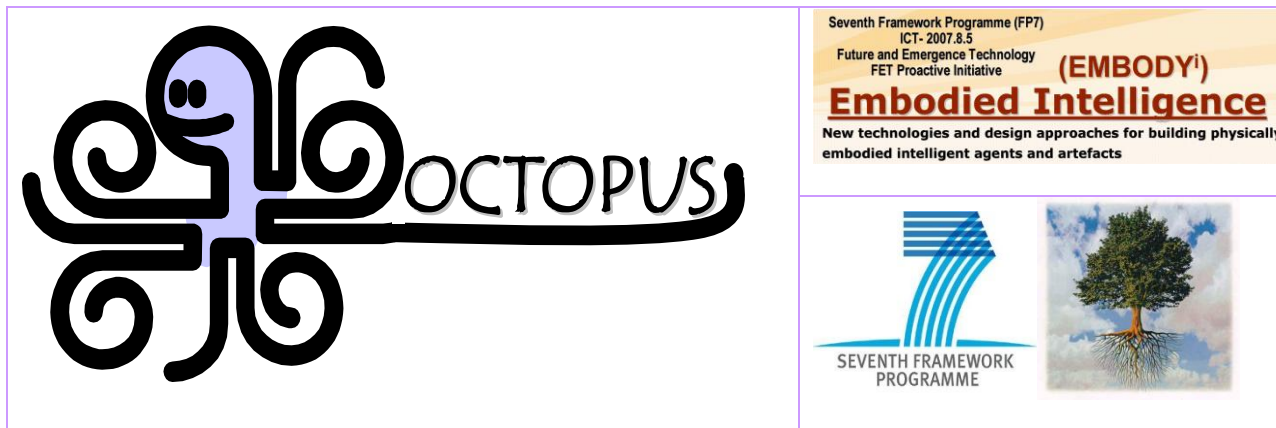

PROJECT DELIVERABLE



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1. Summary

This deliverable is for internal use as a quick reference for the existing knowledge on the octopus biology and a guideline to define the work on the successive WPs 4, 5 and 6. It is also for external use as a guide to aid project evaluation.

Here we aim to describe in detail the biological knowledge on *Octopus vulgaris* with an arm anatomical description (see DoW Task 2.1) and an analysis of the nervous system characteristics and organization (motor and sensory) both at the level of the higher and peripheral motor centres (see DoW Task 2.2). We also provide a summary of the studies of the motor control of the stereotypical goal directed arm movements (see DoW Task 2.3).

2. Introduction

Today there are about 700 species of coleoid cephalopods inhabiting almost every marine environment, from the deep sea to the tide level, from the tropics to the Polar regions. Coleoid cephalopods are molluscs, yet in their morphology, physiology, ecology and even behavior were shaped by a co evolutionary arms race with modern teleost fish. They often developed abilities and properties convergent to vertebrates (Packard 1972). *Octopus vulgaris* "lives fast and dies young"; it grows extraordinarily quickly (by mulluscan standards), they mature after about a year and yet rarely live for more than a two or maybe three years. Despite their short lives octopuses are "brainy" animals and their behavior is complex and diverse. The brain/body ratio of *Octopus vulgaris* is comparable to that of lower vertebrates and they are among the largest of any invertebrate brains. Apart from its behavioral plasticity and high cognitive capacity the large size of the brain is needed to control the animals flexible body and skin. Their brain anatomy and neuronal pathways have been extensively studied (for reviews see: Budelmann et al., 1997; Nixon & Young 2003; Young, 1971).

The octopus is an ideal animal for studying the capabilities of the advanced invertebrate brain because of its complex vertebrate-like behaviors (Hochner et al 2003; 2006). Octopuses have been, and still are, extensively used for training experiments, because they learn rapidly. They also use body posture, swaying, advance and retreat, slower and faster arm movements, skin color, skin smoothness or roughness, eyes and iris permutation to exhibit many subtle signs of their difficulties and frustrations during the learning procedure (Budelmann et al., 1997). A further practical advantage of training *Octopus vulgaris* is that they are solitary animals, are relatively easy to handle in captivity and can withstand surgical procedures (Wells 1978; Young, 1971).

Most important the octopus is an ideal animal model for studying the generation and control of the movements of flexible arms. The knowledge thus gained is of immense relevance to the field of biologically inspired robotics. Data already obtained on the octopus' outstandingly efficient nervous and muscular control of its eight flexible arms (Gutfreund et al., 1996; 1998; 2006; Sumbre et al., 2001) can inspire the design and control of flexible robotic arms. Understanding the central organization of the higher motor centers and the basic sensory-motor

organization of the octopus may now help solving engineering problems related to flexible robotic arms, particularly in simplifying the construction and control of such appendages.

In the vertebrate brain the convergence of the whole body's sensory systems in the central nervous system is one condition for planning motor commands. A fundamental organizational principle is the "somatotopic organization". Extensive studies of microstimulation of higher motor centers have shown the absence of such an organization in the octopus brain. Indeed a motor representation in form of 'overlapping circuits' has been described. These overlapping circuits were shown to be consistent with the functional organization of the octopuses' complex but relatively small brain (Zullo et al, unpublished).

In the octopus, one of the major major questions is, how are complex behavioral capacities and motor and sensory information integrated to generate a specific behavioral response. Furthermore we need to understand how the central and peripheral nervous systems act together to reach this goal. To tackle these questions, it is first necessary to look at the morphological organization, the physiology and the behavioral outputs of the octopus central and peripheral nervous system.

3. Organization of the octopus arm anatomy and control systems

3.1 Description of the octopus arm anatomy and biomechanics

3.1.1 Description of the octopus arm anatomy

The arm consists of four distinct parts:

- 1. The skin or tegument**
- 2. The muscles**
- 3. The nervous system**
- 4. The vascular system**

The skin is composed of two layers (Cloney and Brocco, 1983; Budelmann et al., 1997):

- 1. The epidermis**
- 2. The dermis**

The epidermis consists of a single sheet and is covered by striated cuticle. The dermis of the octopus arm consists of connective tissue, which surrounds the chief muscle bundle in equal thickness on all sides. It is permeated by many blood-vessels, by muscular and nerve strands, and also contains chromatophores and luminous organs.

The arms of octopuses consist of a tightly packed three-dimensional array of muscle fibers. The musculature of the arms of octopuses not only generates the forces required for movement, deformation and changes in stiffness; it also provides the required “skeletal support”. This type of “skeletal support” system is termed “muscular hydrostat”.

The muscles of the arm are classified into (Graziadei 1965, 1971):

1. the intrinsic musculature of the suckers
2. the intrinsic musculature of the arms
3. the acetabulo-brachial musculature that connects the suckers to the arm musculature

The intrinsic musculature of the arm consists of a densely packed, three-dimensional array of muscle fibers surrounding a large central axial nerve cord that extends the full length of the arm.

The nervous system is composed of three distinct parts (Graziadei, 1971):

1. the central or axial nerve
2. the group of ganglion cells situated above each sucker
3. the intramuscular nerves

The peripheral nervous system of the arm endows the arm with both local and central neural control. It contains approximately 5×10^7 neurons for each arm, arranged within one axial nerve cord, five intramuscular nerve cords and the ganglia of the suckers (Fig. 1). The cerebrobrachial tracts of each arm are linked by two groups of fibers that together comprise the interbrachial commissure (Fig. 2). One group of these fibers serves as a direct connection of each tract in the arm to its neighbor, the other group projects circularly to connect all the arm tracts (Graziadei, 1971).

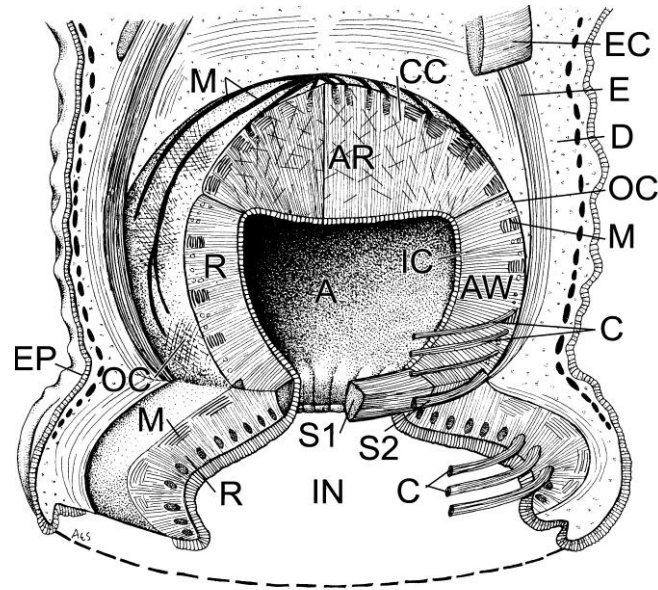


Fig. 1: Schematic cutaway diagram of an octopus sucker. A, acetabulum; AR, acetabular roof; AW, acetabular wall; C, circular muscle; CC, crossed connective tissue fibers; D, dermis; E, extrinsic muscle; EC, extrinsic circular muscle; EP, epithelium; IN, infundibulum; IC, inner connective tissue layer; M, meridional muscle; OC, outer connective tissue layer; R, radial muscle; S1, primary sphincter muscle; S2, secondary sphincter muscle.

The ganglia within a tract share the same structure along the entire length of the arm. Connectives and sensory-motor nerves arise from the ganglionic core. Connectives link the core with the four intramuscular nerve cords and with the sucker ganglia. The sensory-motor nerves are the nerves to the suckers and the nerves of the intrinsic arm muscles. This latter group contains two categories of nerves called “sensory-motor nerves”, probably to stress their involvement in a sensory-motor neural network in the arm (Graziadei, 1971). The chain of ganglia which run the length of the arm innervate the suckers via the ventral roots which innervate the intrinsic arm muscles and thus carry the motor signals that drive arm movements. Two axonal tracts, the cerebrobrachial tracts, run dorsally along the arm nerve cord. These tracts contain axons that transmit efferent signals to the arm and afferent information to the central brain.

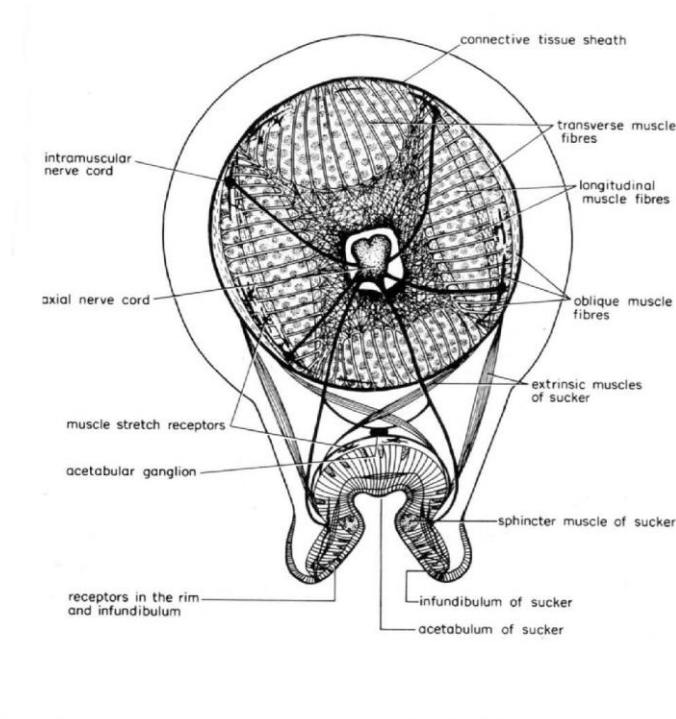


Fig. 2: Muscles and nerves in the arm, shown in transverse section.

3.1.2 Muscle fiber morphology and arrangement

3.1.2.1 Morphology

Electron microscopic observations have revealed that the transverse muscles have sarcomeres of obliquely striated muscles (Kier 1985, 1991). The myosin filaments are very short compared to those found in vertebrates (approximately 1.58 μm (Offer 1987)). Short thick filaments and sarcomeres are characteristic of fibers reaching high strain rates at the cost of a relatively low tensile stress (e.g. Josephson 1975; Van Leeuwen 1991, 1992). The transverse muscle mass of the arms consists of typical cephalopod obliquely striated cells.



Fig. 3: Transverse section of the arm of *Octopus bimaculoides*, showing the major components of the intrinsic arm musculature. Connective tissues are stained red and muscle tissue is stained brown in this preparation. AN, axial nerve cord; ACT, aboral crossed-fiber connective tissue sheet; AR, artery; CT, connective tissue; IN, intramuscular nerve; LM, longitudinal muscle fibers; OCT, oral crossed-fiber connective tissue sheet; OME, external oblique muscle layer; OMI, internal oblique muscle layer; OMM, median oblique muscle layer; TM, transverse muscle fibers; TR, trabeculae. Scale bar: 200 μm , 10 μm -thick paraffin section stained with Picro-Ponceau and Haematoxylin

3.1.2.2 Arrangement

The **axial nerve cord** (AN) is situated on the central axis of the arm, it includes both nerve cell bodies and axons (Fig. 3). Surrounding the AN is a tightly packed mass of muscle and connective tissue consisting of three major groups of muscles: **transverse, longitudinal and oblique**. Muscle fibers in the **transverse muscle mass** (TM) are orientated in planes perpendicular to the longitudinal axis of the arm and extend to insert on **connective tissue layers** (CT) on the oral and aboral sides, and transversely on connective tissues surrounding the oblique muscles. The transverse muscle fibers extending approximately parallel to the sagittal plane originate on the thick crossed-fiber connective tissue sheets located on the oral and the aboral side of the arm. They extend towards the central axis of the arm in longitudinal sheets between bundles of longitudinal muscle. These longitudinal sheets are termed “trabeculae” (Graziadei,

1965). The **longitudinal muscle fibers** (LM), are oriented parallel to the longitudinal axis of the arm and surround the central core of transverse muscle. The fibers extend longitudinally as bundles between the trabeculae of the TM. Three pairs of **oblique muscles** are observed: **external** (EO), **medial** (MO) and **internal** (IO). The external and medial oblique muscles originate and insert on the oral and aboral connective tissue layers. The connective tissue sheets consist of a crossed-fiber array, i.e., their fibers are oriented obliquely to the long axis of the arm in a highly ordered array of both right- and left-handed helixes oriented at an angle of 50-60° to the long axis. The external and medial oblique muscles are also oriented at a similar angle to the long axis of the arm and thus form a composite helical array of muscle and connective tissue fibers oriented as both a right- and a left-handed helix. The internal oblique muscles appear to have their origin and insertion on connective tissues surrounding the transverse muscle. The internal oblique muscles appear to be oriented at a smaller angle to the long axis of the arm, ranging from 40-50° (Kier & Stella, 2007).

A thin layer of muscle fibers arranged circumferentially is also present. This layer is thickest on the aboral side and covers the aboral crossed-fiber connective tissue layer. It extends down each side of the arm as a thin layer, wrapping the external oblique muscle layers, and then inserts on the oral crossed-fiber connective tissue layer (Fig. 4) (Kier & Stella, 2007).

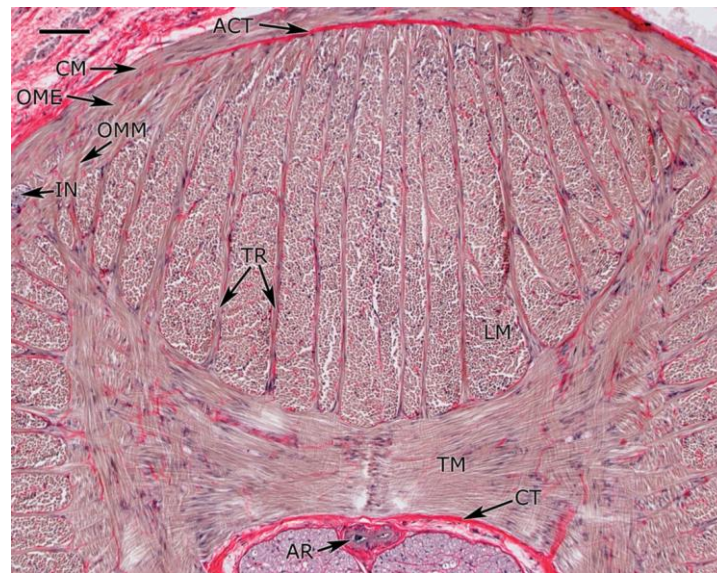


Fig. 4: Transverse section of the arm of *Octopus bimaculoides*; aboral to the axial nerve cord showing the details of the musculature and the connective tissue arrangement. Connective tissues are stained red and muscle tissue is stained brown in this preparation. Fibers of the transverse musculature originate on the aboral connective tissue sheet and extend through the longitudinal musculature, dividing it into elongate bundles. Many of the transverse muscle fibers are visible as they extend through the transverse muscle mass to insert on the connective tissue surrounding the axial nerve cord. ACT, aboral crossed-fiber connective tissue sheet; AR, artery; CM, circular muscle layer; CT, connective tissue; IN, intramuscular nerve cord; LM, longitudinal muscle fibers; OME, external oblique muscle layer; OMM, median oblique muscle layer; TM, transverse muscle fibers; TR, trabeculae. Scale bar: 100 μm , 10 μm -thick paraffin section stained with Picro-Ponceau and Haematoxylin.

3.1.3 Biomechanics

Muscular-hydrostats differ from more common musculoskeletal systems because muscles alternately stiffen and support a structure, or produce movement. The musculature therefore acts both as the effector of movement and as the support for movement.

The most important biomechanical feature of a muscular-hydrostat is that it is a structure of constant volume. Muscle tissue is composed primarily of an aqueous liquid which is practically incompressible at physiological pressure. In a muscular-hydrostat, any change in one dimension will cause a compensatory change in at least one other dimension (Kier & Smith, 1985).

Analysis of arm morphology from the standpoint of biomechanics suggests that the transverse musculature is responsible for elongation of the arms, the longitudinal musculature is

responsible for shortening, and the oblique muscle layers and associated connective tissues create torsion (Kier and Stella, 2007).

In the case of the arms of octopuses, which shows significant elongation, contraction of the transverse muscle decreases the cross-section, and because the appendage is constant in volume, the length must increase. The transverse muscle is antagonized by the bundles of longitudinal muscle, which shorten the appendage and re-extend the transverse muscle fibers. Transverse muscle contraction decreases an area resulting in an increase in length. Due to this musculature action the displacement is amplified and a stretching of the arm is achieved. For instance, a 70% elongation of the tentacles is produced by only a 23% decrease in tentacle diameter (Kier 1982, Kier & Smith 1985, Van Leeuwen & Kier 1997).

In the arms of octopuses, the transverse and longitudinal muscles also play a role in bending movements. Bending results from selective contraction of longitudinal muscle on one side of the arm. In order for bending to occur, the longitudinal compression force must be resisted; otherwise longitudinal muscle contraction would simply shorten the arm. The transverse muscle fibers resist this compression force. Thus, bending movements require simultaneous contraction of longitudinal muscle and transverse muscle (Kier 1982, Kier & Smith 1985, Smith & Kier 1989).

Torsion or twisting movements of the arms are produced by contraction of the helical or oblique muscle layers. The direction of torsion (i.e., clockwise vs. counter clockwise) depends on the handedness of the contracting helical or oblique muscle layer. Torsion in both directions is observed in the arms and both right- and left-handed helical muscle layers are present (Kier and Stella, 2007).

3.2 Description of the unique neural control properties (motor and sensory system) of the arm neuromuscular system

Intracellular recordings evoked post synaptic potentials (**EPSPs**) from **longitudinal** and **transverse muscles fibers** in the octopus arm confirmed that the axons of the motor neurons to the arm muscles leave the axial nerve cord via its dorsal roots, with the field of innervation of each nerve restricted to an ipsilateral area near the ganglion. These findings agree with anatomical descriptions of radial connections from the axial nerve cord to the surrounding

intrinsic musculature via the lateral and dorsal nerve roots (Graziadei, 1971). In addition the dorsal roots also contain sensory afferents from mechanosensory elements in the intrinsic muscles of the arm.

Matzner et al. (2000) have previously reported three types of synaptic responses in octopus arm muscles, indicative of differential slow and fast motor innervations. The core of both slow and fast muscle activation is limited to the region where the dorsal nerve root enters the muscles. The many dorsal roots, all-lying about 0.2 mm apart, suggest that the motor innervation provides a local and continuous control of muscle contraction.

Octopus arm muscle fibers are only ~1 mm long and are also “compact electrotonically”. No significant electrical coupling between muscle cells has been revealed (Matzner et al., 2000; Rokni & Hochner, 2002). Because of this, and as each motor nerve innervates only a small region, we would expect spatiotemporal patterns of muscle activation to be accompanied by similar patterns of activity in the motor neurons in the axial nerve cord. The propagating wave of muscle activity observed during arm extension (Gutfreund et al., 1998; Sumbre et al., 2001; 2005; 2006) should be accompanied by a wave of neuronal activity propagating along the axial nerve cord.

The extraordinary mobility of amputated octopus arms (Wells 1962 b; Wells, 1978; Wells and Wells, 1957; Rowell, 1963; Altman, 1971, Sumbre et al. 2001) indicates the extensive role of the axial nerve cord circuitry in controlling arm movements. The axial nerve cord contains networks that can generate a phase lag between the activity in adjacent ventral and dorsal nerve roots without requiring feedback (Gutfreund et al 2006). Stimuli delivered to the ventral root of the isolated nerve cord can evoke long-lasting neuronal discharges, which are synaptically mediated. However, the activity recorded in the isolated nerve cord was not sufficient to account for a complete whole arm behavior such as arm extension. Instead, this activity could be associated with the local reflexes. A common reflex of the octopus arm is the grip reflex (Rowell, 1963; Wells, 1978; Altman, 1971). Sufficiently strong mechanical or chemical stimulation of a sucker causes the arm to bend and adjacent suckers (both distal and proximal) to serially protract towards the stimulus. Since ventral roots project directly to the sucker apparatus, stimulating a ventral root may mimic the sensory signal (mechanical and

chemical) from a single sucker and the burst of activity in ventral roots from neighboring ganglia may reflect the motor output to neighboring suckers.

It was tested if stimulating the axonal tracts (using the stimulation paradigm that evoked stereotypical arm extension) can generate a propagating pattern of activity in the nerve roots, of amputated or isolated arms (Gutfreund et al 2006, Sumbre et al., 2001). Unlike the fictive rhythmical movements generated by CPGs in the spinal cord, the axial nerve cord of the octopus arm cannot support such fictive arm extension. It therefore appears likely that feedback from muscles (Gutfreund et al., 2006) is part of the feed forward circuitry controlling arm extension (Gutfreund 1998). Morphological studies (Graziadei, 1965) and the evoked nerve activity responses to mechanical stimulation of the muscles, suggest the presence of mechanoreception in the muscles that can drive such a feedback system (Gutfreund et al 2006).

In addition to the axial nerve cord, four other, much smaller, nerve cords have been identified in the arms. These intramuscular nerve cords run along the periphery of the intrinsic musculature, connecting with the main axial nerve cord at regular intervals. Some neurons in these cords innervate local muscle fibers. However, more prominently, small multipolar nerves in the musculature, which may serve as stretch receptors, project to these nerve cords (Graziadei, 1965). Electrophysiological finding by Gutfreund et al (2006) muscle mechanoreception is most sensitive in peripheral muscle fibers agrees with Graziadei's anatomical description. Moreover it suggests an interesting organization in the context of the mechanics of the arm. The mechanosensory system of the intrinsic musculature is preferentially located in the periphery where muscle strain is expected to be stronger during bending of the arm. The peripheral location and the apparently serial local sensory organization make this afferent system ideally suited for sensing bending of the main arm trunk, in terms of bend position and direction (cf. Grillner et al., 1984 for the lamprey). It was suggested that this sensory information is crucial for organizing and executing the motor activity underlying bend propagation in arm extension and reaching movements, a hypothesis that requires further experimental testing.

3.3 Description of the arm sensory receptors

The arm contains a widespread sensory system that allows the animal to collect mechanical and chemical information from the immediate environment and to appropriately react to the stimuli. The suckers contain the most effective mechanical and sensory systems. Each sucker consists of an external structure or **acetabular cup** that is richly innervated by sensory cells, a specific ganglion and a peduncle.

The acetabular cup (Fig.1) consists of an **infundibulum** which bears a series of radial ridges and grooves (Fig. 2). It is covered by a chitinous cuticle or sucker lining that is shed periodically and continuously renewed (Girod, 1884; Naef, 1923; Nixon and Dilly, 1977; Packard, 1988). A rim of loose and folded dermis and epithelium encircles the infundibulum and is separated from it by a circumferential groove. The rim of **loose epithelium** is in turn surrounded by a zone of epithelium that includes cells with inclusions that have the staining characteristics of acid polysaccharides typical of molluscan mucus (Kier and Smith, 1990). At the center of the infundibulum is an orifice that opens into an approximately spherical cavity called the **acetabulum** (Girod, 1884). A cuticle covers both the infundibulum and the acetabulum it is made of a chitin-protein complex secreted by underlying epithelium cells (Hunt & Nixon 1981). The infundibulum has radial folds and small pores that consist of an aggregation of rods forming regular spaces (0.8 μm in diameter, Nixon & Dilly 1977). The cuticle is shed from the infundibulum and the acetabulum simultaneously as a single unit. The suckers are attached to the arm by a short muscular base.

The sucker ganglion is composed of:

- i. motor neurons that innervate the peduncle muscle of the sucker (peripheral reflexes)
- ii. bipolar and multipolar interneurons of unclear function.

Lying opposite the suckers, the arm ganglia contain a higher density of nerve cells, giving the neuronal part of the axial nerve cord its ganglionic appearance. This higher density of nerve cells probably results from the fact that a great part of the axial nerve cord activity is associated with the activity of the suckers (Graziadei, 1971).

The rim of each sucker carries a huge number of primary sensory cells devoted to both chemical and mechanical senses. A single sucker of 3mm diameter carries several tens of thousands of sensory cells (Graziadei 1971, Wells 1978). The whole skin of the octopus is estimated to carry

up to 2.4×10^8 sensory cells. To date 4 types of receptor cells have been identified (Fig: 5). They are all found in the columnar epithelium of the sucker. Two of these organs (type 1 & 3) are presumed to be mechanoreceptors. Type 1 is a round cell buried beneath the surface of the epithelium while Type 3 is a deeply buried multipolar cell with a narrow neck protruding towards the surface. The two other sensory cell types (type 2 & 4) are presumed to be chemical receptors. Both are fusiform with ciliated tips reaching between the epithelia cells to the surface. Type 2 receptors are about 10 times more frequent than all other receptor types.

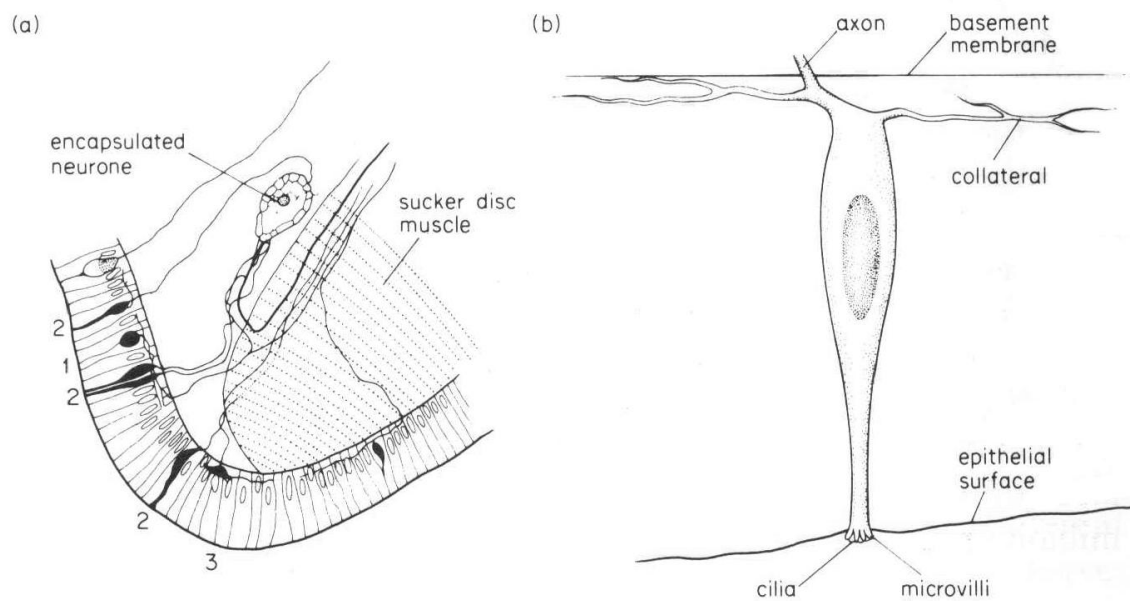


Fig 5: (a) The position of sense organs and an encapsulated neuron in the rim of a sucker. (b) shows a detail of receptor type 2, which is presumed to have a chemosensory function (after Graziadei, 1964; Wells 1978)

Deep receptor-like neurons, described as stellate neurons, are found at many sites within the arms and suckers (Graziadei, 1965b). These are branched neurons that may be proprioceptors monitoring muscle deformation (Wells, 1978). Indeed, recent studies have physiologically demonstrated that mechanosensory information is transmitted from the intrinsic muscles in the axial nerve cord (Gutfreund et al., 2006). The attachment force of a sucker depends on the difference between ambient pressure and the pressure of the water enclosed by the sucker. Measurements show that cavitations on most marine surfaces will occur at pressures between 0 and 2100 kPa (Smith, 1996). This result implies that at sea level (ambient pressure 5

100 kPa), cavitation normally limits a sucker to a maximum pressure differential of 100–200 kPa. However, the ambient pressure increases by 100 kPa for each 10 m of depth; thus, the maximum pressure differential that can be created before reaching the cavitation threshold increases correspondingly (see Smith *et al.*, 1993; Smith, 1996).

For four different species of cephalopod tested on surfaces where cavitations would not be limiting, the pressure differential ranged from 100–270 kPa (Smith, 1991, 1996). Presumably their musculature and mechanics are not capable of creating greater pressure differentials. Smith (1996) showed that decapod suckers could produce pressure differentials that are several times greater than those created by octopus suckers. Smith (1996) measured pressure differentials in decapod suckers that were as high as 830 kPa. This strength advantage is only relevant in deeper water; at depths less than 100 m, cavitations limits tenacity. At sea level, the decapod suckers are limited to the same pressures as octopuses. The cost of this increased strength is likely a loss in dexterity relative to octopus suckers (Kier & Smith, 2002). In an earlier study by Dilly *et al.* (1964) it was shown that a 2.5 kg octopus could exert a maximum pulling force of 18 kg. The maximum force the animal could use to push objects is about 50 % of this. Parker (1921) measured the breaking forces on either 1 or sets of 3 suckers in *Octopus bimaculatus*. He showed that a single sucker of 2 mm diameter had a breaking force of 0.02 kg whereas the breaking force for 3 suckers was 0.07 kg. Larger suckers (6 mm) had breaking forces of 0.14 kg or 0.43 kg.

3.4 Organization of the octopus brain and higher motor centers

3.4.1 Overview on the organization of the octopus brain

The groundwork on the anatomy of the octopus brain was carried out by Young (1971) and his collaborators. Their studies provide a very detailed description of the structure of the nervous system (Young, 1971). Such histological and anatomical analyses reveal the structural basis of the connections between peripheral and central nervous system and between the various brain lobes.

The **octopus brain** is composed of two central neural masses, the supraoesophageal and the suboesophageal masses, which are separated by the oesophagus. These lie inside a

cartilaginous brain capsule. The optic lobe lies adjacent to each eye outside the cartilage (Fig. 6). The following functionally different areas can be distinguished:

- 1) Lower motor centers
- 2) Intermediate motor centers
- 3) Higher motor centers
- 4) Regulators
- 5) Receptor analyzers
- 6) Auxiliary memory centers

The lower, intermediate and higher motor centers were identified on the basis of movements evoked by stimulation of *Sepia* brain (Abbott et al., 1995).

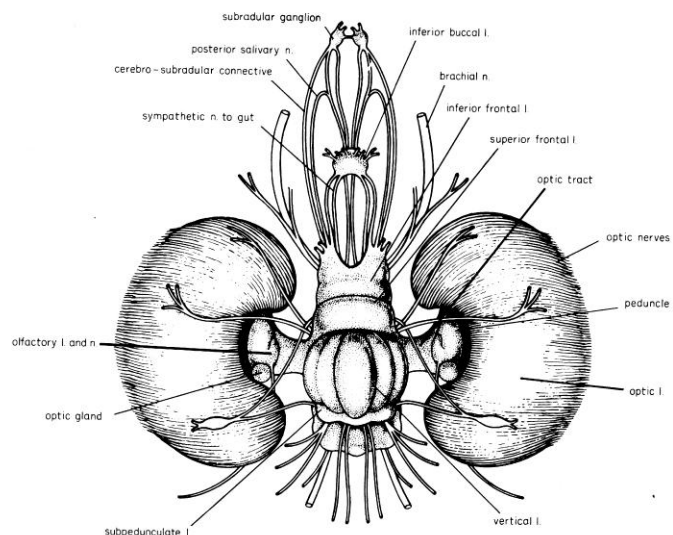


Fig. 6: The brain of octopus vulgaris seen from above. l=lobe, n=nerve (after Young, 1971)

Lower motor centers lie in the suboesophageal lobes and send their commands directly to the motor effectors. Such centers include the fin lobe or the large motor neurons directed to the ganglia of the arms. They are controlled by the higher motor centers and also receive input from arm receptors, thus ensuring a local but limited control of the arm.

Intermediate motor centers indirectly innervate the muscle effectors, the descending path passing through at least one synapse in a peripheral ganglion. These centers lie in the suboesophageal lobes and are comprised of large cells with axons to the arm ganglia. They also

receive input from higher motor centers and arm receptors. The pedal ganglia and palliovisceral lobe belong to this level of motor control.

The higher motor centers can evoke combined and complex, coordinated movements of several groups of muscles. They are located in the supraoesophageal brain mass and are composed of medium sized cells. They include the anterior basal and posterior basal lobes, the latter being divided into the intermediate basal, dorsal basal, median basal and lateral basal lobes. The activating input to these centers comes directly from receptor analyzers and receptor organs and forms the peduncle lobe, which is called the "regulator". Higher motor centers are assumed to work hierarchically through the intermediate and lower motor centers to produce a wide variety of movements and behaviors from different body parts, like fin, arm or mantle (Messenger, 1983).

The peduncle lobes or regulators are situated on the optic tract. They constitute a prime area for the integration of visual input from the optic lobes, vestibular inputs from the statocysts and proprioceptive information from arm, mantle and skin receptors.

The receptor analyzers are the optic lobes, which also play a role as a "memory store", and the system of inferior frontal-posterior buccal lobes. The inferior frontal-posterior buccal lobes receive information from receptor organs, and the peduncle lobes also send a centrifugal projection to the optic lobes. It is thought that this projection functions to select the information from the receptors to be sent to the higher motor centers. The optic lobes have also been defined as "controllers" in the sense that their inner part, the medullary region, can play a role as a higher motor centre in establishing visually directed motor programs.

The **auxiliary memory centers** are comprised of the superior frontal and vertical lobes and some parts of the inferior frontal system. They represent areas of memory recording and control the animal's tendency to attack (Abbott et al., 1995; Young, 1971; Messenger, 1983).

Sensory levels equivalent to the motor levels have not been identified, but it is assumed that there is also some form of hierarchical sensory system (Abbott et al., 1995).

3.4.2 Overview on the organization of the higher motor centers

The anterior basal lobe has been described as the main structure involved in the control of arm movement during the seizing and manipulation of food, the control of head and eye movements during walking and swimming and the control of posture (Young, 1971).

The dorsal basal lobe appears involved in the elaboration of action patterns of defense and avoidance and in coordination with somatic and visceral functions (Young, 1971).

The median basal lobe is thought to function primarily in initiation and control of movement of the funnel and mantle and partly in the control of the chromatophores. It can also play a role in arm movement during some actions, like swimming.

The lateral basal lobes are thought to be involved in control of the chromatophores and skin musculature. Parts of the anterior and median basal lobes are similarly organized to the peduncle lobe. Because of this, together with the peduncle lobe, they have been called the octopus "cerebellum".

The peduncle lobe is also involved in the formulation of movement commands. This lobe was shown to be mainly involved in regulating visually directed motor programs. It does not directly elaborate a motor program, but affects the way the program is executed. The peduncle lobe has a special function in the integration and co-ordination of motor programs through its proximity and extensive interconnection with the optic lobes (Messenger, 1983). In addition to its role in motor integration, the peduncle lobe may also play a role as a **sensory proprioceptive centre**. It receives afferent fibers directly from the arms, head, mantle, skin and even from the suckers. Note that these afferents project directly to the peduncle lobe and do not pass through the lower motor centers (Messenger, 1983). The input from the statocyst is crucial for the orientation of the of the octopuses' eyes. Due to the wiring of the optical nerves we find a chiasms between the upper and the lower part of the octopus eye. Young (1971) and Wells (1978) believed this to be necessary to compensate for the inversion of the image in the eye. Apart from the influence of the statocyst on the orientation of the eye little is known about the integration of multiple sensory inputs.

The fact that the peduncle lobe is the site of multimodal convergence supports its function as an integrative "metacontroller" in the sense that it regulates motor programs set up elsewhere, especially in the optic lobes (Messenger & Tansey, 1979). Young (1976) and Messenger (1983) pointed out that there are common features in the function of the vertebrate cerebellum, from lamprey to man, and that cephalopod peduncle lobe appears to play a similar role (compare, for example, the oculo-motor control loops of cephalopods and mammals and particularly the function of the peduncle lobe and cerebellum in this loop).

This lobe, with its apparent similarity to the cerebellum, provides a further indication of deep similarities between this invertebrate neural system and the more complex vertebrate system that drives articulated limbs.

3.4.2 Overview on the sensory centers

In a bottom-up view of the octopus nervous system (NS) we can identify three main point of integration at different levels. In the arm axonal tract the relatively small number of axons suggested that there is a great deal of processing of motor commands and sensory information at the level of the arm NS itself (Graziadei, 1971; Altman, 1971; Sumbre et al., 2001).

An intermediate integrative point between information coming from central and peripheral nervous system and the superior and inferior centers of the CNS is the suboesophageal mass which receives information from the supraesophageal mass and from the peripheral nervous system of the arm and the rest of the body (Young, 1971).

At a higher level the peduncle lobes constitute a prime area for the integration of visual input from the optic lobes, vestibular inputs from the statocysts and proprioceptive information from arm, mantle and skin receptors. It is a site of multimodal convergence and also plays a role as a sensory-proprioceptive centre.

The distribution of the sensory information coming from the arm at the level of the CNS is widespread (Budelmann and Young, 1985). The inferior frontal system receives fibers from each arm. The superior frontal-subvertical system also receives direct inputs from the arm (Budelmann and Young, 1985). A strong lateralisation of the sensory areas with only a low percentage of fibers also projecting contralaterally has also been shown.

4. Learning and development of behavior

4.1. Learning

4.1.1. Non associative learning

Sensitization, or the increased likelihood of an animal responding to a stimulus, has been demonstrated clearly in *Octopus vulgaris*. Reward or punishment that takes place *before* the presentation of a test shape will respectively decrease or increase the likelihood of an attack on the

stimuli. This has been shown for tactile discriminations (Wells & Wells, 1958; Wells 1967) as well as olfactory stimuli (Chase & Wells, 1986). The importance of sensitization usually dwindles during discrimination training, as longer-term changes become entrenched.

Habituation, one of the simplest forms of learning, is the relatively persistent waning of a response as a result of repeated stimulation without reinforcement. In an experiment with a blind octopus (Wells & Wells, 1956) a plastic cylinder placed on the arm of the animal is passed under the web to the mouth, examined, and rejected. If the same object is repeatedly presented, after a few trials the octopus stops passing it to the mouth. After more trials the octopus spends only a few seconds examining it. Habituation of a visual response has been demonstrated in bay squids (Long et al., 1989), showing the decline of escape jets and ring patterns on the mantle with repeated presentation of a fish predator model. The squids also showed dishabituation to the predator model after a threat stimulus. Recently, habituation has been shown in visual tasks in intact animals (Kuba et al 2006a; 2006b)

4. 1. 2. Associative learning

Associative learning involves long-term changes in behavior as a consequence of an association between particular sets of events, which can be manipulated by an experimenter in the laboratory. There have been a few studies on classical and instrumental (operant) conditioning (Papini & Bitterman, 1991). Classical conditioning was also employed in a color vision study with *Eledone* and *Octopus* (Mikhailoff, 1920; Kühn, 1950) and in a study of "infra-sound".

Most attempts to train octopuses to pull a lever for a food reward failed (Dews, 1959; Crancher et al., 1972). The only successful lever pulling experiment was done by Nixon (1969) who trained the octopuses first to attack a certain shape and then fixed the shape on the lever. In a similar task Crancher et al. (1972) trained an octopus to insert an arm into a feeding tube. All this led Wells (1978) to the conclusion that an octopus cannot learn about the position of its arm.

There are numerous examples for conditioning from the various visual and tactile discrimination experiments (see above). Octopuses learn to attack one of a pair of stimuli when reinforced either with a food reward or with a small electric shock. Learning seems to be very quick with easy discriminations, although octopuses never reach the levels of performance

achieved by mammals in discrimination tasks. In visual discrimination training, the simultaneous presentation of the stimuli achieves higher scores than the successive presentation, which is also true for vertebrates (Sutherland & Muntz, 1959). Visual discrimination experiments have been carried out with various species of *Octopus* (for reviews see: Boal 1991; Hanlon & Messenger, 1998; Mather 1995; Wells 1978). In decapods, *Sepia* (Messenger, 1977b), *Loliguncula* (Allen et al., 1985) and also *Todarodes* (Flores, 1983) have been tested on visual discriminations.

Octopuses also learn successive tactile discriminations, accepting and passing an object to the mouth, or rejecting and pushing away a negative stimulus (see Wells, 1978). Tactile discrimination experiments were also important to study the mechanisms of memory in these animals (Robertson, 1994; Robertson et al., 1994). There is some evidence that in *Octopus* the visual and tactile memories may interact (Messenger, 1983; Allen et al., 1986). Octopuses were trained first with a tactile and then a visual discrimination (plastic balls in black, white, or clear; rough or smooth). It was found that a negatively associated visual memory was sometimes able to interfere with a previously learned positively associated tactile memory (Mackintosh, 1983). It was also shown that if the arms on one side of the body were trained to reject or accept an object it took about 1-3 hours to achieve the same performance on the other side of the body. This is the case for both visual and tactile learning (for a review see: Wells 1978).

Avoidance learning has long been recognized in cephalopods (Uexküll, 1905; Polimanti, 1910) and demonstrated in experiments where hermit crabs carrying anemones or other food species right next to an anemone are offered to various octopuses and the avoidance behavior after being stung is described (Boycott, 1954; Ross, 1971; Hand, 1975; Brooks, 1988). Maldonado (1968, 1969) trained an octopus to leave a dark box for the light to avoid an electric shock. Cephalopods have been tested in several maze experiments, but with little success (Boycott, 1954; Schiller, 1949; Wells, 1978). The octopuses seemed to be unable to remember the series of movements made to achieve a food reward. It is quite possible that they only learned to persist in moving about until they found the food. The failure of *Octopus vulgaris* in experiments that require the utilization of proprioceptive information led Wells (1978) to the conclusion that proprioceptive information is unavailable to higher order learning centers in the brain. To this day there is only one successful maze experiment (Walker et al., 1970), where an octopus was trained to turn left or right in a T-maze to regain entry to the water, but it is not really certain which kind of information

was used by the octopus. The outcome of the maze experiments raises the question how octopuses learn about the spatial relationships in their natural environment as they forage.

A similar interpretation could apply to the bottle-opening behaviour, where the animal is presented with a crab in a glass jar with a plug in it (Pieron, 1911; Boycott, 1954; Cousteau & Diole, 1973; Fiorito et al., 1990). The octopus swam to the jar and enveloped it. After some time the plug came out and eventually one or more arms intruded into the jar and located the crab. The time to get the crab is remarkably long (over 50 seconds) and the time spent outside the jar does not significantly decrease in 20 trials (Fiorito et al., 1990). The time needed to open the jar did decline, but even after 10 days it was over a minute. Even stimulus pre-exposure didn't improve their performance (Fiorito, 1998).

4. 1. 3. Information processes in visual learning

An interesting output of the series of discrimination experiments was that octopuses show some stages of visual information processing similar to mammals. Octopuses show **stimulus generalization**, which can be demonstrated in a "transfer test". For example, octopuses that were trained to attack a small solid square attacked a small outline square much more frequently than a large outline square, even without a reward (Sutherland, 1960, 1969). Other cases of stimulus generalization were shown for size invariance and brightness invariance (Messenger, 1981) and for degrees of roughness (Wells & Young, 1970).

Octopuses also show **receptor generalization**. After being trained to make a visual discrimination using one part of the retina only, the performance was significantly better than chance when the task was presented to a different part of the retina (Munz, 1962). The same applies to **interocular transfer** (Muntz, 1961) and tactile discriminations (Wells, 1959). An octopus trained to reject an object presented to one arm rejected the same object when tested on other arms, even after a time lapse of 20 minutes or.

Cue-additivity has also been demonstrated in octopuses. With two or more relevant cues, the animals learned significantly faster than with only one cue (Sutherland & Mackintosh, 1971; Mackintosh, 1974). This has been tested with combinations of size and shape (Sutherland et al., 1965) and with brightness and orientation (Messenger & Sanders, 1972). Interestingly, the

octopuses do not attend equally to both features of the stimulus. Some learn the discrimination in terms of one cue, some in terms of the other. **Reversal learning** has also been studied in *Octopus* (Mackintosh, 1965), octopuses made fewer errors with progressive reversals, but the performance seems to depend on earlier training regimes, similar to rats. The results of these various experiments suggests that octopuses might be able to form **learning sets**. Studies in this field have just recently begun.

4. 2. Development

Very little is known about the development of behavior in cephalopods. They are difficult to keep and just as difficult to study in the sea. Cephalopods must change their behavior considerably during their life cycle because of their great change in size and life style. Many species have to change their diet as well as foraging areas or dens as they grow larger. The ontogenetic development of behaviors and motor primitives in *Octopus vulgaris* is virtually unstudied. This is mainly due to its development from a planktonic paralarvae (Fig. 7). Recent progress in aquaculture will help to close this gap.

Shoaling seems to start at a certain mantle length in some squid (Hanlon et al., 1987). There is evidence that the activity rhythm is "tuned" or sharpened over the first weeks of life in *Octopus*, but also in animals that are newly settled (Mather, 1984; Wells & Wells, 1970). Very little is known about the development of sexual behavior. Tinbergen (1939) has shown with models that the extended fourth arm and the Zebra bands of the mantle in cuttlefish function as "releasing stimuli" in a way that in the absence of these stimuli every conspecific is treated as a female (see also Hanlon & Messenger, 1988; Messenger, 1970). There is developmental improvement of the jet escape response during the early stages of growth in squid (Gilly et al., 1991).

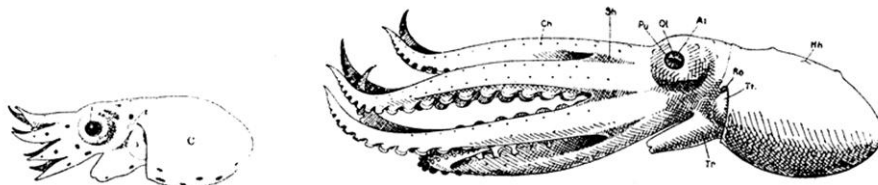


Fig. 7: On the left: a freshly hatched planktonic paralarva. On the right: a freshly settled *Octopus* following a 4 – 8 weeks planktonic stage

Although Decapods already produce most of the adult repertoire of body patterns, there are some differences (Hanlon & Messenger, 1988; 1998). The greatest change in body patterning behavior between hatchlings and adults is the shift in emphasis from crypsis to communication, since reproduction involves signaling. Also, the response to a threatening object changes in an adult octopus or cuttlefish (Packard & Sanders, 1971). There is no evidence so far to suggest that cephalopods learn the use of their different body patterns or learn how to modify them (Warren et al., 1979). On the other hand, it seems quite possible that learning could influence some of the displays used in communication by highly social squids, though experiments are lacking.

Wells (1958, 1962b) found that naive hatchling *Sepia* respond only to moving objects and only to a shrimp shape when it is moved along its long axis. This preference for an elongate figure is like that shown by octopuses for rectangles moved along their long axis and suggests some pre-programming for the detection of prey shaped like fish or prawn. Cuttlefish become less selective once they have attacked prawn five or ten times, which suggests that now a learning program opens which allows them to sample different prey items, maybe by reinforcement learning. Messenger (1973) and later Dickel et al. (1997) and Agin et al (2006a) showed that during the first weeks of life *Sepia officinalis* is not able to learn not to attack prawns that are inaccessible for predation as they are inside a transparent tube. Following this initial stage of life the ability for plasticity and memory slowly develops (Agin et al. 2006b). Their study showed that the animals' performance in the prawn in a tube test of the inhibition of predatory behavior changes radically during the first few weeks of life. Their data shows that a significant increase in acquisition performance takes place between 15 and 21 days of age. The retention curves in 8- and 15-dayold cuttlefish show a monotonic memory process, presumably reflecting the presence of only short-term memory. In 21-day-old cuttlefish, there are two distinct processes, which could be a labile short-term memory, and a subsequent intermediate memory. These memory systems seem to become more effective over the course of post-embryonic development. Moreover, the retention curves obtained in the oldest cuttlefish (30- and 90-day-old) bear a close resemblance to those observed in adults. Dickel et al. (2000, 2001) have shown in cuttlefish that during development an enriched physical and social environment not only has a positive effect on growth rate but also on the acquisition and retention of learning tasks. Further research of this group also showed that there is a crucial time of food imprinting in

hatchling *Sepia* (Darmaillacq et al. 2004; 2006a; 2006b).

5. Biologically driven motor control principles in stereotypical movements

(stereotypical movements kinematics and dynamics analysis, modelling)

Achieving efficient control of flexible structures is extremely complex, both in biological and robotic systems, because such systems have a virtually infinite number of DOFs. This requires special strategies for solving the inverse kinematic and dynamic problems which are believed to be the necessary steps for achieving a proper control of open loop systems. An efficient experimental approach to study this by combining behavioral, kinematical, physiological and modeling techniques was developed. Using this approach have shown that although reaching and fetching movements are very different in nature (bend propagation vs. articulate-like rotation), the control strategies evolved are similarly based on restricting the controlled variables to just 3 DOFs (Gutfreund et al., 1996; 1998; Sumbre et al., 2001; 2005; 2006).

To understand the neural control strategies involved, EMG recording was used to correlate the patterns of muscle activation with the kinematic features of arm movements (Gutfreund et al., 1998, Sumbre et al. 2005, 2006). For the reaching movements, it was found that the variables best correlated with the EMG are the two global variables, peak acceleration and peak velocity, and these two variables can be predicted from the level of muscle activity, regardless of when this activity is measured during the movement. These results suggest that the level of activity is the parameter dictating the extension velocity and that a feed-forward control mechanism may be responsible for generating the movement. In contrast, EMG recording during fetching revealed that the level of activity is not correlated with kinematic parameters, but rather with the length of the segments (Sumbre et al 2005, 2006). This suggests that in fetching, stabilizing the articulated structure against the large normal drag forces (Yekutieli et al 2005a) is the main control variable. This energetic load is probably the price for achieving end-point accuracy.

Combining behavioral and physiological approaches enabled characterizing the unique control strategies that evolved in octopus arms for simplifying the control complexity. It was found that the complexity of visually guided arm extensions in the octopus is reduced by the existence of hard-wired programs for the execution of basic motion patterns. These programs are embedded within the arm neuromuscular system itself (Sumbre et al., 2001). Using electrical stimulation of arms disconnected from the brain evoked arm extension movements that showed identical kinematics to natural arm extensions. Thus it was suggested that brain commands are issued only for scaling, adjusting and combining movements to achieve the desired end result. Using two EMG electrodes to study the dynamic control of fetching reveals an extremely interesting mechanism for a simple calculation of the segment lengths according to where along the arm the object is grasped (Sumbre et al. 2006). The two electrodes recording detected two waves of muscle activation, one starting at the base of the arm and the second close to the grasping site. These waves travel toward each other and their collision point sets the location of the medial joint. This mechanism allows calculating the structure of the articulated arm at the peripheral level with no need for complex representation of the 8 arms in the relatively small brain (Sumbre et al, 2006).

Based on those physiological and kinematic studies a 2D dynamic model of the octopus arm aimed at exploring possible strategies of movement control in this muscular hydrostat was developed (Yekutieli et al., 2005a). The arm was modeled as a multi-segment structure, each segment containing longitudinal and transverse muscles maintaining a constant volume, a prominent feature of muscular hydrostats. The input to the model was the degree of activation of each of its muscles. The model represented the external forces of gravity, buoyancy, and water drag forces. It also included in its description the internal forces generated by the arm muscles and the forces responsible for maintaining a constant volume. This dynamic model was then used to investigate the octopus reaching movements and to explore the mechanisms of bend propagation that characterize this movement. Based on computer simulations it was found that:

- 1) A simple command producing a wave of muscle activation moving at a constant velocity is sufficient to replicate the natural reaching movements with similar kinematic features;
- 2) The biomechanical mechanism that produces the reaching movement is a stiffening wave of muscle contraction that pushes a bend forward along the arm;
- 3) The perpendicular drag coefficient for

an octopus arm is nearly 50 times larger than the tangential drag coefficient. During a reaching movement, only a small portion of the arm is oriented perpendicular to the direction of movement, thus minimizing the drag force.

This model was further used to investigate the neural strategies used for controlling the reaching movements of the octopus arm (Yekutieli et al., 2005b). Sending a simple propagating neural activation signal to contract all muscles along the arm produced an arm extension with kinematic properties similar to those of natural movements. Control of only 2 parameters: the amplitude of the activation signal and the activation traveling time (the time the activation wave takes to travel along the arm) were sufficient to fully specify the extension movement. It was also found that the same kinematics could be achieved by applying activation signals with different activation amplitudes all exceeding some minimal level. This suggests that the octopus arm could use minimal amplitudes of activation to generate the minimal muscle forces required for the production of the desired kinematics. Larger-amplitude signals would generate larger forces that increase the arm's stability against perturbations without changing the kinematic characteristics. These modeling studies suggested that the octopus arm biomechanics might allow independent control of kinematics and resistance to perturbation during arm extension movements.

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