1.31 Behavioral Analysis of Learning and Memory in Cephalopods
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1.31.1 An Historical Overview

The ancient civilizations of the Mediterranean, whose lives were closely linked to the sea, were clearly aware of the existence and beauty of cephalopods. Exquisite motifs and sketches of marine creatures were recurrent in the well-known Middle and Late Minoan pottery (approximately 2000–1000 BC) and also in the probably less famous Chiriquian art, typical of northwest Panama (approximately 300 BC). Both made extensive use of lozenge-shaped figures from which eight waving arms emerged. Independent of their realistic or stylized rendering, common characters such as dots, spots, and circles of various shades and colors convinced archeologists, anthropologists, and art historians that the figures were artistic representations of the same peculiar marine creature: the octopus.

This brings our attention to the fact that, among living organisms, cephalopods have been familiar to man since antiquity. In his *Historia Animalium*, Aristotle (fourth century BC) described the difference in occurrence between the inking response of cuttlefishes (concealment and fear) and that of squids and octopuses (fear only), together with the changing of the patterns of their skin to match the background and disguise themselves from predators and prey. He also mentioned the octopus’s strong curiosity (or stupidity, to put it in his words), which made it an easy catch since a hand shaking under the water surface was sufficient to elicit its exploratory drive (*Aristotle, 1910*). Cephalopods’ behavior even intrigued Darwin, who gave a detailed account of his observations of octopuses and cuttlefishes in the waters of the islands of Cape Verde (*Darwin, 1870*).
Many other anecdotal and popular reports are known from the classic literature (review in Cousteau and Diole, 1973; see also Borrelli et al., 2006).

1.3.1.1 The Role of the Zoological Stations in Cephalopod Research

It is without doubt, however, that the scientific and systematic analysis of cephalopods’ anatomy, physiology, and behavior started near the beginning of the twentieth century, mainly from initiatives of the Zoological Station of Naples (Stazione Zoologica di Napoli) and its outstanding guests.

The Stazione, with its strategic position on the seafront, fleet of fishing boats, and professional personnel, gave foreign scientists the unique opportunity to combine leisure and work as temporary guests of the institute. Since its foundation, octopuses were among its favorite research models; moreover, they figured as a symbol of the Stazione, as is clear from the decorations on the wrought-iron gates of the main entrance to the institute (Figure 1) and from the numerous old postcards of the Naples Aquarium, which often depicted them. This stemmed mainly from the long-standing tradition of Neapolitan fishermen to catch live cephalopods, always considered a prime delicacy (for review, see Lo Bianco, 1909; Lane, 1960).

During this period, the outstanding studies of Giuseppe Jatta (1896) and Adolf Naef (1923, 1928) on the anatomy, systematics, and ontogeny of cephalopods, conducted and published at the Stazione, formed the groundwork for further studies. In addition, the first observations of cephalopods living in captive conditions (in tanks at the Naples Aquarium) gave an idea of the complexity of their behavior and learning capabilities (e.g., Piéron, 1911; for review see Boycott, 1954). Dr. Ariane Droescher referred to a description by Anton Dohrn, who tried to avoid octopuses feeding on lobsters kept in adjacent tanks. Solid cement walls, raised several centimeters above the water surface, separated the aquarium’s tanks. However, his attempts proved to be unsuccessful. In fact, as observed by Dohrn, the “same day, one of [the octopuses] climbed over the wall, attacked the unsuspecting crayfish and, after a short battle, tore him into two pieces! . . . The octopus [could] have seen that the crab [was] set by the keeper into the neighbouring tank, or he [could have] smelled the prey in the circulating water of the tanks. Anyhow, the event shows that the octopus [was] able to deduce from a sensual impression that there [was] a prey that he did not see, to conclude and, finally, to perform an air-jump in the right direction” (Salvini-Plawen, 1979, pp. 218–219).

Research on the biology and behavior of cephalopods was not confined to the Stazione but attracted the attention of and flourished in many other marine laboratories, both in Europe and the United States. As an example, a thorough description of the chromatic changes and behavioral repertoire of *Octopus vulgaris* was carried out by Cowdry (1911) at the Bermuda Biological Station for Research. Similar studies were conducted on *Loligo pealeii* by Williams (1909) at the Marine Biological Laboratory (Woods Hole, MA, USA) and by Stevenson (1934) at the Biological Station of St. Andrews (Scotland). Finally, the behavior and complexity of body patterning of *Sepia officinalis* was described by Bert (1867) at Arcachon (France), by Tinbergen (1939) at the Aquarium of the Zoological Station of Der Helder (The Netherlands), and finally by Holmes (1940) and Sanders and Young (1940), who carried out their studies at the Marine Biological Laboratory in Plymouth (UK).

1.3.1.2 A Research Effort Lasting Over a Century

In order to evaluate the research on cephalopods from the pioneering studies to the present day, we counted the number of publications cited in the Zoological
Record on a yearly basis for over a century, from 1900 to 2006 (Figure 2). Only publications concerning the anatomy and physiology of the central nervous system, the behavioral responses to learning paradigms, or living habits of cephalopods were considered pertinent and were accounted for. The number and distribution of papers over years clearly shows the prominent role achieved by the octopus in the study of learning and memory in cephalopods in the 15 years spanning from 1955 to 1970 (Figure 2).

1.3.1.2.1 The contribution of J. Z. Young
After the Second World War, the insight into the octopus rose mainly by the initiative of the British anatomist and zoologist John Zachary Young. On a yearly basis, JZ (as he was commonly called), together with a plethora of students and co-workers, was hosted by the Stazione Zoologica, where he systematically studied the anatomy and physiology of cephalopods’ nervous system, increasing the knowledge on the behavior and learning capabilities of these animals. Many people were involved in these and related physiological studies: Brian B. Boycott, Francesco Ghiretti, Pasquale Graziadei, Nicolas J. Mackintosh, Hector Maldonado, John B. Messenger, William R.A. Muntz, Andrew Packard, Geoffrey D. Sanders, Norman S. Sutherland, and Martin J. Wells are but a few names.

The impressive bulk of knowledge gained over these years at the Stazione and in other laboratories around the world has confirmed the old view of cephalopod preparations as marine guinea pigs (A. Droescher, personal communication) or as primates of the sea (Kerstitch, 1988). In fact, Young became more and more conscious of the fact that the “brain of the octopus has already abundantly proved its value for the study of behaviour. It is perhaps the type most divergent from that of mammals that is really suitable for study of the learning process” (Young, 1971: p. vii).

Apart from a few other contributions (for review, see Boycott, 1954), when J. Z. Young and Brian

![Figure 2](image-url)  
Figure 2  Number of publications per quinquennium on behavior, learning, and memory in cephalopods (cuttlefish, squid, and octopus) indexed in the Zoological Record from 1900 to 2006.
Boycott started their adventure with *Octopus vulgaris* at the Stazione Zoologica, two major works were available to them: a comprehensive overview of the brains of different cephalopod species (*Sepia officinalis*, *Sepiola robusta*, *Loligo vulgaris*, *Illex coindetti*, *Argonauta argo*, *O. vulgaris*, *Eledone moschata*, *Octopus tuberculata*; Thore, 1939) and an experimental study on the effects of the removal of certain lobes of the brain of *S. officinalis* on the behavior and learning of the cuttlefish (Sanders and Young, 1940).

Their aim was to study the learning capabilities of these animals by combining behavioral observations and lesions of the neural centers in order to disclose the functional organization of the cephalopod brain involved in the control of different behaviors; essentially, the predatory response.

*O. vulgaris* appeared to them as the ideal candidate for a series of reasons. First, because it was easy to maintain in aquaria. A tank of relatively reduced space (30 × 100 × 40 cm) with running seawater and a pair of bricks as shelter was (and still is) sufficient to make an octopus happy and at home. In this type of experimental setting, an initially scared, hiding, and pale octopus, as it commonly appears on the day it is captured becomes a tame, pet-like animal with time (Buitendijk, 1933; Hochner et al., 2006). Second, because of its natural curiosity. A few days in captivity are normally enough for the octopus to show its intrinsic attitude to attend to any object placed in its space (30 × 100 × 40 cm) with running seawater and a pair of bricks as shelter was (and still is) sufficient to make an octopus happy and at home. In this type of experimental setting, an initially scared, hiding, and pale octopus, as it commonly appears on the day it is captured becomes a tame, pet-like animal with time (Buitendijk, 1933; Hochner et al., 2006). Second, because of its natural curiosity. A few days in captivity are normally enough for the octopus to show its intrinsic attitude to attend to any object placed in its aquaria, which is largely a result of its voracity and exploratory drive toward natural (or artificial) objects. Third, because of its resilience to recover from massive brain surgery, contrary to what Young and coworkers experienced with *S. officinalis* (Sanders and Young, 1940).

The advantage of the octopus preparation became clear when Boycott introduced an efficient training technique. *O. vulgaris*’s hunting behavior was utilized as biological drive to teach animals to discriminate between stimuli that were positively and negatively reinforced. In the first experiments, octopuses were presented with crabs alone or associated with a white square; every attack on the latter was negatively reinforced (6–12 V AC). Several dozen trials were enough for the animals to learn the task and to respond correctly in quite a stable and predictive way.

In a series of subsequent experiments, it was demonstrated that octopuses were able to distinguish between different shapes by successive presentation of the two objects. The octopus was allowed to eat the crab when it attacked the positive figure, but an electric shock was delivered to the animal by a probe when it attacked the negative stimulus (Boycott and Young, 1956; for review, see Sanders, 1975; Hanlon and Messenger, 1996). Following this initial set of experiments, the protocol was improved by giving the animal a piece of anchovy as positive reinforcement instead of crabs, which could reduce the octopuses’ predatory response because of satiety (Young, 1961).

### 1.31.1.3 The Breadth of the Studies on Octopus and Other Cephalopods

The versatility of this training protocol allowed a rapid growth of the field up to the 1960s (see also Figure 2). In fact, different research directions emerged from the original study by Boycott and Young (for review, see also Wells, 1965b).

For example, the first were centered on the study of the different sensorial capabilities (orientation in space, vision, chemotaxis, touch, and proprioception) and of the structures devoted to their control using different approaches (e.g., behavioral, ablation, and stimulation). Subsequent attention was focused on disclosing the role of the lobes of the brain (essentially the vertical and the peduncle lobes) in the learning and behavioral responses and in the motor coordination.

All these studies allowed Young and coworkers to produce a model of the brain of a learning (and behaving) octopus (Young, 1964; Clymer, 1973). In addition, these studies demonstrated that the animals were capable of sensitization, habituation, associative learning (passive avoidance, visual and tactile discrimination), and spatial learning (for a review, see Young, 1961; Sanders, 1975; Wells, 1978; Boyle, 1986; Boal, 1996; Hanlon and Messenger, 1996; Hochner et al., 2006). As clearly stated by Wells, “Octopuses can be taught to make a wide variety of tactile and visual discriminations. They learn rapidly under conditions that would lead to learning by mammals, and they achieve similar standards of accuracy of performance. This places them in a different category from many invertebrates, where research has tended to concentrate on demonstrating that the animals can learn at all. In the case of *Octopus* there is now no doubt that the species can learn, whatever definition of learning one cares to employ. One is free to pass on to considerations of *what* these animals can detect and learn about the world around them and what *we* can learn about the organisation of the cephalopod brain from their successes and failures” (Wells, 1965a, p. 115).
The analysis of the literature clearly shows what cephalopod workers (and modern neuroscientists) know well: The octopus, as a model, was almost (and suddenly) abandoned by the end of the 1960s (Figure 2).

This was mainly a result of:

- The relatively poor control by experimenters of the behavioral training procedures (Bitterman, 1966, 1975);
- The lack of appropriate tools to explore the neurophysiological properties of cells within certain lobes of the brain (e.g., amacrine cells in the vertical lobe; Young, 1985); and
- The inability of octopuses to pick up kinesthetic cues and the lack of proprioceptive feedback of the higher centers of the octopus brain (review in Wells, 1978).

Controlled handling, maintenance, and training procedures (Walker et al., 1970) and the thorough knowledge of the behavior and learning capabilities of cephalopods (Maldonado, 1963a; Packard, 1963; Messenger, 1968, 1977; Packard and Sanders, 1969, 1971; Packard and Hochberg, 1977; Hanlon, 1978; Hanlon and Messenger, 1988) have allowed subsequent workers to cope with the difficulties implicit in the training protocols and to produce renovated experimental approaches to the study of the behavioral biology of learning in these animals. This has led to a growing number of publications over the last 20 years (Figure 2) that are not necessarily focused on the octopus model but on different cephalopod species.

### 1.31.2 The Cephalopod Brain and Its Learning Capabilities

Dramatic evolutionary changes in the body plan and in the gross morphology of the nervous system (and of its relative organization) led to the origin and diversification of the phylum Mollusca (Kandel, 1979; Lee et al., 2003).

The nervous system, in particular, varies greatly in complexity and in the number of neurons among taxa (Bullock, 1965a,b,c,d). However, it is in cephalopods that this complexity reaches its highest degree within the phylum – a complexity that can be recognized at three levels. First, the brain size (relative to body weight) is comparable to that of vertebrate brains and positions cephalopods just below higher vertebrates (i.e., birds and mammals; Packard, 1972). Second, an average-sized octopus presents roughly 500 million neurons. More than one-third (roughly 200 million cells) are recruited to form its central nervous system (Young, 1963), a number that appears to be from 200 to 10 000 times higher when compared with the brains of other invertebrates (Apis and Aplysia, respectively). Finally, the degree of complexity of the nervous system is not only limited to the relative size and number of neurons within the brain but also stems from its neuroanatomical organization (for review, see Young, 1971; Budelmann, 1995; Williamson and Chrachri, 2004).

#### 1.31.2.1 General Organization of the Brain

In cephalopods, the ganglia recruited to form the central nervous system may be considered homologous to the labial, buccal, cerebral, pedal, pleural, and visceral ganglia of gastropod mollusks. Contrary to what occurs in the typical molluscan design, in a cephalopod the ganglia are fused together and clustered around the most anterior part of the esophagus (for a review, see Bullock, 1965b; Budelmann, 1995). The agglomeration of the ganglia, which happened by the shortening of the connectives and commissures, form three almost distinct parts: the supra- and the subesophageal masses, and a pair of optic lobes that emerge laterally from the supraesophageal mass (one for each side positioned just behind the eyes).

The supraesophageal mass originated from the ancestral labial, buccal, and cerebral ganglia. The main lobes constituting it are the inferior, superior, and posterior buccal; the inferior frontal, subfrontal, and superior frontal; the vertical; and the basal lobes (Figure 3). Moreover, certain lobes of the supraesophageal mass (e.g., the optic and olfactory lobes) are of more recent neural formation. The subesophageal mass, instead, essentially derived from the paired pedal, pleural, and visceral ganglia that fused together to a different extent within and between cephalopod species (Nixon and Young, 2003). Again, neural masses of more recent origin were added to the ganglia listed above to form the brachial ganglion, mainly devoted to the control of the actions of arms and suckers.

Altogether, this provides cephalopods with the highest degree of centralization compared with any other mollusk and with the vast majority of other invertebrate phyla. Nevertheless, the nervous system keeps the basic invertebrate organization with layers of cell bodies distributed externally and with an inner neuropil.
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(a) Vertical
Subvertical
Precommissural
Posterior basal
Palliovisceral
Peduncle
Plex
Optic

(b) Vertical
Subvertical
Precommissural
Median basal
Posterior basal
Palliovisceral
Peduncle
Plex
Optic
A thorough description of the gross morphology, neuroanatomy, and organization of the cephalopod brain is known for *O. vulgaris* (Young, 1971) and for decapods (Young, 1974, 1976, 1977, 1979; Messenger, 1979). (Reviews on this topic are available in Bullock, 1965b; Budelmann, 1995; Nixon and Young, 2003; but see also Budelmann et al., 1997). In addition, quantitative data on the relative size of the different brain lobes of various species of cephalopods is also available following the contributions of Wirz (1959) and Maddock and Young (1987). Information on the relative size of the lobes in hatchlings has been determined for several Mediterranean species by Frösch (1971; for review see also Nixon and Young, 2003).

Taken as a whole, these studies have shown that the Cephalopoda present a marked diversification of cerebrotypes that should correspond to differences in the habitats occupied within the marine environment (Nixon and Young, 2003; Borrelli, 2007). For example, the brachial and inferior frontal lobes are highly diverse between octopods and decapods, with those in the former being considerably larger than in the latter as a consequence of their benthic lifestyle (and tactile sensorial modality). On the other hand, the vertical lobe (as integrative center, see Sections 1.31.2.2, 1.31.2.3) shows greater variability among species both in relative size and gross morphology (Young, 1979; Maddock and Young, 1987; for a review, see Nixon and Young, 2003).

Finally, as mentioned above, the arms of an octopus contain about two-thirds of the some 500 million neurons in total. The arms can thus work rather autonomously (following a hierarchical functional control of the higher motor centers), as they can generate highly stereotyped movements (Altman, 1971; Sumbre et al., 2001, 2005, 2006).

Moreover, we now know the possible function of roughly 40 lobes within the cephalopod brain by stimulation experiments carried out in *S. officinalis* (Boycott, 1961) and *O. vulgaris* (Boycott and Young, unpublished data: cited in Young, 1963, 1971).

These studies have shown that the supraesophageal mass is responsible for sensory processing and analysis and control of behavior, and it also provides motor commands and coordination to the higher motor centers (i.e., basal lobes). The subesophageal mass, instead, provides the control of particular sets of effectors via intermediate and lower motor centers.

### 1.31.2.2 Neural Substrates of Behavior

The analysis of hundreds of lesion experiments conducted on octopuses (mostly), squids, and cuttlefishes (review in Sanders, 1975; Boyle, 1986) and of several dozen serial histological sections of cephalopod brains allowed Young and coworkers not only to describe the anatomy of the nervous system of these animals (for a review, see Nixon and Young, 2003) but also to unravel the circuit leading to their visual and tactile learning capabilities (Figure 3). In Young’s view, learning and memory are achieved in cephalopods by “a series of matrices of intersecting axes, which find associations between the signals of input events and their consequences” (Young, 1991: p. 200).

#### 1.31.2.2.1 Tactile information

During tactile processing (and learning), the decision to grasp or reject an object by an octopus is made on the basis of the interaction of a network made up by the following eight matrices:

- Lateral inferior frontal lobes
- Median inferior frontal lobe
- Subfrontal lobe
- Posterior buccal lobes
- Lateral superior frontal lobes
- Median superior frontal lobe
- Vertical lobe
- Subvertical lobe

The system, which corresponds to roughly six lobes of the supraesophageal mass, is tuned to take any object touched (i.e., tactile exploratory drive) unless pain signals are conveyed.

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**Figure 3** A schematic representation of the neural pathways for the visual (blue) and tactile (red) sensorimotor systems in the brains of *Sepia officinalis* (a) and *Octopus vulgaris* (b). Possible integrations (or shared pathways) between the two systems are indicated in green. In the supraesophageal mass, several matrices of the two systems overlap, particularly in *Octopus* (see text for details). The optic lobes (bottom) are depicted from horizontal sections. The reciprocal positions and sizes are not in scale. The supra- and subesophageal masses of the two species are drawn or photographed from a sagittal section: (Sepia (a) after Sanders FK and Young JZ (1940). Learning and other functions of the higher nervous centres of Sepia. *J. Neurophysiol.* 3: 501–526. Octopus (b); after Young JZ (1991) Computation in the learning system of cephalopods. *Biol. Bull.* 180: 200–208. Esophagus (es); plexiform zone (plex).
The interaction between the inferior frontal (positive signals) and the subfrontal (negative signals) plays the major role in decision making (i.e., take/reject) by the animal.

Finally, the inferior frontal system (in decapods, the posterior buccal and the lateral inferior frontal lobes; in octopods, the posterior buccal, the lateral and median inferior frontal, and the subfrontal lobes) is qualified as the main site of the long-term memory storage for tactile information, as roughly 60% of the total tactile learning capacity is thought to reside in this brain region (Young, 1983). The remaining quota is distributed between the superior frontal and vertical lobes (approximately 25%) and, to a limited extent, in areas of the subesophageal mass (the remaining 15%; Young, 1983; see also Budelmann and Young, 1985; for a review, see Wells, 1978; Young, 1991, 1995; Williamson, 1995; Williamson and Chrachri, 2004).

In decapods, the so-called inferior frontal lobe is more comparable to the posterior buccal lobe of Octopus than to the inferior frontal lobe sensu stricto (Young, 1971, 1979), which is lacking in cuttlefishes and squids. However, the distribution and interchange of fibers originating from the arms make the inferior frontal lobe of decapods appear close to the median inferior frontal lobe of *O. vulgaris* (see p. 350 and p. 314 of Young, 1971, 1979, respectively).

From a functional point of view, the fact that cuttlefishes and squids detect their prey visually and capture it with the arms or by ejection of the tentacles (depending on the prey species; see a review in Hanlon and Messenger, 1996) makes us assume that they have only a limited need and capacity for learning tactile information. Manipulatory activities related to feeding are considered largely programmed and based on reflexes (but see Halm et al., 2000, 2002, 2003), with some inhibitory pathways (i.e., reciprocal inhibition) from the small amacrine cells that are distributed among the large motor neurons of the buccal and subesophageal centers (Young, 1976, 1991), in close resemblance to the spinal cord of mammals (Young, 1995).

### 1.31.2.2.2 Visual information

Like the tactile learning system, visual stimuli are classified and processed behaviorally by a network composed of the following four matrices:

- Lateral superior frontal lobes
- Median superior frontal lobe
- Vertical lobe
- Subvertical lobe

The optic lobe also plays a major role in the visual learning system but was excluded by Young from the assemblage of matrices because of its location outside of the supraesophageal mass.

Again, like before, the system of matrices is tuned to promote the animal to attack the stimulus unless unpleasant feelings are perceived. At the level of the optic lobes, the cells from the retina reach the outer plexiform zone, where they make contact with a large number of cells (second-order visual cells; see Figure 3) that act as feature detectors. They constitute dendritic fields of various shapes and extensions that allow the recognition of “the relevant features of objects and scenes” (Young, 1995, p. 434) encountered by the animals during their everyday life (see also Deutsch, 1960; Sutherland, 1960; for a review, see Sanders, 1975; Hanlon and Messenger, 1996).

The axons of the second-order cells “form columns proceeding to the center of the [optic] lobe, where they interact in an interweaving matrix of cells and fibers” (Young, 1991, p. 205).

Outputs of the neurons of the optic lobe proceed toward various areas of the brain (Figure 3). Some go directly to the magnocellular lobe, which is considered to act in situations where rapid escape reactions are needed (Young, 1971, 1973, 1991, 1995). Other fibers proceed to the peduncle and basal lobes, thus serving to regulate movements, and finally the third pathway goes toward the core of the visual matrices. It is at this level that the interaction between the lateral superior frontal (promoting the attack) and the median superior frontal and vertical lobes (inhibiting the attack) regulates the animal’s behavior. Finally, according to Young, memory formation for visual experiences and their outcome take place within the optic lobes, with active participation of the supraesophageal centers (Young, 1991, 1995).

As is shown in Figure 3, the neural organization of the visual system of decapods has close affinities to that of octopods (Cajal, 1917; Sanders and Young, 1940; Young, 1973; for a review, see Nixon and Young, 2003; Williamson and Chrachri, 2004). Perhaps the system works in a similar way in cuttlefishes and squids, although it has not yet been extensively studied in the learning context, as has been done for octopuses (see a review in Young, 1991; Agin et al., 2006a).

### 1.31.2.3 How Computation in the Learning System Is Achieved

The idea promoted by Young and colleagues on the existence of multiple matrices in the central nervous
system, working in the control of behavioral responses, found its roots in the pioneering studies of Cajal (1917) and Sanders and Young (1940). The model proposed by Young was deduced essentially on the basis of morphological and experimental evidence (see the review in Young, 1961).

Following Young, the two systems work on similar principles. The information is processed through a series of matrices that allow signals of different types (and meanings) to interact to some extent with each other and to regulate subsequent behavior for attack/take and retreat/reject responses (Figure 3). In addition, the modulation between promotion and inhibition is tuned in order to facilitate exploratory behavior. According to Young, the systems are designed with a close similarity with complex nervous systems such as the mammalian hippocampus and neocortical centers (Young, 1995). This system seems to be limited in that a complete integration (transfer) between visual and tactile information is relegated only at the level of the effectors (Allen et al., 1986), although limited cross-modality has been shown in O. digueti at higher neural levels (Michels et al., 1987). Similar findings are reported for O. vulgaris by Robertson and Young (in preparation, cited by Michels et al., 1987) but unfortunately are unpublished as far as we know.

Wells (1978) published an alternative hypothesis worthy of mention based on the response of animals to an associative learning context. In his opinion, the sensorial inputs (visual system) reach the vertical lobe, where they are modulated on the basis of the effects of positive or negative signals (tactile system). Here the system is tuned to sensitize by raising or lowering the level of response on the basis of previous experiences. In this way, new information is added to the long-term cumulative experience, allowing short-term fluctuations and flexible and adaptive behavior (Wells, 1978).

The hierarchical control of motor patterns (described earlier) with lower (i.e., arms), intermediate (i.e., subesophageal), and higher (i.e., supraesophageal) motor centers (Boycott, 1961; Young, 1971, 1991) has been updated by experimental evidence (Plän, 1987). According to this view, different motor areas work in consensus, contributing to a more democratic concept of neuronal assembly. In other words, parallel central sensorimotor pathways cooperate synaptically to produce a given motor pattern (i.e., behavior; Plän, 1987), which appears to be similar to what has been shown in other invertebrates (for a review, see Getting, 1989; Leonard and Edstrom, 2004; Calabrese, 2007).

These models should be validated (hierarchical vs. democratic) in view of modern experimental approaches.

The compartmentalization (or certain modularity of the system) achieved by the multiple matrices may correspond well to the lifestyle adaptations of cephalopod species in different environments and niches (Nixon and Young 2003; Figure 4), as recently tested by combining ecological and neuroanatomical data (Borrelli, 2007).

As solicited in several occasions by Young himself (1985, 1995), a physiological investigation of the responses of the cells within the various regions of the brain is necessary to disclose the functional characteristics of the system. It is only relatively recently that attention has been focused on studies of this kind, starting from the pioneering studies on S. officinalis (Bullock and Budelmann, 1991) and O. vulgaris (Williamson and Budelmann, 1991) up to the latest findings of Hochner and coworkers (2003, 2006).

Figure 4  Old drawing by Comingio Merculiano (1887, unpublished) of octopuses (Octopus vulgaris), squids (probably Loligo vulgaris), and cuttlefishes (Sepia officinalis) expressing their different behavioral adaptations. Stazione Zoologica Archives, ASZN: Ua.I.506.
The most recent electrophysiological studies in the octopus confirm the view that convergent evolution has led to the selection of similar networks and synaptic plasticity in remote taxa (i.e., cephalopods and higher vertebrates), contributing to the production of complex behavior and learning capabilities (for review, see Hochner et al., 2006). A similar architecture and physiological connectivity of the vertical lobe system (i.e., median superior frontal and vertical lobes) of the octopus with the mammalian hippocampus, together with the large number of small neurons acting as interneurons, suggest a typical structure with high redundancy of connections working with en passant innervations. This makes it possible to create large-capacity memory associations (Hochner et al., 2003, 2006). However, the analogy between the octopus and mammalian systems is not complete, the major differences being in the morphological organization and physiological characteristics (see a review in Bullock, 1963b; Williamson and Chrachri, 2004; Hochner et al., 2006).

Finally, it is important to underline that a system similar to Octopus has been found in S. officinalis, but with differences emerging at various levels (morphological, physiological, and behavioral; Agin et al., 2006a; Graindorge et al. 2006b; B. Hochner, personal communication). The present results, although promising, must be taken with caution since they are preliminary at this stage.

Last, it is worth mentioning that a computer simulation model of the predatory response of O. vulgaris (attack behavior sensu Maldonado, 1963a; Packard, 1963; for a review, see also Borrelli et al., 2006) has been developed (Clymer, 1973).

In the model, a mnemon (i.e., a visual feature with associated memory value resulting from experience; Young, 1965) 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 (i.e., attack command) is 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 combined in the model and determine the final attack/retreat response (Clymer, 1973).

The model proposed by Clymer was based on the knowledge of O. vulgaris’s predatory behavior (also as a result of the discrimination experiments) as deduced by Young (1964, 1965) and Maldonado (1963b). Interestingly, the results produced from the 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 (Clymer, 1973). In addition, the model has been recently reviewed and modified on the basis of the most advanced findings on neural networks and learning in simulated environments (Myers, 1992).

1.31.3 Learning in Cephalopods

Many reviews centered on the biology and learning capabilities of cephalopods have been published during the course of the last 50 years. An analysis of the literature indexed in both the Zoological Record and the Web of Science (from 1950 to 2007) selected roughly 100 reviews regarding the topic. Several other reference works (e.g., The Mollusca by Wilbur, 1983–1988) with chapters relevant to the subject must also be taken into consideration.

Over the last few decades, different workers have attempted to synthesize the knowledge on the behavioral biology of cephalopods and its flexibility (Packard and Hochberg, 1977; Hanlon, 1988; Mather, 1995, 2007; Boal, 1996; Messenger, 1996, 2001; Williamson and Chrachri, 2004; Borrelli et al., 2006; Hochner et al., 2006, to cite just a few). The most significant and comprehensive reviews published on cephalopod biology, learning, and memory are those by Young (1961), Sanders (1975), Wells (1978), Boyle (1986), and Hanlon and Messenger (1996).

In this chapter, we are deliberately not summarizing the information provided by the papers listed above, as this would necessarily result in redundancy. Our aim is simply to offer the reader with a general overview of what is known on the subject, which phenomena/mechanisms have been described and analyzed in detail, and which cephalopod species have been chosen as models for studies on the learning and memory capabilities of this taxon (Table 1). In the following pages, we focus our attention on the results and directions of the most recent advancements on the behavioral biology of learning and memory in cephalopods.

As thoroughly reviewed by Hanlon and Messenger (1996), various forms of learning (Table 1) 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. In essence, a large number of the entities proposed by Moore (2004) in his cladogram of learning processes have been shown in some of the 780 cephalopod species known to date. It is a pity that
Table 1  Breadth of the learning paradigms shown in cephalopods, by species

<table>
<thead>
<tr>
<th></th>
<th>Habituation</th>
<th>Sensitization</th>
<th>Classical conditioning</th>
<th>Instrumental conditioning</th>
<th>Avoidance learning</th>
<th>Spatial learning</th>
<th>Mazes and problem solving</th>
<th>Social learning</th>
<th>Perceptual processes in visual learning</th>
</tr>
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<tbody>
<tr>
<td>Sepia officinalis</td>
<td>X</td>
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<td>Todarodes pacificus</td>
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<tr>
<td>Octopus vulgaris</td>
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<td>Octopus maorum</td>
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<td>Octopus maya</td>
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<td>Enteroctopus dofleini</td>
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<td>Eledone moschata</td>
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</table>

Data are arranged following Hanlon RT and Messenger JB (1996) Cephalopod Behaviour. Cambridge: Cambridge University Press. The learning capabilities (√) by species are deduced from reviews (Sanders GD [1975] The cephalopods. In: Corning WC, Dyal JA, and Willows AOD [eds.] Invertebrate Learning. Cephalopods and Echinoderms, pp. 1–101. New York: Plenum Press, and Hanlon RT and Messenger JB [1996] Cephalopod Behaviour. Cambridge: Cambridge University Press), with the exception of more recent findings (X) that are also described in the text. It is important to underline that certain paradigms, such as discrimination learning, are here classified as classical conditioning, although they could also be considered as cases of operant (or Thorndikian) conditioning (see Moore, 2004).
learning (and behavior) studies are still restricted to a limited number of representatives (Table 1). In fact, a detailed description of the behavioral repertoire is available for only roughly 30 species (review in Hanlon and Messenger, 1996; Borrelli et al., 2006). Another problem inherent to the cephalopod literature is that some phenomena have been relegated to anecdotal accounts (e.g., tool use: Power, 1857; Pliny the Elder, 1961) and that experimentally controlled observations on such capabilities remain to be done.

1.31.3.1 Sensitization

Following our everyday practice with octopuses, the daily presentation of food increases the chance of the animal attacking, so that its predatory performance (measured as the time to attack the prey from its appearance in the tank) improves with time. This is a clear case of sensitization, similar to what was tested empirically in the past by Wells and coworkers, who showed that food could enhance the probability to attack the stimuli (as much as shock depressed it; Wells, 1967a, cited in Hanlon and Messenger, 1996), or as was demonstrated in conditions where chemotactic behavior was studied (Chase and Wells, 1986).

This phenomenon has recently been confirmed in our laboratory for O. vulgaris following the classic acclimatization phase to the experimental setting. The continuous administration of a reward (food) over days improves the predatory performance that reaches a steady-state level during consecutive attacks. However, it is difficult to rule out whether different speeds in the attack curves (when interindividual variability is evaluated) may be related to food preferences, novelty, or familiarity toward food items, or to individual differences in the capability to cope with contextual learning processes, and so on (see also Section 1.31.4.1).

Finally, it could be extremely interesting to explore whether the relative length of time in the laboratory has an effect on octopuses’ performance in a learning paradigm, say, for example, on the individual preferences in a simultaneous visual discrimination task. This concept should not be underestimated, also considering that as the positive learning process (see Section 1.31.4.1) proceeds with time, differences between individuals may be reduced to a few clear-cut responses and other characteristics of the subjects may emerge. Such processes may be easily extended to other cephalopods, although significant differences between species are expected as a result of different lifestyles and adaptive capabilities (for a review, see Packard, 1972; Nixon and Young, 2003).

1.31.3.2 Various Forms of Associative Learning

A new set of learning protocols (or variations on the theme) has recently been developed successfully in cephalopods. We provide a few examples below.

Painting quinine on the carapax of prey items (crabs, shrimps) was sufficient to show simple and rapid taste aversion learning in S. officinalis (Darmaillacq et al., 2004). This produced significant shifts in prey choice that were retained over the long term (for at least 3 days; Darmaillacq et al., 2004).

Calvé (2005) showed that the cutout of a bird (predator) gliding over individual cuttlefishes elicited startling reactions of different intensities. The startling stimulus significantly affected the cuttlefishes’ hunting behavior, although evidence suggests that the animals habituated to it (Calvé, 2005).

Plastic spheres were utilized in successive visual discrimination tasks in order to test whether classical conditioning could change the species-specific predatory (or hunting) behavior; the results suggest that autoshaping occurs in S. officinalis (Cole and Adamo, 2005).

All the cases mentioned above represent innovations in the practice of learning studies with Sepia and clear additions to the classic prawn-in-the-tube training procedure pioneered by Sanders and Young (1940) and modified by successive authors (Wells, 1962; Messenger, 1973; see also Chichery and Chichery, 1992a,b; Figure 5). Notwithstanding, this procedure promoted a large number of studies on associative learning in the cuttlefish and on the biological machinery involved (e.g., Agin et al., 2000, 2001, 2003; Bellanger et al., 1997, 1998, 2003, 2005; Halm et al., 2003). Recent findings in S. officinalis strongly support the view that learning not to attack prey trapped in a transparent tube (inhibitory learning) corresponds to associative learning (Agin et al., 2006b; Purdy et al., 2006).

The number of training protocols available for Octopus is traditionally greater than for other species, mainly as a result of the animal’s behavioral flexibility and feasibility of experimental studies with this species (see also Table 1). Moreover, recent protocols such as passive avoidance, additional problem-solving tasks (e.g., jars with multiple openings, black boxes; Borrelli, 2007), and even habituation tests have extended the repertoire of training paradigms that may be utilized to find answers to the fundamental question of how and to what extent O. vulgaris is capable of learning to modify its behavioral response.
1.31.3.3 Development of the Learning Capabilities

The prawn-in-the-tube protocol, which was and is still utilized to study associative learning in *S. officinalis*, disclosed important details on how cuttlefishes' behavioral plasticity changes during ontogeny. The analysis of this phenomenon made it possible to find significant correlations with the development (and maturation) of the neural circuitry, considered to play a role in the processing of behavioral responses and learned changes (Wells, 1962; Messenger, 1973; Chichery and Chichery, 1992a,b; Dickel et al., 1997, 1998, 2001; Agin et al., 2006a,c; for review see also Dickel et al., 2006).

In a similar way, the predatory efficiency of *Loligo* spp. on copepod swarms is reported to improve with age. It was found that the mastery of copepod capture develops progressively from the initial basic attack type up to more specialized strategies that effectively extend the range of capture to both longer and shorter distances, culminating in the adult-like prey capture behavior (Chen et al., 1996).

Similar changes of behavior with experience have also been described for octopuses; the optimization of the site and number of holes drilled in bivalve preys, for example, distinguishes juvenile from adult performance (see a review in Mather, 1995, 2007).

Figure 5  Two examples of classic tasks utilized to study learning and memory recall in *Octopus* and *Sepia*. (a)–(d) A sequence of frames taken from video recordings of the problem-solving experiment in *O. vulgaris* (classic jar). Courtesy of Mr. M. Schumacher, RS-Film. (e) A sketch of the prawn-in-the-tube protocol utilized with *S. officinalis* (after Wells MJ (1962) Early learning in Sepia. Symp. Zool. Soc. Lond. 8: 149–169).
1.31.3.4 Spatial Learning

Spatial learning was originally tested in cephalopods using mazes, which led to a heated debate as to whether octopuses were capable or not of learning a detour (Bierens de Haan, 1926; Buytendijk, 1933; Schiller, 1949; Boycott, 1954; Wells, 1965a; but see Walker et al., 1970; Moriyma and Gunji, 1997).

During the last few decades, studies on the problem-solving abilities of these animals have been frequently confused and erroneously attributed to spatial learning processes (Piéron, 1911; Fiorito et al., 1990, 1998b; but for critiques, see Mather, 1995; Hanlon and Messenger, 1996).

Nevertheless, spatial learning *sensu stricto* has been shown in several species over the last few years. Apart from the pioneering studies of Mather (1991) on *O. vulgaris*, learning to orient and navigate in space is reported for *O. h expired to the two motor components of the task with trials, thus suggesting that learning is involved in solving the problem (Fiorito et al., 1990, 1998b).

The switch between the two modalities (from visual to tactile) that controls and determines the octopus' performance in the task is not automatic and occurs at different instances in different animals. This further supports the view that learning of the task is required in order to sort out the motor programs (probably species specific) that are necessary to solve the paradigm. In fact, the pulling action, which is required by an animal dealing with the operandum, is already present in the species’ behavioral repertoire, as the same technique is adopted by octopuses preying upon bivalves (McQuaid, 1994; Fiorito and Gherardi, 1999; Steer and Semmens, 2003). Moreover, the deterrent may be compared to the so-called speculative pounce (e.g., Yarnall, 1969), a common foraging strategy in which animals mostly use a tactile-driven manipulation and blind exploration of the sea bottom in search of edible items hidden under rocks or in crevices (for a review, see Hanlon and Messenger, 1996; Borrelli et al., 2006). Different tasks (e.g., jars with multiple openings and boxes with drawers) have recently been designed to further explore *O. vulgaris*’ problem-solving capabilities.

Similar objects could be used with other octopod species, facilitating comparative analysis of the taxon’s behavioral flexibility. In addition, such tasks may provide more general information on the biological correlates involved in the two modalities (visual and tactile) that govern such behavioral responses.

1.31.3.5 Other Learning Capabilities

Problem solving and social learning have mostly been studied in *O. vulgaris*. These learning paradigms have often been debated and criticized for not providing clear evidence of learning in these animals (Mather, 1995, 2007; Hanlon and Messenger, 1996; but see also Biederman and Davey, 1993; Suboski et al., 1993).

1.31.3.5.1 Problem solving

The task traditionally utilized to test the problem-solving capabilities of *O. vulgaris* (Fiorito et al., 1990), derived from the pioneering experiments by Piéron (1911), uses the well-known skills of octopuses with jars (Cousteau and Diolé, 1973). In the classic experiment, the animal is faced with a transparent glass jar, closed with a plug, containing a live prey. The octopus generally attacks the object at first glance, with the attack elicited by visual cues (the sight of the prey). The physical contact with the jar makes the animal switch to the tactile-driven exploration of the stimulus (Figure 5). While manipulating the jar, the octopus must solve two problems: the operandum (i.e., removing the plug by pulling), and the detour (i.e., the blind exploration of the inside of the jar with the arms to reach for the prey).

It was shown that octopuses spent significantly less time on the two motor components of the task with trials, thus suggesting that learning is involved in solving the problem (Fiorito et al., 1990, 1998b).

During the last few decades, studies on the problem-solving abilities of these animals have been frequently confused and erroneously attributed to spatial learning processes (Piéron, 1911; Fiorito et al., 1990, 1998b; but for critiques, see Mather, 1995; Hanlon and Messenger, 1996).

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Similar objects could be used with other octopod species, facilitating comparative analysis of the taxon’s behavioral flexibility. In addition, such tasks may provide more general information on the biological correlates involved in the two modalities (visual and tactile) that govern such behavioral responses.

1.31.3.5.2 Social learning

Fiorito and Scotto (1992) provided experimental evidence of social learning in *O. vulgaris*. They showed that naïve animals were able to discriminate and choose between two stimuli following observation
of trained conspecifics. The observer octopuses appeared to follow the model witnessed, and their choice seemed to be stable over time. The original work was replicated to test the role of the neural circuit considered to be involved in social learning (Fiorito and Chichery, 1995) and to study the attention and memory retrieval capabilities of this kind of learning after a pharmacological interference (Fiorito et al., 1998a).

The results of social learning in *O. vulgaris* were debated and criticized in a number of publications (e.g., Biederman and Davey, 1993; Hanlon and Messenger, 1996).

Future studies are required to reply to the many questions that remain open on this peculiar learning capability, since it is possible that certain other factors may act in favor of or against the outcome of the observation of the conspecific’s behavior by naïve observers (e.g., reproductive drive, social dominance, territoriality; for review, see also Boal, 2006). This, at least, appears to be the case in *S. officinalis*, where social experience promotes (Warnke, 1994) or inhibits (Boal et al., 2000b) social facilitation in the feeding behavior of cuttlefishes, probably depending on the relative age of the animals.

### 1.31.4 Neglected Issues in the Study of Cephalopod Learning

As amply discussed above, most of our knowledge on the learning capabilities and memory recall in cephalopods derives from experiments conducted in the laboratory, the sole exception being the field studies on *O. vulgaris* carried out in the waters of Bermuda (Mather, 1991; Mather and O’Dor, 1991). Mather and colleagues demonstrated that octopuses acquire information and keep memories of their surroundings to navigate during foraging trips (i.e., spatial learning; Mather, 1991; see also Hanlon and Messenger, 1996; Forsythe and Hanlon, 1997). Moreover, *O. vulgaris* is capable of behavioral plasticity in lifestyle resulting from changes in foraging needs and predation pressure (Mather and O’Dor, 1991).

Despite the growing number of publications on the habits and adaptive responses of cephalopods in the wild (e.g., Smale and Buchanan, 1981; Moynihan and Rodaniche, 1982; Roper and Hochberg, 1988; Mather and Mather, 1994; Hanlon et al., 1999), our inference on learning in cephalopods is still largely biased by laboratory evidence. Whether this is the result of the behavioral flexibility (neural and behavioral) of the cephalopod system to the new context (captive situation), or whether it corresponds to the animals’ real needs in the wild, still remains an open question.

#### 1.31.4.1 Effect of Acclimatization: Contextual Learning

Following capture, the animals are generally immediately brought by the fishermen to the laboratory, where they are exposed to the experimental setting (i.e., a novel environment). In the case of an octopus, the animal is thus constrained to a tank that represents a foraging area about 300 times smaller than that required by the animal in natural conditions. In addition, the scenery to which the animal is exposed is somewhat dull and uniform, independent of whether it is designed to provide an enriched or an impoverished environment (e.g., Dickel et al., 2000; Anderson and Wood, 2001; Poirier et al., 2004). We may therefore hypothesize that the new context has only a poor resemblance to the seascape the cephalopod had experienced until then.

This is true not only for octopuses but also for other cephalopod species. A rough analysis of the literature on cephalopods published over the last 10 years has provided exhaustive examples and confirms this view.

The animal must adapt to this novel environment, which is achieved in a variable amount of time and is generally referred as acclimatization. During the acclimatization phase, a common practice is to expose cephalopods to live prey (for exceptions, see, e.g., Boletzky and Hanlon, 1983; Boal, 1993; Koueta et al., 2006), not only to maintain the animals in captivity but also to test their recovery in motivation to attack (i.e., well-being; for a review, see Boyle, 1991). In addition, the animals are exposed to experiments that study their predatory behavior (attack/not attack or take/reject responses). Thus, they must be able to face the task and plastically adapt their species-specific predatory behavior to the new context. As already mentioned above, this phase takes a variable length of time and depends (from species to species) on the animals’ previous experience, the individual variability resulting from biological and possibly ecological factors (differences in age, sex, maturity, etc.), and the common practice of experimenters, to cite a few examples.

It has been recently demonstrated that during the acclimatization phase, the animal is exposed to a positive learning process, which is a form of contextual learning. As described by Maldonado (1963a,b, 1964), at the beginning of acclimatization (or
training), the time spent by an octopus to attack the stimulus is relatively long, and the behavior (in terms of types of attack) is highly variable. However, as the animal becomes more and more accustomed to the experimental setting (or paradigm), its attacks on the stimulus become faster and faster, a process known as the positive learning process. Moreover, this reduces the types of attack curves to nearly a stereotype, that is, the full attack in the octopus (Maldonado, 1963a; Packard, 1963) and the tentacle attack in decapods (e.g., S. officinalis; Messenger, 1968; Loligo vulgaris. Neill and Cullen, 1974; L. pealeii and L. plei. Kier, 1982; Illex illecebrosus: Foyle and O’Dor, 1988).

In sum, during the positive learning process (sensu Maldonado, 1963a), behavioral syndromes are generally reduced to a few broad types representing populational differences (within a species). These differences are recognized to play important ecological and evolutionary roles, mainly during the adaptation to environmental changes (Sih et al., 2004a,b).

In addition, there is evidence in O. vulgaris that this phenomenon is directly linked to a more general form of contextual learning, as recently shown in other invertebrates (e.g., Tomsci et al., 1998; Liu et al., 1999; Haney and Lukowiak, 2001; Menzel, 2001; Law et al., 2004; Skow and Jakob, 2006; Zhang et al., 2006).

A series of factors may interfere in this process, such as:

- The time of day at which the experiment is conducted (which does not necessarily correspond to the animal’s peak of activity in natural conditions);
- The feeding regime;
- The experimental setting to which the animal is exposed; and
- The prey types utilized in captivity that do not necessarily correspond to those fed upon in the wild.

Concerning this last point, for example, the animals’ performance in the new context may be affected by individual dietary preferences derived from previous feeding habits (e.g., for S. officinalis Darmailaq et al., 2006), which have also been shown to influence the performance in visual and tactile discrimination tasks during associative learning (e.g., for O. vulgaris: Messenger and Sanders, 1972; Bradley and Messenger, 1977).

### 1.31.4.2 Neophobia/Neophilia and the Shy–Bold Continuum

Another neglected issue in the study of learning in cephalopods is whether novelty may interfere with the animals’ decision-making processes (Greenberg and Mettke-Hofmann, 2001). In other words, familiar objects or prey types should be preferred to novel ones, and the natural exploratory drive, connected to cephalopods’ voracious appetite, should be reduced by the presence of novel stimuli.

In *O. vulgaris*, for example, the behavioral flexibility (in terms of learning capabilities) of this species corresponds to ecological plasticity, where opportunistic behaviors and reduced neophobia are exhibited (see also Section 1.31.2.3). *O. vulgaris* seems to show less feeding specialization and a higher versatility in foraging than other cephalopods. This appears to be related to the changes in the lifestyle during ontogeny (Hanlon and Messenger, 1996; Nixon and Mangold, 1998) and to the frequent horizontal and vertical migrations by the different age classes moving in the water column (e.g., Oosthuizen and Smale, 2003). Therefore, octopuses have to deal with different environments both at a small and a large time scale (seasons and life history, respectively) that expose them to potentially different degrees of complexity. Under such circumstances, a low neophobia is expected for *O. vulgaris*, as results from current experimental work.

On the other hand, ecological stereotypy may favor individuals (or species) that contrast novel situations by exhibiting less flexible behaviors. This should be investigated.

A consequence of the neophobia/neophilia behavioral types discussed above, at any level (from the individual to the species), leads to the shy–bold continuum (or in the cephalopod sense, to the approach–withdrawal axis sensu; Packard, 1963).

This has been shown in several cephalopods: *S. officinalis* (Hanlon and Messenger, 1988; Calvé, 2005), *Euprymna tasmania* (Sinn and Moltschaniwskyj, 2005), *O. vulgaris* (Packard, 1963; Borrelli, 2007), and *O. rubescens* (Mather and Anderson, 1993).

As described by Calvé (2005), cuttlefishes are classified as shy when they mostly remain inactive and when, following stimulation, respond by inking and jetting away. At the opposite end of the spectrum, bold animals appear active in the tank and interact more with humans (i.e., are tame; Buytendijk, 1993; Hochner et al., 2006). Of course, individuals will respond differently to the same tests on the basis of whether their behavioral traits tend more toward one or the other of the opposite shy–bold extremes of the axis.

Similar findings have been shown for *O. rubescens*, where individuals were classified into three major
behavioral components (activity, reactivity, avoidance; Mather and Anderson, 1993).

As already mentioned, the individual differences of behavioral traits in cephalopods confirm the phenomenon to be widespread among different taxa, ranging from invertebrates to vertebrates (e.g., Armitage, 1986; Kagan et al., 1988; Coleman and Wilson, 1998; López et al., 2005; Mettke-Hofmann et al., 2005; for a review, see also Gosling, 2001; Sih et al., 2004b; Mettke-Hofmann et al., 2005).

In planning future studies testing neophobia/neophilia in cephalopods, the importance of population differences (i.e., genetic polymorphism) and individual experience on environmental factors should be considered, as has been done in other animals. For example, it could be interesting to test how the response of individuals captured with different fishing methods in the wild would appear along the shyness continuum in laboratory conditions, taking advantage of what is known in the pumpkinseed sunfish (Coleman and Wilson, 1998). Moreover, Coleman and Wilson (1998) discovered that animals that behaved boldly in threatening contexts did not act necessarily the same when exposed to novel foods (i.e., foraging contexts). Would it not be intriguing to find similar – or even contrasting – results in cephalopods?

### Table 2

Summary of studies on the time course of memory recall in *Sepia officinalis* (So) and *Octopus vulgaris* (Ov)

<table>
<thead>
<tr>
<th>Paradigm</th>
<th>Species</th>
<th>Training</th>
<th>STM</th>
<th>MTM</th>
<th>ARM</th>
<th>LTM</th>
<th>PSD</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prawn-in-the tube</td>
<td>So</td>
<td>1-t</td>
<td>20 m</td>
<td>48 h</td>
<td>+</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Prawn-in-the tube</td>
<td>Ov</td>
<td>1-t</td>
<td>5 m</td>
<td>24 h</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Prawn-in-the tube</td>
<td>So</td>
<td>Massed</td>
<td>1 d</td>
<td>2 d</td>
<td>2</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prawn-in-the tube</td>
<td>Ov</td>
<td>Massed</td>
<td>2 d</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instrumental conditioning</td>
<td>So</td>
<td>Spaced</td>
<td>14 d</td>
<td>24 h</td>
<td>–</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avoidance</td>
<td>So</td>
<td>Spaced</td>
<td>3 d</td>
<td>4 w</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avoidance</td>
<td>Ov</td>
<td>Massed</td>
<td>16 w</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avoidance</td>
<td>So</td>
<td>Spaced</td>
<td>1 d</td>
<td>24 h</td>
<td>–</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avoidance</td>
<td>Ov</td>
<td>Massed</td>
<td>1 h</td>
<td>8 h</td>
<td>1 d</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


### 1.31.5 Memory in Cephalopods

Despite the considerable number of studies published on the extent of memory recall and on the effects of its impairment induced by experimental interference (for a review, see Sanders, 1975; Wells, 1978), very little is known on the ability of cephalopods to encode and retrieve information.

From the classic works of Sanders and Young (1940) and Schiller (1949), it was shown that cuttlefishes and octopuses are capable of short- and long-term memory, although differences emerged between the two species and among paradigms (Table 2). It is astonishing that in many cases the memory trace was reported to last for a very long time (e.g., in octopus for weeks, according to Boal, 1991; Fiorito and Scotto, 1992; for months, according to Sanders,
Many unpublished observations carried out in our laboratory confirm this view. However, it was found that this remarkably long memory trace is not common to all individuals that learn a given task (Sanders, 1970). The reasons behind this surprising result of only certain individuals having a particularly long memory should be further investigated.

Unfortunately, the systematic analysis of the memory phases (Table 2), together with the time course of retention and memory consolidation (and perhaps reconsolidation), in cephalopods remains insufficient, especially when compared with the knowledge currently available for other taxa (for a review, see, e.g., McGaugh, 2000; Dudai, 2004).

Whether the memory recall observed in cephalopods corresponds to a more phylogenetically conserved consolidation mechanism is an issue that has been tested in *Octopus vulgaris* using several approaches (Maldonado, 1968, 1969; Zarrella, unpublished data). The data suggest that the establishment of long memory traces, to learn conditioned or associative responses, is maintained in *Octopus* following anesthetic treatments and protein synthesis inhibition (Zarrella, unpublished data) applied before or after training (massed intervals). In contrast, electroconvulsive shocks cause significant deficits in retention of previously learned paradigms (spaced intervals: Maldonado, 1968, 1969).

Moreover, protein synthesis inhibition was found to impair memory recall for the prawn-in-the-tube protocol in *S. officinalis* (Agin et al., 2003).

The above studies, although limited in number and species studied, show how promising it could be to test the role of the biological machinery in the establishment of long-term memories in cephalopods, especially when considering the contrasting evidence between species that has emerged so far.

### 1.31.6 Concluding Remarks

Whoever has had the chance to interact with a cephalopod, in the tank or at sea, remains struck by the richness of its behavioral repertoire, its distinct personality traits, and its penetrating gaze, which make it an extraordinary and fascinating creature. There is no doubt that cephalopods are learning animals, although it is difficult to give an objective view of the variety and extent of their learning capabilities.

What is still lacking in the field is more communication and exchange of ideas among cephalopod researchers and the focus on common aims or objectives that could strengthen the work in these animal models. The contribution to the knowledge of the behavioral biology in cephalopods should not be restricted to a handful of workers but be of interest to a greater number of scientists.

Through our behavioral analysis of learning and memory in cephalopods, we hope to have contributed to increasing the understanding and scientific interest and awareness of these animals.

As a last note, the study of cephalopods, despite the long historical tradition, suffers from the lack (or incomplete) availability of tools that are now available for other invertebrate models (such as *Aplysia, Aplysia, Caenorhabditis, and Drosophila*). The approach and direction of studies such as those pioneered and masterly conducted by Maldonado and coworkers on *Chasmagnathus* should be the example (see the review in Romano et al., 2006). The recent work on *Octopus* genomics (Ogura et al., 2004; Choy et al., 2006) has contributed outstanding results that give us hope for the future.

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