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Article

Alternative Sites of Synaptic Plasticity in Two Homologous "Fan-out Fan-in" Learning and Memory Networks

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Summary

Background: To what extent are the properties of neuronal networks constrained by computational considerations? Comparative analysis of the vertical lobe (VL) system, a brain structure involved in learning and memory, in two phylogenetically close cephalopod mollusks, *Octopus vulgaris* and the cuttlefish *Sepia officinalis*, provides a surprising answer to this question.

Results: We show that in both the octopus and the cuttlefish the VL is characterized by the same simple fan-out fan-in connectivity architecture, composed of the same three neuron types. Yet, the sites of short- and long-term synaptic plasticity and neuromodulation are different. In the octopus, synaptic plasticity occurs at the fan-out glutamatergic synaptic layer, whereas in the cuttlefish plasticity is found at the fan-in cholinergic synaptic layer.

Conclusions: Does this dramatic difference in physiology imply a difference in function? Not necessarily. We show that the physiological properties of the VL neurons, particularly the linear input-output relations of the intermediate layer neurons, allow the two different networks to perform the same computation. The convergence of different networks to the same computational capacity indicates that it is the computation, not the specific properties of the network, that is self-organized or selected for by evolutionary pressure.

Introduction

There is no easy way to experimentally test the involvement of computation algorithms in neural processing. In principle, this could be achieved by showing that different networks that support similar computation are interchangeable without apparently affecting behavior. One possibility is to use genetic engineering tools to manipulate the networks and study the effect on behavior. However, an exciting alternative is to look for situations in which evolutionary or self-organization processes have created computationally analogous networks. Here, we present a comparative neurophysiological analysis of two homologous learning and memory networks in phylogenetically close species. We reveal differences between the network that suggest that different neural network properties may converge to a similar computational capacity. The network properties are thus likely to be constrained by the computational/behavioral requirements.

The modern Cephalopod mollusks, such as *Octopus vulgaris* and *Sepia officinalis* studied here (phylum Mollusca, class Cephalopoda, subclass Coleoidea), demonstrate very complex behaviors. Yet, they are invertebrates, and their brains are organized relatively simply as a set of distinct, interconnected lobes [1, 2]. Of particular interest to us is the large vertical lobe (VL) in the supraesophageal part of the central brain. In both species the VL is implicated in complex behaviors involving learning and memory [3–6].

Although there are differences in the gross morphology of the VL of octopus and cuttlefish [2, 7], the two species show similarities in the anatomical connectivity, especially at the level of the first two synaptic layers and the neuron types. Figures 1A and 1B schematically show the fan-out fan-in (diverging-converging) bisynaptic arrangement in the VL system of both octopus and cuttlefish, based on the anatomical research of Young [2], Boycott [8], Gray [9], and Shomrat [6]. Input to the VL derives from the superior frontal lobes (SFL) that are thought to be the site of multimodal sensory integration [10, 11]. Neurons from the SFL (SFLn, numbering 1.8 million in the octopus) project to the VL, where they diverge at a ratio of ~1/14 to innervate en passant millions of small amacrine interneurons (AM, numbering 25 million in the octopus). These AM then converge at a ratio of ~380/1 to innervate via a special serial synaptic structure [2, 9] onto merely tens of thousands of large efferent neurons (LN, numbering 65,000 in octopus) that project out of the VL. The existence of an additional direct pathway from SFLn to LN had been hypothesized in the 1960s (2). However, such direct connections could not be found by either previous electron microscopy (EM) studies [9] or our current physiological experiments.

Here, we physiologically test and compare the synaptic connectivity and plasticity of the first two synaptic layers in this proposed fan-out fan-in network in the VL of the cuttlefish and octopus. Our physiological results support the anatomical scheme suggested by Gray (Figures 1A and 1B) as the main connectivity network explaining the input-output properties of the VL. We demonstrate that despite physiological and anatomical similarity, there is a dramatic difference in the organization of synaptic plasticity of the VL systems of these two phylogenetically close species. However, our results also show that, because of the special neurophysiological properties of the AM neurons (namely the linearity of their input-output relation), the difference in the organization of synaptic plasticity may be of only little computational consequence.

Results

We used a slice preparation to study the electrophysiological properties of the VL network in the two species and measured the responses to stimulation of the SFL tract as depicted schematically in Figure 1C (LFP rec. and inset). Stimulation of the SFL tract evoked a similar local field potential (LFP) waveform

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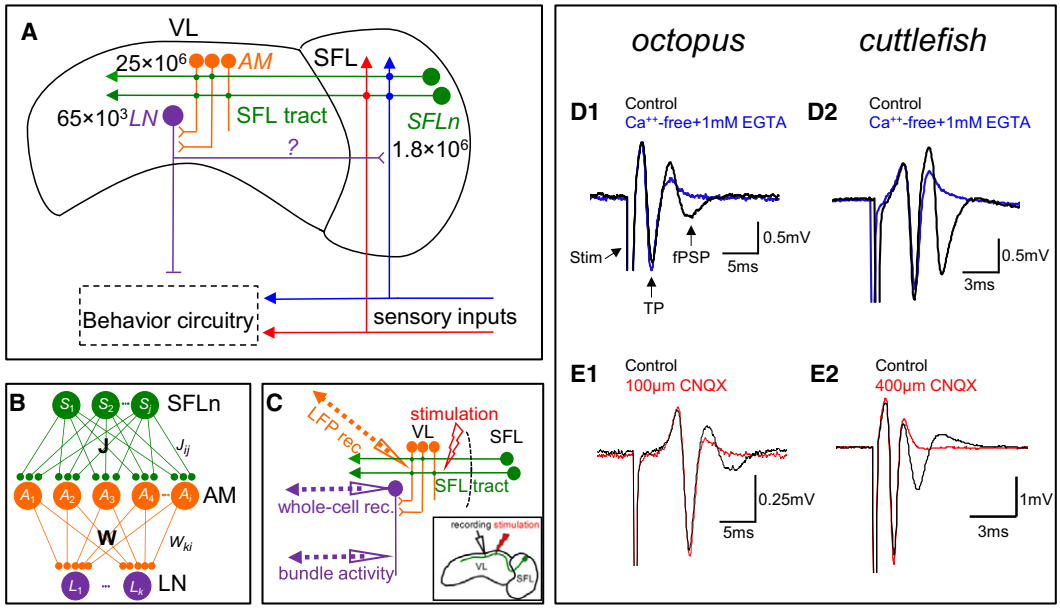

Figure 1. The Organization of the VL System and Basic Properties of the LFP Recorded Close to the SFL Tract in Octopus and Cuttlefish

(A) A schematic wiring diagram of the basic connectivity and types of cells in the SFL-VL system in octopus and cuttlefish (Numbers for octopus. For more details see text).

(B) A schematic description of the fan-out fan-in architecture of the VL network. S_i, A_i and L_i denote the i^{th} neuron in the SFLn, AM, and LN populations, respectively. The matrices J and W denote the strengths of the synaptic connections between the SFLn and AM and between the AM and LN neurons, respectively (see text and Supplemental Computational Considerations for further information).

(C) Schematic presentation of the location of the stimulating electrode at the SFL tract and the three sites and modes of recording (LFP rec., whole-cell rec., and bundle activity). Inset: A drawing of the slice preparation (~6 mm in length) showing one possible location of stimulating and recording electrodes (~0.5–1.0 mm apart) along the SFL tract that runs below the outer layer of AM cell bodies.

(D1 and D2) Superimposed LFPs in control and after blocking fPSPs in Ca^{2+}-free EGTA ASW in octopus (D1) and cuttlefish (D2). The following abbreviations are used: Stim, stimulus artifact; TP, tract potential; fPSP, postsynaptic field potential.

(E1 and E2) similar to D1-2 showing blockade of fPSP by AMPA-like glutamatergic receptor antagonists. The examples are from different experiments.

in the VL area in cuttlefish and octopus, demonstrating the similar organization in the two species (Figure 1, octopus, D1 and E1; cuttlefish, D2 and E2). Following the stimulation artifact (*Stim*, Figure 1D1) an LFP waveform, composed of a triphasic tract potential (TP, Figure 1D1) generated by the action potentials propagating along the SFL axon tract, was followed by a mainly negative-going field potential (fPSP, Figure 1D1). This field potential, maintained the same waveform irrespective of its amplitude (i.e., no population spikes, see [12]) and disappeared when the physiological solution (artificial sea water [ASW]) was replaced by a Ca^{2+}-free solution (Figures 1D1 and 1D2) and in the presence of AMPA-type receptor blockers (CNQX, DNQX, or kynurenate, Figure 1E1 and 1E2; see [12]). This pharmacology and the LFP waveform characteristics indicate that it is a glutamatergic postsynaptic field potential (fPSP) generated by the synaptic input into the inexcitable AM interneurons [12, 13].

To characterize the synaptic input converging onto the large efferent neurons (LN), we stimulated the SFL tract (Figures 1C; see Supplemental Experimental Procedures) and recorded the response both intracellularly from LN (Figure 1C, whole-cell rec.) and as extracellular efferent spiking activity in the bundles of the LN axons (Figure 1C, bundle activity). Figure 2A shows intracellular whole-cell recordings from LN in the octopus (A1) and cuttlefish (A2). In addition to the EPSP evoked from the SFL tract, the cells appeared to be bombarded with a high frequency of spontaneous EPSPs. Some cells in the octopus, but none found so far in the cuttlefish, received mainly inhibitory inputs (data not shown).

Our physiological results are in close agreement with the anatomical organization suggested by Gray [9] for the octopus and depicted in Figure 1A, which describes the main input-output connections of the VL. Consistent with Gray's proposal of a bisynaptic connection from SFL to LN, the latency of the intracellularly recorded EPSP after SFL tract stimulation was significantly longer than that of the glutamatergic fPSP (octopus: EPSP, 10.84 ± 0.66 ms, n = 4; fPSP, 5.27 ± 0.22, n = 14; cuttlefish: EPSP, 7.58 ± 0.65 ms, n = 17; fPSP, 3.9 ± 0.48 ms, n = 14; in each species $p \leq 0.001$, two-tailed t test). The average latencies differences were 4.37 ± 0.70 ms and 3.68 ± 0.81 ms in the octopus and cuttlefish, respectively. These delays fit an additional monosynaptic delay between the AM fPSPs and the LN EPSPs. Moreover, blocking the glutamatergic fPSP inhibited the tract-evoked activity in LN axon bundles (Figure S1). Thus, consistent with the anatomy and Gray's connectivity model [9] (Figures 1A and 1B), the SFLn do not appear to make monosynaptic connections to the LN and the input to the LN is driven bisynaptically via the AM interneurons. Note that LN output was blocked by both cholinergic (see below and Figure 2) and glutamatergic inhibitors (Figure S1), thus ruling out the possibility of a strong direct excitatory LSFn→LN connection.

The all-or-none spikelets evoked by synaptic inputs (Figures 2A1, 2A2, arrowheads) or by current injection [13] were relatively small. This indicates that full action potentials are generated at the axon or dendrite, electronically far from an inexcitable cell body. It thus appears that the VL neurons maintain properties commonly found in invertebrate, especially

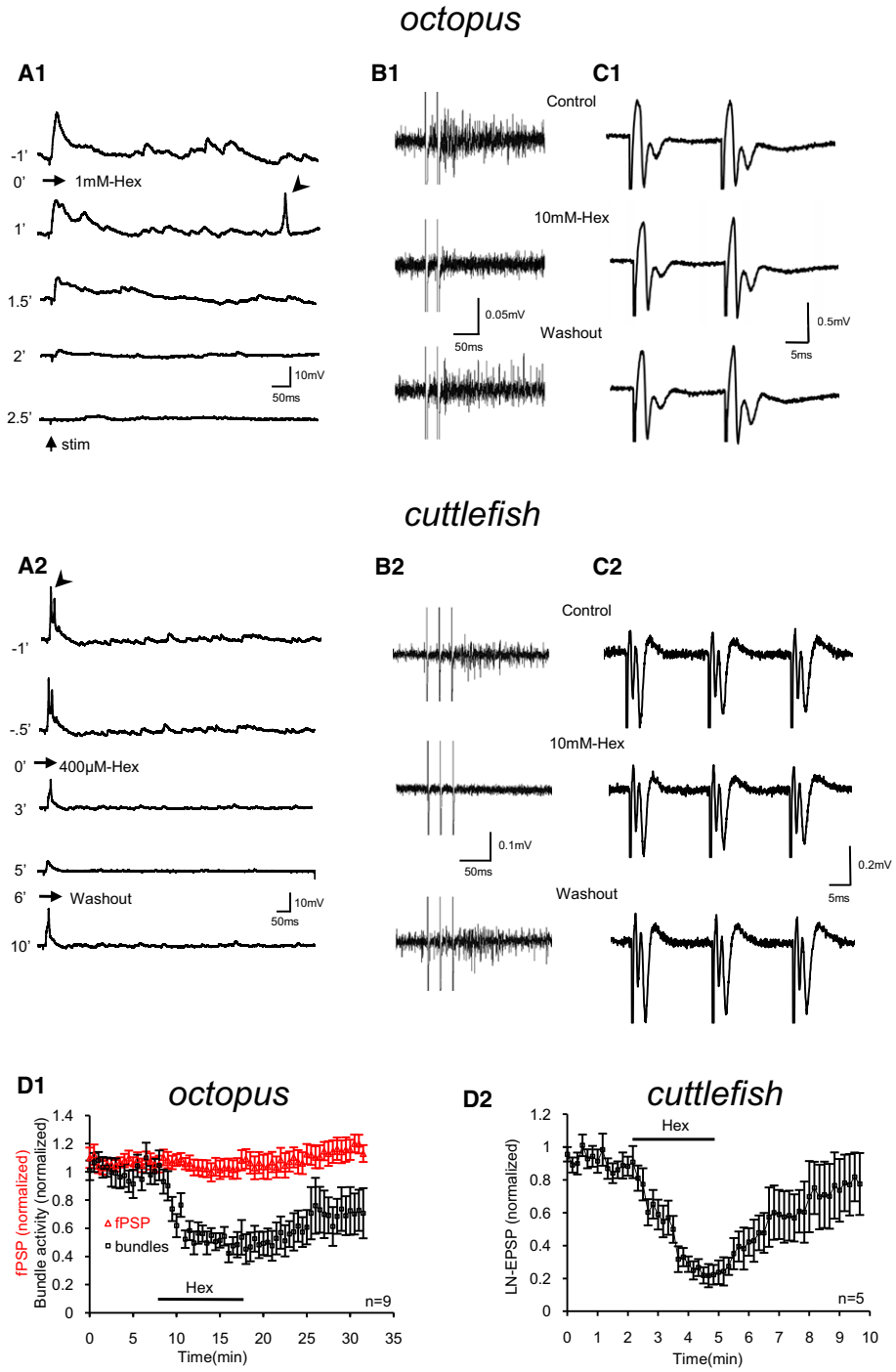

Figure 2. The Synaptic Inputs to the LN Are Most Likely Cholinergic in Both Octopus and Cuttlefish

(A1 and A2) Hexamethonium blocked the spontaneous and SFL tract-evoked EPSPs recorded intracellularly from LN in octopus (A1) and cuttlefish (A2) (the arrowheads point to two examples of spikelets).

(B1 and B2) Hexamethonium blocked the burst of action potentials recorded extracellularly from LN axonal bundles both in octopus (B1) and cuttlefish (B2). Note that twin (B1 and C1) or triplet (B2 and C2) stimuli were sometimes used to obtain a clearly measurable bundle response.

(C1 and C2) Hexamethonium had no effect on the TP and fPSPs in both octopus (C1) and cuttlefish (C2). Records were obtained simultaneously with bundle activity shown in B1 and B2.

(D1) Summary of nine experiments in octopus as exemplified in B1 and C1. Black curve indicates normalized integrated bundle activity; red indicates fPSP amplitude. Here and in subsequent figures, in each experiment the responses were normalized to the average of three to ten test responses at the beginning of the experiments and the error bars depict the standard error of the mean. (D2) Summary of five whole-cell recording experiments in cuttlefish showing the inhibition of the evoked EPSPs by hexamethonium (0.4–0.8 mM) and recovery after washout. The individual experiments in D1 and D2 are aligned with respect to time of hexamethonium administration.

See Figure 1C for the different recording modes.

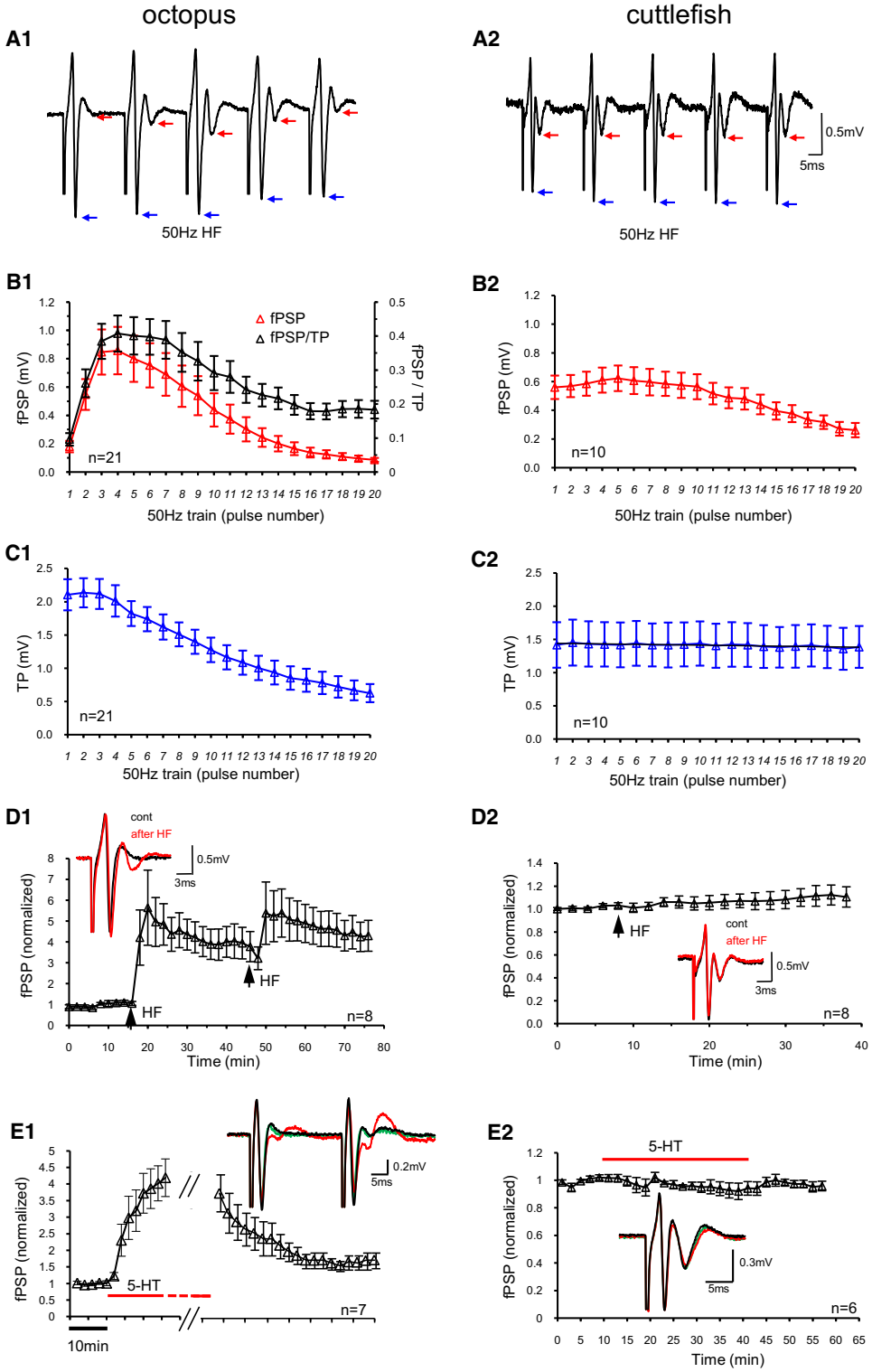

Figure 3. Short- and Long-Term Synaptic Plasticity at the SFLn→AM Connection Differed Dramatically in Octopus and Cuttlefish

(A1 and A2) Short-term plasticity in octopus (A1) and cuttlefish (A2), In octopus the first five fPSPs in the 50 Hz HF stimulation trains (taken from first of the four trains of 20 stimuli used for LTP induction) showed a robust synaptic facilitation followed by synaptic depression. Note in contrast the large and stable fPSPs in the cuttlefish. Red arrows mark the negative peaks of the fPSPs. Blue arrows mark peak negativity of TP.

(B1 and B2) Summary of 21 experiments from octopus (B1) and 10 from cuttlefish (B2) showing fPSP amplitude (mV) during the 20 pulses of the HF train. The black curve in the octopus (B1) shows the fPSP normalized to the corresponding TP potentials in the 20 pulse train shown in C1.

(C1 and C2) TP amplitude (mV) during the experiments in octopus (C1) and cuttlefish (C2), respectively.

(D1 and D2) Summary of eight experiments from octopus and cuttlefish showing the development, maintenance and saturability of LTP in octopus (D1) but no significant change in the cuttlefish (D2). LTP was induced by four HF trains (20 pulses, 50 Hz, 10 s interval), fPSPs were normalized to the averages of ten

arthropod neurons (in other mollusks full overshooting spikes are rather common) [14]. Also consistent with the invertebrate monopolar morphology of the neurons, and with an inexcitable neurite and cell body, the EPSP amplitudes recorded in the LN cell bodies were large relative to the spikelets (Figures 2A1, 2A2) [14].

In order to measure the activity of a large number of LN neurons simultaneously over extended periods, we recorded the activity in the LN axon bundles extracellularly (Figure 1C, bundle activity) in both octopus and cuttlefish (Figures 2B1 and 2B2, respectively). Finding an SFL tract-evoked response in the LN axonal output enabled us, as shown below, to characterize the input-output relationship of the VL in a technically simple and straightforward manner.

To characterize the neurotransmitters involved in the fast excitatory transmission to the LN, we examined the effect of the nicotinic antagonist hexamethonium on synaptic transmission. Hexamethonium is a known blocker of acetylcholine receptors in mollusks and also blocks neuromuscular transmission in the octopus arm [15]. As shown in Figure 2A, hexamethonium (0.1–10 mM) inhibited evoked and spontaneous EPSPs in the LN in both octopus (Figure 2A1) and cuttlefish (Figures 2A2 and 2D2). It also inhibited the SFL tract-evoked bursts of action potentials recorded extracellularly from the LN axon bundles (Figures 2B1 and 2D1, octopus; 2B2, cuttlefish). However, hexamethonium did not affect the glutamatergic fPSPs (Figures 2C1 and 2D1, red curve). These results suggest that the excitatory synaptic input to the large LN cells in both species is cholinergic. Thus, as in other invertebrates [14], fast excitatory transmission in the central nervous system of octopuses and cuttlefish is not mediated solely by glutamatergic synaptic transmission as in vertebrates.

Short- and Long-Term Synaptic Plasticity

Despite the similarities in the anatomy and physiology of the octopus and cuttlefish VL described above, there were also marked differences. Figures 3A–3C illustrate the differences in the short-term synaptic dynamics of the fPSPs in octopus (Figures 3A1–3C1) and cuttlefish (Figures 3A2–3C2). These properties were analyzed in the first of the four high-frequency (HF) trains (20 pulses, 50 Hz) typically given for long-term potentiation (LTP) induction (see below). During the HF stimulation, the fPSP in the octopus showed a robust synaptic facilitation in the first few stimuli (Figures 3A1, red arrows, and 3B1). This was followed by a slower synaptic depression later in the train. This depression frequently caused complete suppression of the fPSP by the end of a train of 20 pulses (Figure 3B1, red curve). In contrast, the fPSP in the cuttlefish was large and relatively stable during most of the stimulation train (Figures 3A2, red arrows, and 3B2).

The differences in the fPSP dynamics are partially due to differences in the dynamics of the presynaptic TP (blue arrows in Figures 3A1 and 3A2). The dynamics of the amplitudes of the TP during the 50 Hz train in the octopus and cuttlefish are shown in Figures 3C1 and 3C2. In the octopus, the average TP amplitude began declining after the third stimulus (Figure 3C1). Normalizing the fPSP amplitude to that of the TP showed that the decline in TP explained most, but not all, of the fPSP depression; significant depression was still evident in the normalized fPSP (Figure 3B1, black curve). Yet the 1.9-fold facilitation of the fPSP (normalized to the TP) at the end of the train suggests that, at the level of a single axon, the robust transient facilitation was not followed by synaptic depression. In the cuttlefish, the more stable behavior of the fPSPs during the train correlated with the stable TP amplitude during the entire train (Figure 3C2). Therefore, the 0.5-fold depression at the end of the train in the cuttlefish fPSP (Figure 3B2) was not due to modulation of TP amplitude and, unlike the octopus, reflects a slow and delayed activity-dependent synaptic depression. Thus, the difference in fPSPs dynamics between the species appears to be due to differences in the excitable properties of the SFLn axons or terminals, and presynaptic and possibly postsynaptic mechanisms. That the TP has a faster time course in the cuttlefish than in the octopus is consistent with differences in the excitable properties of the SFLn axons (Figures 1D1 and 1E1, cf. 1D2 and 1E2).

In line with the differences in short-term synaptic plasticity between octopus and cuttlefish, we also found fundamental differences in the long-term plasticity of the SFLn→VL synaptic inputs (cf. insets and averages in Figures 3D1 and 3D2). Although octopuses showed a robust LTP (~4-fold enhancement) that saturated after four HF tetanization trains (20 pulses, 50 Hz, 10 s interval, Figure 3D1), the cuttlefish showed no long-term modifications in TP or fPSP amplitudes or time course (Figure 3D2). Moreover, as shown in Figure S2, although in octopus LTP induction changed the slope of the linear dependence of fPSP on TP amplitude, in cuttlefish this linear relationship was not modified following HF stimulation. In the cuttlefish, a number of different stimulation protocols were tested and none induced long-term facilitation of the fPSP (e.g., 0.5–1 s trains at 10 to 100 Hz) nor depression of the fPSP (e.g., 1 to 50 Hz for extended duration). It should be emphasized that in the octopus, induction of saturated LTP was not accompanied by changes in synaptic dynamics (12) in any way resembling those in the cuttlefish. Thus, it is unlikely that LTP was somehow induced in cuttlefish before the recordings.

Serotonin Neuromodulation

Serotonin (5-HT) is a powerful neuromodulator in mollusks [16]. We have recently shown that 5-HT robustly induces facilitation of the SFL input to the VL in the octopus, and it reinforces LTP induction, possibly serving as a reward signal [17]. Although in the octopus the facilitation requires higher 5-HT concentration than in *Aplysia* (100–200 μM versus 10–20 μM; see [17]), this enhancement is mediated, at least in part, presynaptically, similar to the sensory-motor synapses in *Aplysia californica* [18]. The involvement of additional *Aplysia*-like postsynaptic mechanism [19, 20] has yet to be tested. Unlike *Aplysia*, 5-HT in the octopus has mainly short-term modulatory effects (Figure 3E1, see also [17]). However, we found that 5-HT at concentrations between 10 μM and 1 mM had no significant effect on the SFL tract-evoked fPSP in cuttlefish (Figure 3E2).

test fPSPs at the beginning of the experiments. The insets show a superimposition of LFP traces before (black) and after HF stimulation (red). Note a clear enhancement of the fPSP in octopus but not in the cuttlefish.

(E1 and E2) 5-HT induced short-term facilitation of SFLn→AM synapses in octopus (E1) but not cuttlefish (E2). (E1) Summary of seven experiments in octopus demonstrating the facilitatory effect of 100–200 μM 5-HT for at least 20 min and its reversal on washout. (Experiments are aligned at onset of 5-HT washout). (E2) Summary of six experiments in cuttlefish (100–200 μM 5-HT, for ~30 min). Insets in E1-E2; LFP traces before (green), in the presence of 5-HT (red), and after washout (black). (Note that due to lack of effect not all three colors are apparent in the E2 inset).

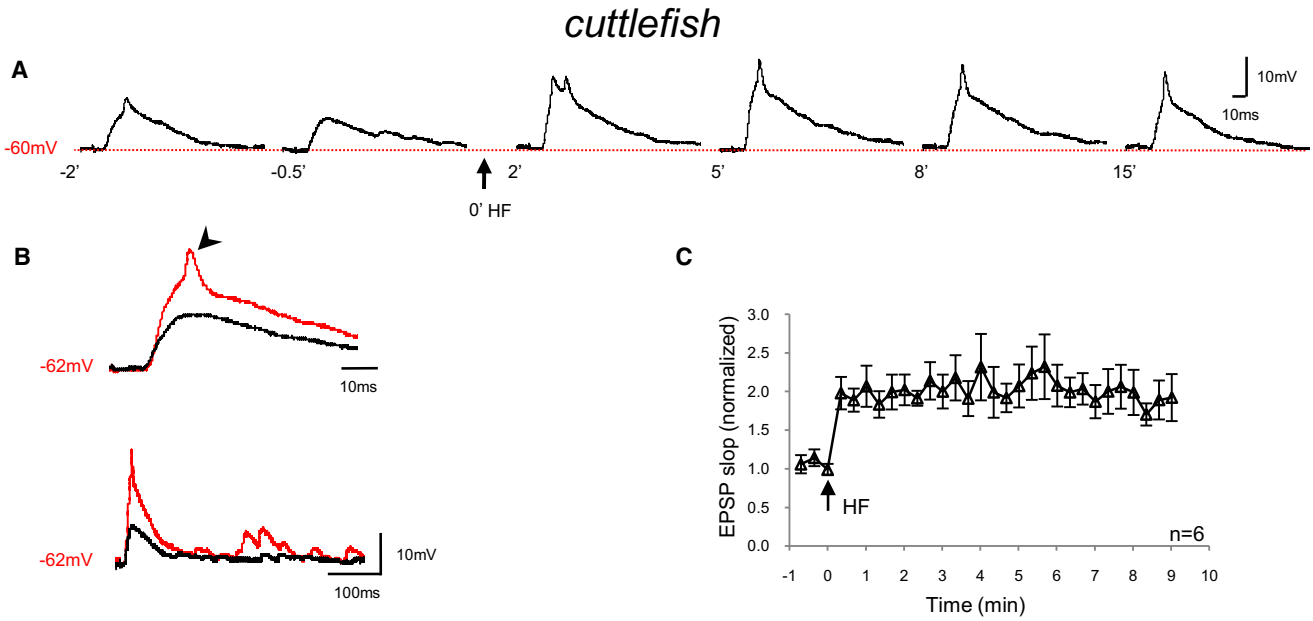

Figure 4. Activity-Induced LTP of the EPSP in the LN of Cuttlefish

(A) Whole-cell intracellular recordings showing EPSP and spikelets (arrowhead in B) evoked by stimulation of the SFL tract. HF stimulus at time 0 induced LTP of the EPSP.

(B) Superimposition of evoked EPSPs before (black) and after HF (red). Note the fast (upper traces) and slower (lower traces) time scales.

(C) Summary of six intracellular recordings experiments as in (A). LTP is expressed as an increase in slope of EPSP onset normalized to control.

Plasticity at the Input to the Efferent LN

In contrast to the lack of LTP at the SFL input to the cuttlefish VL, we found LTP of the cholinergic connection to the LN in the cuttlefish. The LTP in cuttlefish was demonstrated by intracellular recording of EPSPs (Figures 4A and 4B). The average results of six such experiments in the cuttlefish are shown in Figure 4C. The large enhancement of the EPSP slope and amplitude did not decay and the synapse was still facilitated 10 min after stimulation of the SFL tract with the HF protocol as above (Figures 4A and 4C).

Intracellular recordings are limited to several minutes, and the results may be affected by washout phenomena. Therefore, we studied these plastic changes by recording extracellularly from the LN axon bundles (Figures 5A2–5C2). A clear enhancement of the evoked spiking activity was found in all four experiments in cuttlefish (Figure 5B2). Following the HF, the response recorded extracellularly stabilized at an enhanced level for at least the duration of recording (~90 min; Figure 5A2). Thus, the activity-dependent potentiation in cuttlefish is a long-lasting phenomenon, demonstrating LTP of the cholinergic synapses onto the LN. The long-term potentiation of a central cholinergic synapse in cuttlefish VL is a novel finding and demonstrates that long-term potentiation is not an exclusive property of glutamatergic synapses.

To examine whether the cholinergic synapses onto the LN are also plastic in the octopus, we recorded the extracellular response of LN neurons to tract stimulation before and after HF stimulation. Figures 5A1 and 5B1 show a stable and reproducible potentiation of the efferent bundles activity in the octopus (Figure 5B1, black curve). This result is expected due to the LTP in the SFLn→AM synapses and, indeed, the intracellularly recorded tract-evoked PSPs were potentiated (n = 3, data not shown). However, these experiments are not sufficient to address the question of whether there is *additional* plasticity at the cholinergic synapses onto the octopus LN.

Testing for synaptic plasticity at the input to the LN directly was difficult due to our inability to exclusively stimulate a group of AM neurons extracellularly; the SFL tract intermingles with the AM neurites in the ventral part of the outer layer of AM cell bodies (see schemas in Figure 1C). We therefore recorded the fPSP simultaneously with activity in the LN axon bundles and quantified the integrated bundle activity evoked by a test stimulus to the SFL tract (see Supplemental Experimental Procedures). As expected for the octopus, this axon bundle LTP was accompanied by a clear potentiation of the fPSP (Figures 5A1, inset, and 5B1, red curve).

We then tested whether the input-output relationship between fPSP amplitude and bundle activity was modified by LTP. We did this by correlating the integrated activity in the bundles with the amplitude of fPSPs generated by various stimulus intensities. We then induced LTP in the SFL tract and tested this correlation again. As can be seen in Figure 5C1, the relationship between the fPSP and bundles activity did not change significantly following LTP induction as quantified by the similarities between the slopes of the linear fits in Figure 5C1 (black, before LTP induction, and red, after LTP induction; slope = 0.89, $r = 0.82$, $p < 5 \times 10^{-8}$ before LTP and slope = 0.71, $r = 0.76$, $p < 7 \times 10^{-7}$ after the induction of LTP; $p \leq 0.58$ for the difference between two correlation coefficients).

The conclusion that the linear input-output relation of the octopus VL did not change following LTP is supported by the similar relative LTP of the fPSP and integrated root activity shown in Figure 5B1. Thus, this HF protocol for activity-dependent potentiation did not induce a clear long-term modification at the synaptic inputs to the octopus LN, and most if not all of the roots activity LTP can be attributed to fPSP facilitation.

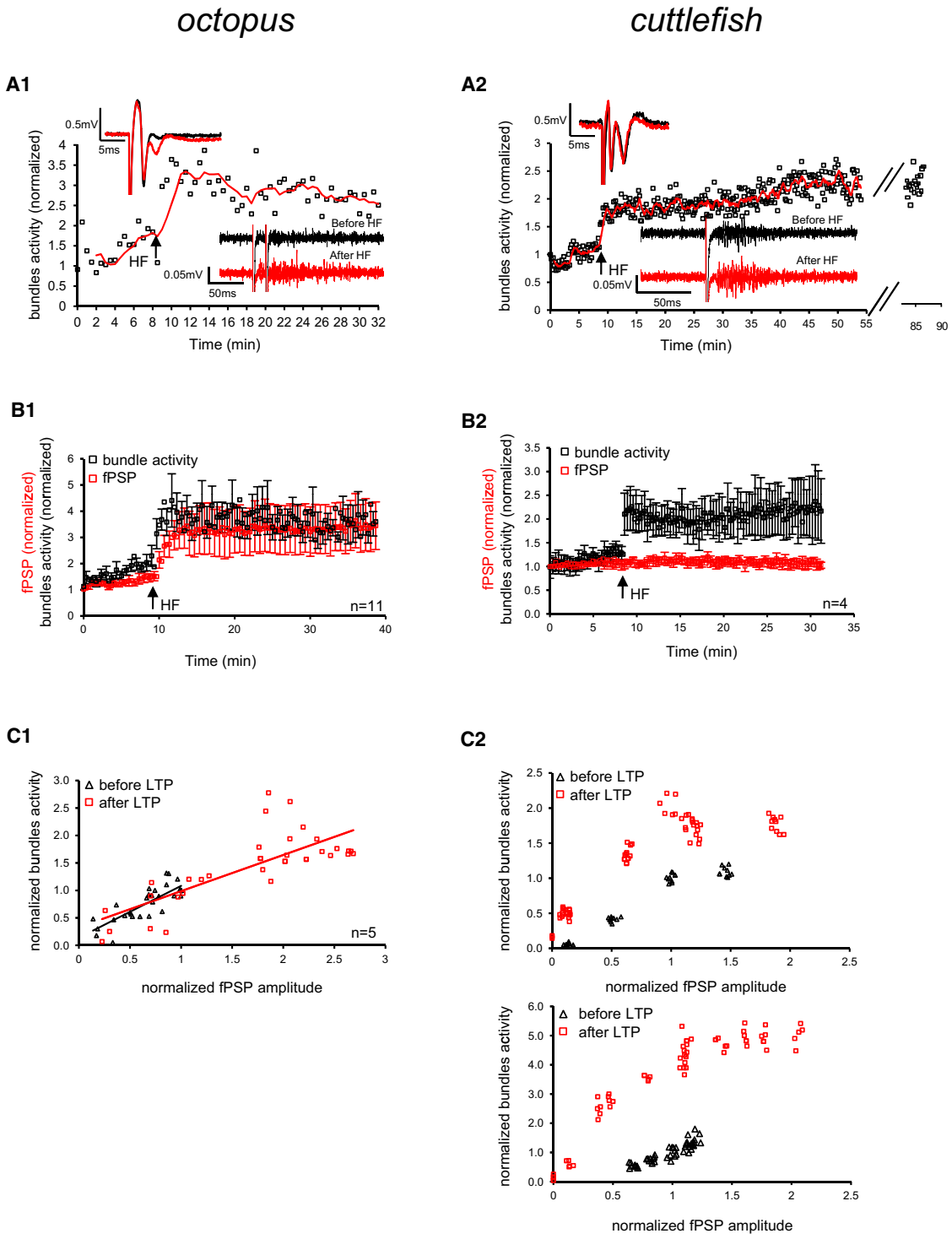

Figure 5. Input-Output Relationships in the VL

Extracellular recordings from the LN axon bundles show LTP of the VL output in both octopus (left) and cuttlefish (right).

(A1 and A2) The development and maintenance of LTP as measured by the SFL tract-evoked integrated bundles activity (red line is a moving average of five test pulses). Upper insets: superimposition of LFP before (black) and after (red) HF. Lower insets: activity of the LN axon bundles before (black) and after (red) HF.

(B1 and B2) Summary of the experiments of the type shown in A1 (octopus) and A2 (cuttlefish).

(C1 and C2) The VL input-output relationship before (black) and after (red) LTP induction. The relationship is expressed as the correlation between the integrated activity in LN axon bundles and the fPSP amplitude generated by various stimulus intensities. The fPSP amplitudes and the bundles activity were normalized to those obtained in controls by a test pulse at the intensity used for LTP induction. In the cuttlefish (C2) two examples are shown. LTP did not affect the linear relationship in the octopus (C1), whereas in cuttlefish a clear change was evident. Following LTP induction in the cuttlefish (C2, red), the linearity is apparent at intensities lower than 1. This result suggests LTP specificity because only the tetanized connections underwent LTP (see [12]).

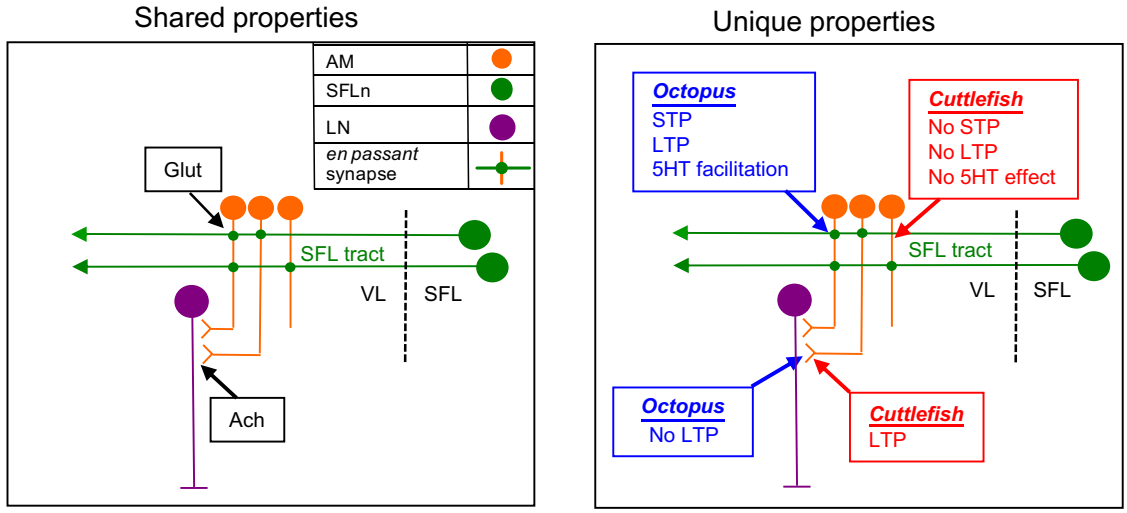

Figure 6. Summary of the Comparative Analysis of the VL Systems of *Octopus vulgaris* and *Sepia officinalis*
Left: shared properties. Right: differences between the two species. STP is used as an abbreviation for short-term plasticity.

In sharp contrast to the octopus, this input-output relationship in the cuttlefish VL showed a clear change in the dependence of integrated activity in the LN axonal bundles on fPSP amplitude (Figure 5C2, two examples). This change was expected as the intracellular recordings revealed that LTP occurs at the synaptic input to the LN (Figure 4).

Discussion

The schemas in Figure 6 summarize the similarities and differences between the homologous VL networks in the octopus and the cuttlefish. The network connectivity pattern (left) is based on Gray's EM study [9] that described the main connectivity pattern in the VL as a simple feed-forward connection of SFLn→AM→LN. This description is supported by the physiological input-output properties of the VL, analyzed here. Remarkably, although the network architecture and the electrophysiological and synaptic connectivity of the constituent neurons are similar in the two species, the sites of synaptic plasticity are dichotomously different. The octopus shows short-term and long-term plasticity and neuromodulation at the SFL→AM glutamatergic synapse and no plasticity at the AM→LN cholinergic synapse. The opposite is true for the cuttlefish: we found no plasticity in the SFL→AM synapse but LTP at the AM→LN connection.

The fan-out fan-in architecture seen in these two VL networks is widespread in neuronal networks. However, to the best of our knowledge, plasticity in such networks in adult brains is restricted to the fan-in layer, as we found in the cuttlefish. For example, the locust mushroom body does not show synaptic plasticity in the first layer of synapses that are input onto the many small Kenyon cells [21]. In contrast, spike time-dependent plasticity (STDP) has been demonstrated in the fan-in layer [22]. Similarly, LTP has been found in extrinsic neurons of the mushroom body of the honeybee [23–25]. Another well studied example is the cerebellar cortex. In the cat, 10^6 mossy fibers innervate 10^9 granular cells, which converge onto 10^6 Purkinje cells [26]. Plasticity in the parallel fibers, the axons of the granular cells in the fan-in layer, has been the subject of intense research for more than three decades [27]. In machine learning, it is common to construct fan-out fan-in networks for nonlinear classification problems. In such networks the inputs are projected to a higher dimension space via a nonlinear transformation and are then linearly projected to a lower dimensional space [28]. In these models, the nonlinear transformation in the fan-out layer is typically fixed, whereas the fan-in connections are plastic, enabling learning [28].

The locus of plasticity in the VL system of the octopus thus stands in sharp contrast to other neuronal networks, both natural and artificial (see Supplemental Discussion). This difference is particularly surprising given the standard locus of plasticity in its phylogenetically close relative, the cuttlefish. Does this unique organization of the octopus VL necessarily mean a different functional implication? An exciting possibility is that in this system, the same functionality can be achieved in the two dichotomously different networks. According to this view, the computation achieved by this network is invariant to the locus of plasticity.

To explore this possibility, we consider the input-output relationship of the AM neurons. Figure S2 depicts the dependence of the fPSP on TP. Note that in both species the fPSP shows an approximately linear relationship with the input (TP amplitude). Because the AM neurons are inexcitable [12, 13, 29], these results indicate that the membrane potential of the AM neurons is a linear function of their input. Moreover, Figure 5C demonstrates that the LN axon bundles activity is also a linear function of the fPSP. These results indicate that the output of the AM neurons is a linear function of the input, a result that has profound computational implications.

To gain insight into these implications, we consider a schematic example, in which the VL network is characterized by a single SFLn neuron (S), a single AM neurons (A) and a single LN neuron (L). Assuming that the synaptic input to a neuron is the sum of the presynaptic activities weighted by the synaptic efficacies, and denoting the synapse from S to A by J, the input to neuron A is given by $I_A = J \cdot S$. If the output of the AM neuron is a linear function of its input, $A = a \cdot I_A$, where a characterizes the transfer function of the neuron, then the input to the LN neurons is given by $I_L = a \cdot J \cdot W \cdot S$, where W denotes the synaptic strength from A to L (Figure 1B). Thus, the dependence of the input to the LN neuron I_L on the input

S is mediated by the product of the two synaptic efficacies, *J* and *W*. In other words, a change in the value of *J* is computationally equivalent to a change in the value of *W*.

In the Supplemental Computational Considerations, we show that if the input-output relation of the AM neurons is linear, then in the general fan-out fan-in architecture of Figure 1B, the same computation and learning can be achieved independently of the locus of plasticity. According to this view, the different evolution or self-organization of the locus of plasticity in the two systems is permissible because in this type of system, with a linear input-output relationship of neurons in the intermediate layer, computation and learning is indifferent to the locus of plasticity.

The possibility that computation in the VL is invariant to the locus of plasticity is reminiscent of very interesting studies of heterogeneity in the pyloric circuit of the stomatogastric ganglion of the crab *Cancer borealis* [30, 31]. Substantial variability in the level of expression of different ionic channels and the strengths of the synaptic connections was demonstrated and found to have little effect on network dynamics. Our study suggests that the computation of the network may be invariant to much grosser features such as the locus of plasticity. Moreover, in the case of the stomatogastric ganglion, the heterogeneity is between individuals of the same species, possibly as a result of a homeostatic regulation that converges to one of multiple solutions with the same target neural activity [32]. In contrast, in the case of the modern cephalopods, the heterogeneity is between species as a result of a selection process converging to one of several possible solutions having the same computational capabilities.

Conclusions

These differences in network organization, discovered in phylogenetically close species and in networks with homologous function, cell types, connectivity, and architecture, is compatible with the idea that computational constraints influence the emergence of network properties [33, 34]. Moreover, it indicates that it is the computation capacity of the network, not the specific properties, that is self-organized or selected for by evolutionary pressure.

Experimental Procedures

Available online as Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, Supplemental Discussion, and two figures and can be found with this article online at doi:10.1016/j.cub.2011.09.011.

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References

1. Budelmann, B.U. (1995). The cephalopods nervous system: What evolution has made of the molluscan design. In The nervous system of invertebrates: An evolutionary and comparative approach, O. Breidbach and W. Kutsuch, eds. (Basel: Birkhauser Vergal), pp. 115–138.
2. Young, J.Z. (1971). The anatomy of the nervous system of Octopus vulgaris (Oxford: Clarendon Press).
3. Boycott, B.B., and Young, J.Z. (1955). A memory system in Octopus vulgaris Lamarck. Proc. R. Soc. Lond. B Biol. Sci. *143*, 449–480.
4. Fiorito, G., and Chichery, R. (1995). Lesions of the vertical lobe impair visual discrimination learning by observation in Octopus vulgaris. Neurosci. Lett. *192*, 117–120.
5. Graindorge, N., Alves, C., Darmaillacq, A.S., Chichery, R., Dickel, L., and Bellanger, C. (2006). Effects of dorsal and ventral vertical lobe electrolytic lesions on spatial learning and locomotor activity in Sepia officinalis. Behav. Neurosci. *120*, 1151–1158.
6. Shomrat, T., Zarrella, I., Fiorito, G., and Hochner, B. (2008). The octopus vertical lobe modulates short-term learning rate and uses LTP to acquire long-term memory. Curr. Biol. *18*, 337–342.
7. Nixon, M., and Young, J.Z. (2003). The brain and lives of cephalopods (Oxford: Oxford University Press).
8. Boycott, B.B. (1961). The functional organization of the brain of the cuttlefish Sepia officinalis. Proc. R. Soc. Lond. B Biol. Sci. *153*, 503–534.
9. Gray, E.G. (1970). The fine structure of the vertical lobe of octopus brain. Philos. Trans. R. Soc. Lond. B Biol. Sci. *258*, 379–394.
10. Wells, M.J. (1978). Octopus (London: Chapman and Hall).
11. Young, J.Z. (1991). Computation in the learning-system of cephalopods. Biol. Bull. *180*, 200–208.
12. Hochner, B., Brown, E.R., Langella, M., Shomrat, T., and Fiorito, G. (2003). A learning and memory area in the octopus brain manifests a vertebrate-like long-term potentiation. J. Neurophysiol. *90*, 3547–3554.
13. Hochner, B., Shomrat, T., and Fiorito, G. (2006). The octopus: A model for a comparative analysis of the evolution of learning and memory mechanisms. Biol. Bull. *210*, 308–317.
14. Bullock, T.H., and Horridge, G.A. (1965). Structure and function in the nervous systems of invertebrates (San Francisco: Freeman).
15. Matzner, H., Gutfreund, Y., and Hochner, B. (2000). Neuromuscular system of the flexible arm of the octopus: Physiological characterization. J. Neurophysiol. *83*, 1315–1328.
16. Glanzman, D.L. (2010). Common mechanisms of synaptic plasticity in vertebrates and invertebrates. Curr. Biol. *20*, R31–R36.
17. Shomrat, T., Feinstein, N., Klein, M., and Hochner, B. (2010). Serotonin is a facilitatory neuromodulator of synaptic transmission and "reinforces" long-term potentiation induction in the vertical lobe of Octopus vulgaris. Neuroscience *169*, 52–64.
18. Hochner, B., Klein, M., Schacher, S., and Kandel, E.R. (1986). Action-potential duration and the modulation of transmitter release from the sensory neurons of Aplysia in presynaptic facilitation and behavioral sensitization. Proc. Natl. Acad. Sci. USA *83*, 8410–8414.
19. Li, Q., Roberts, A.C., and Glanzman, D.L. (2005). Synaptic facilitation and behavioral dishabituation in Aplysia: Dependence on release of Ca2+ from postsynaptic intracellular stores, postsynaptic exocytosis, and modulation of postsynaptic AMPA receptor efficacy. J. Neurosci. *25*, 5623–5637.
20. Li, H.L., Huang, B.S., Vishwasrao, H., Sutedja, N., Chen, W., Jin, I., Hawkins, R.D., Bailey, C.H., and Kandel, E.R. (2009). Dscam mediates remodeling of glutamate receptors in Aplysia during de novo and learning-related synapse formation. Neuron *61*, 527–540.
21. Jortner, R.A., Farivar, S.S., and Laurent, G. (2007). A simple connectivity scheme for sparse coding in an olfactory system. J. Neurosci. *27*, 1659–1669.
22. Cassenaer, S., and Laurent, G. (2007). Hebbian STDP in mushroom bodies facilitates the synchronous flow of olfactory information in locusts. Nature *448*, 709–713.
23. Keene, A.C., and Waddell, S. (2007). Drosophila olfactory memory: Single genes to complex neural circuits. Nat. Rev. Neurosci. *8*, 341–354.
24. Masse, N.Y., Turner, G.C., and Jefferis, G.S.X.E. (2009). Olfactory information processing in Drosophila. Curr. Biol. *19*, R700–R713.
25. Menzel, R., and Manz, G. (2005). Neural plasticity of mushroom body-extrinsic neurons in the honeybee brain. J. Exp. Biol. *208*, 4317–4332.

26. Ito, M. (1984). The cerebellum and neural control (New York: Raven Press).
27. Ito, M. (2002). The molecular organization of cerebellar long-term depression. Nat. Rev. Neurosci. *3*, 896–902.
28. Vapnik, V.N. (1998). Statistical learning theory (New York: John Wiley & Sons, Inc.).
29. Hochner, B. (2010). Functional and comparative assessments of the octopus learning and memory system. Front Biosci (Schol Ed) *2*, 764–771.
30. Goaillard, J.M., Taylor, A.L., Schulz, D.J., and Marder, E. (2009). Functional consequences of animal-to-animal variation in circuit parameters. Nat. Neurosci. *12*, 1424–1430.
31. Prinz, A.A., Bucher, D., and Marder, E. (2004). Similar network activity from disparate circuit parameters. Nat. Neurosci. *7*, 1345–1352.
32. Liu, Z., Golowasch, J., Marder, E., and Abbott, L.F. (1998). A model neuron with activity-dependent conductances regulated by multiple calcium sensors. J. Neurosci. *18*, 2309–2320.
33. Kaschube, M., Schnabel, M., Löwel, S., Coppola, D.M., White, L.E., and Wolf, F. (2010). Universality in the evolution of orientation columns in the visual cortex. Science *330*, 1113–1116.
34. Miller, K.D. (2010). Neuroscience. π = visual cortex. Science *330*, 1059–1060.