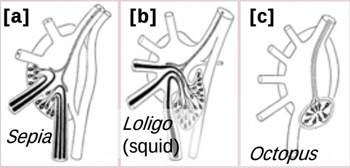


dissection — α-tubulin immunostaining

- How do stellate ganglia develop ? as a result of what molecular mechanisms ?
- How do they eventually get connected to the brain ?
- May they provide information about the CNS history within Lophotrochozoa ?

Giant axons

Stellate ganglia in cephalopods exhibit various degrees of complexity and of axon fusion, depending of life habit: [a] and [b] are pelagic and able of rapid muscular contractions, [c] is benthic and is a poor swimmer.



giant fibers — giant axons — typical axons

- How do axons fuse ?
- Which genetic or molecular differences could explain the functional adaptations of stellate ganglion throughout the evolution of cephalopods ?

Chromatophores

- How the chromatophore pattern is controlled? [a]
- What controls axon guidance throughout stellate ganglia to chromatophores ?
- How bilateral pattern is set up without any central nervous axis ? [b]

LOOKING FOR HOMOLOGOUS GENES AND GENE EXPRESSION

Among genes known to participate to the nervous system formation in Metazoans **few have been identified in the Mollusca** yet.

According to our problematic (see questions above), we focus on genes involved in

neuronal determination
neuronal specification
axonal growth

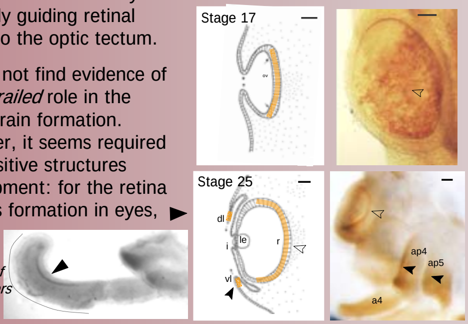
during embryonic development.

Our aim is to characterise the genes, to determine their expression patterns and identify their function by extinction and/or surexpression.

Engrailed,...

In vertebrates, *engrailed* is involved in organizing the mid-hindbrain boundary or in correctly guiding retinal axons to the optic tectum.

We did not find evidence of an *engrailed* role in the *Sepia* brain formation. However, it seems required for sensitive structures development: for the retina and lids formation in eyes, ► in arms

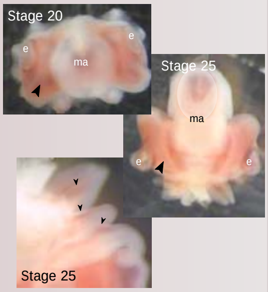


arm 4 at stage 25

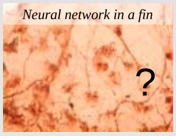
Baratte et al. 2007- Gen. Gene Evol

Pax6,...

Pax-6 is a master gene in eye development. In cephalopods, it is expressed in eyes, olfactory organs, brain and arms.



...and other candidate genes under identification

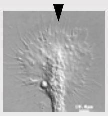


- Neuroectodermic determination and neuronal specification

  the NK family [*Nk1 / Nk2 / Nk6*]

- Attraction and/or repulsion of growth cone

ligand	*receptor(s)*
semaphorins	plexins, neuropilins
netrins	DCC
ephrins	ephrin receptors



- Median line establishment

slit	robo

PERSPECTIVES

By providing a better understanding of the molecular mechanisms underlying the development of cephalopod nervous structures, this project will improve the knowledge of the nervous system evolution among Lophotrochozoans. The nervous peculiarities of cephalopods among molluscs offer a unique opportunity to investigate how genes produce diversity in neural organisation. Beside traditional invertebrate models (*Drosophila*, *C. elegans*) belonging to ecdysozoan, lophotrochozoan models are essential for the comparative study of molecular mechanisms and pattern formation as representative of the bilaterian diversity.

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