

The brain of *Octopus vulgaris*: shedding light on neural network and neuromodulatory fingerprint

A thesis submitted to the Open University, Milton Keynes, for the degree of

DOCTOR OF PHILOSOPHY

by

Federica Pizzulli

Discipline: Life Health and Chemical Sciences

Affiliated Research Centre:

Stazione Zoologica Anton Dohrn

Naples – Italy

September 2023

Abstract

Octopus has evolved a brain and nervous system which for its centralization, number of neural cells, size, and variety of behavioural outputs, make it surprisingly comparable to a small mammal. In addition, as all cephalopods, it shows remarkable learning and memory abilities that contribute to considering octopus as an emerging model for the study of cognition (Edelman & Seth, 2009; Zarrella et al., 2015; Marini et al., 2017).

Main aim of this PhD project is to contribute to the description of *Octopus vulgaris* brain complexity through neural network analysis and the identification and mapping of molecules with neuromodulator activity, in order to create an integrated map of its brain.

Based on the morpho-functional analysis of Young (1971), I applied Rubinov and Sporns (2010) approach to describe segregation and integration of octopus neural connectivity in the brain. Median basal lobes, subvertical lobe and optic lobes were identified as the most connected regions. In addition, typical connectivity measurements, such as rich club and small world properties, showed remarkable similarities with those observed in humans and other mammals.

Furthermore, this study systematically explored the existence of key neurochemical entities such as acetylcholine, biogenic amines (histamine, serotonin, norepinephrine, dopamine, tyramine, octopamine), glutamate, GABA, glycine, and tachykinin in the cephalopod nervous system. Through *in silico* analysis I identified 60 (for *Euprymna scolopes*) and 81 nucleotidic sequences for *O. vulgaris* encoding orthologous genes related to receptors, transporters, and enzymes belonging to neuromodulator synthesis and metabolism. A selection of these genes was successfully localized in the octopus nervous system through *in-situ* hybridization chain reaction.

Finally, this research delves into neuronal tracing methodologies, exploring new approaches and guidance for further application of tracing methods in the brain.

Overall findings shed light on the neuromodulatory pathways, gene expression, and brain connections in *O. vulgaris*. These provide the groundwork for future research allowing a comprehensive analysis of the neural modulatory systems in cephalopods.