Learning and memory in *Octopus vulgaris*: a case of biological plasticity

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Here we concisely summarize major aspects of the learning capabilities of the cephalopod mollusc *Octopus vulgaris*, a solitary living marine invertebrate. We aim to provide a backdrop against which neurobiology of these animals can be further interpreted and thus soliciting further interest for one of the most advanced members of invertebrate animals.

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The octopus: a 'model' of the brain

About fifty years ago an English zoologist and neuroanatomist, John Zachary Young, published 'A model of the brain' [1]. The book is an account of decades of studies on predatory responses and learning abilities of several species of cephalopods, mainly the common octopus, *Octopus vulgaris*. In its aim, J.Z. Young tried to answer to the question 'how do brains work'. He adopted the approach utilized by 'communication engineers' and cybernetics: the 'brain' is acting as the computer of a homeostat [1].

In the model, a mnemon (i.e. a visual/tactile feature with associated memory value resulting from experience; [2,3••]) is activated by a given input (visual and/or chemo-tactile) to a specific set of classifying cells and switched on/off on the basis of other inputs that depend on the taste-pain circuits. The output of these units is summed up to produce an overall attack strength (i.e. predatory response), in contrast to the opposite inputs that build a retreat. These 'strengths' are combined to determine the final attack/retreat responses.

The 'model' is the result of hundreds of experiments where the predatory response of *O. vulgaris* has been dissected to deciphering its neural control ([4]; for review see also: [5,6••,7,8,9,10•,11,12,13]). It is noteworthy to mention that in several occasions the 'model' found its cybernetic application [14–16]. The mnemon model developed by Clymer [14] is based on a visual feature with associated memory value resulting from experience that is activated by a given visual input to a specific set of classifying cells and switched on/off on the basis of other inputs that depend on the taste-pain circuits. The output of these units, corresponding to the attack command, is further summed-up to produce an overall attack strength, in contrast to the opposite units (retreat command) that in a similar way build an overall retreat strength. These values, or strengths, are then combined and determine the final attack/retreat response [14]. Interestingly, the results produced by Clymer's model are comparable to those obtained from proper experiments with live animals, including the responses resulting from short- and long-term changes in behavior and interference on learning performance when spacing between trials is reduced in time [14]. In a similar way, Myers developed a modified 'cybernetic circuit' based on octopus' mnemon taking into account findings on neural networks and learning in simulated environments [15].

The octopus: a cephalopod

The common octopus is one of the most famous representatives of the class Cephalopoda (i.e. nautilus, cuttlefish, squid and octopus), a numerically small but ecologically and scientifically significant taxon of invertebrates belonging to the phylum Mollusca. The richness of behavioral capabilities of these animals fascinates human beings since the antiquity [6••,17]. Together with other cephalopod species, octopuses also represent a very important resource for human consumption [18].

The class Cephalopoda includes about 700 exclusively marine-living species considered to have rivaled fishes during evolution [19••,20•]. Cephalopods demonstrate a refined and extraordinary ability to adapt their morphology and behavioral repertoire to their living environment [6••,20,21]. Examples among many are: (i) special locomotion including fast jet propulsion, bipedal and tiptoeing [22–24]; (ii) active changes of body patterning achieving crypsis, polyphenism, mimicry and communication including hidden channels [5,25–27]; (iii) special

physiological, neural and behavioral characteristics acquired during evolution (e.g. [19••]). The extraordinary adaptive/plasticity of their physiology and behavior may have contributed greatly to their success [6••,28,29].

Cephalopods are also well known amongst neuroscientists for their contribution to fundamental understanding of the nervous system functioning [3••,30,31,32,33•]. These animals are also emerging models for biology, genomics, neuroscience, cognition and robotics [34•,35,36,37,38].

The octopus: a regulated 'laboratory animal'
Octopuses and their allies have been included from 1st January 2013 in the Directive 2010/63/EU that regulates the use of animals for scientific purposes [39,40]. As a consequence, the invertebrate research in the EU experienced a paradigm shift. In fact, the Directive covers the use of 'live cephalopods' (i.e. hatched juveniles and adults) in the legislation regulating experimental procedures likely to cause pain, suffering, distress or lasting harm [41•,42•]. Under the Directive 2010/63/EU in all Member States cephalopods have the same legal status as vertebrates in relation to their experimental use in research, testing and education [41•,42•,43,44,45]. The outstanding position of cephalopods among the list of regulated animals derives from the assumptions (see Table 1) advocated by the EFSA Panel at the time of the revision of the former EU regulatory document [46,47].

Directive 2010/63/EU is a milestone for invertebrate research because it is the first time particular types of research involving an entire class of invertebrates are regulated in the same way as scientific projects involving vertebrates. As reviewed by Fiorito [42•], although regulation presents obvious challenges, there are several areas where neurophysiological and behavioral neuroscience research is required to address key questions. Current efforts at the international scale are provided with the aim to strengthen the scientific community, and to facilitate the dissemination and adoption of a consensus on the best practices. Such important approach may result in an international coordination of research projects and in facilitating the growth of a 'network for improvement of cephalopod welfare and husbandry in research, aquaculture and fisheries' (Cephs*In*Action; COST Action FA1301: http://www.cost.eu/COST_Actions/fa/Actions/FA1301).

The octopus: a learning animal
Learning and memory appear to occur in all cephalopod species and has been studied with various details in some key species such as the cuttlefish and the octopus (review for example in [6••,48•]). This is considered to be one of the most advanced examples of behavioral plasticity among invertebrates [6••,21,49•]. Sophisticated behavioral repertoire (e.g. individual and social learning, behavioral syndromes, problem solving, communication through hidden channels) and its plasticity parallel those of higher vertebrates; these are related with a highly sophisticated nervous system that–despite the molluscan design–achieves vertebrate-like functional complexity. The neural system organization seems to be also correlated with species-specific lifestyle [20•,50,51]. Finally, the flexibility of the behavioral repertoire of cephalopods is supported by evident cellular and synaptic plasticity at the level of the central and peripheral nervous system, and of the neuromuscular junctions (review in [32•]).

As mentioned above, learning has been studied for decades in the octopus ([6••]; for review see for example [52•]).

Table 1

Summary of judgments on the capacity of experience of pain and distress in cephalopods as assessed by the Scientific Panel on Animal Health and Welfare on the Revision of the Directive 2010/63/EU. These assumptions are based on several scientific contributions [44] and extended by recent works (for review see [42•]). References included in the Notes are provided as further support to the statements provided. Most of the data provided herein comes from octopus and in some cases from cuttlefish.

Criterion	Judgement	Notes
Higher brain centres (c.f. cerebral cortex)	YES	e.g. Vertical lobe in octopus is considered comparable to limbic lobe [52•,70]. Studies are in progress investigating self-awareness and consciousness ([21,35••]; e.g.: [71,72])
Presence of nociceptors	Likely (but not proven)	Neurophysiological afferent recording studies in progress
Nociceptors project to higher neural centres	Likely (but not proven)	Require development of *in vivo* brain recording techniques (but see: [73,74,75])
Behavioral responses	YES	Avoidance of electric shock and other noxious (not necessarily painful stimuli ([57];e.g. [66]).
Receptors for opioids found in the nervous system	Likely (very limited data)	Enk-like peptides, peripheral δ receptors; Opioid growth factor receptor-like protein 1*; Kappa-type opioid receptor*. No direct 'pain' studies are currently available.
Action of analgesics	Not studied	Requires investigation and objective criteria, plus studies on drug delivery

* Gene sequences identified in G. Fiorito laboratory at the Stazione Zoologica Anton Dohrn (Italy) by assembling and annotating *O. vulgaris* transcripts coming from several sources (RNA-seq experiments and [76•,77,78]).

Various forms of learning have been demonstrated in cephalopods, from simple sensitization, to associative learning and problem solving, to more complex forms such as spatial and social learning and tool use (review in [6••]). In essence, a large number of the entities proposed by Moore [53••] in his cladogram of learning processes have been shown (e.g. habituation, classical and instrumental conditioning, associative and spatial learning, perceptual processes in visual learning [6••]) in some cephalopod species. Associative learning paradigms have been used in laboratory experiments of cephalopod learning. In *O. vulgaris*, consolidated long-term memory is controlled by the vertical lobe (a lobe in the brain [54,55•]), while short-term memory is stored in more distributed neural networks, a vertebrate-like pattern of separate memory storage sites [56•,57].

Learning can be also critical to the survival of juvenile forms [58–60]. Vicarious learning, i.e. the capability of learning from conspecifics, has been shown for *O. vulgaris* in the laboratory [61•] and appears to be somehow modulated by neural centers such as the vertical lobe [62]. Despite criticisms proposed to the original finding, the capability of learning from others is documented in octopuses and also recently in other cephalopod species [63•]. Octopuses and cuttlefishes can demonstrate conditional learning [64,65]. As reviewed by Huffard [49•], male octopuses in the wild exhibit mating tactics consistent with their size-based chances of winning agonistic contests, suggesting learning of rank followed by conditional use of mate guarding. In these field observations, mate guarding is not exhibited by small males and thus unlikely to win contests in the local population. By contrast, males appear to mate, guard and maximize mating opportunities only if they are large enough to win contests with other nearby individuals.

Table 2 summarizes several training paradigms successfully utilized with *O. vulgaris* to test learning and memory recall capabilities in this species (for review see also: [6••,21]).

Some of these have been also applied in learning studies aimed to decipher the biological machinery involved in the modulation of *O. vulgaris* behavioral plasticity [66]. In particular, the relationship between learning processes and gene expression in octopus has been evaluated by analyzing changes of some genes (e.g.: *stathmin: Ov-stm, tyrosine hydroxylase: Ov-TH, dopamine transporter: Ov-dat, octopressin: Ov-OP, cephalotocin: Ov-CT*) in response to fear conditioning (learned fear) and social interaction (innate fear). A differential pattern on down-regulation and up-regulation of gene expression in different regions of the octopus central nervous system resulted in these studies as a consequence of either innate or learned fear.

Table 2

Training paradigms utilized with *Octopus vulgaris* to assess learning and memory recall capabilities [6••,49•,51,52•,57,65,79,80].

	Training paradigm and/or Stimuli
Habituation	e.g. jar
Sensitization	Artificial and natural prey items
Classical conditioning	Plastic spheres having different brightness as stimuli; discriminanda differing in orientation, brightness, size or shape (i.e. rectangle, circle, square, diamond); barrel-shaped objects
Avoidance learning	Discriminanda having several shapes including plastic spheres
Spatial learning	Mazes
Problem solving	Mazes, jar and boxes with simple and multiple openings
Social learning	Discriminanda, problem solving
Perceptual processes	Discriminanda that differed in orientation, brightness, roughness, size or shape (rectangle, circle, square, diamond)

For example, in response to learned fear an increase of the expression of *stathmin* and *Ov-TH* was observed, while *Ov-stm* was the sole to increase significantly as the consequence of the 'innate fear'. In parallel, *Ov-stm* and *Ov-dat* decreased their expression in the subesophageal mass (i.e. center of motor control) in response to fear conditioning, while no effect appears to be caused by social interaction. Finally, learned and innate fear paradigms induced an increased expression of *Ov-stm* in the optic lobes (i.e. centers of visual-sensory processing). Instead, *Ov-dat* and *Ov-TH* exhibit an opposite pattern in response to fear conditioning and social interaction.

The increased expression of *Ov-stm* in octopuses subjected to innate and learned fear suggests that in octopus this gene plays a role similar to what is known in vertebrate brain. In mammals, it is known that amygdala enriched stathmin is required for the expression of innate fear and the formation of memory for learned fear [67–69]. Interestingly, *Ov-stm* undergoes in the octopus to a negative regulation in response to fear conditioning. This suggests that the synaptic architecture may be able to change, and that these changes could be related to variations in microtubule dynamics. This result opens the way to a fascinating working hypothesis that requires further studies to understand the relationship between microtubule dynamics, synapse formation, and plasticity of neurons in the octopus.

Conclusive remark

This review does not aim to provide a detailed description of the most recent results obtained by the application of several learning paradigms for studying the biological and

cellular mechanisms underlying learning in *O. vulgaris* and other cephalopod species.

As reviewed by Borrelli and Fiorito [6••] and despite the considerable number of studies published on the extent of learning and memory recall, and on the effects of its impairment induced by experimental interference, very little is known about the ability of cephalopods to encode and retrieve information. From the classic works of Young and co-workers, it is known that cuttlefishes and octopuses are capable of short-term and long-term memory. In many cases the memory trace was reported to last for a very long time: in octopus for weeks to months (review in [6••]). A systematic analysis of the memory phases, the time course of retention and memory consolidation, and possible reconsolidation in octopuses and cephalopods still appear insufficient when compared with the knowledge currently available for other taxa. Whether the memory recall observed in cephalopods corresponds to a more phylogenetically conserved consolidation mechanism or to a characteristic of the neural-network is an issue that has to be tested.

We hope to have provided enough elements to facilitate the continuous regrowth of interest for these fascinating highly 'flexible' animals.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
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