

# The Neurophysiological Basis of Learning and Memory in Advanced Invertebrates

## The Octopus and the Cuttlefish

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### INTRODUCTION

It is commonly believed that invertebrates should be used for studying general questions in neurobiology only when their specific features, such as “a small number of large identifiable neurons,” generation of simple stereotypical behaviors, or their amenity to genetic manipulation, provide a special experimental advantage. These characteristics aid in unraveling cellular and synaptic processing, as well as the neuroethology of relatively simple behaviors. In contrast, invertebrates are thought not to be very useful for examining the neuroethological bases of complex behavior.

However, this approach—ignoring the potential of invertebrates for the analysis of complex behavior—has neglected three facts. First, some invertebrates exhibit behaviors as complex as those of vertebrates and, correspondingly, possess large nervous systems. Second, the nervous systems of invertebrates showing complex behavior (some insects and the modern cephalopods (Coleoidea) octopus, squid, and cuttlefish) still show the typical “simple” invertebrate organization and thus are more amenable to exploration of the neural processes involved in complex brain functions. Third, unlike vertebrates, certain invertebrates may have highly distinct brain structures—like the mushroom bodies of insects and the vertical lobe of cephalopods. The great anatomical differences from the rest of the nervous system indicate special functions and, indeed, both structures subserve the learning component of these animals’ complex behaviors. Thus, comparative functional analysis of these

structures may reveal general principles of the neuronal organization of complex behavior and its evolution.

Research on modern cephalopods has shown that this is indeed the case. These invertebrates show behavioral repertoires comparable to those of higher vertebrates, including behaviors associated with the intelligent hunting and defense behaviors of solitary animals.<sup>1–6</sup> Their learning and memory behaviors are also similar to those of vertebrates.<sup>2–4,6–9</sup> However, their brains maintain the much simpler invertebrate organization.<sup>10</sup> This unique combination of a simple nervous system and complex behavior is especially advantageous for tackling the central question of how a nervous system controls complex behaviors. Comparing the cellular processes and neuronal circuitry of their learning and memory systems with those of other invertebrates and vertebrates may advance our understanding of their evolution and function. This comparative evolutionary approach can determine whether mechanisms subserving cognitive functions evolved convergently across widely diverse phyla or, alternatively, whether evolutionarily primitive mechanisms mediating simple forms of behaviors are conserved in more advanced animals and integrated in mediating complex behaviors.

Another important feature of the modern cephalopods has been revealed through fruitful collaborations between roboticists and biologists. In the approach commonly termed “bioinspired robotics,” engineers are trying to use biological principles to design more efficient robotic hardware (morphology, material, and actuation) and software (processing of

sensory information, decision making, and motor control).<sup>\*</sup> Analysis of the motor control strategies used to drive the cephalopods' long, flexible, and highly skilled arms is helping roboticists design robots with flexible arms for use in confined spaces.<sup>11</sup>

## THE CEPHALOPOD NERVOUS SYSTEM

The nervous system of modern cephalopods (Coleoidea) is uniquely divided into three parts: the central brain (50 million cells in *Octopus vulgaris*); the two optic lobes (each 80 million cells in octopus); and the peripheral nervous system that, uniquely for the octopus due to its eight long flexible arms, is numerically the largest, containing 320 million nerve cells. The central brain comprises tens of lobes in which the cell bodies of the monopolar neurons lie in the outer region of each lobe, whereas their processes form the central neuropil, as is usual in invertebrate ganglia (Figure 24.1).<sup>13,14</sup>

Early experiments indicated that the vertical lobe (VL) is not involved in simple motor functions. Stimulating it or the superior frontal lobes in cuttlefish or octopus evoked no obvious effects, whereas stimulating other parts of the brain caused movements of various body parts.<sup>15,16</sup> Removing the VL also did not appear to affect the animal's general behavior.<sup>9,17,18</sup> Deficiencies were revealed only when animals were required to learn new tasks. After removal or lesions of the VL, octopuses continued to attack crabs despite receiving electrical shocks, unless the intertrial interval was less than approximately 5 min.<sup>17</sup> The VL appears to be specifically involved in long-term and more complex forms of memory.

Lesions in the ventral part of the VL in the cuttlefish (*Sepia officinalis*) led to a marked impairment in the acquisition of spatial learning, whereas lesions in the dorsal part of the VL impaired long-term retention of spatial learning.<sup>19</sup> An interesting result obtained by Sanders and Young in 1940,<sup>20</sup> but with only two animals, was that removal of the cuttlefish VL did not affect the ability of the animals to attack a prawn as long as it remained in the cuttlefish's field of vision. Today, we interpret this result as

<sup>\*</sup>Note the difference between bioinspiration and biomimetics. Currently, bioinspiration seems a more reasonable approach because we still cannot produce artificial materials even close to the properties of the active materials evolved by natural selection. For example, roboticists are far from producing an active synthetic polymer yielding a strain and speed like myofilaments.

suggesting that the cuttlefish VL is involved in working memory.

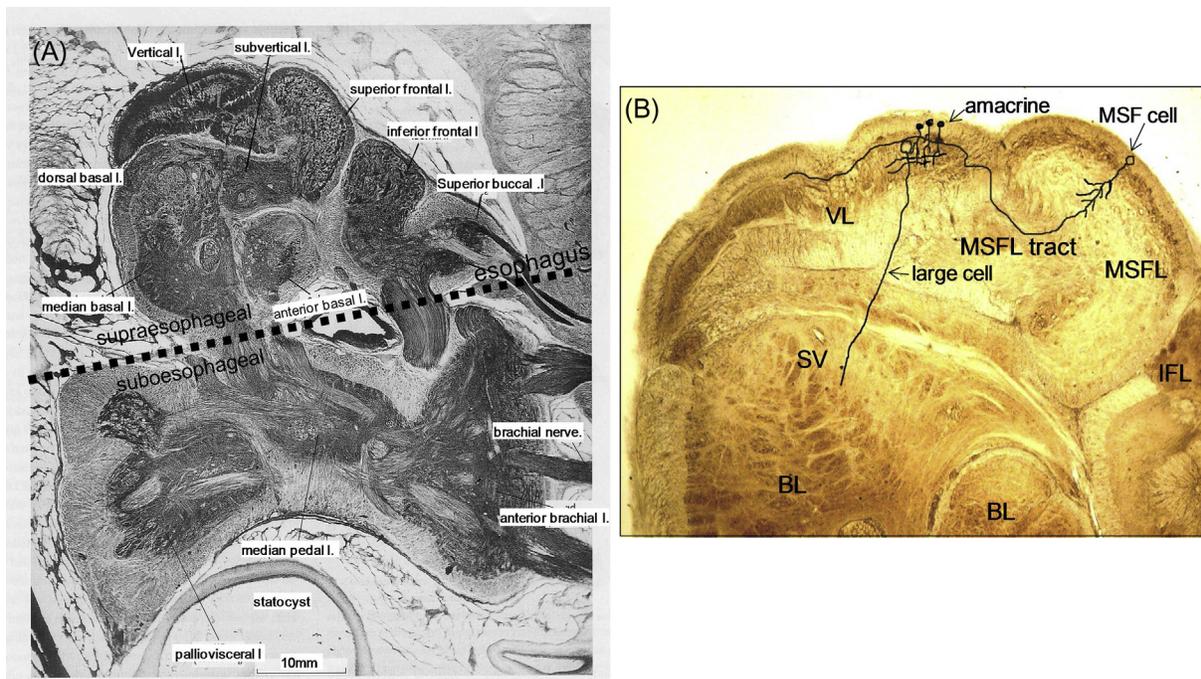
A complex form of learning demonstrated by octopuses is observational learning. A naive octopus learns to attack a previously positively rewarded target after only four observations of a trained octopus attacking the same target. This is much faster than it takes to train the demonstrator octopus.<sup>7</sup> A lesion study has demonstrated that the VL is important for this advanced form of learning.<sup>21</sup>

Finally, the morphological structure of the VL appears relatively simple, but its unique matrix-like organization led Boycott and Young<sup>17</sup> to postulate that the median superior frontal lobe (MSFL)–VL and inferior frontal networks (Figure 24.1) form associative networks for learning and memory. Wells<sup>2</sup> and Young<sup>22</sup> further suggested that the VL matrix is analogous to the mammalian hippocampus memory system and the insect mushroom bodies.<sup>22–24</sup> The VL system is thus an exciting site for exploring the neuronal circuitry and physiological mechanisms involved in various forms and phases of learning and memory.

## ANATOMY OF THE VERTICAL LOBE SYSTEM

The octopus VL contains only two types of neuron, amacrine cells and large efferent neurons, both of which are morphologically typical invertebrate monopolar neurons (Figure 24.2).<sup>13,25,26</sup> Twenty-five million amacrine cells, whose approximately 5- $\mu\text{m}$  diameter makes them the smallest neurons in the octopus brain, converge and synapse onto only approximately 65,000 large neurons ( $\sim 15\text{-}\mu\text{m}$  diameter). The amacrine cells are intrinsic interneurons because their processes remain within the VL (Figures 24.1 and 24.2). In contrast, the large neurons are efferent neurons whose axons form the only output of the VL, leaving organized axon bundles or roots that are easy to identify and record from in brain slice preparations (Figure 24.1B).

The octopus VL receives inputs from the MSFL, which is thought to integrate visual and taste information.<sup>13</sup> Note that although we use the acronym MSFL collectively, the superior frontal lobe (SFL) in the cuttlefish (Decapoda) is not divided into lateral and median parts as in the octopus. The octopus MSFL contains only one morphological type of neuron, whose 1.8 million axons project to the VL in a distinct tract running between the VL neuropil and its outer cell body layer (Figure 24.1). This arrangement allows each MSFL axon to make *en passant* synapses with many amacrine neurons along the VL (Figure 24.2). Young<sup>13</sup> postulated a direct connection to the large



**FIGURE 24.1 The morphological organization of the octopus central brain.** (A) Sagittal section of the sub- and supraesophageal lobes of *Octopus vulgaris* showing the dorsally located superior frontal (MSFL)–vertical lobes (VL) system and the organization of the lobes discussed in the text. (B) Unstained median sagittal section through the dorsal part of the supraesophageal brain mass with superimposed schematic drawing of the three types of neurons in this system and their connections. This area in the supraesophageal lobes (see panel A) forms the main part of the VL slice preparation. BL, basal lobes; IFL, inferior frontal lobe; SV, subvertical lobe. Source: Panel A modified from Nixon and Young.<sup>12</sup>

neurons, but this was not supported by Gray's<sup>25</sup> electron microscopic study nor by recent physiological results. The unusual matrix-like organization of the MSFL–VL complex is shared by the inferior frontal lobe (Figure 24.1), which is involved in chemotactile memory.<sup>2,22</sup>

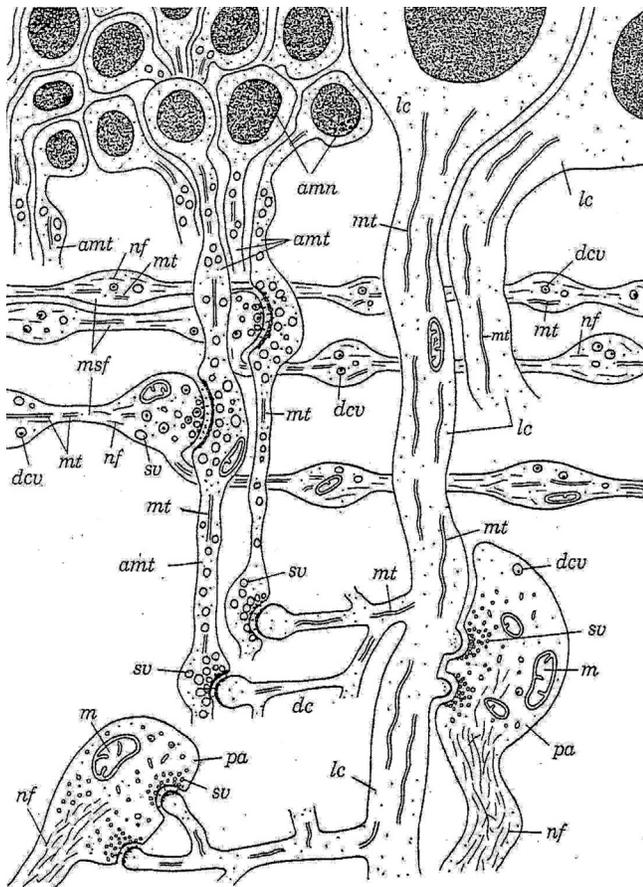
Although little is known about the targets of the axons of the large neurons, Young<sup>13</sup> suggested that some of the large VL neurons send their axons back into the lateral SFL, creating a recurrent loop between the VL and the SFL. Modern tracing techniques have clearly revealed such connections in cuttlefish (N. Graindorge, thesis). Such recurrent excitatory connections are computationally attractive because they may create a reverberatory circuit, which may subserve working memory by maintaining ongoing electrical activity.

Testing the hypothesis that the VL–SFL system forms associative networks for learning and memory clearly requires physiological characterization of these brain circuits and their plastic properties. New experimental preparations, such as the isolated brain, brain slice preparations, and a preparation for stimulating–recording in freely moving animals,<sup>16,27,28</sup> now allow neurophysiological exploration of this anatomically unique system.

## NEUROPHYSIOLOGY OF SFL INPUT TO THE OCTOPUS VERTICAL LOBE

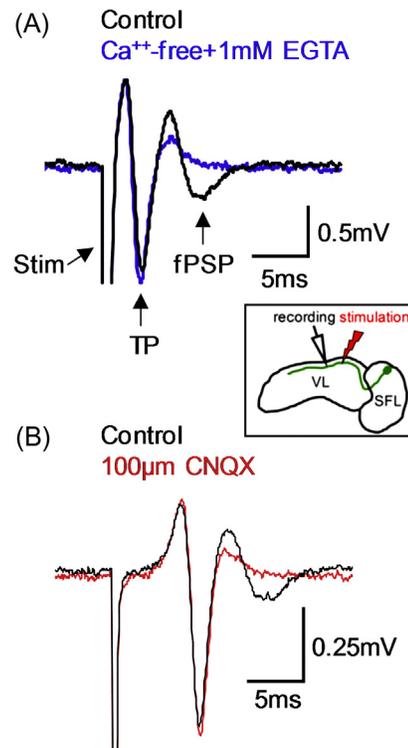
The VL and the inferior frontal lobe are unique structures in the cephalopod brain. Their large numbers of neurons are organized in layers with their processes aligned more or less in parallel, resembling vertebrae brain organization. The insect mushroom body is similarly organized; relatively few projecting neurons originating from the antennal lobe innervate a large number of small amacrine interneurons *en passant*.<sup>28</sup> This organization pattern, typical for “fan-out fan-in” networks, is quite unusual in the invertebrate nervous system. It is highly suggestive that it occurs in invertebrate brain structures involved in higher cognitive functions associated with learning and memory.

Unlike the more random organization of typical invertebrate ganglia, this layered organization generates a measurable external electric field near the active neurons. This field potential shows local field potentials (LFPs; spontaneous, ongoing background activity), evoked potentials and event-related potentials, and a compound field potential similar to an electroencephalogram.<sup>29</sup> Bullock<sup>30</sup> suggested that the existence of such a compound field potential indicates the high level of complexity of the octopus brain.



**FIGURE 24.2** Diagram showing what is thought to be the basic circuitry of the vertical lobe. amn, amacrine cells; amt, amacrine trunk; dc, dendritic collaterals of large cell; dcv, dense-core vesicle; lc, body or trunk of large cell; m, mitochondrion; msf, median superior frontal axon; mt, microtubule; nf, neurofilaments; pa, possible "pain" axon input to the large cell; sv, synaptic vesicles. Source: Reproduced from Gray<sup>25</sup> with permission.

The amount of current flowing in the extracellular space due to neuron activity, such as an action potential, is usually tiny. Because the extracellular impedance is very low (approximately three or four orders of magnitude lower than the neurons' input resistance), only summed field potentials generated by many neurons become large enough to be reliably detected against the background electrical noise. Such summation occurs only if the neurons are synchronously active and their currents flow in the same direction; otherwise, they would cancel each other out. This condition is achieved only when the neurons have long axons running parallel. Such an arrangement, which is common in vertebrate brains, occurs in the MSFL–VL system, in which the MSFL axonal input to the VL is organized in tracts. The cell bodies of the millions of amacrine interneurons lie in the outer zone of the VL lobe, whereas their axons project in parallel into the lobe, perpendicular to the MSFL axonal tract



**FIGURE 24.3** (A) Superimposed LFPs in control and after blocking postsynaptic field potentials (fPSP) in  $\text{Ca}^{2+}$ -free EGTA in artificial seawater. Stim, stimulus artifact; TP, tract potential. (B) Similar to panel A, showing blockade of fPSP by AMPA-like glutamatergic receptor antagonists CNQX. Inset: Schematic slice with a typical placement of the recording and stimulating electrodes.

(Figures 24.1 and 24.2). Such an architecture would be expected to generate a significant LFP, like that generated close to the Schaffer collaterals in the hippocampus.

Stimulating the SFL tract with short current pulse evokes a typical LFP waveform (Figure 24.3), a triphasic (positive–negative–positive) tract potential (TP) generated by the volley of action potential propagating along the stimulated axons in the SFL tract. The delay after the stimulus artifact depends on the distance between stimulus and recording electrodes. A mainly negative LFP follows immediately after the second positive wave of the TP. This potential is most likely a glutamatergic postsynaptic field potential (fPSP) because it disappears in zero-calcium physiological solution (Figure 24.3A) and is blocked by AMPA-like antagonists such as CNQX (Figure 24.3B), DNQX, or kynureinate.<sup>31</sup>

The short latency between the peak negativity of the TP and the fPSP onset ( $\sim 3$  msec; Figures 24.3A and 24.3B) suggests a monosynaptic delay. The physiological results thus agree well with Gray's anatomical scheme (Figure 24.2), in which the terminals of the SFL

axons synapse directly on the amacrine dendrites. That is, the first synaptic layer of the VL is likely a glutamatergic synaptic input from the SFL neurons onto the amacrine interneurons.

Recording LFPs from slices prepared from the cuttlefish VL show similar LFP characteristics to those found in octopus.<sup>32</sup> Thus, cuttlefish appear to have similar membrane properties and connectivity patterns despite significant differences in the overall anatomy of the two VL systems. The octopus VL is composed of five lobuli, whereas in the cuttlefish, the VL is a dome-shaped structure with no distinct enfoldings as in the octopus. However, the amacrine cells and the large neurons of the two systems are quite similar.

The first layer of the VL is similarly organized to the stratum radiatum of the hippocampus. In both cases, the collaterals/tract terminals synapse *en passant* with the dendrites of the pyramidal/amacrine cells, respectively. It is therefore not surprising that the LFP in the VL (presynaptic TP followed by fPSP; Figure 24.3) is similar to that evoked at the stratum radiatum by stimulating the Schaffer fiber collaterals in CA1. However, because the amacrine cells are inexcitable (i.e., they do not generate regenerative action potentials),<sup>8</sup> the fPSP exhibits an amplitude-independent waveform with no population spikes,<sup>27</sup> unlike the fPSP of the hippocampal CA1 pyramidal neurons.<sup>33</sup>

The amacrine cells innervate the large efferent neurons. In the octopus, this synaptic connection is achieved via specialized "serial" synapses (Figure 24.2),<sup>25</sup> in which the MSFL synaptic terminals contact the amacrine dendrite, which in turn is the presynaptic input to the large cells' (neurons) spines. To investigate the nature of this input to the large neurons, infrared differential interference contrast microscopy aided intracellular recording from their cell bodies, as well as extracellular recording of their spiking activity in their axon bundles. Figure 24.4A shows excitatory postsynaptic potentials (EPSPs) evoked by stimulating the MSFL tract of an octopus and spontaneous EPSPs in a large neuron. Although the fine anatomical details in the cuttlefish are not as well-known as in the octopus, intracellular recording from the large neurons in the cuttlefish VL revealed similar neurophysiological properties (Figure 24.5C) as those in the octopus (Figure 24.4A). Like most invertebrate neurons, the cell bodies of the large neurons are inexcitable, as demonstrated by the small non-overshooting spikelets (arrowheads in Figures 24.4A and 24.5C). These decrease in size passively as they pass along the single neurite to the cell body from a distant spike initiation zone at the junction between the dendritic tree and the axon.

In both cuttlefish and octopus, the synaptic input to the large neurons is cholinergic, with both evoked and spontaneous EPSPs being blocked by hexamethonium

(Figures 24.4A and 24.4B), a muscarinic receptor antagonist that also blocks the synaptic potential at the neuromuscular junctions of the octopus arm.<sup>34</sup> Hexamethonium also blocked both spontaneous and evoked spiking activity recorded from the large neuron axonal bundles (Figures 24.4B and 24.4D). As would be expected for glutamatergic synapses, the fPSP of the first synaptic layer in octopus and cuttlefish was unaffected by cholinergic antagonists (Figure 24.4C). Thus, the cholinergic synapse must be the amacrine-to-large neuron synapse. Both cholinergic and glutamatergic antagonists blocked the large neuron output as shown by recording from their axon bundles. These findings suggest that there is no strong direct connection from MSFL axons to the large neurons and that the main connections within the VL are the MSFL inputs onto the amacrine cells, which in turn innervate the large efferent neurons.<sup>32</sup>

The VL system of cuttlefish and octopus thus appears to be organized as a simple feed-forward fan-out fan-in type of network. This type of network architecture is frequently found among biological and artificial networks that learn to classify inputs when endowed with the synaptic plasticity that creates learning ability.<sup>35</sup> The first fan-out synaptic layer may create high dimensionality neural representations of the incoming sensory information in a form suitable for further processing at the fan-in layer.<sup>32</sup> If the VL system of octopus and cuttlefish possesses this architecture and it functions as a learning and memory network, then it is essential that there be synaptic plasticity at one or more of its synaptic sites.

### NEURONAL OUTPUT FROM THE VERTICAL LOBES OF OCTOPUS AND CUTTLEFISH DEMONSTRATES ACTIVITY-DEPENDENT LONG-TERM POTENTIATION

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The organization of the cephalopod lobes in the central brain offers a relatively simple means of measuring the input/output relationship of the VL stimulating the MSFL tract and of measuring the VL output by either recording intracellularly from the large neuron cell bodies or recording their spiking activity in the axonal bundles leaving the VL. Applying four high-frequency (HF) trains to the MSFL tract (20 pulses at 50 Hz) induces a robust activity-dependent LTP in the output of the VL in both octopus and cuttlefish (Figures 24.5A1 and 24.5B1 and Figures 24.5A2 and 24.5B2 respectively). Thus, the region in the coleoids brains associated with learning and memory is endowed with a property universally believed to be essential for networks mediating

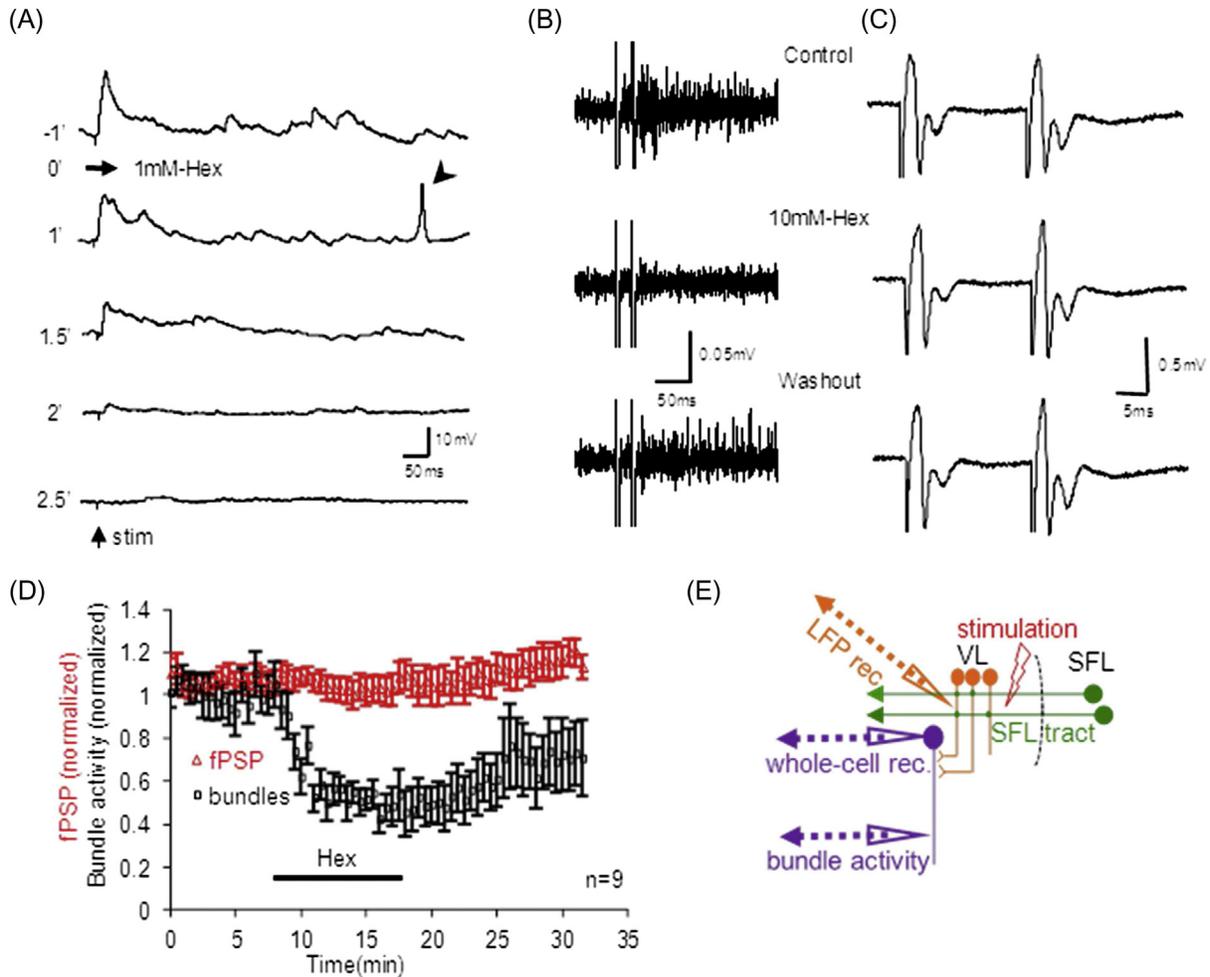


FIGURE 24.4 The synaptic inputs to the large cells are most likely cholinergic. (See panel E for the different recording modes). (A) Hexamethonium blocked the spontaneous and SFL tract-evoked EPSPs recorded intracellularly from a large neuron in the octopus (arrowheads indicate spikelet). (B) Hexamethonium blocked the burst of action potentials recorded extracellularly from large neuron axonal bundles. Twin stimuli were used to obtain a clearly measurable bundle response (B and C). (C) Hexamethonium had no effect on the TP and fPSPs. Records were obtained simultaneously with bundle activity shown in panel B. (D) Summary of nine experiments in octopus as exemplified in panels B and C. Black curve, normalized integrated bundle activity; red curve, fPSP amplitude. Here and in subsequent figures, responses were normalized to the average of 3–10 test responses at the beginning of the experiments. Source: Modified from Shomrat et al.<sup>32</sup>

behavioral learning and memory. What are the mechanisms of this neural plasticity? Are they important for cephalopod behavior?

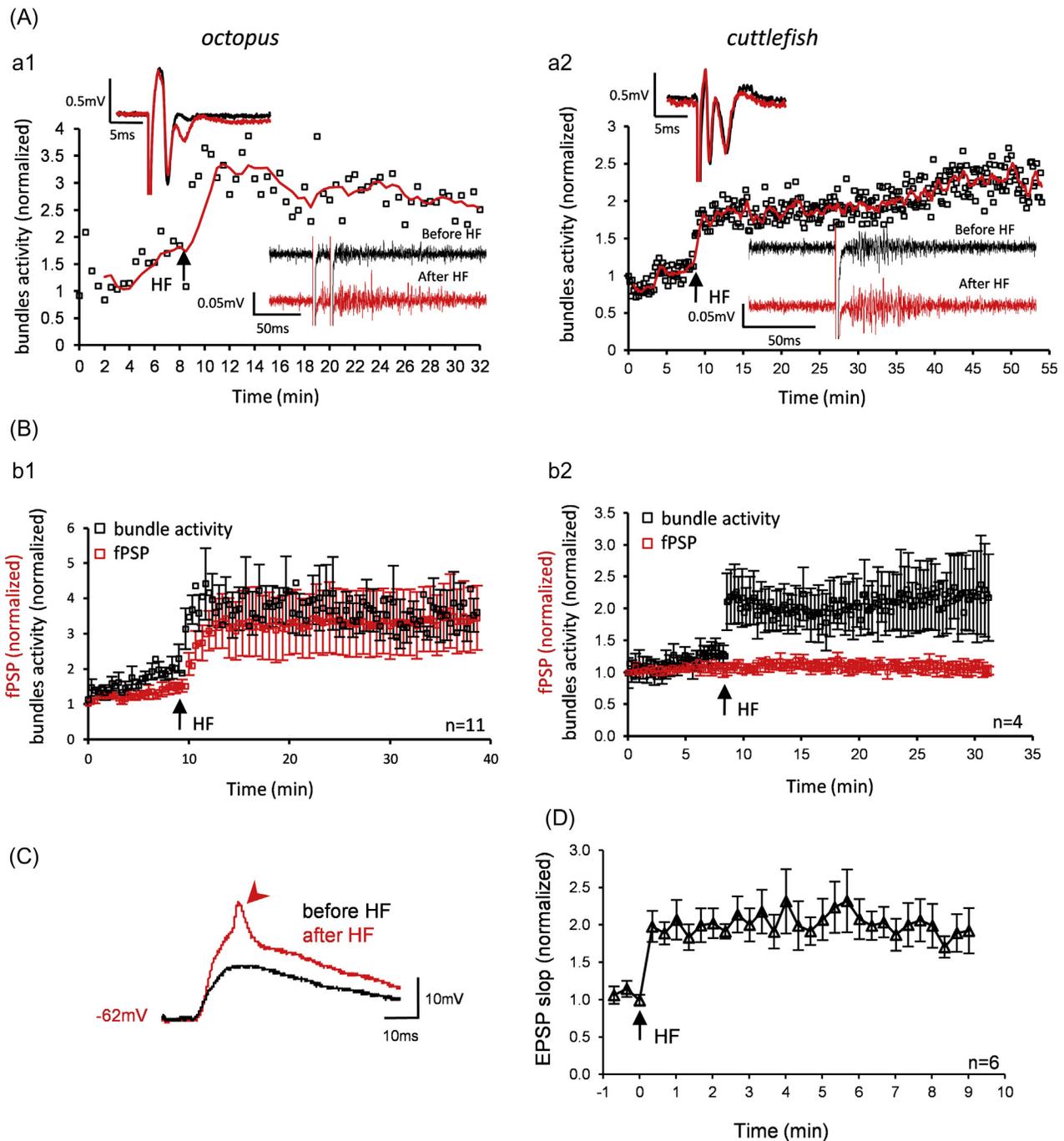
### SYNAPTIC PLASTICITY IN THE VERTICAL LOBES OF OCTOPUS AND CUTTLFISH

Examining the neurophysiological properties of synaptic transmission in the VL network revealed activity-dependent synaptic plasticity. Surprisingly, the synaptic plasticity is located at different sites in the VL of the octopus and cuttlefish.

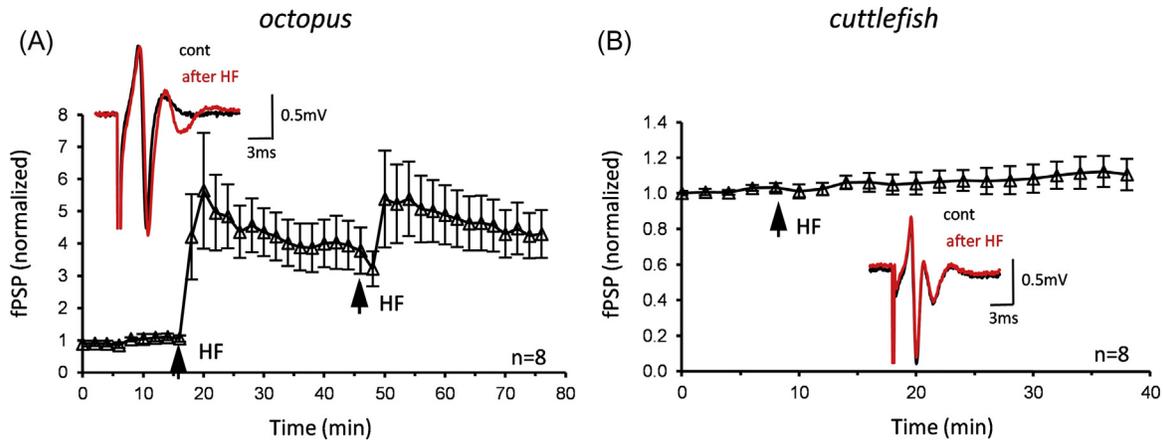
The local field potential recorded at the MSFL tract of the octopus demonstrates a very robust activity-

dependent plasticity. Tetanization led to a long-term potentiation (LTP) with an approximately fourfold increase in fPSP amplitude without affecting the amplitude of the presynaptic TP (Figure 24.6A, inset). The potentiation of the glutamatergic synaptic input to the amacrine neurons is long term. Tetanizing a second time showed that this LTP is a saturated phenomenon because no further long-term enhancement was obtained (Figure 24.6A, second HF). Surprisingly, the exact same experiments in the cuttlefish VL reveal no activity-dependent plasticity (Figure 24.6B).

Does activity-dependent plasticity occur only at the fan-out glutamatergic connections of the octopus VL (Figures 24.1 and 24.5)? As described previously, in the octopus, HF tetanization, which induced LTP of the fPSP, also caused a long-term increase in the extracellular



**FIGURE 24.5** Input/output relationships of the VL show LTP both in octopus (A1 and B1) and in cuttlefish (A2 and B2). (A1 and A2) Extracellular recordings from the axon bundles of the large cells. The development and maintenance of LTP as measured by the integrated activity in the axon bundles evoked by MSFL tract stimulation. Top insets: Superimposed LFPs before (black) and after (red) high-frequency (HF) stimulation. Bottom insets: activity of the large cells axon bundles before (black) and after (red) HF stimulation. (B1 and B2) Summary of experiments of the type shown in panels A1 and A2, respectively. The red curves show the fPSP amplitude. Note the absence of LTP of cuttlefish fPSP. The black curves show the integrated spiking activity recorded from the large cell axonal bundles. Both animals show LTP of the bundle activity. (C) Activity-induced LTP of the EPSP in the large cells of cuttlefish. Whole-cell intracellular recordings showing facilitation of the EPSP and a spikelet (red arrowhead) after HF stimulation of the MSFL tract. (D) Summary of six intracellular recording experiments as in panel C. LTP is expressed as an increase in slope of EPSP onset normalized to control. Source: *Modified from Shomrat et al.*<sup>32</sup>



**FIGURE 24.6** Long-term potentiation at the MSF-to-amacrine connections differs dramatically in octopus (left) and cuttlefish (right). (A) Summary of eight experiments showing the development, maintenance, and saturability of LTP in octopus. (B) Summary of eight experiments showing no significant change in the cuttlefish. LTP was induced by 4 HF trains (20 pulses, 50 Hz, 10-sec intertrain interval). fPSPs were normalized to the average of 10 test fPSPs at the beginning of each experiment. Inset: LFP traces before (black) superimposed on those obtained after HF stimulation (red). Source: *Modified from Shomrat et al.*<sup>32</sup>

spiking activity of the large neuron axon bundles (Figures 24.5A1 and 24.5B1). This result was not surprising: facilitation of the synaptic input to the amacrine cells (the fPSP) should increase their cholinergic input to the large neurons and thus enhance their output (i.e., increase the bundle activity induced by tract stimulation (Figure 24.5B1)). Therefore, this result does not reveal whether there is also synaptic plasticity at the input to the large neurons.

Analyzing the relationship between the amplitude of the fPSP and bundle activity showed this to be linear and unaffected by LTP induction.<sup>32</sup> Because the same fPSP amplitude gave rise to the same level of bundle activity irrespective of LTP, LTP in the octopus VL must occur only at the first fan-out synaptic layer. Indirect support for this interpretation is also apparent in Figure 24.5B1, which shows that the average relative increase in the fPSP amplitude was similar to the relative increase of the average bundle activity of the large neurons. Importantly, this result shows (1) that in the octopus only the glutamatergic input to the amacrine cells undergoes LTP and (2) that the output of the VL (the bundle activity) is linearly related to the input to the VL (the fPSP). This finding has important computational consequences (discussed later).<sup>32</sup>

In the cuttlefish, the site of synaptic plasticity mediating the activity-dependent increase in the VL output (Figures 24.5A2 and 24.5B2) is the cholinergic synaptic input to the large neurons. Intracellular recordings from the large neurons clearly show that HF stimulation of the SFL tract leads to a robust enhancement of the EPSP recorded from the large neuron (Figures 24.5C and 24.5D). Thus, whereas in the octopus the increase in VL output involves LTP of the glutamatergic connection onto the amacrine interneurons (the fan-out connections),

in the cuttlefish the LTP occurs at the converging or fan-in cholinergic connections of the amacrine neurons onto the large neurons (see connectivity scheme shown in Figure 24.12).

## WHAT DO THE VERTICAL LOBES OF OCTOPUS AND CUTTLEFISH COMPUTE?

It is possible that these differences between octopuses and cuttlefish mediate different behaviors yet to be systematically examined. However, computational considerations suggest a quite different possibility. The VL of both octopus and cuttlefish shows a linear relation between the fPSP amplitude and the integrated level of spiking activity in the axonal bundle of the large neurons (Figure 24.5B1)—that is, a linear input/output relationship of the VL (for a detailed explanation, see Shomrat *et al.*<sup>32</sup>). This linear input/output relationship has important computational consequences. In a linearly operating fan-out fan-in network, similar computation capacity is obtained regardless of whether the plasticity is localized at the fan-out or the fan-in layer.<sup>32</sup>

This computational consideration may answer the puzzling question of why these differences in network organization are found in phylogenetically close relatives possessing similar unusual characteristics (flexible body, similar sensory modalities, chromatophore system, and subcutaneous muscular system) and sharing comparable evolutionary adaptation to the same ecological niche and way of life (e.g., both are mainly solitary predators). If the two networks have indeed evolved to the same computational capacity, either via evolutionary selection or via “self-organizational” mechanisms, then computational constraints rather

than specific neural properties may determine the physical network properties.<sup>32</sup>

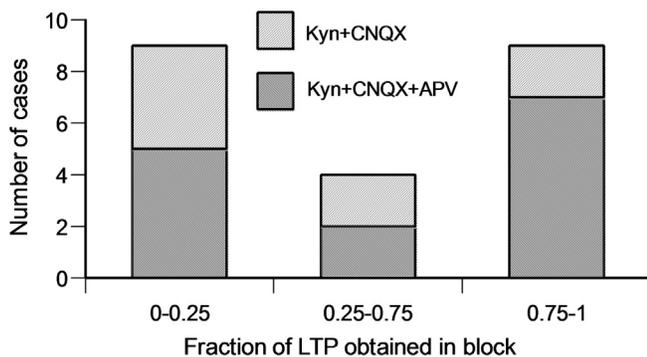
## MECHANISM OF LTP INDUCTION IN THE OCTOPUS VERTICAL LOBE

To understand the functional role of the VL network in learning and memory, we need to determine whether activity-dependent synaptic plasticity is mediated by associative or nonassociative mechanisms. That is, does the synaptic plasticity in the VL fulfill Hebb's rule for associative plasticity? This rule states that the synaptic connection between pre- and postsynaptic cells strengthens only when both are simultaneously and sufficiently active.<sup>36</sup> The NMDA channel, whose discovery was one of the most exciting breakthroughs in modern neuroscience, shows such a coincidence-detecting gating property. We therefore explored the possible involvement of such Hebbian mechanism in the fan-out stage in the octopus. Direct tests for the involvement of an NMDA-like receptor in the postsynaptic current (fPSP) or in LTP induction gave negative results; neither APV, which blocks NMDA-like current in cephalopod chromatophore muscles,<sup>37</sup> nor MK-801 blocked these phenomena<sup>27</sup> (Shomrat and Hochner, unpublished data).

The crucial test for whether the octopus VL has evolved an NMDA-independent Hebbian plasticity is to give the tetanization after completely blocking the postsynaptic response and then, after washing out the antagonists, to check whether LTP was induced. Such experiments show that the LTP in the octopus VL uses both associative and nonassociative induction mechanisms.<sup>27</sup> In slightly less than half the experiments, LTP

induction was largely blocked by glutamatergic antagonists (Figure 24.7, left); in the other experiments, there was hardly any blocking effect and LTP appears to have occurred in the absence of a postsynaptic response. If the two processes thus revealed were evenly distributed among the different synaptic connections in the VL, we would expect a normal distribution of the results. The bimodal distribution in Figure 24.7 can only be explained by the two types of plasticity being segregated in anatomically different regions, as in the hippocampus (CA3 vs. CA1).<sup>38</sup> Such differentiation has not been demonstrated morphologically in the octopus VL.

Characterizing associative/Hebbian-type activity-dependent synaptic plasticity requires determining whether LTP results from an increase in the amount of transmitter released or an increase in postsynaptic response or both—an issue that is still not completely resolved in the various hippocampal synapses. Detailed analysis of the changes in the properties of synaptic transmission accompanying LTP induction in the VL suggests that the expression of LTP is mainly, if not exclusively, presynaptic. That is, it occurs in the MSFL neuron terminals at their synapses with the amacrine neurons.<sup>31</sup> It is conceivable that this synapse, with its Hebbian type of LTP induction (Figure 24.7), involves some sort of retrograde messenger, such as nitric oxide (NO). This would transfer the association signal from the postsynaptic cell back to the presynaptic terminals to induce the LTP.<sup>39</sup> Supporting this possibility, drugs blocking NO production do interfere with tactile and visual learning in the octopus.<sup>40,41</sup> Furthermore, NO has been shown to be an important neuromodulator in invertebrates, a well-studied example being the digestive system of gastropods.<sup>42</sup>

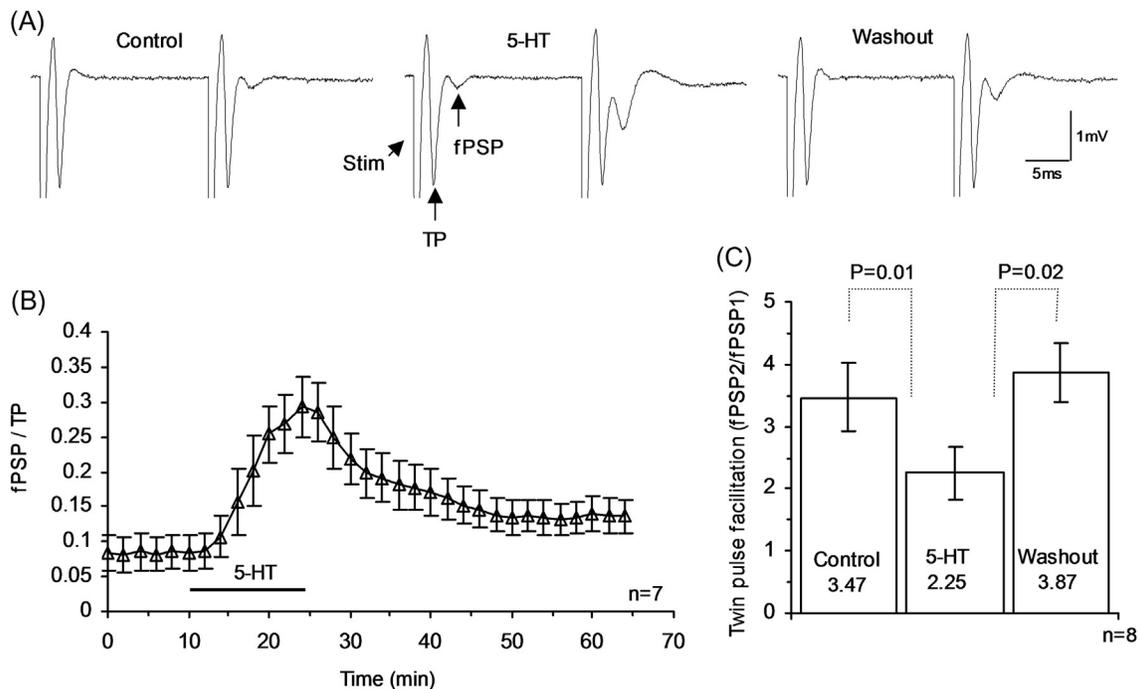


**FIGURE 24.7** Dependence of LTP induction on postsynaptic response. Summary of 22 experiments in which the response during HF tetanization was completely blocked by a mixture of 20 mM kynurenate plus 200  $\mu$ M CNQX and, in some experiments, also 200  $\mu$ M APV. Three ranges of LTP (expressed as the percentage of total LTP) were induced in the presence of the blocking mixture. The distribution shows a higher proportion of either largely blocked (0–25%) or hardly blocked (75–100%) LTP. Source: Reproduced from Hochner et al.<sup>27</sup>

## NEUROMODULATION IN THE VERTICAL LOBE

The highly dynamic properties of neural networks mediating learning and memory are achieved not only by various types of activity-dependent plasticity but also by neuromodulators. Neuromodulators may feed in negative or positive heterosynaptic reward signals that modulate the activity-dependent (homosynaptic) processes.<sup>43</sup> Such organization is well documented in both invertebrates and vertebrates and has most likely evolved to support supervised learning mechanisms.<sup>44–46</sup>

The first neuromodulator tested in the octopus VL was serotonin. Serotonin is a well-known neuromodulator in mollusks, involved in both short- and long-term facilitation of the sensorimotor synapse in the defensive reflex of *Aplysia californica*.<sup>47,48</sup> Immunohistochemistry showed serotonin reactivity in



**FIGURE 24.8** 5-HT induces short-term synaptic facilitation in the octopus. (A) Records from one experiment. Responses to stimulation by twin pulses. Stim, stimulation artifact; TP, tract potential. (B) Summary of seven experiments demonstrating the facilitatory effect of 100–200  $\mu$ M 5-HT and its reversal on washout. Test stimuli were applied every 10 or 20 sec. (C) 5-HT reversibly reduced the level of paired-pulse facilitation measured as the ratio of the second to the first fPSP amplitudes. Source: Modified from Shomrat et al.<sup>49</sup>

nerve terminals in the VL neuropil but not in the cell bodies, indicating that serotonin may convey signals to the VL from other brain or body areas.<sup>49</sup> In cuttlefish VL, Boyer *et al.*<sup>50</sup> described distributed serotonin reactivity mainly in fibers in the neuropile, but in contrast to the octopus VL, some cell bodies were also labeled.

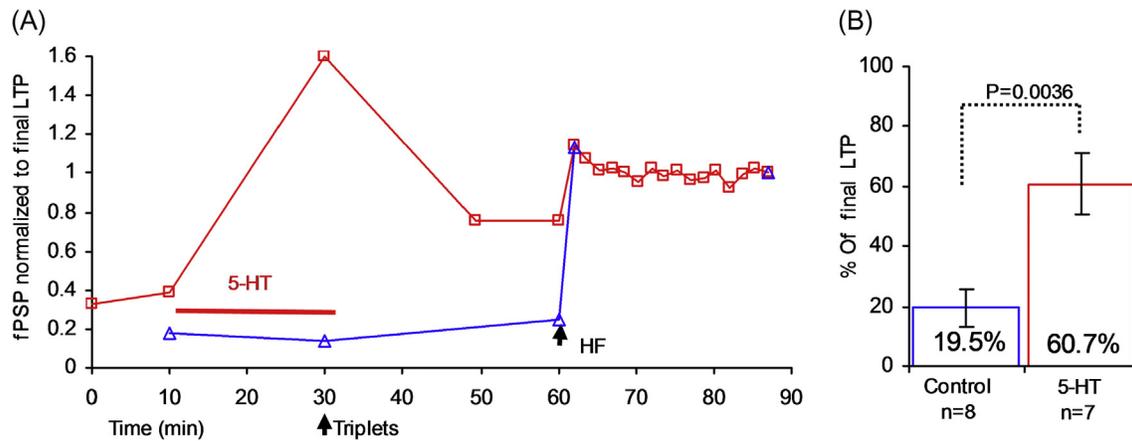
The short-term modulatory effects of serotonin on octopus VL function are shown in Figure 24.8. Serotonin (5-HT) at 100–200  $\mu$ M caused an average of approximately 3.5-fold facilitation of the fPSP. This synaptic connection in the cuttlefish VL, which lacks activity-dependent synaptic plasticity (Figure 24.6B), did not show any modulation by 5-HT.<sup>32</sup> In the octopus, the 5-HT facilitation involves presynaptic modulation of transmitter release because it was accompanied by a reversible reduction in twin-pulse facilitation (i.e., a reduction in the ratio of the second fPSP amplitude to that of the first in twin-pulse stimulation; Figures 24.8A and 24.8C). Repeated exposure to 5-HT did not lead to long-term modulation in the octopus VL, unlike its long-term effects on the *Aplysia* sensorimotor synapse. Also in contrast to *Aplysia*, c-AMP does not appear to be a major second messenger in 5-HT-induced fPSP facilitation in the octopus VL.<sup>49</sup>

It is intriguing that 5-HT shows only a robust short-term facilitatory effect on a synaptic connection undergoing long-term activity-dependent facilitation. The serotonergic system in the octopus VL may have

adapted to provide a modulatory signal to the VL rather than inducing long-term plasticity changes as in *Aplysia*. Based on the experiment shown in Figure 24.9, Shomrat *et al.*<sup>49</sup> suggested that this modulatory signal may serve as a reinforcing effect on LTP induction. In this experiment, tetanization trains of only 3 rather than 20 pulses (at 50 Hz) were applied. This reduced intensity induced only a partial and very modest LTP (19.5% of the final LTP). Similar triplet stimulation in the presence of 5-HT, which caused a robust short-term fPSP facilitation, induced a much higher LTP (60.7%; Figure 24.9B). The simple explanation is that 5-HT augments LTP indirectly by enhancing synaptic activity. That is, the serotonergic system of the VL appears to reinforce the induction of activity-dependent plasticity, and 5-HT may thus convey reward signals to the learning and memory network in the VL, as does dopamine in mammals<sup>46</sup> and mollusks<sup>45,51</sup> and octopamine in insects.<sup>52</sup>

### ARE THE OCTOPUS VERTICAL LOBE AND ITS LTP INVOLVED IN BEHAVIORAL LEARNING AND MEMORY?

The octopus VL, with its simple circuitry, may be the preparation of choice for investigating how neurons participate in storing memories and how



**FIGURE 24.9 5-HT reinforced activity-dependent LTP induction.** (A) Partial LTP was induced by a triplet pulse stimulation protocol. 5-HT (red trace, open squares) caused large short-term facilitation. After 30-min washout (60 min), HF stimulation revealed the residual LTP. HF stimulation gave rise to greater facilitation of the fPSPs in the control experiments (blue trace, open triangles), indicating less recruitment of LTP than in the presence of 5-HT. (B) Summary histogram showing the percentage of the final LTP measured at the end of the experiments exemplified in panel A. LTP was induced by the triplet stimulation protocol with and without 5-HT. Source: Modified from Shomrat et al.<sup>49</sup>

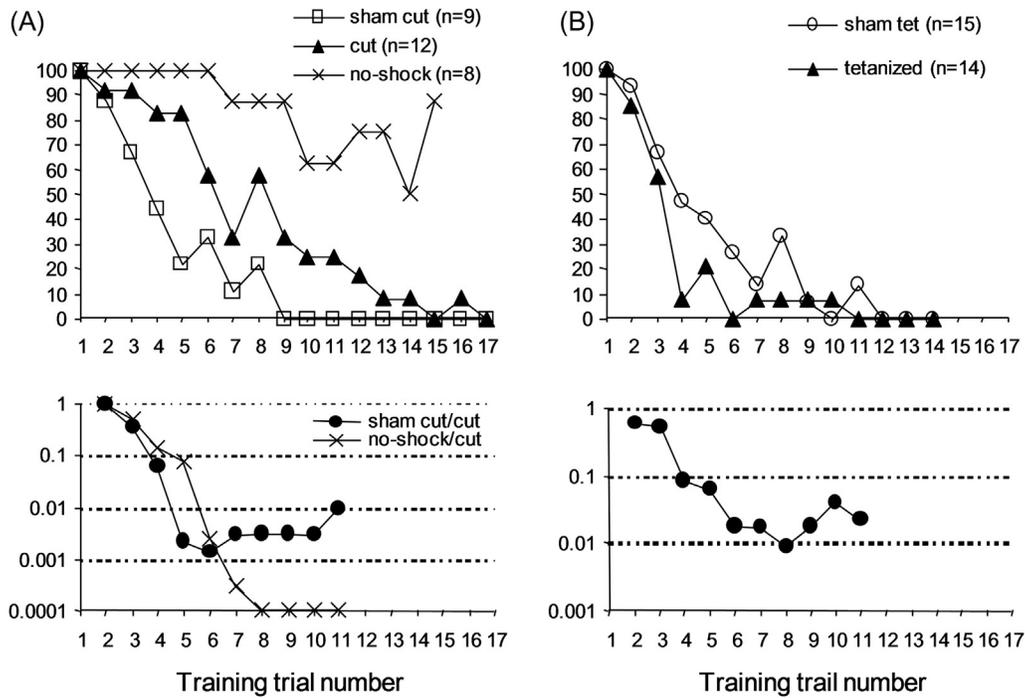
memories are retrieved or forgotten. Pioneering steps in this direction were made by Young, Boycott, Wells, and colleagues during the second half of the 20th century. Their behavioral, morphological, and lesion experiments showed that the octopus VL is important for learning and memory but is not the sole brain lobe involved in these functions.<sup>2</sup> The discovery of activity-dependent long-term plasticity and its neuromodulation in the VL provides physiological support for a VL function in learning and memory. However, a direct experimental test was required to confirm the involvement of these physiological processes in learning behavior.

To directly test the involvement of the LTP in the VL in learning and memory, Shomrat *et al.*<sup>28</sup> followed the experiments of Moser *et al.*<sup>53</sup> These showed that artificial saturation of LTP, induced in the rat hippocampus by tetanization, impaired spatial learning when the tetanization was applied prior to learning. This technically challenging experiment in the rat is much simpler in the octopus because the VL lies most dorsally in the brain and is relatively easily accessible to a large electrode for global tetanization. Such tetanization induced on average 56% of the available LTP.

Shomrat *et al.*<sup>28</sup> then tested whether this reduction in the available synaptic plasticity affected a learning task given 75 min after recovery from anesthesia and tetanization. The learning task was a passive avoidance task in which a mild electric shock “taught” the octopus to stop attacking a red ball. The results of LTP saturation by the tetanization were contrasted with the effects of transecting the medial SFL tract to the VL, which disconnects the VL system from its sensory input. In contrast, inducing strong LTP of the glutamatergic synaptic connection of the SFL axons onto the

amacrine cells may be viewed as “short-circuiting” the VL (see VL circuitry in Figures 24.4E and 24.12). Neither tetanization nor transection eliminated the ability of the octopuses to learn the task (Figure 24.10). Transection slowed the learning rate relative to that of sham-operated animals (Figure 24.10A). Unexpectedly, saturating the LTP had the opposite effect, enhancing the learning process relative to that of nontetanized animals (Figure 24.10B). Thus, LTP of specific synaptic connections in the VL does not appear to be involved in short-term learning. The VL output most likely controls the process mediating short-term learning that occurs outside the VL, with transection reducing and LTP enhancing the rate of learning by modulating the VL output.

Testing for long-term memory 24 hr after training produced more easily understandable results. Both treatments severely impaired long-term memory (Figure 24.11). Sham-operated and control animals did not demonstrate perfect memory of the task, with approximately 70% of the animals attacking the ball on their first trial. However, they demonstrated a robust saving or recollection as they stopped attacking the ball in the following test trials (Figure 24.11, open symbols). The transected animals (Figure 24.11A) remembered almost nothing of the avoidance task they had learned the day before, whereas the tetanized animals showed severe impairment (Figure 24.11B). This difference was probably due to the tetanization not completely saturating the LTP.<sup>28</sup> These results suggest that memories acquired soon after tetanization or transection are not consolidated. In contrast, a memory acquired before tetanization or transection, such as to attack a crab or a white ball (associated with positive reward in pretraining), was not impaired.<sup>28</sup> The results



**FIGURE 24.10** Transection slowed and tetanization enhanced short-term learning of an avoidance task. (A) The MSLF-transected animals showed significantly slower learning curves than the sham controls, with a significant difference from the fourth testing trial on (bottom panel shows cumulative Fisher's test between the different experiments; see Shomrat *et al.*<sup>28</sup>). Nevertheless, the transected animals stopped touching the red ball significantly faster than no-shock controls. (B) Tetanized animals learned faster than the sham-operated animals, and by the eighth trial the level of cumulative Fisher's test fell below 0.01 (0.0094; bottom panel). Source: Modified from Shomrat *et al.*<sup>28</sup>

confirm earlier lesion experiments showing that previous memories were not affected by lesions of the VL,<sup>17</sup> similar to results obtained in mammals and even humans with a severed hippocampus.<sup>54,55</sup>

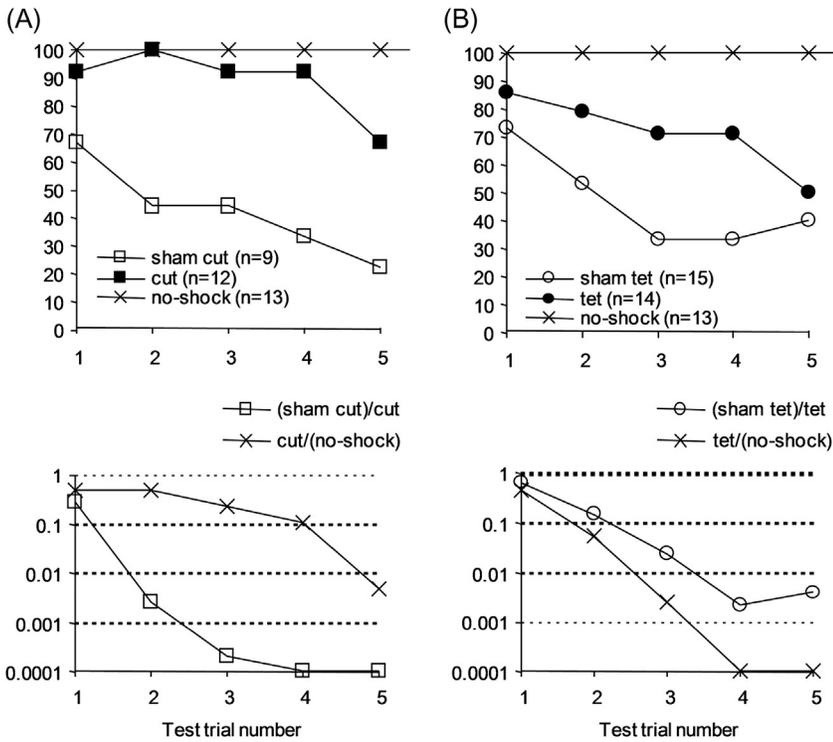
These experiments clearly demonstrate that the VL and its LTP are not important for the actual storage of long-term memory. Short- and long-term memory traces appear to be stored outside the VL, possibly in the circuitry mediating the attack behavior. Instead, the VL plays an important role in controlling the consolidation of short-term memory into long-term memory occurring elsewhere.

## A SYSTEM MODEL FOR OCTOPUS LEARNING AND MEMORY

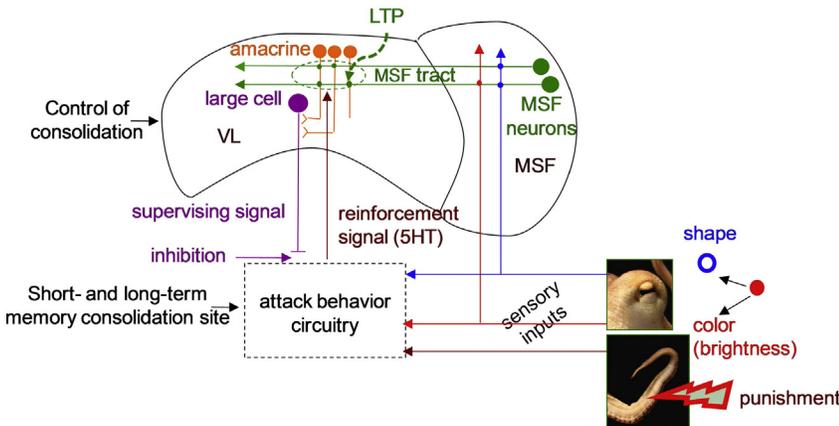
We conclude by proposing a tentative model for the octopus learning and memory system incorporating the physiological and behavioral findings that we have presented in this chapter (Figure 24.12). The rationale behind this model is summarized in Table 24.1.

To explain how the model functions, we consider the passive avoidance task used by Shomrat *et al.*<sup>56</sup> The octopus learns to refrain from attacking a red ball (actually dark because octopuses are color-blind)

by receiving an electric shock on its arms when it attacks. The information on shape and brightness is fed into the attack behavior circuitry that activates the natural attack behavior. This information feeds in parallel to the MSFL. Each quality (brightness and shape) is then transferred by a different set of MSFL neurons to the VL, most likely creating a sparse representation of each sensory quality in the matrix-like connections of the MSFL neurons with the amacrine interneurons. Those amacrine cells receiving inputs from both qualities are more likely to undergo LTP due to their higher level of activity. The "dark versus round" association is reinforced if they are conjugated with the pain signal conveyed to the VL by the serotonergic system. The strengthening of this association during training, in turn, creates a long-term enhancement of the amacrine cell input to the set of large neurons driven by this mutual sensory representation. The VL output generally inhibits the tendency to attack and can be regarded as an inhibitory supervising signal. The enhancement of the output generated by the red ball representation in the VL now specifically inhibits attacking the dark ball. It is not clear whether similar mechanisms mediate positive reward learning, *viz.*, by decreasing the inhibitory drive of the VL.



**FIGURE 24.11 Tetanization and transection impair long-term recall tested 24 hr after training.** The animals were given five test trials without electric shock. Testing revealed no significant difference in long-term memory but impairment in recall in consecutive tests both in transected (A) and in tetanized (B) animals. By the fifth test trial, the experimental animals showed some retention. Cumulative Fisher's exact test between the treated and the sham groups and the treated groups and the noncontingent controls are shown in the bottom panels (see explanation in the legend to Figure 24.10). Source: Modified from Shomrat et al.<sup>28</sup>



**FIGURE 24.12 A tentative model for the octopus learning and memory system.** For explanation, see text and Table 24.1.

**TABLE 24.1 The Five Basic Findings and Their Interpretation on which the Octopus Learning and Memory Model Is Based**

Experimental Finding	Conclusion
1. Transection and tetanization did not affect behavior; octopuses still showed their stereotypical attack behavior.	Sensory inputs feed in parallel to the VL system and to the circuits controlling behavior.
2. Both tetanization and transection did not erase old memories.	Long-term memory is stored outside the VL.
3. Tetanization accelerated learning (decreased the tendency to attack) and transection slowed learning (increased the tendency to attack). However, both treatments did not prevent the attack behavior.	The output of the VL modulates the rate of short-term learning that takes place outside the VL system, possibly by inhibiting the attack-mediating circuit.
4. Both treatments prevented the consolidation of short-term into long-term memory.	The LTP in the VL system is crucial for the consolidation of long-term memory outside the VL system.
5. Serotonin reinforced LTP induction.	Serotonin conveys the reward/punishment signal to the VL, which reinforces LTP of specific synapses active during the signal.

## CONCLUSION

The results presented in this chapter show that cephalopods are valuable animals for exploring the neural mechanisms subserving complex behaviors. Cephalopod behaviors are mediated by a unique embodiment<sup>57</sup> that includes a unique segregation of the nervous system between three large components: the arms, the eyes, and a central brain. However, the cephalopod nervous system still maintains the basic features of invertebrate morphology and neurophysiology. Thus, as we have summarized here, the neural complexity of cephalopods appears to have been achieved by building complex networks from simpler invertebrate elements.<sup>58</sup> Most likely, evolution and self-organization have shaped simpler neural networks to control complex behavior, and these simpler networks provide a more accessible opportunity for assessing how neural networks are embedded in the organization of complex behaviors. The large but simply organized fan-out fan-in network of cephalopods' vertical lobe is a vivid demonstration of this idea.

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