

# Brain development and learning in cephalopod species

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Cephalopods, a group that includes squids, octopuses, and cuttlefish, are renowned for their advanced nervous systems and remarkable behavioral complexities, making them ideal subjects for comparative neurobiological research. This study explores key aspects of cephalopod neurobiology through two distinct approaches. The first experiment investigates neuronal development during embryogenesis in *Loligo vulgaris*. Using immunostaining and HCR-FISH techniques, we tracked the expression patterns of neuronal markers such as *Elav-like-1*, *tetraspanin-8*, *LIM* domain proteins, *e2f3*, *asc*, *FoxD1* and *GATA-2* across developmental stages. Our findings reveal progressive differentiation, migration, and maturation of cells expressing these markers, enhancing understanding of cephalopod brain formation. *Elav-like-1*, crucial for gene regulation in the nervous system, was found across most lobes during early embryogenesis. *LIM* domain proteins expressing cells showed progressive differentiation in subvertical and vertical lobes during specific developmental stages. *E2f3* and *asc* expression in proliferating cells near the nervous system suggested the presence of stem cells and neuronal progenitors in the lateral lips, indicating potential neuronal fate determination and migration.

The second experiment examined the effects of dopamine modulator on learning and memory in the cuttlefish *Sepia bandensis*. Using a behavioral assay known as "prawn in a tube," we tracked the cuttlefish's interest in prey across multiple training sessions. The expected pattern for effective learning was a decrease in interest, reflecting an understanding that the prey is unattainable. However, the consistent slight upward trend in interest seen in the control suggests that *S. bandensis* did not learn to lose interest in the inaccessible prey over time, indicating a lack of observed memory retention. The results also indicated that the number and duration of training sessions might not have been sufficient to induce learning. The experiment with dopamine agonists and antagonists showed no clear pattern, with some animals showing increased or decreased interest.

This research contributes to a comparative understanding of complex nervous system development and cognitive processes across species. By elucidating brain development dynamics in *L. vulgaris* and exploring dopamine impact on cognition in *S. bandensis*, we advance knowledge in cephalopod neurobiology, opening the way for future studies on neurotransmitter roles and cognitive evolution.

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