

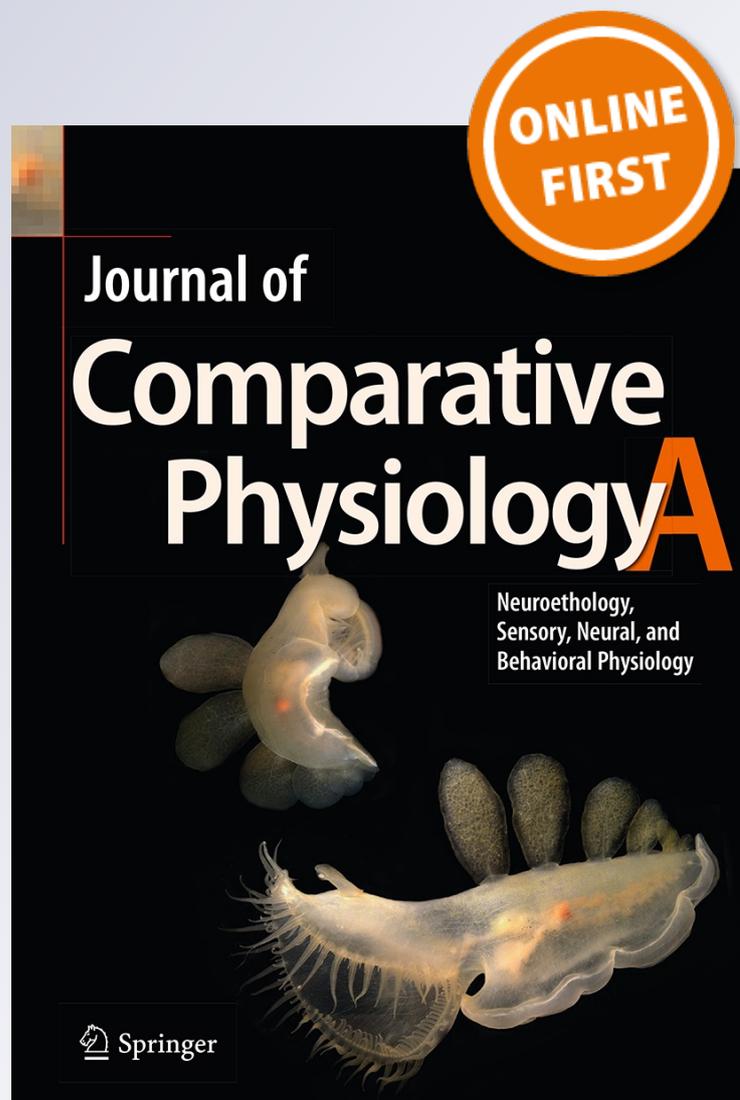
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The vertical lobe of cephalopods: an attractive brain structure for understanding the evolution of advanced learning and memory systems

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Abstract In this review we show that the cephalopod vertical lobe (VL) provides a good system for assessing the level of evolutionary convergence of the function and organization of neuronal circuitry for mediating learning and memory in animals with complex behavior. The pioneering work of JZ Young described the morphological convergence of the VL with the mammalian hippocampus, cerebellum and the insect mushroom body. Studies in octopus and cuttlefish VL networks suggest evolutionary convergence into a universal organization of connectivity as a divergence-convergence ('fan-out fan-in') network with activity-dependent long-term plasticity mechanisms. Yet, these studies also show that the properties of the neurons, neurotransmitters, neuromodulators and mechanisms of long-term potentiation (LTP) induction and maintenance are highly variable among different species. This suggests that complex networks may have evolved independently multiple times and that even though memory and learning networks share similar organization and cellular processes, there are many molecular ways of constructing them.

Keywords Invertebrate learning and memory · Long-term potentiation · Evolution of complex brain · Learning and memory network

Abbreviations

5-HT	Serotonin
AM	Amacrine interneuron
fPSP	Postsynaptic field potential
LFP	Local field potential
LTD	Long-term depression
LTP	Long-term potentiation
LN	Large efferent neuron
MYA	Million years ago
NMDAR	NMDA-like receptors
NO	Nitric oxide
NOS	Nitric oxide synthase
OA	Octopamine
SFL	Superior frontal lobe
TP	Tract potential
VL	Vertical lobe

Introduction

Invertebrates capable of complex behaviors have distinct brain structures whose organization differs from the rest of their nervous system. These structures have been found to be involved in processing multimodal sensory information and establishing memory traces. Prominent examples are the mushroom bodies of insects (Farris 2013; Heisenberg 2003) and the vertical lobe (VL) of modern cephalopods (Coleoidea) (Hochner et al. 2006; Hochner and Shomrat 2013). These structures maintain the main characteristics of invertebrates ganglia, where the cell bodies of the invertebrates typical unipolar neurons are organized in an outer cell bodies layer that surrounds an internal neuropil (Bullcock and Horridge 1965; Hochner 2010). However, in contrast to the typical invertebrate ganglia they are characterized by a very large number of small intrinsic interneurons

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and a stratified anatomical organization, resembling vertebrate organization. This 'vertebrate-like' type of organization has been an inspiring indication that these unique invertebrate structures will help us understand the structure and function of neural networks mediating memory acquisition and other cognitive functions. Indeed, the octopus and cuttlefish VLs have proven to be valuable models for understanding the connectivity, the neural plasticity and the functional involvement of this brain structure in learning and memory (Boycott and Young 1955; Maldonado 1965; Sanders 1975; Shomrat et al. 2008; Brown and Piscopo 2013). Cephalopods have the advantage that they have evolved nervous systems to control the most complex, flexible and advanced behaviors of all invertebrates (Amodio and Fiorito 2013). On the other hand, they belong to the Mollusca phylum (see cladogram in Fig. 1a), with the largest range of behavioral complexity, thus allowing direct comparison of simple and advanced systems (Kandel 1976; Hochner and Shomrat 2013, 2014). Some molluscs, like clams, hardly move, passively filter food and possess only a few tens of thousands of neurons. The octopus, in contrast, is a freely moving, fast predator, whose nervous system contains around half a billion neurons—10 times that of a mouse (Hochner 2012).

The study of the mechanisms of learning and memory in a variety of molluscs allows us to determine whether and how basal molluscan mechanisms of plasticity have been conserved, adapted or lost during the evolution of the more complex behaviors demonstrated by the octopus and other modern cephalopods (Coleoidea). This approach in this most diversified group of animals might shed light on the processes that are involved in the evolution, development and self-organization of complex behaviors and brains (Shomrat et al. 2011). Such comparison takes advantage of the fact that learning and memory mechanisms have been intensively studied in other classes of molluscs, like the gastropods *Aplysia californica* and *Lymnaea stagnalis*. These provide a good reference for basal molluscan learning and memory mechanisms (see Menzel and Benjamin 2013 for a comprehensive survey of invertebrate learning and memory). For example, simple defensive reflexes in *Aplysia* have for many years served as a leading model for understanding the mechanism of simple form of short- and long-term memory (Kandel 2001). This system was important for demonstrating the involvement of modulation of ion channels and vesicular release machinery in the serotonin mediation of behavioral sensitization and dishabituation, respectively (Hochner et al. 1986a, b), each process mediated by the biochemical cascades of different second messengers (Ghirardi et al. 1992). This system also enabled Dash and collaborators (Dash et al. 1990) to discover the importance of cAMP-dependent genes for establishing long-term memory traces. This appears to be a universal

mechanism for the mediation of protein synthesis-dependent long-term memory, both in vertebrates and invertebrates. Also, the feeding systems of *Aplysia* and *Lymnaea* have been important for understanding learning and memory mechanisms of more complex behaviors than the gill and siphon withdrawal reflex described above. In the feeding system, the nitric oxide system is involved in the mediation of learning processes (Kemenes et al. 2002; Susswein and Chiel 2012).

We, therefore, believe that studying cephalopods, with their complex and flexible behavior, is an unprecedented opportunity for a research that can reveal biological principals that are important for the evolution of mechanisms that mediate complex brain functions.

Evolutionary aspects of cephalopods nervous system

Kocot et al. (2011) and Smith et al. (2011) (but see Stöger et al. 2013; Haszprunar and Wanninger 2012) used transcriptome and genome data from all major lineages of molluscs (except Monoplacophora) to propose a topology for Mollusca. Interestingly, a sister-taxon relationship between Gastropoda and Bivalvia is supported. This grouping contains most (>95 %) molluscan species. Importantly, the result that gastropods are a sister group to bivalves, and not cephalopods, has important implications for our understanding of cephalopods evolution and relationships. The possible independent evolution of highly cephalized morphologies in gastropods and cephalopods underscores the need for comparative study across these groups.

There are at least three alternate possible explanations for the large investment in brains organization seen in cephalopods. Homology would support an ancient origin of this organization that is conserved across the Cephalopoda. Alternatively, certain cephalopod groups could have independently derived individual and unique organizations under different evolutionary pressures. And finally, shared features could result from convergent evolution of analogous brain organizations in derived forms. Long-standing ambiguities in cephalopod systematics have been resolved through modern molecular techniques combined with morphological and paleontological data (Strugnell et al. 2005, 2006; Lindgren et al. 2004; Allcock et al. 2015) and help to place these hypotheses into context.

For the purpose of this review it should be remembered that the evolutionary divergence between cephalopods and other molluscs (including the gastropods mentioned above) occurred around 550 million years ago (MYA) (Stöger et al. 2013; Grasso and Basil 2009) (see cephalopod cladogram in Fig. 1b). About 416 million years ago (Kröger et al. 2011) the nautilids and coleoids diverged,

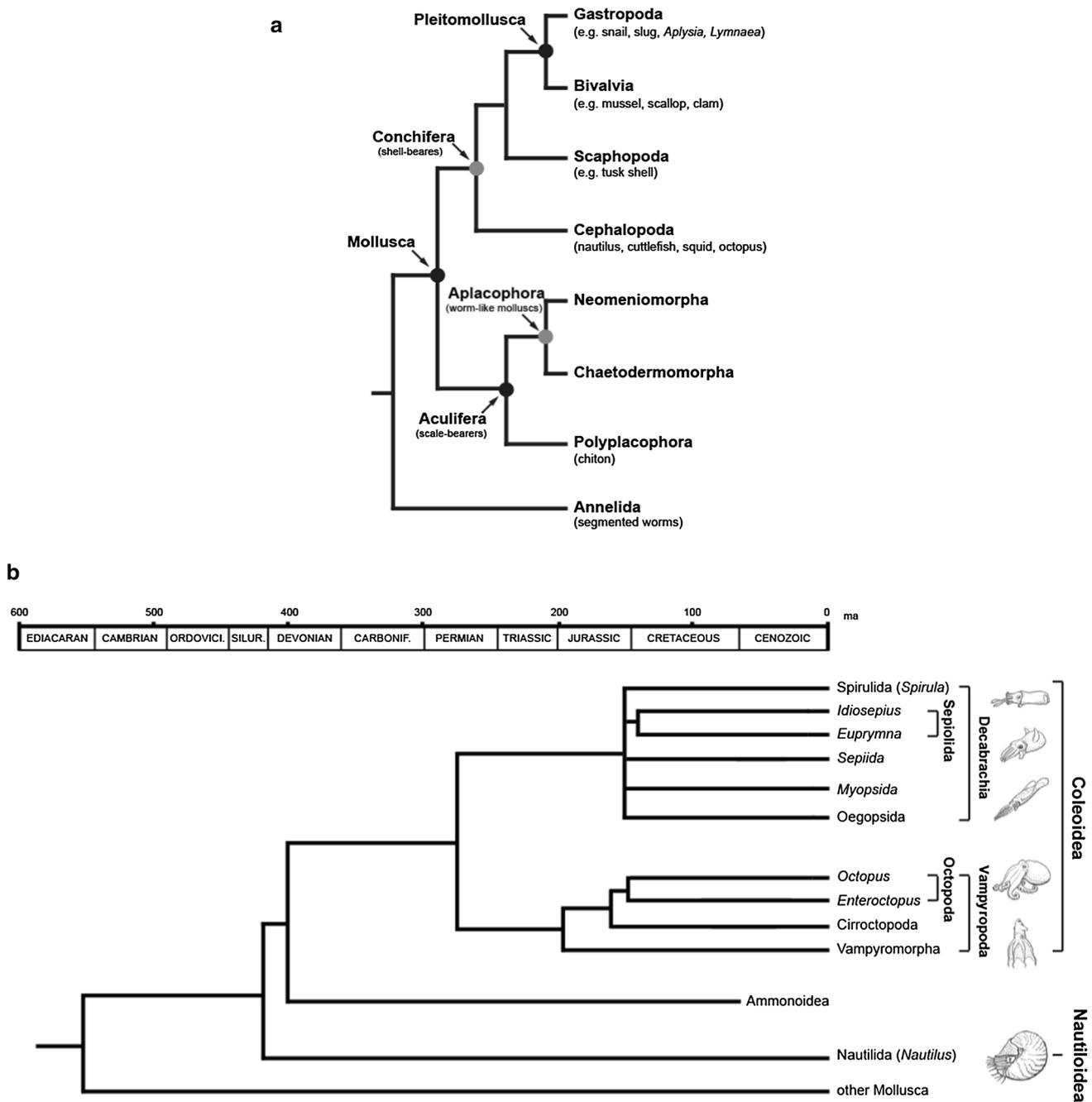


Fig. 1 a A recently suggested deep molluscan phylogeny based on phylogenomics study. Relationships among major lineages of Mollusca were consistent between analyses with multiple outgroups. *Black circles* represent nodes with bs (bootstrap support) = 100 and pp (Bayesian inference posterior probability) = 1.00. *Gray circles*

represent nodes with bs = 100 and pp \geq 0.98. Note that cephalopods and gastropods, both with the most developed central nervous systems among Mollusca, are not considered sister groups (modified from Kocot et al. 2011). **b** Cladogram of relationships among cephalopods (modified from Kröger et al. 2011)

the latter internalizing their shells. Squids, cuttlefish and octopuses, the main subject here, all belong to the subclass Coleoidea, but not to the same superorder. The octopus belongs to the superorder Vampyropoda (order Octopoda), that diverged around 280 MYA from a lineage that underwent a period of intense diversification at around

170 MYA, giving rise to the superorder Decabrachia (ten arms), that includes squids and cuttlefish. The squid and the cuttlefish each belong to different orders: Teuthoidea (suborder Myopsida) and Sepioidea respectively (see Hanlon and Messenger 1996; Kröger et al. 2011; Grasso and Basil 2009).

With the rise of bony fish in the Mesozoic, cephalopods evolved and radiated in direct competition with these new and fast, visual predators—resulting in what has been termed a “cognitive radiation” (Chamberlain 1990; Aronson 1991; Packard 1972). However, numerous prior extinction events and subsequent periods of diversification and competition may have already produced coleoids with fast and flexible behaviors. In addition, a significant pressure on developing cephalopods during the Mesozoic was, in addition to some early fishes, probably other cephalopods (Grasso and Basil 2009). Thus, there were already many substantive pressures on the cephalopod brain prior to the radiation of fishes in the Mesozoic. That modern nautilids express more complex behavior that previously hypothesized substantiates the notion that the complex cephalopod brain may pre-date the Mesozoic (Grasso and Basil 2009; Crook and Basil 2013). The evolution of the cephalopods is especially interesting because the sub-class of “old” cephalopods, Nautiloidea, diverged from the evolutionary lineage of the “modern” cephalopods (Coleoidea) about 430 MYA. As nautilus have not evolved greatly since, they offer the unprecedented opportunity of studying a “living fossil”. Behavioral learning and memory and navigation have recently been demonstrated in *Nautilus pompilius* (Crook and Basil 2008; Crook et al. 2009), and neurobiological studies are also currently underway (J. Basil and B. Hochner, personal communication).

The octopus vertical lobe

Figure 2b shows the connectivity matrix suggested by Young and Gray (Gray 1970; Young 1971) based on their pioneering electron microscopy studies. The physiological findings described below nicely support their connectivity model. Figure 2 shows the wiring diagram of the superior frontal lobe (SFL)-VL system of octopus, the area described and discussed below. Also marked on the network are the locations of the various pharmacological properties, activity-dependent synaptic plasticity and neurotransmitters.

As the anatomical organization of the VL resembles that of the hippocampus (Young 1991), exploring its physiological properties in a slice preparation turns out to be technically similar in many ways. As mentioned above, the general connectivity of the VL is achieved by a basic fan-out fan-in, two-synaptic layer network (Fig. 2a). The 1.8 million SFL neurons diverge (‘fan-out’) and innervate *en passant* 25 million amacrine interneurons (AMs), which comprise about half of the number of neurons in the central part of the brain of an octopus. At the output of the VL, the AMs converge (‘fan-in’) onto merely 65,000 large efferent neurons (LNs), presumed to be the only output of the VL (Young 1971). The VL network is thus organized similarly to feed forward two-layer artificial networks proven to produce intelligent functions such as classification, provided

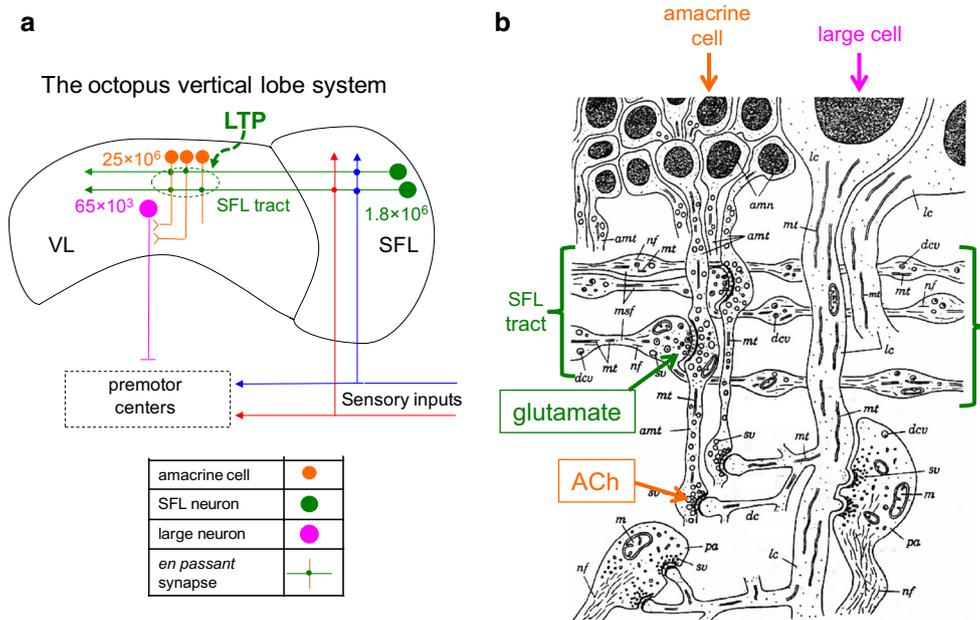


Fig. 2 The organization of the neural elements of the vertical lobe system. **a** Wiring diagram of the VL connectivity. The inset table gives the labeling of the various network components of the VL system. **b** Diagram showing what is thought to be the basic circuitry of the VL adapted from Gray (1970). Cell types and their transmitters

are marked in the corresponding colors (*amn* amacrine interneurons (AM), *amt* amacrine trunk, *dc* dendritic collaterals of large neurons (LN), *dcv* dense-core vesicle, *lc* body or trunk of LN, *m* mitochondrion, *sfl* median superior frontal axon, *mt* microtubule, *nf* neurofilaments, *pa* possible “pain” axon input to the LN, *sv* synaptic vesicles)

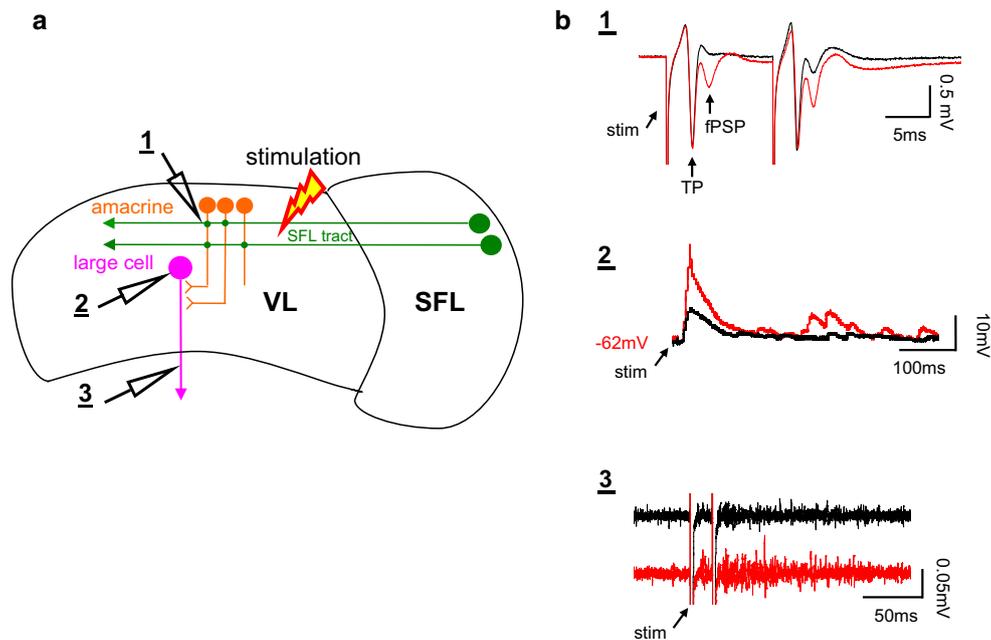


Fig. 3 Three electrophysiological recording configurations used for characterization of the vertical lobe physiology. **a** A schematic wiring diagram of the basic connectivity and types of cells in the SFL-VL system of the octopus and cuttlefish (color labeling as in Fig. 2a). The arrows represent the location of the stimulating electrode at the SFL-VL tract and the three sites and modes of recording; (1) local field potential (LFP) recording; 2) whole-cell recording; 3) extracellular recording of spiking activity). **b** Examples of traces recorded in the three recording modes (**a** 1, 2, 3) evoked by stimulation of the SFL tract. Each panel displays two traces: before (*black*) and after (*red*)

the induction of LTP. 1) Evoked LFP composed of the tract potential (TP) and the following postsynaptic field potential (fPSP) (note the paired pulse stimulation mode). The fPSP is generated by the synapses between the SFL axon terminals and the amacrine interneurons (AMs) and it shows a robust LTP (example from octopus). 2) Whole-cell intracellular recording from the large neurons (LN) showing LTP of the evoked and spontaneous EPSPs (cuttlefish). 3) Extracellular recording showing LTP in the spiking activity of the LN axons (octopus; note the paired pulse stimulation)

that one of the two synaptic layers is endowed with synaptic plasticity (Vapnik 1998; Shomrat et al. 2011).

Three basic neurophysiological methods have been instrumental for characterizing the VL network properties, including the input–output relationship of the VL (Fig. 3). First, due to the laminar organization of the incoming SFL fibers, it is possible to record an evoked local field potential (LFP) close to the axonal tract connecting the SFL with the VL (Fig. 3, recording labeled 1 in 3a and 3b). A tract potential (TP) generated by the presynaptic spiking activity in the axonal tract is followed by the postsynaptic field potential (fPSP) resulting from the synaptic input into the AMs. Due to the similarity in connectivity, this local field potential has similar characteristics to that recorded, for example, in the CA1 region of the hippocampus near the layer where the Schaffer collaterals innervate the dendrites of CA1 pyramidal cells (Kandel et al. 2012). The converging input from the AMs into the LNs can be monitored by intracellular whole-cell recording (Fig. 3, recording labeled 2 in 3a and 3b). The extracellular spiking activity of the VL output can also be conveniently recorded by placing suction electrodes on

the axons of the LNs that are organized in distinct rootlets (bundles) exiting from the VL's ventral end (Fig. 3, recording labeled 3 in 3a and 3b).

Pharmacological characterization of the synaptic connections assisted in identifying the transmitters and the physiological connectivity in the VL (Shomrat et al. 2011; Hochner et al. 2003). Postsynaptic blockers of AMPA-type glutamatergic synapses, such as CNQX, DNQX or kynurenic acid, blocked both the synaptic input to the AMs and the spiking output of the LNs. Hexamethonium and other cholinergic receptor blockers inhibited the excitatory input to the LNs and also the output of the VL via the LN axonal rootlets. These findings suggest that the fan-out input to the AMs is glutamatergic, while the fan-in input to the LNs is cholinergic. Moreover, the connectivity scheme, depicted in Fig. 2a, is supported by the experiments that show that either cholinergic or glutamatergic inhibitors blocked the output of the VL via the LNs axons (Shomrat et al. 2011), thus suggesting that there are no direct connections from the SFL neurons to the LNs. These findings support the connectivity scheme suggested by Gray (Gray 1970) (Fig. 2b).

Robust activity-dependent LTP of the synaptic input to the VL in octopus was first demonstrated in a slice preparation by Hochner et al. (Hochner et al. 2003) using the LFP generated by the incoming axons from the SFL (Fig. 3b1, TP) that is followed by a postsynaptic field potential (Fig. 3b1, fPSP) generated by the synaptic input to the AMs. As in the hippocampus, this LFP recording revealed a robust activity-dependent LTP (induced by 4 trains of 20 stimuli at 50 Hz). However, because the AM are likely inexcitable interneurons (Hochner et al. 2006), the LTP involves augmentation of a pure post-synaptic field potential without a recruitment of population spikes as typical for the LFP recording of hippocampal LTP. The level of LTP in the octopus VL, on average a fourfold increase in the amplitude of the synaptic field potential, is, to our knowledge, the largest activity-dependent LTP documented.

Hochner et al. (2003) could not find any indication that the LTP induction mechanism in the octopus VL is NMDA-mediated. This is a crucial finding, as NMDA-like receptors are thought to be the major coincidence detector molecules evolved specifically for mediating associative (Hebbian) plasticity (Kandel et al. 2012; Glanzman 2010). Interestingly, this likely NMDA-independent LTP in the octopus VL involves an exclusively presynaptic mechanism (Hochner et al. 2003). It thus resembles the presynaptic expression of the non-associative LTP of mossy fiber synaptic input to the CA3 pyramidal cells of the hippocampus, which are also non-NMDA dependent (Yeckel et al. 1999; Kandel et al. 2012). The octopus LTP induction mechanism shows postsynaptic response-dependence, indicative of Hebbian type LTP in only about half the synaptic connections between the SFL neurons and the AMs (Hochner et al. 2003). This suggests two different mechanisms of LTP induction, non-Hebbian and Hebbian, interestingly resembling the difference between the mossy fibers non-associative LTP to that of the associative LTP of the Schaffer collaterals inputs in the CA3 and CA1 regions, respectively, of the hippocampus (see Kandel et al. 2012).

The mechanisms of LTP induction and expression in the VL are still under investigation, but emerging results suggest that induction involves activity-dependent activation of protein kinase C and expression is mediated by constitutive activation of nitric oxide synthase (NOS) (Turchetti-Maia et al. 2014). The involvement of nitric oxide (NO) in mediating the octopus LTP may explain the Hebbian postsynaptic dependence together with presynaptic expression and maintenance of the VL LTP. For example, activation of NOS postsynaptically in the AMs would generate NO that would diffuse retrogradely to the presynaptic SFL neuron terminals, elevating the probability of transmitter release by activating soluble guanylyl cyclase. This is a commonly accepted scheme for NO-mediated plasticity both

in mammals and invertebrates (Garthwaite 2008; Prast and Philippu 2001).

In conclusion, it seems that, while the activity-dependent nature of LTP induction and the expression mechanisms seem to converge with cellular mechanisms similar to those in mammals, the molecular mechanisms mediating these converged processes appear to be based on adaptation of molecular mechanisms more commonly reported for mediation of invertebrate long-term plasticity (nitric oxide system) (Moroz and Kohn 2011) rather than the NMDA system commonly found in vertebrates. It should be noted that the issue of the involvement of NO in mammalian LTP is still unresolved due to conflicting findings (Andersen et al. 2006; Padamsey and Emptage 2014). Even though NMDA is most likely not involved in LTP of the octopus VL (we are careful here, as proving that something is absent is methodologically very tricky), NMDA-like receptors (NMDAR) do exist in molluscs and have even been implicated in the simple learning in the defense reflex of *Aplysia californica* (Lin and Glanzman 1994). Of more significance for the octopus VL, NMDAR-like immunoreactivity has been demonstrated in octopus and cuttlefish VLs (Di Cosmo et al. 2004) and an APV-sensitive NMDA-like current was identified in the chromatophore muscle cells of squids (Lima et al. 2003). Thus, lack of NMDAR-like gene product is likely not the reason for convergence to a non-NMDA mediated LTP, but rather selection of an alternative mechanism.

In contrast, the NO system is involved in experience-dependent modulation of gastropod (Korshunova and Balaban 2014; Susswein and Chiel 2012; Kemenes et al. 2002) and octopus (Robertson et al. 1994, 1996) behaviors. However, the NO function has been adapted from induction and expression of neuronal plasticity (e.g. Kemenes et al. 2002) to expression of the activity-dependent LTP in the VL of the octopus (Turchetti-Maia et al. 2014). These findings support the idea that receptors and enzymes may have been conserved throughout molluscan evolution, but how they are implemented in functional cascades in different biological systems and species is highly modular and adaptive; much as Sossin and Abrams (2009) proposed for the modular implementation of protein kinases in plasticity in gastropod molluscs. This suggestion is further supported below, where we describe dramatic differences between octopus and cuttlefish VL (Shomrat et al. 2011) and adaptation of molluscan neuromodulation systems (Shomrat et al. 2010).

Modulatory signals in the octopus vertical lobe

Theoreticians and physiologists generally accept that neural networks involved in learning and memory possess neural

plasticity mechanisms that are subjected to up- and down-regulation by neuromodulators. Such a mechanism is important for enabling ‘supervised learning’, as it can facilitate or inhibit learning depending on reward contingencies. Prominent examples for such modulatory systems are the dopaminergic system in mammals (Schultz 2010) and octopamine and dopaminergic neurons in insects (Giurfa 2006; Perry and Barron 2013; Burke et al. 2012; Waddell 2013).

In the octopus VL, serotonin (5-HT), a well-documented facilitatory neuromodulator in molluscs (Kandel 2001), also facilitates the glutamatergic synaptic connection between the SFL neurons and the AMs—the same synaptic connections showing activity-dependent LTP (Shomrat et al. 2010). We have compared the thoroughly studied modulatory effects of 5-HT in the gill- and siphon-withdrawal reflex of *Aplysia* (Kandel 2001) to the effects observed in octopus VL. In the octopus, 5-HT causes pre-synaptic facilitation at a higher concentration (~100 μM) than in the sensory-motor synapse of *Aplysia* (10 μM , see Shomrat et al. 2010 for a possible mechanistic interpretation for this concentration difference). A further difference is that in the octopus prolonged exposure to 5-HT does not lead to an intermediate-term facilitation of the synaptic connection between the SFL and the AMs (Shomrat et al. 2010), while in *Aplysia* it does (Kandel 2001; Antonov et al. 2010). A possible delayed development of 5-HT induced long-term facilitation as described in *Aplysia* (e.g. after 24 h) (Mauelshagen et al. 1998; Sutton et al. 2002; Antonov et al. 2010) still remains to be tested.

However, while 5-HT appears to have “lost” its long-term modulatory capabilities in the octopus VL, serotonergic inputs to the VL may indirectly enhance the activity-dependent induction of LTP. In the presence of 5-HT, a modest tetanic stimulus (e.g., 4 trains of 3 pulses, instead of 20), that under control conditions would induce only a partial or no LTP, can lead even to a fully saturated LTP (Shomrat et al. 2010). This finding, together with the immunohistochemical results (Shigeno and Ragsdale 2015; Shomrat et al. 2010), suggests that 5-HT may convey modulatory signals from other lobes to the VL. This serotonergic input may thus serve to transmit reinforcement signals that facilitate, for example, the induction of a long-term association between stimuli that are temporally coupled with a punishment. This punishment (e.g. electric shock to the arms) may activate the serotonergic input to the VL (Shomrat et al. 2008 and see proposed model in Hochner and Shomrat 2013, 2014).

As with the LTP mechanisms discussed above, the results suggest that the serotonergic system in molluscs is versatile and may be adaptively implemented to achieve various modulatory functions. This idea has been nicely demonstrated in the phylogenetic differences in the expression of 5-HT modulation that were correlated with behavioral differences, even at the level of homologous sensory

neurons in various Aplysiidae species. This includes a complete loss of 5-HT excitatory effects (Wright et al. 1996; Wright 1998) similar to the “loss” of the intermediate-term 5-HT effect in the octopus VL.

Octopamine (OA), an excitatory neuromodulator in the feeding system of gastropods (Vehovszky et al. 2004; Wenzell et al. 2009), has conserved its short-term excitatory effect in the VL of the octopus and was recently shown to provoke a short-term facilitatory effect in the VL, similarly to 5-HT. However, unlike 5-HT, OA (100 μM) attenuates LTP induction; high-frequency stimulatory trains in the presence of OA induce only short-term facilitation. Thus, as with the other neuromodulators, octopamine function has been adapted to have a specific effect on the activity-dependent long-term plasticity and, in contrast to 5-HT, octopamine inhibits LTP induction. It, therefore, suggests that 5-HT and OA convey opposing reinforcement signals (Greenwood et al. 2009; Shomrat et al., in preparation).

So far we have not found long-term depression (LTD)-like mechanism, either by repeated low-frequency stimulation as found in mammals (Collingridge et al. 2010), or by the neuropeptide Phe-Met-Arg-Phe-amide (FMRFamide), that induces long-term synaptic depression in *Aplysia* (Montarolo et al. 1988; Shomrat et al., unpublished data). The effect of OA is, therefore, an important mechanistic addition, as it is difficult to conceptualize a learning and memory network without a depotentiation mechanism (e.g. Turrigiano 1999).

Cephalopod vertical lobes exhibit dramatically different synaptic organization

The gross anatomy of the cuttlefish *Sepia officinalis* VL is quite different from that of octopus (Nixon and Young 2003; Young 1979), yet their circuits share the same three neuronal elements (Fig. 2). In both animals the connectivity is organized in a similar fan-out fan-in network (Fig. 2a) typical of the organization of a theoretical classification network (Vapnik 1998). Pharmacological analysis has shown that the same transmitters are used for the fast synaptic transmission in both species: the connection of the SFL to AMs is glutamatergic and the connection of the AM to the LNs is cholinergic (Shomrat et al. 2011).

Therefore, it was surprising that these two networks show dichotomical differences in short- and long-term synaptic plasticity (Shomrat et al. 2011). In the octopus, short- and long-term synaptic plasticity are localized at the glutamatergic synaptic connection (Figs. 2, 3b). In contrast, in the cuttlefish this synaptic connection does not show any robust plasticity. Also, the neuromodulator 5-HT (see above) does not have any modulatory effect on this fan-out layer in the cuttlefish VL.

Nonetheless, intracellular whole-cell recordings from the LNs of the cuttlefish VL (Fig. 3b2) or extracellular recording of the spiking activity from the rootlets of the LNs axons (Fig. 3b3) do reveal LTP, suggesting that in the cuttlefish plasticity occurs at the cholinergic fan-in synaptic connection (Shomrat et al. 2011). Thus, the octopus–cuttlefish comparison provides a unique example of homologous networks in animals in the same phylogenetic sub-class, whose input–output relations show robust LTP localized to a different synaptic site and mediated by different neurotransmitters.

Such differences in homologous networks (Bullock and Horridge 1965; Young 1988; Shomrat et al. 2011) would be less expected if the neural network properties were genetically determined. However, the two networks may have evolved to carry out different learning and memory processes specific to the different lifestyles of the octopus and the cuttlefish (Hanlon and Messenger 1996). Alternatively, these two networks may possess similar computational capabilities despite their differences. Computational considerations show that, if the input/output relationships are linear, the same computational capabilities are expected, regardless of whether the plasticity lies at the fan-out or fan-in synaptic connection (Shomrat et al. 2011). As tests of the input/output relationships of the VL suggest that they are linear in both animals, the results and the theoretical analysis support the idea that networks may self-organize to implement a particular behaviorally relevant computation (Shomrat et al. 2011). That is, the emerging computational properties of the network, rather than the specific properties of the neurons, determine the relevance of the network components for a specific behavior.

Conclusion

We have described the main physiological properties of the vertical lobe system of *Octopus vulgaris* and to some degree also that of the cuttlefish *Sepia officinalis*. With respect to the topic of this issue, celebrating Excellence in Invertebrate Neuroscience, our main objective was to show that the cephalopods are highly suited for addressing the question of how the nervous system mediating complex cognitive functions has evolved. Our results are consistent with the idea that the cephalopod VL shares global cellular properties with the complex learning and memory systems of vertebrates and insects. Yet, the molecular mechanisms that mediate synaptic plasticity and neuromodulation in the VL are based on extensive adaptations of conserved molluscan mechanisms. This comparison of advanced and more primitive species, together with the robust variability in the organization of the VL plasticity amongst related species, suggests that there are many independent ways

to construct a complex and efficient learning and memory system.

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