

The Octopus: A Model for a Comparative Analysis of the Evolution of Learning and Memory Mechanisms

BINYAMIN HOCHNER^{1,*}, TAL SHOMRAT¹, AND GRAZIANO FIORITO²

¹ *Department of Neurobiology, Institute of Life Sciences and the Interdisciplinary Center for Neural Computation, Hebrew University, Jerusalem, 91904 Israel; and* ² *Neurobiology Laboratory, Stazione Zoologica 'Anton Dohrn' Villa Comunale, I-80121 Naples, Italy*

Abstract. Comparative analysis of brain function in invertebrates with sophisticated behaviors, such as the octopus, may advance our understanding of the evolution of the neural processes that mediate complex behaviors. Until the last few years, this approach was infeasible due to the lack of neurophysiological tools for testing the neural circuits mediating learning and memory in the brains of octopus and other cephalopods. Now, for the first time, the adaptation of modern neurophysiological methods to the study of the central nervous system of the octopus allows this avenue of research. The emerging results suggest that a convergent evolutionary process has led to the selection of vertebrate-like neural organization and activity-dependent long-term synaptic plasticity. As octopuses and vertebrates are very remote phylogenetically, this convergence suggests the importance of the shared properties for the mediation of learning and memory.

Introduction

The octopus and its relatives the cuttlefish and squid are representatives of the modern Coleoidea, a group of the molluscan class Cephalopoda. These are invertebrates that separated from their ancient ancestors, the Belemnoidea, probably around 380 million years ago (reviews in Clarke *et al.*, 1988; Teichert *et al.*, 1988). The old cephalopods are virtually extinct, their last and only living representative being the shell-bearing *Nautilus* (Nautiloidea).

The modern cephalopods (coleoids) have changed dramatically over the course of their evolution; their body structure and way of life differ enormously from those of *Nautilus*

and other molluscs. These evolutionary changes were most likely driven by the selection forces imposed by competition with teleosts (bony fish) and reptiles that radiated in the sea just as modern cephalopods began to develop (Packard, 1972).

The development of sophisticated motor, sensory, and cognitive capabilities, such as excellent vision (for review see Budelmann, 1996; Muntz *et al.*, 1999; Williamson and Chrachri, 2004), highly efficient flexible arms (Yekutieli *et al.*, 2002; Flash and Hochner, 2005), and the ability to learn rapidly (Wells, 1978; Mather, 1995; Hanlon and Messenger, 1996; Nixon and Young, 2003) allowed the dramatic changes in cephalopod behavior necessary for successful competition with the vertebrates (Packard, 1972; O'Dor and Webber, 1986). Both the peripheral and central nervous systems of the modern cephalopods acquired new features to achieve these capabilities. In some cases, systems emerged that are unique to modern cephalopods (*e.g.*, the chromatophore system; for review see Packard *et al.*, 1988; Demski, 1992), while other systems evolved analogous in structure and function to those of vertebrates (*e.g.*, camera eyes and statocysts; see also Hochner, 2004).

This natural selection of analogous systems in phylogenetically remote animals is defined as a convergent evolutionary process (Packard, 1972). Convergent evolution of basic organizational principles may be due to physical constraints or to functional requirements (Eisthen and Nishikawa, 2002; Nishikawa, 2002). Examining convergence to understand complex brain functions is especially exciting when phylogenetically remote animals like cephalopods and vertebrates exhibit similar forms of complex behaviors (*e.g.*, Demski, 1992; Ogura *et al.*, 2004). Here evolutionary convergence may help reveal the basic functional principles of complex biological systems and the major constraints that led to their specific evolution.

Received 6 December 2005; accepted 21 March 2006.

* To whom correspondence should be addressed. E-mail: bennyh@lobster.lsb.huji.ac.il

Octopus Behavior

The first known record of the behavioral capabilities of octopuses and their close relatives dates back to Aristotle, who defined octopuses as “stupid” animals, since their strong exploratory drive made it easy for people to catch them by simply waving their hands underwater (Aristotle, 1910). Modern research on octopus behavior and learning started in 1947 with Professor John Z. Young, a neuroanatomist and physiologist, who initiated a systematic analysis of the neural correlates of the predatory behavior of these animals (Boycott, 1954; Boycott and Young, 1955a). During the following 30 years, an impressive research effort, carried out mainly at the Stazione Zoologica ‘A. Dohrn’ in Naples, Italy, produced a significant number of papers establishing the octopus as a leading invertebrate model for the analysis of complex behaviors (reviews in Sanders, 1975; Hanlon and Messenger, 1996). However, according to S.P.R. Rose, the use of *O. vulgaris* was “instructive [for] the study of its behaviour [but] the octopus did not prove to be God’s organism for the elucidation of memory. Despite Young’s enthusiasm [. . .] his research programme became an experimental dead-end” (Rose, 2003). Since then, however, modern behavioral and electrophysiological approaches have been adapted to researching the complex octopus brain (see below).

Apart from the evolutionary significance of the octopus, several practical reasons make it highly suitable as a subject for research on the neural bases of complex behavior. Firstly, it takes only a few days for an octopus to adapt to captivity. This adaptation, or acclimatization, can easily be perceived by humans, as it involves a clear transition from a frightened, hiding octopus to a pet-like animal that behaves in a friendly way and attends to any event occurring in its aquarium area. It involves the acquisition of recurrent positive experience in obtaining a reward (*i.e.*, food) that makes the octopus attack more often and faster, thus resulting in a “positive learning process” (Young, 1956; Maldonado, 1963a). In its extreme, this form of adaptation may affect the octopus’s performance and introduce complications due to possible interactions between the animal and the experimenter or the experimental context (Boycott, 1954; Dews, 1959; Crancher *et al.*, 1972). This “tameness” of the octopus has stimulated important discussions on the proper training procedures in octopus research (Bitterman, 1966; Bitterman *et al.*, 1975) since in some cases it could have caused failure to demonstrate associative learning (Boal, 1993). Several studies have suggested how to overcome this problem (Boycott, 1954; Papini and Bitterman, 1991; Boal, 1996).

A second reason for the octopus’s suitability for research on the neural basis of complex behavior is that the animal shows highly stereotypic predatory behavior, which is easy

to activate (*e.g.*, by offering a crab tied to a string) and to quantify (Boycott and Young, 1955a). Furthermore, due to the octopus’s natural “curiosity,” this pattern of behavior can be initiated by various artificial targets, thus creating conditions ideal for visual discrimination experiments (Fiorito and Scotto, 1992). Third, octopuses are resilient to invasive surgery and recover rapidly following lesions in their central nervous system under deep anesthesia (Boycott and Young, 1955a; Young, 1971; Sumbre *et al.*, 2001). Due to these advantages, the behavior of *O. vulgaris* has been extensively studied, and its learning abilities have been characterized in captivity. In our view, the most comprehensive of a series of reviews on the learning capabilities of these animals are those by Sanders (1975); Wells (1978); Boyle (1986); Mather (1995); and Hanlon and Messenger (1996).

As solitary hunters, octopuses are exploratory, attending to any novel object in their visual field. They show sensitization, habituation, associative learning including visual and tactile discriminative capabilities, and spatial learning (a few relevant references are as follows: Goldsmith, 1917; Boycott and Young, 1955a, b; Sutherland, 1959, 1961; Young, 1959; Muntz *et al.*, 1962; Dilly, 1963; Mackintosh and Mackintosh, 1963; Rhodes, 1963; Wells, 1964; Mackintosh, 1965; Crancher *et al.*, 1972; Fiorito *et al.*, 1990; Mather, 1991; Papini and Bitterman, 1991; Boal, 1993; Moriyama and Gunji, 1997; Boal *et al.*, 2000). Octopuses demonstrate a sophisticated and extraordinary ability to adapt their behavioral repertoire to the current environment and circumstances (Maldonado, 1963b; Packard, 1963; Maldonado, 1964; Packard *et al.*, 1988), and this may have contributed greatly to their success and that of other cephalopods in their evolutionary competition with fish (Packard, 1972; O’Dor and Webber, 1986; Aronson, 1991).

The octopus also shows observational learning (Fiorito and Scotto, 1992)—that is, the capability of utilizing information from conspecifics on how to solve a visual discrimination task. Observational learning induces a memory trace that is stable for at least 5 days after the observation phase. Individual and social learning and memory recall appear all to share common neural pathways (Fiorito and Chichery, 1995; Fiorito *et al.*, 1998).

In summary, there has been an impressive amount of research over the last 50 years on the learning capabilities of octopuses; by our count, about 200 papers on different aspects of learning of *O. vulgaris* appeared between 1950 and 1980. Unfortunately, the last 20 years saw a decline in this field of research, mainly due to the lack of appropriate neurophysiological tools to test the neural circuits in this animal (Young, 1985). Only recently, with the development of new and suitable methods, has this avenue of research regained momentum.

Organization of the Octopus Brain

As neuroscientists, we are not surprised to find that the octopus's advanced behavior is associated with dramatic developments in the structure of its nervous system. Due to an encephalization of the ganglionic masses in cephalopods, the octopus central nervous system is more similar to the vertebrate brain than to the ganglionic chain of its close relatives like the gastropods and bivalves (Young, 1971; Kandel, 1976; Budelmann *et al.*, 1997). The size of the modern cephalopod nervous system (normalized to body weight) lies within the same range as vertebrate nervous systems—smaller than birds and mammals but larger than fish and reptiles (Packard, 1972). In comparison with lower molluscs, the cephalopods show an extreme change in the number and organization of nerve cells. For example, the *Aplysia* nervous system contains about 20,000 neurons, whereas the nervous system of the octopus comprises about 500 million cells (Young, 1963).

The octopus nervous system is divided morphologically into three main parts and functionally into a hierarchy of

functional levels. Two major parts of the nervous system, the optic lobes and the nervous system of the arms, lie outside the brain capsule. The two large optic lobes contain 120 to 180 million neurons. The arm nervous system, containing two-thirds of the total 500 million neurons in the octopus nervous system, may function rather autonomously, as it can generate coordinated stereotypical movements (Altman, 1971; Sumbre *et al.*, 2001, 2005; Sumbre *et al.*, 2006). The central brain contains 40 to 45 million cells. Lying inside the cartilaginous brain capsule and surrounding the esophagus (Fig. 1A), the brain comprises 40 lobes that maintain a basically invertebrate organization with outer cell body layers and inner neuropil. The evolution of a massed central brain most likely occurred through shortening the connectives between various ganglia, decreasing the distance between the various integration centers to speed up the computational interactions between them (Budelmann, 1995). Stimulation and lesion experiments have helped assign possible functions to several of these lobes (reviewed in Wells, 1978; Boyle, 1986; Young, 1991, 1995).

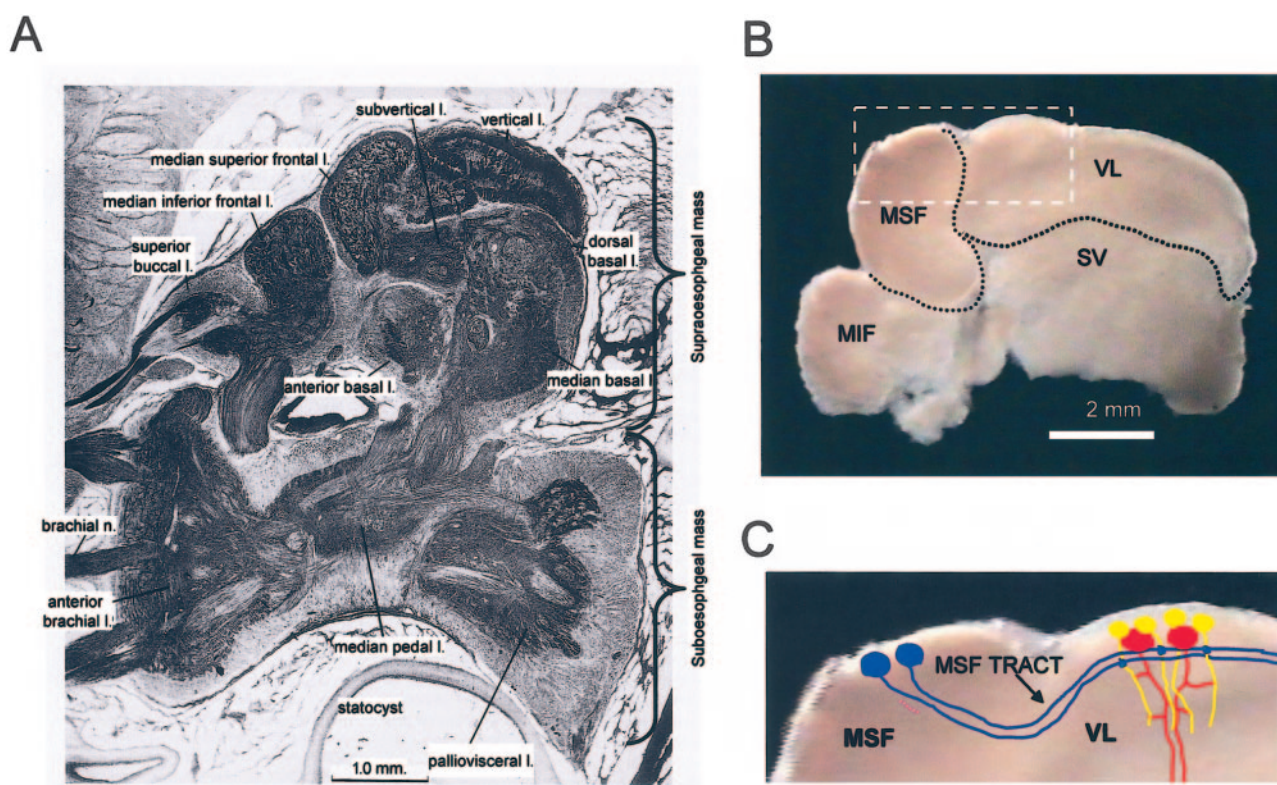


Figure 1. The slice preparation and the basic circuitry of the MSF-VL system. (A) A sagittal section in the central brain of octopus showing the sub- and supraesophageal masses. Note the location of the vertical and median superior frontal lobes (modified from Nixon and Young, 2003, by permission of Oxford University Press). (B) An image of a slice used in the physiological experiments. A sagittal slice from the medial part of the supraesophageal brain mass showing the vertical lobe (VL) and median superior frontal lobe (MSF) located dorsally to the median inferior frontal (MIF) and subvertical (SV) lobes. (C) The area within the white rectangle in B with a superimposed circuitry schema. MSF neurons (blue) innervating the VL via the MSF tract are shown schematically, as are the amacrine cells (yellow), which synapse onto the large efferent cells (red) (see Young, 1971).

Certain areas of the cephalopod brain are particularly interesting with respect to evolutionary convergence because they show a strikingly similar morphological organization to areas of the vertebrate brain that mediate similar functions. For example, the three cortical layers of the cephalopod optic lobe are organized similarly to the deeper layers in the vertebrate retina (Young, 1971). This similarity in the integrational layers is all the more striking because the mechanisms of transduction and physiological responses to light are totally different (*e.g.*, Hardie and Raghu, 2001). As in other invertebrates, the membrane potential of the octopus photoreceptor cells is depolarized (positive potential change) in response to light, whereas in vertebrates it is hyperpolarized (negative potential change). These opposite responses to light are mediated by two different second messenger cascades.

Similarly, the structure of the peduncle lobe, in which small granular cells give rise to arrays of thin parallel fibers, resembles the arrangement in the folia of the vertebrate cerebellum (Hobbs and Young, 1973; Young, 1976; Woodhams, 1977). The peduncle, together with higher motor centers in the basal lobes, receives inputs from both the visual and gravitational (statocysts) systems and has cerebellar-type effects on motor function (Messenger, 1967a,b). The parallel and linear organization of small-diameter fibers in the vertebrate and the octopus systems suggests the importance of this type of organization for the timing computations needed to integrate visual and gravitational information for body motor and eye coordination. Finally, the vertical lobe (VL), the highest in the central brain hierarchy, resembles the vertebrate hippocampus, both in its involvement in learning and memory and in its morphological organization (see below).

If these architectural similarities are the outcome of convergent evolutionary processes, they may highlight the importance of connectivity, rather than cell structure or cellular and biophysical properties in brain function.

Neurobiology of Learning and Memory

Specific lobes in the octopus supraesophageal mass are involved in learning and memory

Possibly exceeding the level of functional specificity of the vertebrate brain, specific areas in the octopus brain are dedicated to learning and memory. In particular, the VL appears essential for long-term learning and memory, a role first revealed in lesion experiments by Boycott and Young (1955a) and Maldonado (1965) (see also Wells, 1978; Young, 1991). Removal of the VL did not appear to affect the behavior of the animal; the lesioned animals stayed in their homes, came out to attack crabs, ate, walked, and swam normally. Similarly, stimulating the VL or the superior frontal lobes of animals produced no obvious effects, whereas stimulating other parts of the brain caused move-

ments of some part of the body. Stimulation and lesions produced similar results in cuttlefish (Boycott, 1961). Thus, the vertical and superior frontal lobes do not seem to be engaged in any basic motor functions.

Behavioral deficiencies after lesions or removal of the VL were revealed only when the animals had to learn new tasks or perform tasks, such as visual discrimination, learned before the operation. For example, after removal or lesion of the VL, an octopus continues to attack crabs in spite of receiving electrical shocks, unless the crab is presented several minutes after the shock (Boycott and Young, 1955a). Moreover, Fiorito and his colleagues showed that the VL is important for observational learning. A naive octopus needs to observe a trained octopus attacking a previously positively rewarded target only four times (much faster than it takes to train the demonstrator octopuses) before the observer itself prefers attacking the same target (Fiorito and Scotto, 1992). Lesioning or ablating the VL impairs observational short-term learning and affects long-term memory performance (Fiorito and Chichery, 1995). Taken together, all these experiments show that the VL is specifically involved in long-term and more complex forms of memory. Further indirect support for the involvement of the VL in learning was obtained in cuttlefish by showing a positive correlation between VL development and learning performance (Messenger, 1973; Dickel *et al.*, 2001). As far as we know, the classic work by Sanders and Young (1940) is the only study in which removal of the cuttlefish's VL was tested. The results demonstrated clear impairment in the ability of the animals to hunt, while the effect on learning was less significant.

The VL is composed of only two types of typical invertebrate monopolar neurons (Fig. 1); 25 million small amacrine interneurons (the smallest in the octopus brain, 6 to 10 μm dia) converge onto only 65,000 large neurons ($\sim 17 \mu\text{m}$ dia). The axons of the large cells form the only output of the VL, as the processes of the amacrine interneurons remain within the lobe. The lobe receives only two inputs, of which the largest numerically is the 1.8 million axons from the median superior frontal (MSF) lobe (Fig. 1; Young, 1971).

The MSF lobe, containing only one type of neuron, is thought to integrate sensory information (Young, 1971). The MSF neurons convey their integrated signals to the VL via a distinct tract running between the VL neuropil and its outer cell body layer (Fig. 1C). Similarly to the interaction between the Schaffer collaterals and the pyramidal cells in the hippocampal CA1 region, the VL amacrine cell neurites cross the MSF tract perpendicularly, enabling each of the 1.8 million MSF axons to make *en passant* synapses with many of the 25 million amacrine neurons in the VL (Fig. 1C, see Gray, 1970; Young, 1971). A similar organization is found in the projections from the median inferior frontal lobe to the subfrontal lobe, which plays a role in tactile learning (Fig. 1A; Wells, 1978; Young, 1991, 1995).

Drawing on extensive behavioral investigations, morphological data, and lesion studies, Boycott and Young (1955a) and Wells (1978) and their colleagues hypothesized organizational schemes for the visual and tactile learning and memory systems in the octopus brain. Although there are differences in the interpretation of the results (see Wells, 1978), it is clear that these systems consist of multiple matrices of intersecting axons, whose synapses may form associations between conjunctions of sensory signals (visual or tactile) and their outcomes (Wells, 1978; Young, 1991).

An additional interpretation arises from the numerical distribution of cells in the central brain of the octopus. The VL and the subfrontal lobes contain most of the cells in the central brain, ~ 25 and ~ 5 million cells, respectively, of a total of ~ 40 million cells. Most of them ($\sim 99\%$) are the smallest amacrine interneurons in the octopus nervous system (Young, 1971). In addition, uniquely to *O. vulgaris*, the VL is composed of five cylindrical gyri (Young, 1971), which, as in vertebrate brains, may increase the VL volume. It is tempting to speculate that the special selection pressures acting on the octopus to advance its memory led to the development of especially small brain neurons in an enlarged and folded lobe in order to aggregate such a large number of individual cells in the typical connectivity of the "learning and memory" lobes. The unique morphology of these lobes, which are remarkably different from the other, more typically molluscan parts of the octopus brain, suggest the importance of these features for neural networks involved in learning.

In summary, several morphological features in the learning and memory areas of the octopus brain show convergence to the organization of areas with similar functions in

vertebrate brains. To further explore this convergence, we need to physiologically characterize these brain circuits and their plastic properties. This will determine whether there has been cellular and functional convergence or whether older molluscan cell properties have been conserved. Such experiments, which are described below, have only recently become possible with the development of an *in vitro* slice preparation of the VL-MSF system, which allows intracellular recording from the small cells in the VL and extracellular recording of field potentials (Hochner *et al.*, 2003).

Electrophysiological characterization of neurons in the learning and memory area reveal properties common to invertebrates

The *in vitro* slice preparation permits intracellular recording in whole-cell configuration from the cells of the MSF-VL system (Fig. 2A) by using differential interference contrast optics together with the infrared video microscopy. This technique was developed for recording in live brain tissue of vertebrates. It allows better visualization of single neurons (Stuart *et al.*, 1993) and has been recently employed in slice preparations of cephalopod optical lobes (Chrachri and Williamson, 2004, 2005). Combining these methods with the whole-cell recording configuration allows characterization of the membrane properties and synaptic inputs of both the small amacrine cells and the large neurons in the outer cell body layer of the VL.

The slice is superfused with oxygenated artificial seawater at room temperature, and the method used for whole-cell recording is described in detail in Rokni and Hochner (2002). An example of a recording in whole-cell configu-

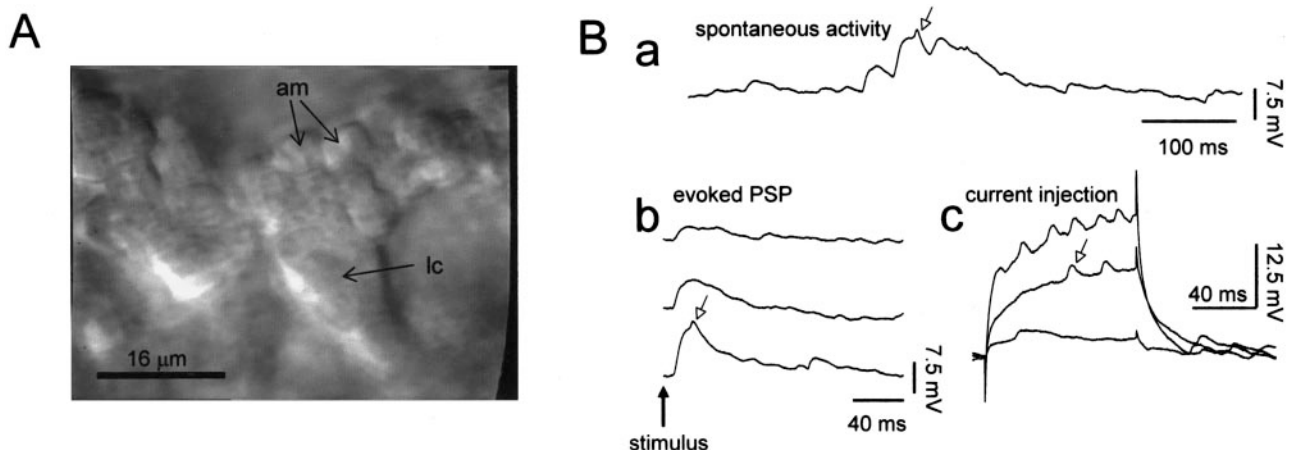


Figure 2. A slice preparation allows intracellular recording from the cells in the vertical lobe. (A) High-magnification image taken with infrared differential interference contrast optics, showing the amacrine cells (*am*) and the large cells (*lc*). (B) Intracellular recording in whole-cell configuration from a large cell in the vertical lobe. (Ba) Demonstration of the intense spontaneous, mainly excitatory, synaptic activity that can reach threshold for initiation of a small decremental spike (arrow). (Bb) Stimulation of the median superior frontal lobe (MSF) tract (at the beginning of each trace) generates intensity-dependent EPSPs which can initiate a spikelet. (Bc) Intracellular stimulation (0.04, 0.1, 0.2 nA, respectively) generates only a train of spikelets.

ration from a large VL cell is shown in Figure 2. There is high spontaneous, mostly excitatory, synaptic activity (Fig. 2Ba). Typical for invertebrate neurons, no overshooting action potentials could be detected at the cell body, neither by current injection (Fig. 2Bc) nor due to synaptic input (Fig. 2Bb). There were only spikelets (arrows) that were possibly decremental spikes generated at electrically remote sites.

The inexcitable nature of the neuron cell bodies in the VL and the MSF can also be demonstrated in whole-cell recordings from the cell bodies of enzymatically dissociated neurons. Such recordings were made from cell bodies dissociated together with neurites 50–150 μm long. Figure 3A shows the membrane potential responses to injections of current pulses into the cell body of an MSF cell. The voltage responses demonstrate graded active membrane properties and delayed rectification, but no spiking activity. The voltage clamp experiment in Figure 3B shows that there is no voltage range in which a large net inward current is recorded. This suggests that the active currents in this part of the cell are not sufficient to support generation of action potentials.

Taken together, these findings indicate that the morphological and electrical features of the neurons in these higher brain areas of the octopus conserve typical invertebrate properties of neurons — that is, a non-spike-generating cell body extends a neurite to the neuropil, where the dendritic tree ramifies and the axon begins its projection. The “integrate and fire zone” in efferent cells such as in the MSF or the large VL cells is most likely located somewhere along

the neurite, as often found in arthropod neurons. Cells in other areas of the octopus brain are similarly electrically inexcitable (Williamson and Budelmann, 1991; Miyan and Messenger, 1995). Note, however, that overshooting spikes in the cell bodies are a common feature in other molluscs (Kandel, 1976).

Field potential recording in the learning and memory area reveals a robust activity-dependent long-term potentiation

As in vertebrate preparations like the hippocampus slice, the MSF-VL slice preparation contains many cells of the same type, organized in parallel and in the same orientation. This feature allows recording of a coherent field potential when the cells are synchronously active. (The field potential is a linear summation of the small potential generated by the voltage drop of the extracellular current of each cell over the extracellular resistance. When cells are randomly oriented, the cells' currents cancel each other out.) That field potentials can be recorded in octopus brain slices may be related to the fact that compound electrical activity resembling the vertebrate EEG can also be recorded from the octopus brain. T.H. Bullock remarked that such EEG-like activity could be recorded in the octopus brain but not in other, less developed invertebrates (Bullock, 1984; Bullock and Basar, 1988). It is likely that the requirements of an advanced nervous system generate certain constraints of cell organi-

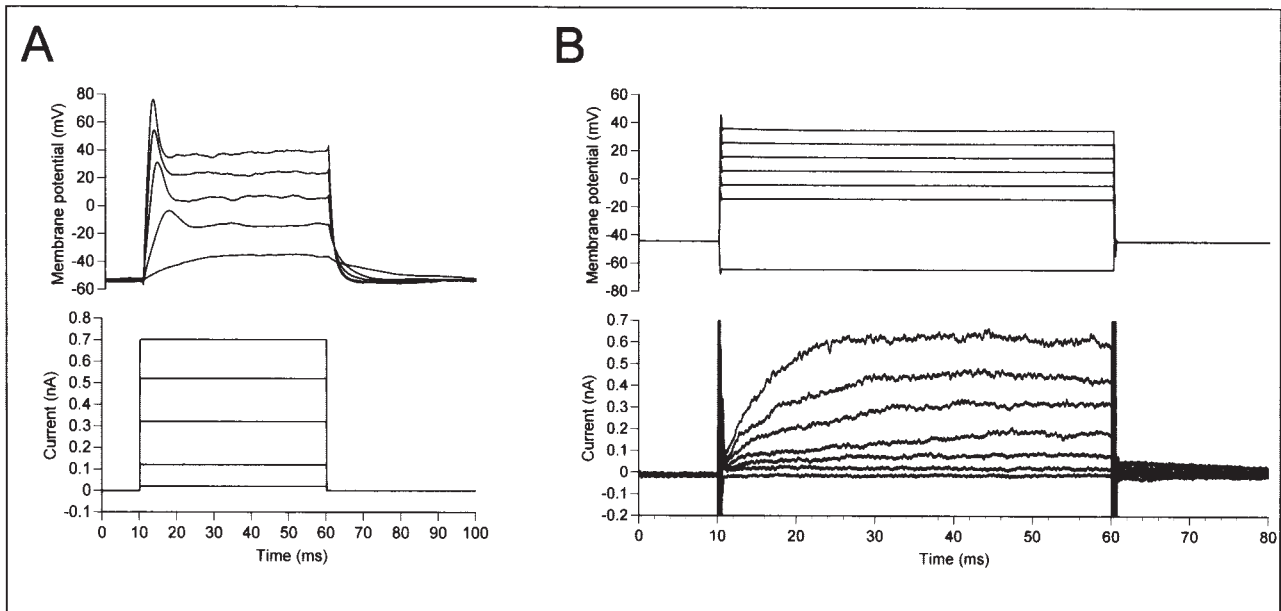


Figure 3. The inexcitable properties of small cells from the octopus brain. Examples for whole-cell current clamp (A) and voltage clamp (B) in dissociated cells from the MSF. (A) Only graded responses are induced by current injections. (B) Another cell voltage-clamped in a continuous single-electrode voltage-clamp mode. The membrane potential was held at -43 mV and stepped to different membrane potentials. Note the absence of net inward current and a prominent delayed rectification.

zation and orientation that allow extracellular recordings of compound and field potentials.

Due to the organization of the MSF-VL system, stimulating the tract from the MSF lobe generates typical field potentials in the VL close to the MSF tract (Fig. 1C). Such stimulation evokes a large positive-negative biphasic potential shortly after the stimulus artifact (Fig. 4A). This field potential is composed of activity in the axonal tract, followed by a second, smaller, mainly negative-going wave, which is a synaptic field potential (fPSP). As in vertebrates, this synaptic potential is a glutamatergic AMPA-like response, as it is blocked by CNQX and kynurenatate (Hochner *et al.*, 2003).

Tetanic stimulation of the MSF tract leads to a large and enduring increase in the synaptic field potential (Fig. 4A, B), suggesting an activity-dependent long-term enhancement of the synaptic connections. These results indicate the existence of robust plastic properties at the main synaptic input to the vertical lobe, which are similar to long-term potentiation (LTP) in the vertebrate brain. Co-operativity, specificity, gradation, and saturation—typical for Hebbian synapses—have also been demonstrated. The induction mechanisms of the LTP involve both postsynaptically-dependent and -independent mechanisms, as complete block

of the postsynaptic response with CNQX and kynurenatate blocked LTP induction in only about half the cases (Hochner *et al.*, 2003). None of these induction mechanisms seem to involve a vertebrate-like *N*-methyl-D-aspartate (NMDA)-like receptor, because the induction was not affected by 2-amino-5-phosphonovalerate or MK-801 (Hochner *et al.*, 2003). These findings are more significant because NMDA-like currents have been discovered in the chromatophore muscle cells of squid (Lima *et al.*, 2003). NMDA-like immunoreactivity has also been reported in the brains of cuttlefish and octopus (Di Cosmo *et al.*, 2004). However, as discussed in Hochner *et al.* (2003), the octopus LTP appear to more closely resemble the NMDA-independent LTP of the mossy fibers synapse in the CA3 region of the mammalian hippocampus (review by Nicoll and Schmitz, 2005).

Are plastic mechanisms of more primitive molluscs conserved in the octopus MSF-VL system?

Numerous studies in *Aplysia* have shown that serotonin (5-HT) plays a role in both short- and long-term sensory-motor synaptic plasticity, which are important in simple forms of learning and memory (Hawkins *et al.*, 2006).

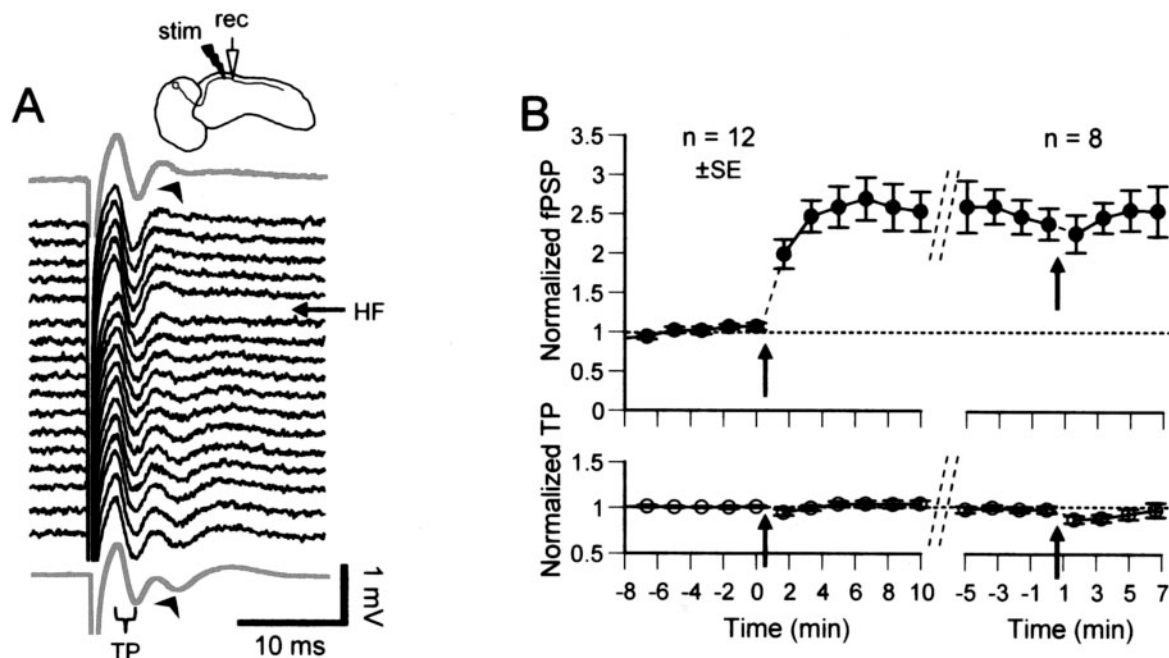


Figure 4. Field potential recording in the slice preparation reveals long-term potentiation in the median superior frontal lobe (MSF) to vertical lobe synapses. (A) *Inset* showing the experimental arrangement of the stimulating (stim) and recording (rec) electrodes near the MSF tract. The stimulus artifact was followed by a large tract field potential (TP) and a small postsynaptic field potential (fPSP) (arrowheads). Raw data traces (1/10s) show the development (in downward order) of a robust facilitation of the fPSP after 4 high-frequency tetani (HF, 20 stimuli at 50 Hz, 10-s interval) to the MSF tract (arrow HF). Gray traces; averages before tetanization (upper) and after (lower). (B) Time course of long-term potentiation development, maintenance, and saturability of 12 control experiments. In each experiment, the fPSP and TP amplitude are normalized to the averages before the HF. Arrows indicate 4 HF tetani. In 8 of the 12 experiments, a second HF was delivered (modified from Hochner *et al.*, 2003, used with permission from the American Physiological Society).

Shomrat *et al.* (2005) recently showed that 5-HT, localized immunohistochemically in the relevant area in octopus VL, also has a robust facilitatory effect on the fPSP amplitude, albeit in higher concentrations ($\sim 100 \mu M$) than found in *Aplysia*. Hochner *et al.* (2003) were previously unable to demonstrate any effects of 5-HT, probably because they used lower concentrations. The current preliminary results suggest that, in contrast to its effects in other molluscs, 5-HT has only short-term effects in the octopus VL; repeated application of 5-HT does not lead to long-term potentiation as it does in *Aplysia* (Montarolo *et al.*, 1986).

What are the molecular mechanisms that mediate learning and memory processes in octopuses?

Several pharmacological studies in cephalopods have shown the effects of drugs on behavioral learning and memory and thus suggest plausible biochemical mechanisms for the cellular mediation of these processes. There are indications for a possible involvement of nitric oxide (NO) in both learning and memory in octopus (Robertson *et al.*, 1994, 1996) and cuttlefish (Halm *et al.*, 2003). Moreover, extensive biochemical and immunohistochemical studies have demonstrated the presence of an NO system in the brain of cephalopods (review, Palumbo, 2005). Acetylcholine may be involved in visual learning, as Fiorito *et al.* (1998) found that scopolamine impairs octopus visual learning. Agin *et al.* (2003) reported that injecting a protein synthesis inhibitor, cycloheximide, interfered with cuttlefishes' retention of a behavioral task if it was injected between 1 and 4 h after training. Similarly, cytochalasin D blocks touch learning in *O. vulgaris* (Robertson, 1994). Although the site or sites of a drug's action are not clear in all these studies, it appears fruitful to investigate the effects of these modulators on the physiological properties of the MSF-VL slice preparation and its short- and long-term plastic properties.

Concluding Remarks

In this review we have introduced our invertebrate model—the cephalopod mollusc *Octopus vulgaris*. The nervous system of this invertebrate does not consist of “a small number of large identifiable neurons,” nor is it “amenable to genetic manipulation”—properties that make some invertebrates ideal for studying general questions in neurobiology. On the contrary, our interest in this unique invertebrate is based on its well-documented complex behavioral repertoires that we and others have shown to be comparable to those of vertebrates.

The findings emerging from recent electrophysiological studies in the octopus suggest that a convergent evolutionary process has led to the selection of similar networks and synaptic plasticity in evolutionarily very remote species that evolved to similar behaviors and modes of life. These evolutionary

considerations substantiate the importance of these cellular and morphological properties for neural systems that mediate complex forms of learning and memory. In particular, the similarity in the architecture and physiological connectivity of the octopus MSF-VL system to the mammalian hippocampus and the extremely high number of small interneurons in its areas of learning and memory suggest the importance of a large number of units that independently, by *en passant* innervation, form a high redundancy of connections. As these features are found in both the octopus MSF-VL system and the hippocampus, it would appear that they are needed to create a large capacity for memory associations.

Nevertheless, the convergence is not complete—not all features of the octopus MSF-VL system are analogous to those of the hippocampus. The octopus VL still maintains the basic morphological organization of invertebrate ganglia, with monopolar neurons whose cell bodies are organized in an outer layer and which send their processes into an internal neuropil (Bullock and Horridge, 1965). In addition, biophysical analysis reveals the inexcitable nature of the cell body membrane of the VL and MSF neurons, more typical of invertebrate neurons. Our findings thus suggest that the “language” of communication between neurons, which is determined by cell structure and membrane properties, is more species-specific. In contrast, it is the network structure and wiring, together with some kind of activity-dependent synaptic plasticity, that are fundamental for brain structures devoted to learning and memory.

Acknowledgments

Supported by the Smith Family Laboratory, the Hebrew University, and US-Israel Binational Science Foundation (to BH) and by Istituto Banco di Napoli, Fondazione (to GF). We thank Dr. Jenny Kien for suggestions and editorial assistance, and Dr. Letizia Zullo and Dr. Michael Kuba for their valuable help.

Literature Cited

- Agin, V., R. Chichery, E. Maubert, and M.-P. Chichery. 2003. Time-dependent effects of cycloheximide on long-term memory in the cuttlefish. *Pharmacol. Biochem. Behav.* **75**: 141–146.
- Altman, J. S. 1971. Control of accept and reject reflexes in the octopus. *Nature* **229**: 204–206.
- Aristotle. 1910. *Historia Animalium*, Tr. by D'Arcy Wentworth Thompson. Clarendon Press, Oxford.
- Aronson, R. B. 1991. Ecology, paleobiology and evolutionary constraint in the octopus. *Bull. Mar. Sci.* **49**: 245–255.
- Bitterman, M. E. 1966. Learning in the lower animals. (Comment). *Am. Psychol.* **21**: 1073.
- Bitterman, M. E., W. C. Corning, J. A. Dyal, and A. O. D. Willows. 1975. Critical Commentary. Pp. 139–145 in *Invertebrate Learning: Cephalopods and Echinoderms*. Plenum Press, New York.
- Boal, J. G. 1993. An assessment of complex learning in octopuses. Ph.D. dissertation, The University of North Carolina at Chapel Hill.

- Boal, J. G. 1996.** A review of simultaneous visual discrimination as a method of training octopuses. *Biol. Rev.* **71**: 157–190.
- Boal, J. G., A. W. Dunham, K. T. Williams, and R. T. Hanlon. 2000.** Experimental evidence for spatial learning in octopuses (*Octopus bimaculoides*). *J. Comp. Psychol.* **114**: 246–252.
- Boycott, B. B. 1954.** Learning in *Octopus vulgaris* and other cephalopods. *Pubbl. Stn. Zool. Napoli* **25**: 67–93.
- Boycott, B. B. 1961.** The functional organization of the brain of the cuttlefish *Sepia officinalis*. *Proc. R. Soc. Lond. B Biol. Sci.* **153**: 503.
- Boycott, B. B., and J. Z. Young. 1955a.** A memory system in *Octopus vulgaris* Lamarck. *Proc. R. Soc. Lond. B Biol. Sci.* **143**: 449–480.
- Boycott, B. B., and J. Z. Young. 1955b.** Memories controlling attacks on food objects by *Octopus vulgaris* Lamarck. *Pubbl. Stn. Zool. Napoli* **27**: 232–249.
- Boyle, P. R. 1986.** Neural control of cephalopod behavior. Pp. 1–99 in *The Mollusca*, A.O.D. Willows, ed. Academic Press, Orlando, FL.
- Budelmann, B. U. 1995.** The cephalopods nervous system: what evolution has made of the molluscan design. Pp. 115–138 in *The Nervous System of Invertebrates: An Evolutionary and Comparative Approach* O. Breidbach, and W. Kutsch, eds. Birkhauser Verlag, Basel.
- Budelmann, B. U. 1996.** Active marine predators: the sensory world of cephalopods. *Mar. Freshw. Behav. Physiol.* **27**: 59.
- Budelmann, B. U., R. Schipp, and S. von Boletzky. 1997.** Cephalopoda. Pp. 119–414 in *Microscopic Anatomy of Invertebrates*, Vol. 6A, *Mollusca*, F.W. Harrison and A. Kohn, eds. Wiley-Liss, New York.
- Bullock, T. H. 1984.** Ongoing compound field potentials from octopus brain are labile and vertebrate-like. *Electroencephalogr. Clin. Neurophysiol.* **57**: 473–483.
- Bullock, T. H., and E. Basar. 1988.** Comparison of ongoing compound field potentials in the brains of invertebrates and vertebrates. *Brain Res.* **472**: 57–75.
- Bullock, T. H., and G. A. Horridge. 1965.** *Structure and Function in the Nervous Systems of Invertebrates*. Freeman, San Francisco.
- Chrachri, A., and R. Williamson. 2004.** Cholinergic and glutamatergic spontaneous and evoked excitatory postsynaptic currents in optic lobe neurons of cuttlefish, *Sepia officinalis*. *Brain Res.* **1020**: 178.
- Chrachri, A., and R. Williamson. 2005.** Dopamine modulates synaptic activity in the optic lobes of cuttlefish, *Sepia officinalis*. *Neurosci. Lett.* **377**: 152.
- Clarke, M. R., and E. R. Trueman. 1988.** Evolution of recent cephalopods: a brief review. P. 331 in *The Mollusca*, vol. 12, *Paleontology and Neontology of Cephalopods*, K.M. Wilbur, ed. Academic Press, New York.
- Crancher, P., M. P. King, A. Bennet, and R. B. Montgomery. 1972.** Conditioning of a free operant in *Octopus cyaneus* Gray. *J. Exp. Anal. Behav.* **17**: 359–362.
- Demski, L. S. 1992.** Chromatophore systems in teleosts and cephalopods: a levels oriented analysis of convergent systems. *Brain Behav. Evol.* **40**: 141.
- Dews, P. M. 1959.** Some observations on an operant in the octopus. *J. Exp. Anal. Behav.* **2**: 57.
- Di Cosmo, A., M. Paolucci, and C. Di Cristo. 2004.** N-methyl-D-aspartate receptor-like immunoreactivity in the brain of *Sepia* and *Octopus*. *J. Comp. Neurol.* **477**: 202–219.
- Dickel, L., M.-P. Chichery, and R. Chichery. 2001.** Increase of learning abilities and maturation of the vertical lobe complex during postembryonic development in the cuttlefish, *Sepia*. *Dev. Psychobiol.* **39**: 92–98.
- Dilly, P. N. 1963.** Delayed responses in *Octopus*. *J. Exp. Biol.* **40**: 393–401.
- Eisthen, H. L., and K. C. Nishikawa. 2002.** Convergence: obstacle or opportunity? *Brain Behav. Evol.* **59**: 235.
- Fiorito, G., and R. Chichery. 1995.** Lesions of the vertical lobe impair visual discrimination learning by observation in *Octopus vulgaris*. *Neurosci. Lett.* **192**: 117–120.
- Fiorito, G., and P. Scotto. 1992.** Observational learning in *Octopus vulgaris*. *Science* **256**: 545–547.
- Fiorito, G., C. von Planta, and P. Scotto. 1990.** Problem solving ability of *Octopus vulgaris* Lamarck (Mollusca, Cephalopoda). *Behav. Neural Biol.* **53**: 217–230.
- Fiorito, G., C. Agnisola, M. d'Addio, A. Valanzano, and G. Calamandrei. 1998.** Scopolamine impairs memory recall in *Octopus vulgaris*. *Neurosci. Lett.* **253**: 87–90.
- Flash, T., and B. Hochner. 2005.** Motor primitives in vertebrates and invertebrates. *Curr. Opin. Neurobiol.* **15**: 660–666.
- Goldsmith, M. 1917.** Acquisition d'une habitude chez le Poulpe. *C.R. Acad. Sci. Ser. III Life Sci.* **764**: 737–738.
- Gray, E. G. 1970.** The fine structure of the vertical lobe of octopus brain. *Phil. Trans. R. Soc. Lond. B* **258**: 379–394.
- Halm, M. P., M. P. Chichery, and R. Chichery. 2003.** Effect of nitric oxide synthase inhibition on the manipulative behaviour of *Sepia officinalis*. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **134**: 139.
- Hanlon, R. T., and J. B. Messenger. 1996.** *Cephalopod Behaviour*, Cambridge University Press, Cambridge.
- Hardie, R. C., and P. Raghu. 2001.** Visual transduction in *Drosophila*. *Nature* **413**: 186–193.
- Hawkins, R. D., E. R. Kandel, and C. H. Bailey. 2006.** Molecular mechanisms of memory storage in *Aplysia*. *Biol. Bull.* **210**: 174–191.
- Hobbs, M. J., and J. Z. Young. 1973.** A cephalopod cerebellum. *Brain Res.* **55**: 424–430.
- Hochner, B. 2004.** Octopus nervous system. In *Encyclopedia of Neuroscience*, 3rd ed. G. Adelman and B. Smith, eds. Elsevier B.V. Amsterdam. [CD-ROM.]
- Hochner, B., E. R. Brown, M. Langella, T. Shomrat, and G. Fiorito. 2003.** A learning and memory area in the octopus brain manifests a vertebrate-like long-term potentiation. *J. Neurophysiol.* **90**: 3547–3554.
- Kandel, E. R. 1976.** *Cellular Basis of Behavior: An Introduction to Behavioral Neurobiology*. W.H. Freeman, San Francisco.
- Lima, P. A., G. Nardi, and E. R. Brown. 2003.** AMPA/kainate and NMDA-like glutamate receptors at the chromatophore neuromuscular junction of the squid: role in synaptic transmission and skin patterning. *Eur. J. Neurosci.* **17**: 507–516.
- Mackintosh, N. J. 1965.** Discrimination learning in the octopus. *Anim. Behav. Suppl.* **1**: 129–134.
- Mackintosh, N. J., and J. Mackintosh. 1963.** Reversal learning in *Octopus vulgaris* Lamarck with and without irrelevant cues. *Q. J. Exp. Psychol.* **15**: 236–242.
- Maldonado, H. 1963a.** The positive learning process in *Octopus vulgaris*. *Z. Vgl. Physiol.* **47**: 191.
- Maldonado, H. 1963b.** The visual attack learning system in *Octopus vulgaris*. *J. Theor. Biol.* **5**: 470.
- Maldonado, H. 1964.** The control of attack by *Octopus*. *Z. Vgl. Physiol.* **47**: 656–674.
- Maldonado, H. 1965.** The positive and negative learning process in *Octopus vulgaris* Lamarck: influence of the vertical and median superior frontal lobes. *Z. Vgl. Physiol.* **51**: 185–203.
- Mather, J. A. 1991.** Navigation by spatial memory and use of visual landmarks in octopuses. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **168**: 491–497.
- Mather, J. A. 1995.** Cognition in cephalopods. *Adv. Study Behav.* **24**: 317–353.
- Messenger, J. 1973.** Learning performance and brain structure: a study in development. *Brain Res.* **58**: 519–523.
- Messenger, J. B. 1967a.** The effects on locomotion of lesions to the

- visuo-motor system in octopus. *Proc. R. Soc. Lond. B Biol. Sci.* **167**: 252–281.
- Messenger, J. B. 1967b.** The peduncle lobe: a visuo-motor centre in octopus. *Proc. R. Soc. Lond. B Biol. Sci.* **167**: 225–251.
- Miyan, J. A., and J. B. Messenger. 1995.** Intracellular recordings from the chromatophore lobe of *Octopus*. Pp. 415–429 in *Cephalopod Neurobiology*, J.N. Abbott, R. Williamson, and L. Maddock, eds. Oxford University Press, Oxford.
- Montarolo, P. G., P. Goelet, V. F. Castellucci, J. Morgan, E. R. Kandel, and S. Schacher. 1986.** A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in *Aplysia*. *Science*. **234**: 1249–1254.
- Moriyama, T., and Y. P. Gunji. 1997.** Autonomous learning in maze solution by *Octopus*. *Ethology* **103**: 499–513.
- Muntz, W. R. A., N. S. Sutherland, and J. Z. Young. 1962.** Simultaneous shape discrimination in *Octopus* after removal of the vertical lobe. *J. Exp. Biol.* **39**: 557–566.
- Muntz, W. R. A., S. N. Archer, M. B. A. Djamgoz, E. R. Loew, J. C. Partridge, and S. Vallerga. 1999.** Visual systems, behaviour, and environment in cephalopods. P. 467 in *Adaptive Mechanisms in the Ecology of Vision*. Kluwer Academic Publishers, Dordrecht.
- Nicoll, R. A., and D. Schmitz. 2005.** Synaptic plasticity at hippocampal mossy fiber synapses. *Nat. Rev. Neurosci.* **6**: 863.
- Nishikawa, K. C. 2002.** Evolutionary convergence in nervous systems: insights from comparative phylogenetic studies. *Brain Behav. Evol.* **59**: 240.
- Nixon, M., and J. Z. Young. 2003.** *The Brain and Lives of Cephalopods*. Oxford University Press, Oxford.
- O'Dor, R. K., and D. M. Webber. 1986.** The constraints on cephalopods: why squid aren't fish. *Can. J. Zool.* **64**: 1591.
- Ogura, A., K. Ikeo, and T. Gojobori. 2004.** Comparative analysis of gene expression for convergent evolution of camera eye between octopus and human. *Genome Res.* **14**: 1555–1561.
- Packard, A. 1963.** The behaviour of *Octopus vulgaris*. *Bull. Inst. Oceanogr. (Monaco)* **1D**: 35–49.
- Packard, A. 1972.** Cephalopods and fish: the limits of convergence. *Biol. Rev.* **47**: 241–307.
- Packard, A., E. R. Trueman, and M. R. Clarke. 1988.** The skin of cephalopods (coleoids): general and special adaptations. P. 37 in *The Mollusca*, Vol. 11, *Form and Function*, K.M. Wilbur, ed. Academic Press, New York.
- Palumbo, A. 2005.** Nitric oxide in marine invertebrates: a comparative perspective. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **142**: 241.
- Papini, M. R., and M. E. Bitterman. 1991.** Appetitive conditioning in *Octopus cyanea*. *J. Comp. Psychol.* **105**: 107–114.
- Rhodes, J. M. 1963.** Simultaneous discrimination in octopus. *Pubbl. Stn. Zool. Napoli* **33**: 83–91.
- Robertson, J. D. 1994.** Cytochalasin D blocks touch learning in *Octopus vulgaris*. *Proc. Biol. Sci.* **258**: 61–66.
- Robertson, J. D., J. Bonaventura, and A. P. Kohm. 1994.** Nitric oxide is required for tactile learning in *Octopus vulgaris*. *Proc. Biol. Sci.* **256**: 269–273.
- Robertson, J. D., J. Bonaventura, A. Kohm, and M. Hiscat. 1996.** Nitric oxide is necessary for visual learning in *Octopus vulgaris*. *Proc. Biol. Sci.* **263**: 1739–1743.
- Rokni, D., and B. Hochner. 2002.** Ionic currents underlying fast action potentials in the obliquely striated muscle cells of the octopus arm. *J. Neurophysiol.* **88**: 3386–3397.
- Rose, S. P. R. 2003.** *The making of memory: from molecules to mind*. Anchor Books, New York.
- Sanders, F. K., and J. Z. Young. 1940.** Learning and other functions of the higher nervous centres of *Sepia*. *J. Neurophysiol.* **3**: 501.
- Sanders, G. D. 1975.** The cephalopods. Pp. 139–145 in *Invertebrate Learning*, W.C. Corning, J.A. Dyal, and A.O.D. Willows, eds. Plenum Press, New York.
- Shomrat, T., N. Feinstein, M. Klein, and B. Hochner. 2005.** The involvement of serotonin and octopamine in short- and long-term plasticity in the octopus vertical lobe. Abstract 655.10 [Online: Abstract Viewer/Itinerary Planner]. Society for Neuroscience, Washington, DC.
- Stuart, G. J., H. U. Dodt, and B. Sakmann. 1993.** Patch-clamp recordings from the soma and dendrites of neurons in brain slices using infrared video microscopy. *Pflug. Arch.* **423**: 511–518.
- Sumbre, G., Y. Gutfreund, G. Fiorito, T. Flash, and B. Hochner. 2001.** Control of octopus arm extension by a peripheral motor program. *Science* **293**: 1845–1848.
- Sumbre, G., G. Fiorito, T. Flash, and B. Hochner. 2005.** Neurobiology: motor control of flexible octopus arms. *Nature* **433**: 595–596.
- Sumbre, G., G. Fiorito, T. Flash, and B. Hochner. 2006.** Octopuses use a human-like strategy to control precise point-to-point arm movements. *Curr. Biol.* **16**: 767–772.
- Sutherland, N. S. 1959.** Visual discrimination of shape by octopus: circles and squares, and circles and triangles. *Q. J. Exp. Psychol.* **11**: 24–32.
- Sutherland, N. S. 1961.** Discrimination of horizontal and vertical extents by *Octopus*. *J. Comp. Physiol. Psychol.* **54**: 43–48.
- Teichert, C., M. R. Clarke, and E. R. Trueman. 1988.** Main features of cephalopod evolution. P. 11 in *The Mollusca*, Vol. 12, *Paleontology and Neontology of Cephalopods*, K.M. Wilbur, ed. Academic Press, New York.
- Wells, M. J. 1964.** Tactile discrimination of shape by *Octopus*. *J. Exp. Psychol.* **16**: 156–624.
- Wells, M. J. 1978.** *Octopus*. Chapman and Hall, London.
- Williamson, R., and B. U. Budelmann. 1991.** Convergent inputs to *Octopus* oculomotor neurones demonstrated in a brain slice preparation. *Neurosci. Lett.* **121**: 215–218.
- Williamson, R., and A. Chrachri. 2004.** Cephalopod neural networks. *Neurosignals* **13**: 87.
- Woodhams, P. L. 1977.** The ultrastructure of a cerebellar analogue in octopus. *J. Comp. Neurol.* **174**: 329–345.
- Yekutieli, Y., G. Sumbre, T. Flash, and B. Hochner. 2002.** How to move with no rigid skeleton? The octopus has the answers. *Biologist (Lond.)* **49**: 250–254.
- Young, J. Z. 1956.** Visual responses by *Octopus* to crabs and other figures before and after training. *J. Exp. Biol.* **33**: 709–729.
- Young, J. Z. 1959.** Extinction of unrewarded responses in *Octopus*. *Pubbl. Stn. Zool. Napoli* **31**: 225–247.
- Young, J. Z. 1963.** The number and sizes of nerve cells in *Octopus*. *Proc. Zool. Soc. Lond.* **140**: 229.
- Young, J. Z. 1971.** *The Anatomy of the Nervous System of Octopus vulgaris*. Clarendon Press, Oxford.
- Young, J. Z. 1976.** The 'cerebellum' and the control of eye movements in cephalopods. *Nature* **264**: 572–574.
- Young, J. Z. 1985.** Cephalopods and neuroscience. (Naples Suppl.) *Biol. Bull.* **168**: 153–158.
- Young, J. Z. 1991.** Computation in the learning system of cephalopods. *Biol. Bull.* **180**: 200–208.
- Young, J. Z. 1995.** Multiple matrices in the memory system of octopus. Pp. 431–443 in *Cephalopod Neurobiology*, J.N. Abbott, R. Williamson, and L. Maddock, eds. Oxford University Press, Oxford.