Biology and Life History of Cephalopods

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Nervous Cnidarians started it all…

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The Cnidaria are usually described as having neither a brain nor a central nervous system, but just a simple nerve net that reacts to stimuli and commands muscular cells. If nervous cells are monophyletic, then the Cnidarians are the simplest living animals that possess them, albeit with a "loose" architecture. The Bilateria sit on this evolutionary innovation that became very elaborate in the "higher phyla". This story can be told in a different way, though.
The cnidarian life cycle comprises a bilateral planula, with a front and a rear part, a radial benthic polyp that often forms modular colonies, and a radial planktonic medusa that is usually a single individual (with noticeable exceptions in the Siphonophora).

1: Planulas can have a brain and a central nervous system. The planula of the hydrozoan *Clava multicornis* has a frontal concentration of nervous cells and a nervous chord that departs from it and reaches the posterior end. This is the simplest, and the first, central nervous system. According to some theories on metazoan evolution, paedomorphic planulas that attained sexual reproduction resemble the simplest Bilateria: acoelous turbellarians (Acoelomorpha).

2: Polyps usually have a nervous net (and a head). Once the planula has found a settling spot, it metamorphoses into a primary polyp and the central nervous system becomes a network. Radial polyps receive stimuli from all directions and are not polarized as bilateral organisms, such as the planula and the rest of the bilateria. Polyps are mostly benthic and can have very simple sense organs that receive stimuli in form of vibrations, being also able to perceive chemicals dissolved in the water. It is suggestive that the solitary and freshwater *Hydra* is the paradigmatic cnidarian in textbooks: the exception becomes the rule! *Hydra*, however, has a "head" in the form of a concentration of nervous cells around the mouth (some call it a "brain").

3: Medusae have elaborate sense organs and a circumesophageal centre of control (nerve ring). Medusae are free living and swim with an open body cavity that is lined with striated muscles (a mesoderm) and do have a circle of tentacles on the bell margin, where, according to species, sense organs

*for Professor **Ferdinando Boero** see:*
http://www.dipartimentodibiologia.unina.it/personale/ferdinando-boero/
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are present in form of eyes, statocysts, or rhopalia (compound sense organs comprising both eyes and statocysts). Some cnidarian eyes are rather simple, but others (e.g. those of the Cubozoa) are very complex. Pax Genes, the control genes that command the formation of eyes, are shared by the Cnidaria and the rest of the Metazoa. Medusae do have two nerve rings along the umbrellar margin, directly connected with the sense organs. They receive stimuli from the sense organs and send commands to the striated muscles of the subumbrellar cavity and to the tentacles. An interstitial Hydrozoan, Otohydra, has a closed subumbrellar cavity that becomes a brood pouch, the mouth is encircled by the tentacles and the ring of statocysts around it is connected with a nerve ring that is a circumesophageal brain. The two rings of medusae are a brain, and this architecture is optimal for a radial organism that, being a predator, performs elaborate behaviors.

4: The evolution of the nervous system started with the Cnidaria and deserves more attention in comparative neurology: to understand the apexes, the roots must be considered carefully, and fully understood.

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NeuroCeph - Cephalopod Neurophysiology
a CephRes2020 Virtual Event Focus on Session

Main Organizer: Dr Letizia Zullo (Italy)
Co-Organizers: Prof. Binyamin Hochner (Israel); Dr Michael Kuba (Japan)

Cephalopod body and nervous system have been study 'models' in Neuroscience from before the early twentieth century. Many fields of investigations from Behavior to Neuroanatomy and Neurophysiology have been developed so far. In recent years, cephalopods turned to be exciting animals for understanding the independent evolution of neurophysiological processes involved in mediation of complex behavior and as an outstanding demonstration for highly efficient motor behavior in soft-bodied animals. We will attempt at presenting the major ‘hot’ topics, including some newest discovery and challenges in the field of brain and muscle Neurophysiology with a special attention on the current methodological advances and bottlenecks.
Spanning from single cell to collective recording of excitable cells activity, we aim to favour a collaborative bench-work aiming at providing new neurophysiology methodological strategies for data collection and interpretation to be beneficial to cephalopod research.

[*abridged from the original proposal*]

A novel NO-dependent 'molecular-switch' mediates memory acquisition in the vertical lobe of *Octopus vulgaris*

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The octopus vertical lobe (VL), a brain area that controls the sophisticated learning of this invertebrate, demonstrates a robust activity-dependent long-term potentiation (LTP) which was shown to be important for memory acquisition (Shomrat et al, 2008).
We show here that the presynaptic expression of LTP involves activation of nitric oxide synthase (NOS) and that nitric oxide (NO)-dependent reactivation of NOS functions as a 'molecular switch' mediating the very long, protein synthesis-independent, LTP maintenance (> 10h). While NADPH-diaphorase histochemistry supports the presence of NOS in the VL, we could not find any indication for the involvement of the canonical NO-dependent cGMP cascade in LTP. Additionally, NO-donors and NO-scavengers had no effect.
These negative results suggest the possible involvement of processes that function at high NO concentration (e.g., s-nitrosylation). We then measured NO concentration amperometrically and found that induction of LTP is accompanied by a long-term increase in the amperometric signal that corresponded to NO's oxidation potential (750 mV). This increase, on μM ranges, was much higher than the one found for the activation of cGMP cascade. We therefore hypothesize that a process such as s-nitrosylation could serve as an effective mediator of a local retrograde message for ensuring specificity in presynaptic LTP.

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Deciphering Octopus brain spiking activity

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Octopuses possess the most complex brain organization outside of vertebrates. They exhibit a rich behavioral repertoire that involve various forms of learning. The octopus' central brain integrates a large amount of sensory information from the optic lobes and the peripheral nervous system of the arms and issues commands to lower motor centers. It was shown that the octopus appears not to use a somatotopic representation of body parts in the higher motor centers (the basal lobes) in the central brain. This raises the question whether there is a somatotopic representation of the sensory information in these higher brain centers.

In the current work, different stimulation modes (tactile and visual) were given to freely behaving animals while extracellular multi unit recordings from different areas in the central brain were acquired. Here we developed a computational methodology to perform spike sorting and clustering analysis that enables characterizing the neuronal units that are activated by the different simulation modes. The results suggest that as was found with motor representation (Zullo et al., 2009) there is no somatotopic organization of sensory information in the higher motor centers. Moreover, the clustering analysis suggests that units representing different sensory modalities are integrated into different clusters depending on the stimulus delivered to the animal.

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Unrestrained EEG recording in Cephalopods

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The present study aims to establish the first-ever electro potential recordings from the brains of behaving unrestrained octopuses, and the first-ever multi-channel recordings from a cephalopod. Previous studies (Brown et al 2006, Zullo et al 2009) were limited to animals with implanted wired single-channel electrodes directly connected to an amplifier and recording device outside of the water. An octopus has eight sensitive and maneuverable arms, which can grab and extract an electrode even if just a fragment protrudes from the skin, yielding displacement of the probe, unstable recordings, electrode damage, and abortion of the experiment.
The neurologger is fully contained within the animal, and does not rely on protruding cables, so that the animal can't tamper with wires or corrupt the recording by altering the location of the electrodes.
We have been able to collect the first multi-channel EEG recordings from the brains of behaving unrestrained octopuses. Our current research has found differences in the EEG power spectrum during different activity states. Using the neuro-loggers together with standard electrodes embedded trough the cartilaginous capsule into the brain, we can EEG activity with electrodes either remaining above the brain or inside the brain. This and future recordings will help us to better understand the evolutionary origins of sleep behaviour and chronobiology. Additionally, knowing the activity cycle of these animals will be important for any attempts regarding sustainable marine culturing of this highly important species.

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Insight into the connectome of the octopus learning and memory system

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Cephalopod molluscs exhibit sophisticated cognitive abilities mediated by a pivotal brain structure: the vertical lobe (VL). It is believed that the VL shows a connectivity organization typical of learning and memory network, however the anatomical considerations are limited to the work of Young and Gray in the 1940-70's.
The current study aims to produce detailed description of the synaptic connections of the various neurons comprising the VL, using recent progress of volume electron microscopy imaging. Accordingly, an *O. vulgaris* VL was fixed, serially sliced (30nm sections), imaged with a Scanning Electron Microscope at 4 nm pixel resolution and aligned into a traceable 3D stack (260x390x30µm). Extraordinary rich and highly dense synaptic profiles, with various type and combinations of synaptic vesicle types, are observed across the VL neuropil. All synaptic outputs of several superior frontal lobe (SFL) axons, the main input to the VL, were reconstructed revealing that in addition to the known connection to the amacrine interneurons (AM), they also innervate two yet undescribed cell types or subtypes.
Our results show that the AMs are structurally exceptionally simple receiving just a single synaptic input from only one SFL axon, thus suggesting an interneuron with no integration role. In contrast, the newly uncovered SFLs postsynaptic partners display complex dendritic arborizations receiving multiple synaptic inputs from the SFL axons. Overall our results reveal a larger diversity in VL cell types together with a connectivity scheme more complicated than previously described including unprecedented mono-presynaptic input in central synapse.

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Visual coding and functional organization in the octopus optic lobe

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Cephalopods are highly visual animals who use vision to detect predators and prey, as well as to drive unique behaviors like their rapid body pattern camouflage displays. Strikingly, the cephalopod visual system evolved independently from those of other highly visual species, so that both the eye and the underlying neural circuitry are evolutionarily distinct. However, there have been no direct recordings of visual responses in the cephalopod central nervous system, so it is unknown how this independently evolved visual system encodes the visual world. In this study, we used two-photon calcium imaging to record visually evoked responses in the primary visual processing center of the octopus central brain, the optic lobe, to identify the features extracted by the octopus visual system and determine how information is organized in the central brain. Similar to most visual systems, we found neurons with spatially localized receptive fields responsive to both ON and OFF stimuli locally clustered within the optic lobe that were organized in a retinotopic order. We also identified unique properties, such as size suppression for light stimuli but size summation for dark, that may have evolved to suit the specific demands of the visual world of the octopus. This study represents the first insight into the neural coding and organization of this highly capable but poorly understood visual system.

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Octopus arm biomechanics

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Cephalopods are highly evolved marine invertebrates that colonized almost all the oceans of the world at all depths. This imposed the occurrence of several modifications of their brain and body whose muscle component represents the major constituent. Moreover, modern cephalopods are soft-bodied animals that manifest, during movements, considerable variations in their body shape alongside with reversible modification of their stiffness/softness ratio. These properties are especially interesting for their translational aspects. Hence, studying their muscle physiology may give important hints not only in the context of animal biology but also in the emerging field of soft robotics. Here we present the major features of the octopus arm musculature. We will show their physical constraints within the arm embedding and how these muscles are adapted to work in relation to their use in motion. This information may provide a bench work for designing new soft materials with muscle-like properties employed in soft robotics.

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