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A Learning and Memory Area in the Octopus Brain Manifests a Vertebrate-Like Long-Term Potentiation

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Hochner, Binyamin, Euan R. Brown, Marina Langella, Tal Shomrat, and Graziano Fiorito. A learning and memory area in the octopus brain manifests a vertebrate-like long-term potentiation. *J Neurophysiol* 90: 3547–3554, 2003. First published August 13, 2003; 10.1152/jn.00645.2003. Cellular mechanisms underlying learning and memory were investigated in the octopus using a brain slice preparation of the vertical lobe, an area of the octopus brain involved in learning and memory. Field potential recordings revealed long-term potentiation (LTP) of glutamatergic synaptic field potentials similar to that in vertebrates. These findings suggest that convergent evolution has led to the selection of similar activity-dependent synaptic processes that mediate complex forms of learning and memory in vertebrates and invertebrates.

INTRODUCTION

Comparative analysis of brain mechanisms of invertebrates with sophisticated behavior like that of cephalopods (Hanlon and Messenger 1996) may advance our understanding of the neural processes involved in mediating complex behaviors. The octopus is ideal for such comparative study because it and the other modern cephalopods have evolved from shell-protected, slowly moving scavengers into agile, mobile predators sharing their environment and way of life with fish and competing successfully with them (Packard 1972). Octopus behavior is mediated by a highly developed centralized brain (Young 1971) of which the vertical lobe (VL) is involved in learning and memory (Sanders 1975; Wells 1978). Removal of the VL severely impairs long-term memory recall and the ability to learn new tasks (Boycott and Young 1955). Removing the VL also impairs the octopus' unique ability to learn a task by watching a previously trained demonstrator octopus perform it (Fiorito and Chichery 1995; Fiorito and Scotto 1992).

The VL contains only two types of morphologically identified neurons: approximately 25 million small (approximately 5 μm) amacrine interneurons that synapse onto only approximately 65,000 "large" (approximately 15 μm) efferent cells (Gray 1970; Young 1971) (Fig. 1, *A* and *B*). Young (1995) suggested that these neurons and their connections with the 1.8 million afferents from the medial superior frontal lobe (MSF; Fig. 1, *A* and *B*) form an association matrix analogous to the vertebrate hippocampus. Here we show that a slice preparation from the VL system can be used to examine the neural processes underlying the octopus' outstanding learning abilities. We found that the VL manifests long-term potentiation (LTP) similar to that in vertebrates, suggesting a convergent evolution of the synaptic processes underlying complex forms of learning.

METHODS

Animals

Specimens of *Octopus vulgaris* from the Bay of Naples, Italy, were held in individual aquaria in synthetic seawater (Aquarium Systems) as previously reported (Matzner et al. 2000).

Slice preparation

The preparation is an adaptation from Williamson and Budelmann (1991). The animals were deeply anesthetized in cold seawater containing 2% ethanol. The supraoesophageal mass was removed through a dorsal opening of the cranium (Young 1971). To soften its tough fibrous sheath, the brain was treated for 30 min at room temperature in 1% protease type IX (Sigma) in artificial seawater (ASW). ASW composition was (in mM) 460 NaCl, 10 KCL, 55 $MgCl_2$, 11 $CaCl_2$, 10 glucose, and 10 HEPES (pH 7.6). The brain was then glued to a vibratome stage with Histoacryl glue and supported from three sides with agar blocks. The vibratome bath was filled with a cold (4–8°C) 50:50 mixture of ASW and isotonic solution of $MgCl_2$. The brain sheath was removed and 400-μm sagittal slices were cut. The slices were maintained for $\leq$5 days at 15°C in culture medium (L15 with L-glutamine, adjusted to seawater salt concentration) and antibiotics (penicillin, 100 iu; streptomycin, 0.1 mg; amphotericin B, 0.25 μg/ml). Physiological experiments started 1 day after slice preparation.

Physiological experiments

The experimental chamber was continuously perfused with oxygenated ASW at room temperature at a rate of approximately 3.5 ml/min (approximately 2 volume changes/min). Drugs and experimental solutions were introduced via the perfusion system and took approximately 1–3 min to reach the recording site. Field potentials were evoked by stimulating the MSF tract (Fig. 1, *B* and *C*) with Teflon-insulated 0.08-mm-diam bipolar stainless steel electrodes. DC coupled extracellular recordings were made with glass microelectrodes filled with ASW (4–7 MΩ). The MSF tract was stimulated with test pulses of 0.2 ms every 10 s. Three to four tetanization trains were usually delivered by the same electrode. For higher intensities, the pulse duration was increased to 0.3 ms during the tetanization. LTP

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experiments were started only if a synaptic field potential (fPSP) could be detected under control conditions—sometimes by pair or triple pulse facilitation. In experiments where the fPSP of a single test pulse was small, we measured the second fPSP of twin pulses (20-ms interval), which was highly facilitated. Each series of experiments was intermittently accompanied by control LTP experiments. In these control experiments, tetanization was delivered following a period of ≥5 min recording, and the presence and level of LTP was determined by recording fPSP for at least a further 8 min.

Data analysis

Tract and synaptic field potentials were measured from trace averages (usually 10 traces of test responses = 100-s time period). The amplitude of the MSF tract potential (TP) was measured from the positive to the negative peaks. The fPSP amplitude was measured from the positive peak at the onset of the fPSP to its negative peak. We usually measured the area of the fPSP, defined as the area enclosed by a line connecting the two positive peaks at the beginning and end of the fPSP (Fig. 1*C*, hatched area in *bottom* gray trace). In some cases, fPSPs were scaled to correct changes of <20% in TP amplitude during an experiment. This procedure should not introduce a large error due to nonlinearity (see Fig. 2*A*). Normalized LTP was expressed relative to the average fPSPs before tetanization. Because of the linear relation between fPSP amplitude and area (see RESULTS and Fig. 2), either fPSP amplitude or area was used for calculating the normalized LTP values.

RESULTS

The large number of cells in the octopus VL and their laminar organization allows the recording of field potentials as in vertebrate brains. Stimulating the MSF tract evoked a large triphasic field potential near the tract (Fig. 1*C*, TP), whose amplitude declined with increasing distance between the stimulating and recording electrodes This decline probably results from the divergence of the MSF axons in the VL complex (Gray 1970; Young 1971). A second potential followed the tract potential (Fig. 1*C*, arrowheads); its presence, amplitude, and waveform varied in different preparations and locations.

This second potential, which we characterize below as an fPSP, undergoes a robust and enduring potentiation after a few short trains of high-frequency (HF) stimulation of the MSF tract. Figure 1*C* shows the gradual appearance of a prominent second negative potential following four tetanization trains (20 stimuli, 50 Hz, 10-s interval). Figure 1*D* summarizes 21 control LTP experiments (see METHODS) and depicts the level of potentiation relative to control as a function of the significance level (*P*) using an unpaired *t*-test. The average LTP in all the experiments was 2.01 ± 0.91 (mean ± SD, $P < 0.0001$, 1-sample 2-tailed *t*-test for difference from a hypothetical mean of 1). Four of the 21 experiments did not show significant LTP ($P > 0.1$), while 12 experiments showed a very high level of significance ($P < 0.0006$), with LTP > 1.5 (circled in Fig. 1*D*).

Figure 1*E* shows the average level and time course of

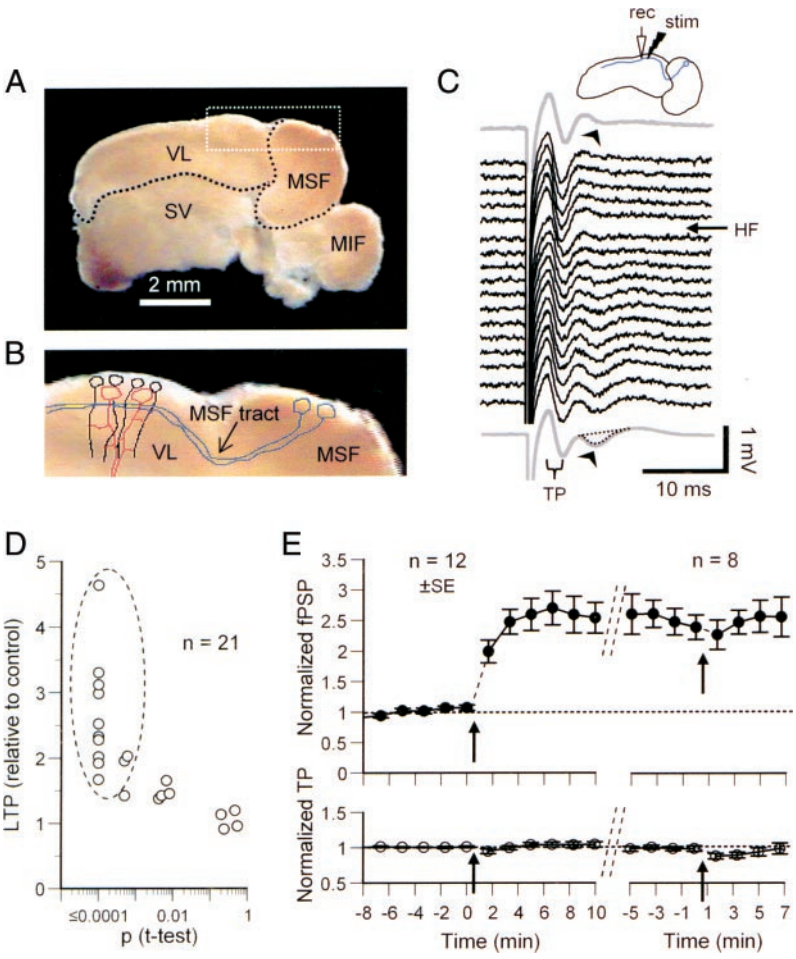

FIG. 1. Properties of the octopus vertical lobe (VL) slice preparation. *A*: sagittal slice from the medial part of the supraoesophageal brain mass showing VL and medial superior frontal lobe (MSF) located dorsally to the medial inferior frontal (MIF) and subvertical (SV) lobes. *B*: area within the white rectangle in *A* with a superimposed schema showing the MSF neurons (blue) innervating the amacrine cells (black). The amacrine cells synapse onto the large efferent cells (red) (Young 1971.). *C*: *Inset*: experimental arrangement of the stimulating (stim) and recording (rec) electrodes near the MSF tract. Stimulus artifact was followed by a large tract field potential (TP) and a small postsynaptic field potential (fPSP) (arrowheads). Raw data traces (1/10 s) show the development (in downward order) of a robust facilitation of the fPSP after 4 high-frequency trains (HF, 20 stimuli at 50 Hz, 10-s interval) to the MSF tract (arrow HF). Gray traces, averages before tetanization (*top*) and after (*bottom*). The hatched area is the fPSP area. *D*: distribution of LTP levels as a function of their significance in 21 control experiments. LTP level is expressed relative to control and is calculated by dividing the average fPSP (area or amplitude) of 30–90 responses (5–15 min) after the HF, by the average of 30–60 responses before the HF. Significance of change was estimated by an unpaired *t*-test. *E*: time course of LTP development, maintenance, and saturability of 12 control experiments (circled in *D*). Experiments are aligned to time of HF. In each experiment, the fPSP (area or amplitude) and TP amplitude are normalized to the averages before the HF. Arrows indicate 4 HF tetani. Time-break lines: in 8 of the 12 experiments, a 2nd HF was delivered. These are aligned at 5 min before the 2nd HF. fPSP and TP are shown at the same scale to allow comparison of relative changes.

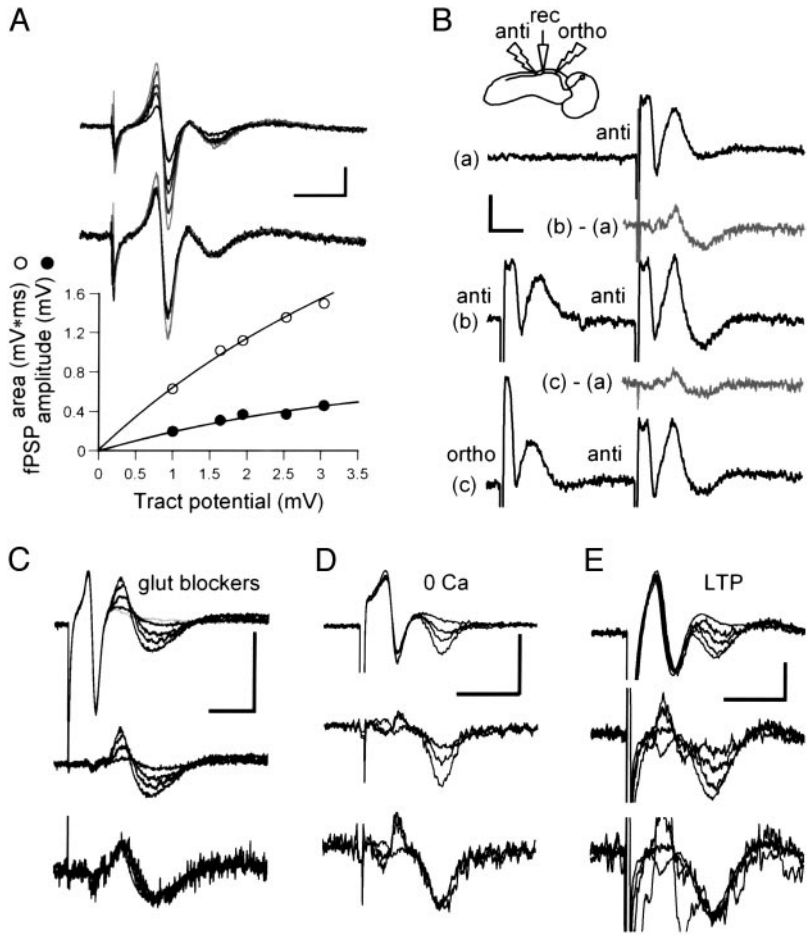

FIG. 2. Characterization of the fPSP (traces are averages of 2–10 responses). *A*: relationship between TP amplitude and fPSP area and amplitude. *Top*: superposition of average responses obtained at 5 stimulus intensities. *Middle*: a good fPSP's overlap after fPSP amplitude normalization. *Bottom*: sub-linear relationship of fPSP area (○) and amplitude (●) to TP. The fitted lines are derived from the relation $fPSP = K_1 \times TP/(K_2 + TP)$, assuming a linear relation between TP amplitude and synaptic conductance and saturation of fPSP due to reduction in driving force for synaptic current. *B*: paired pulse facilitation (PPF) of fPSPs and cross-PPF between ortho- and antidromic stimulation. *a*: single antidromic stimulation. *b*: PPF of paired antidromic stimuli. *b-a*: subtraction of *a* from *b* shows the level of PPF. *c*: cross-PPF of antidromic by orthodromic stimulus. *c-a*: subtraction shows the level of cross-PPF that is lower than in PPF (*b-a*). *C*: fPSP is blocked (200 μM CNQX + 20 mM kynurenate) with no change in fPSP waveform. *Top*: superposition of 5 responses obtained during rinsing out the blockers. *Middle*: the same traces after subtraction of the maximally blocked trace (gray in *top*) showing the isolated biphasic fPSP. *Bottom*: amplitude normalization of the subtracted traces to show the invariance of fPSPs shapes. *D*: similar to *C* during the blockade in Ca^{2+}-free ASW. *E*: similar to *C* during the development of LTP. Calibrations: 5 ms; 0.5 mV; 0.2 mV in *B*.

development of the potentiation in these 12 experiments. The potentiation took about 5 min to develop with the average potentiation level being 2.56 ± 0.83. This potentiation was maintained for the duration of the experiment (≤5 h). Our tetanization protocol resulted in a saturated level of potentiation, as demonstrated by the lack of further potentiation following a second tetanization episode (Fig. 1*E*, *right*; $n = 8$). The LTP does not involve clear presynaptic modification of MSF axon excitability because, in contrast to the fPSP potentiation, the average normalized TP did not change significantly during the experiments except for a transient decline just after the tetanizations (Fig. 1*E*, *bottom*).

This activity-modulated potential appears to be an fPSP because *1*) it disappeared in Ca^{2+}-free ASW (Fig. 2*D*); *2*) it showed a robust, frequency-dependent modulation, such as paired pulse facilitation (PPF, Fig. 2*B*); and *3*) it is also most likely a glutamatergic fPSP because it was blocked by CNQX, kynurenate (Fig. 2*C*), and DNQX, and occluded by glutamate (1 mM, data not shown).

The characteristics of the fPSP suggest that it derives from postsynaptic currents. Both the amplitude and the area of the fPSP were linearly related to the tract potential. However, a large fPSP (e.g., following LTP or PPF) may demonstrate some sub-linear relations (Fig. 2*A*), which could be attributed to changes in synaptic driving force (see Fig. 2). The intensity-dependent changes in fPSPs were not accompanied by changes in fPSP waveform, as demonstrated by superimposing the amplitude normalized fPSPs (Fig. 2*A*) and by the significant linear correlation between the amplitude and area of the fPSPs ($r = 0.9693$, $P < 0.0064$). The fPSP waveform was also unaffected by treatments that modulate postsynaptic responses (Fig. 2, *C–E*); the fPSPs, recorded during recovery from CNQX and kynurenate block, could be superimposed when normalized with respect to amplitude (Fig. 2*C*). Similar results were obtained when the fPSP diminished in low Ca^{2+} ASW (Fig. 2*D*) or when the fPSP was robustly augmented during LTP development (Fig. 2*E*). These findings all suggest that the fPSP represents a glutamatergic synaptic current with no population spikes.

Morphological studies by Gray (1970) indicate that the MSF tract innervates the amacrine cells in the VL (Fig. 1*B*) similarly to the innervation of hippocampal pyramidal cells in CA1 by the Schaeffer collaterals. His findings therefore predict that an fPSP could be evoked by antidromic stimulation of the MSF tract, and we confirm this in Fig. 2*Ba*. Moreover, the presence of cross PPF (Fig. 2*B* and 4 other experiments), in which orthodromic stimulation facilitated the antidromic responses (or vice versa), provides direct physiological support for the *en passant* mode of innervation indicated in Gray's study. In an *en passant* synapse, the synaptic connection is made somewhere along the presynaptic axon, allowing the synapse to be activated by action potentials traveling in both directions; therefore the pairing for PPF can be achieved by appropriately timing the ortho- and antidromic stimuli.

LTP maintenance did not require the ongoing test stimulation because it was maintained without test stimuli for ≥130 min. In the several cases where this was tested, the LTP was not erased by temporarily blocking the fPSPs for 10–25 min by perfusing the preparation with low Ca^{2+} (5 experiments) or with CNQX and/or kynurenate (3 experiments). We examined the effect of 5-HT on the fPSP, because it is involved in both short- and long-term synaptic plasticity in other molluscs (Kandel 2001) and is present in the octopus brain (Messenger 1996). We did not detect any significant effects in 13 experiments using 10–50 μM 5-HT perfused for periods ranging from 4 to 30 min.

LTP involves presynaptic expression

Examination of the fPSPs evoked by trains of HF stimulation (50 Hz) revealed profound dynamic properties—facilitation of the fPSP in the first three to four pulses followed by a depression (Fig. 3*A*, *top*). This characteristic dynamic undergoes a marked modification after LTP induction, as shown in Fig. 3*A* (*middle*). Comparing individual fPSPs in the two trains (Fig. 3*A*, *bottom*) shows that there is a robust potentiation of the first fPSP, which disappears gradually by the fourth fPSP, while depression is evident from the eighth stimulus onward (cf. *top* and *middle*). Figure 3, *B* and *C*, shows an analysis of the average of 7 experiments. The relative potentiation is not the same for all 20 fPSPs in the train, as would be expected were the site of modification postsynaptic. Instead, the difference between the control and potentiated fPSPs (Fig. 3*B*, black dots) changes from positive in the first three pulses into negative by the fifth stimulus. Importantly, summing the areas of the individual fPSPs in the trains revealed no significant changes following LTP (Fig. 3*C*). This result suggests that LTP in the VL involves presynaptic modification, as was inferred from changes in PPF in the hippocampus mossy fiber synapses (Zalutsky and Nicoll 1990). Moreover, the constancy of the integrated fPSPs area (Fig. 3*C*) suggests that LTP modification in the octopus is entirely presynaptic, since it is difficult to reconcile this result with conventional postsynaptic modifications. The most parsimonious explanation is that the synaptic depression during HF stimulation results from exhaustion of the transmitter pool available for release, and while LTP involves a robust change in the release dynamics, there is no change in the size of this pool and in the kinetics of its replenishment. In only one case did we find that the relative potentiation was similar for all fPSPs in the train.

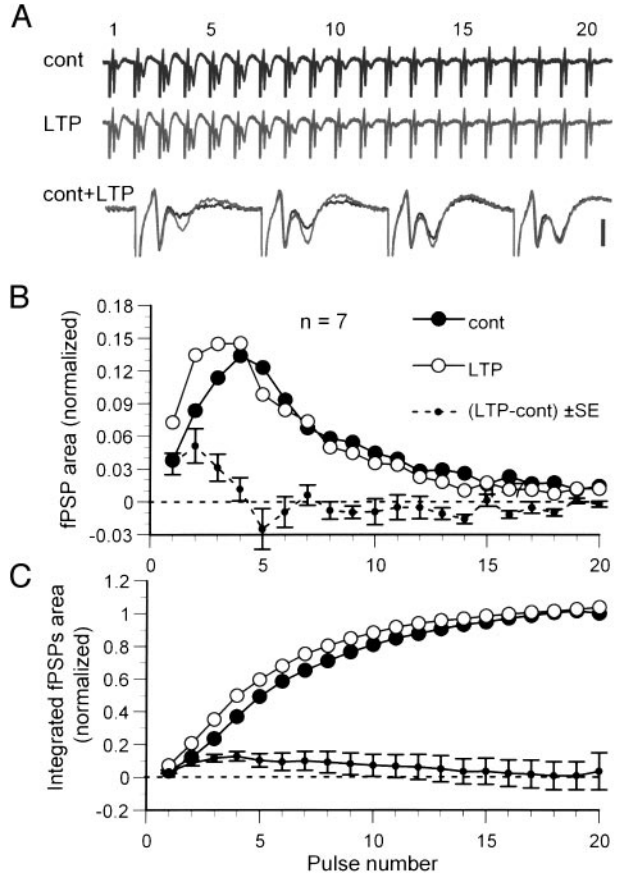

FIG. 3. Dynamic properties of the synapses suggest that LTP involves presynaptic modifications. *A*: dynamic properties of the fPSP as revealed during a 50-Hz train of 20 stimuli (cont, black trace) and its modification after LTP (LTP, gray trace). Note the robust facilitation of the fPSPs that is followed by depression. *Bottom* (cont + LTP): superposition of the 1st 4 pulses in the trains shows increase of only the 1st 3 fPSPs. *B*: summary of 7 experiments. fPSP areas are normalized to the sum of fPSP areas in the control trains. Average control (●) and the LTP (○). Average differences (LTP − cont) ± SE (black dots) are shown to demonstrate the pulses with significant difference from 0 (broken line). *C*: integrated areas of the fPSPs in the respective trains (demonstrated by the running sums, symbols as in *B*). Total area did not differ significantly from controls after LTP induction.

Octopus LTP showed several features of both Hebbian and non-Hebbian activity-dependent synaptic enhancement

COOPERATIVITY. Cooperativity was demonstrated by the presence of an intensity threshold for LTP induction. The three train *insets* in Fig. 4*A* show the first 9 of the 20 stimuli in each train. Train (1) in Fig. 4*A* caused some postsynaptic responses (arrows) but no LTP (see *inset* and HF arrow (1). Increasing the pulse duration within the train from 0.15 to 0.2 ms led to an increase in amplitude of the TP and fPSPs [Train (2)], and consequently, to a significant LTP (*inset* and HF (2). Three such trains resulted in a further facilitation (3). These results demonstrate the existence of threshold and gradation in LTP induction. Since an increase in TP amplitude most likely indicates recruitment of more MSF fibers (e.g., Figs. 2*A* and 4*C*), it is reasonable to assume that LTP induction requires the cooperative activation of a minimal group of MSF fibers in addition to the HF stimulation. Threshold was demonstrated in two of three trials, so it is not yet clear that this is a general feature of LTP in the octopus VL.

SPECIFICITY. In the experiment in Fig. 4*B*, two test fPSPs were generated by orthodromic and antidromic volleys in the MSF tract. The absence of cross-PPF suggests that each stimulus activated different groups of MSF fibers. In two experiments, orthodromic tetanization caused LTP of only the orthrodromically stimulated fibers, while antidromic tetanization caused facilitation of the antidromically evoked fPSP. In a third experiment, where some cross-PPF was demonstrated before HF (e.g., Fig. 2*B*), some partial cross-LTP was also induced in the responses evoked by the nontetanized electrode.

Figure 4*C* shows a further method of assessing specificity. The dependence of fPSP area on TP amplitude was tested by varying the pulse intensities. Results from three experiments were pooled by normalizing the fPSP area and TP amplitude to

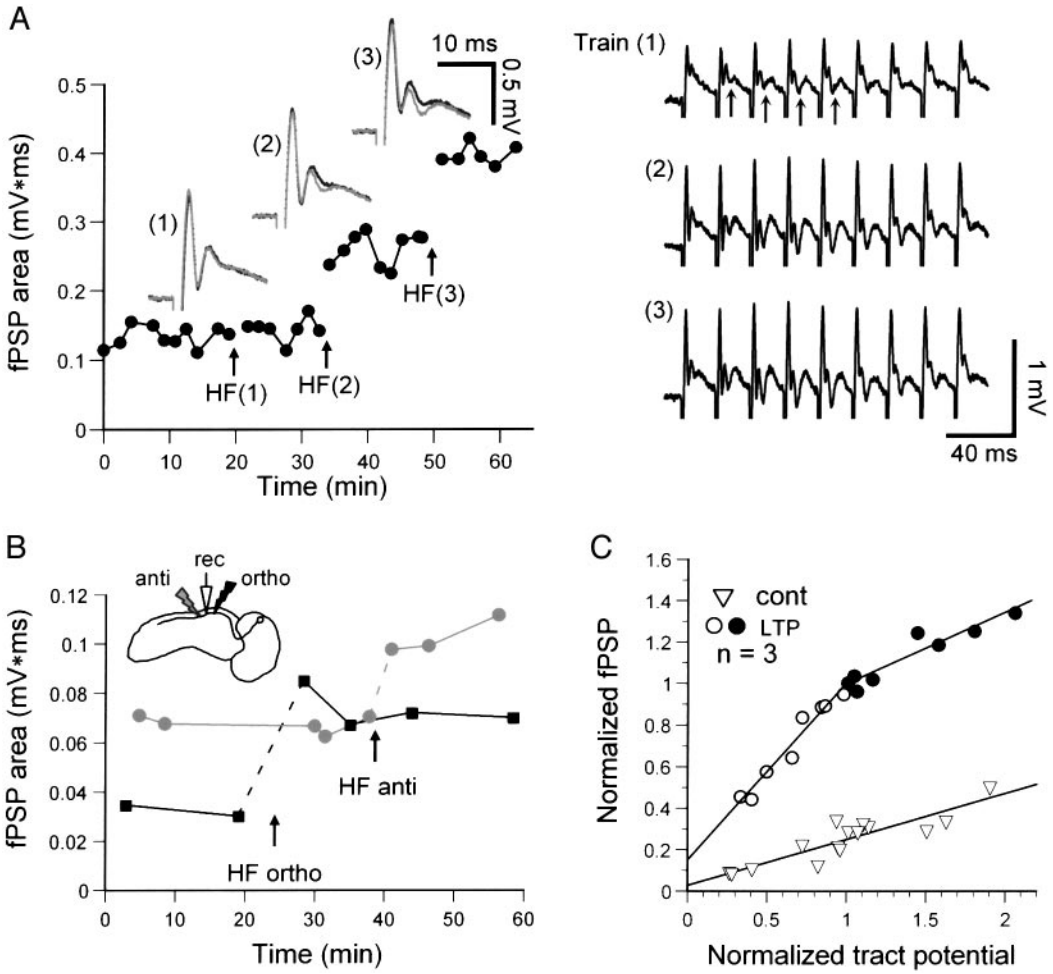

FIG. 4. Cooperativity, gradation, and specificity of LTP induction. *A*: threshold intensity for LTP induction. One tetanization train (pulse duration 0.15 ms) was given at time marked HF(1). This train evoked fPSPs [marked in the *right panel* as Train (1)]. Here the 9 first stimuli of the train are shown (several fPSPs are marked with arrows). No potentiation was induced, as seen in the graph and *inset (1)*, which shows superposition of the average field potentials before (black) and after the train (gray). A single HF train with pulse duration 0.2 ms [Train (2), given at time marked HF(2)], caused higher release and led to significant facilitation [*inset (2)*]. Three such trains [the 1st is shown in Train (3)] were given at time HF(3) and further facilitation was obtained [*inset (3)*]. *B*: plot of the test fPSPs evoked by ortho- and antidromic stimulation. *Inset:* experimental arrangement. Tetanization caused LTP only of the tetanized input. (HF-ortho and HF-anti respectively). *C*: 3 experiments in which the dependence of fPSP on TP amplitude was examined before (△) and after LTP induction (○ and ●). fPSP and TP are normalized to those obtained by the intensity of the HF stimulation. Control results are scaled by the average LTP (LTP = 4). Three regression lines are fitted to the control (△, $r = 0.8937$, $P < 0.0001$), after LTP at the range of TP ≤ 1 (○, $r = 0.9754$, $P \leq 0.0015$), and TP > 1 (●, $r = 0.9459$, $P \leq 0.0028$). Following LTP, the slope increased only at TP potentials equal to or less than the tetanization amplitude (i.e., TP ≤ 1). Dependence at higher amplitudes remained close to the pre-LTP slope. This suggests that only the tetanized axons were potentiated.

those evoked by the tetanization stimulus in each experiment. The linear dependency of the fPSP on TP amplitude increased following LTP, providing that TP amplitude remained <1, which was the tetanization TP amplitude. Following LTP, the slope below 1 increased 3.89-fold (cf. ○ and △; difference between slopes $t = 7.13$; $P < 0.0001$), while the slope above 1 (●) increased only by a factor of 1.55 (difference from the slope below 1, $t = -5.55$, $P < 0.0001$) and differed less significantly from the control slope ($t = 2.07$, $P = 0.0523$). These results support specificity as they suggest that fibers recruited by intensities above that of the HF stimulation were not potentiated and therefore their dependence on TP amplitude remained more similar to control values.

CALCIUM IONS ARE ESSENTIAL FOR LTP INDUCTION. LTP induction was blocked when Ca^{2+} was reduced to a level that inhibited the fPSPs during the train. Figure 5*A* shows the average results of six experiments where the fPSPs were completely blocked during the HF stimulation in Ca^{2+}-free ASW. Following rinsing back ASW, the recovered average test fPSPs showed some augmentation relative to control although this was not significant. However, now a second HF stimulation led to the induction of LTP.

N-METHYL-D-ASPARTATE–INDEPENDENT INDUCTION OF LTP. We found no strong indication for the involvement of conventional *N*-methyl-D-aspartate (NMDA)-like receptors in the LTP. In 10 of 19 experiments, LTP was obtained in the presence of APV and/or MK-801 up to a concentration of 200 μM. In the experiments where LTP was not induced in the presence of APV, LTP was also not induced after APV had been washed out. Moreover, we did not find any effects of APV on fPSPs in

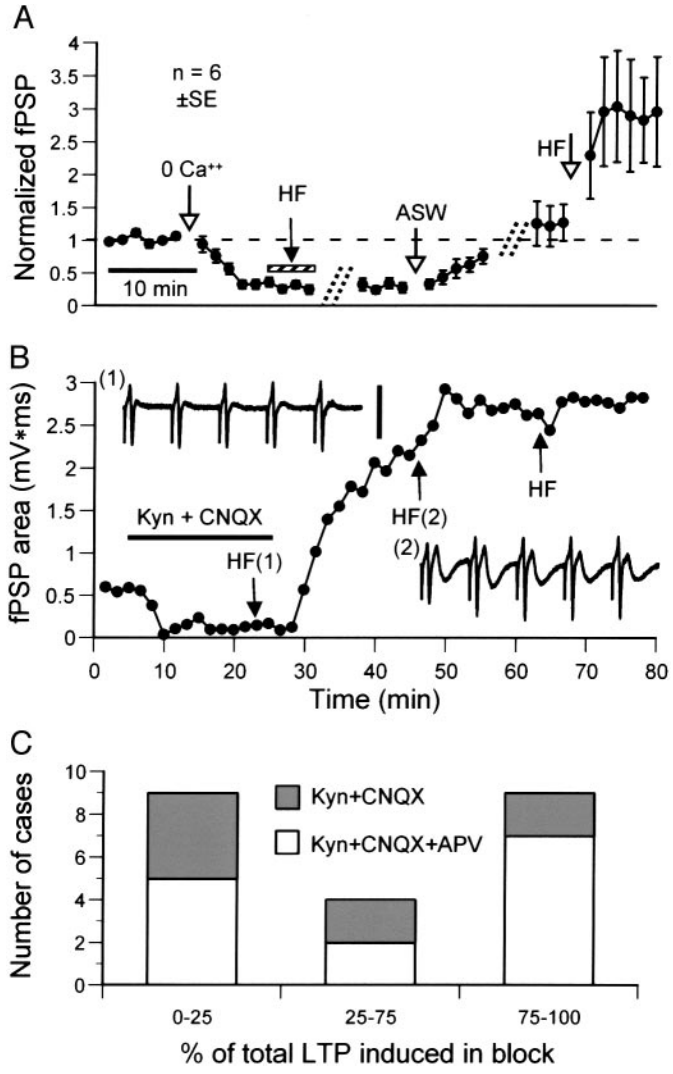

FIG. 5. Dependence of LTP induction on synaptic transmission and postsynaptic response. *A*: summary of 6 experiments where release was completely blocked during the 4 HF trains in Ca^{2+}-free artificial seawater (ASW). To average the experiments, they were divided into 3 sections that were aligned at (empty arrows) *1*) start of perfusion with Ca^{2+}-free ASW, and the HF was given at different time in each experiment depending when fPSP was blocked; *2*) returning to ASW, and *3*) the 2nd HF given after recovery in normal ASW. HF during the block did not induce significant LTP while HF following the recovery led to LTP induction. *B*: an experiment where the response during HF [*inset (1)*] was completely blocked by a mixture of 20 mM kynurenate plus 200 μm CNQX. After rinsing, the test fPSP was highly potentiated with respect to control, and additional HF [*inset (2)*] led only to small additional potentiation. *C*: summary of 22 experiments such as that shown in *B*. Experiments with normal and half Ca^{2+} concentration are pooled. Inclusion of 200 μm APV in the blocking mixture is indicated. The histogram categories depict three ranges of LTP (expressed as the percent of total LTP) 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.

normal ASW, or in low-Mg^{2+} ASW (5 mM Mg^{2+}, 5 experiments). In *Aplysia* neurons, this Mg^{2+} concentration was found to eliminate the voltage dependence of a glutamate-induced current (Dale and Kandel 1993). In contrast to APV, MK-801 had some inhibitory effects on both the tract and postsynaptic field potentials. As these effects may be associated with the known influence of MK-801 on excitability (e.g., Rothman 1988), we cannot tell whether its lack of effect on LTP induction is also associated with lack of effect on fPSPs. The failure of these drugs to block LTP does not exclude the possibility that the dynamic properties of the synapse (Fig. 3) overcame the APV competitive inhibition. Because MK-801 is a noncompetitive blocker, the lack of effect of this drug on LTP induction is more conclusive. Note that an APV and MK-801 sensitive NMDA-like current has been found in squid chromatophore muscle cells (Lima et al. 2003).

DOES LTP INDUCTION DEPEND ON THE POSTSYNAPTIC RESPONSE?

To ensure postsynaptic block of the highly facilitated fPSPs during the HF stimuli, we had to use a powerful blocking mixture of 200 μM CNQX and 20 mM kynurenate (Mellor and Nicoll 2001; Yeckel et al. 1999). As we sometimes detected fPSPs during HF stimulation even under these conditions (probably due to the facilitation during the train and the competitive nature of these inhibitors), Ca^{2+} concentration was also halved in some experiments to ensure a complete block. This Ca^{2+} concentration in itself was not sufficient to block HF fPSPs.

Figure 5*B* shows an example of an experiment where the HF response was completely blocked [*inset (1)*], nevertheless, a major part of the total LTP was induced by this HF stimulation. This is shown by the recovery of the fPSP to a highly potentiated level following washout of the drug and the marginal additional LTP obtained by the second HF (HF (2)) that evoked very large responses [*inset (2)*]. This result indicates clearly that some form of LTP can be induced in the absence of fPSPs, and thus most likely without the strong postsynaptic depolarization that is an important component in Hebbian-type associative induction of LTP. Nevertheless, this does not exclude other postsynaptic mechanisms (see DISCUSSION).

In 22 such experiments, we quantified the level of fPSP-independent LTP induction by calculating the fraction of total LTP induced during the block. For example, in the experiment in Fig. 5*B*, it was estimated to be 80%. The results of these experiments are summarized in Fig. 5*C*; various levels of LTP, from complete block to full LTP, were obtained under conditions where no fPSPs were detected during the HF stimulation. It is unlikely that residual NMDA-like currents mediate the induction of LTP, since there were no clear differences in the distribution of LTP levels when the blocking mixture included APV (Fig. 5*C*; χ^2 for 2 variables; $P \leq 1$). The distribution of the LTP levels shows a symmetric bimodal distribution with higher proportion of cases with either low or high levels of LTP. Assuming a random distribution of the two forms of LTP in the VL, with and without postsynaptically response-dependent induction mechanisms, we would predict that 94.75% of the cases should appear in the 25–75% range. The chance that the observed distribution fits this prediction is <0.0001 (1-sample χ^2 test). These results show that there are two distinct forms of LTP in the MSF synapses. One form most likely requires glutamatergic fPSPs for induction and thus may contribute to Hebbian-type associative induction. The other is induced in the absence of fPSPs; this suggests non-Hebbian mechanisms, but the exact mechanism of this form of LTP awaits examination with intracellular recording and imaging techniques. The bimodal distribution of these two forms of LTP can be explained if these two forms of LTP segregated in different areas in the VL, which so far do not appear to be morphologically differentiated.

DISCUSSION

Here we present the first analysis of field potential recording from a slice preparation of the octopus VL. This area of the octopus brain is involved in complex forms of long-term learning and memory (Boycott and Young 1955; Fiorito and Chichery 1995) and shows some structural analogy to the hippocampus (Young 1995). The VL shows a robust activity-dependent synaptic plasticity that resembles aspects of vertebrate LTP. This finding may indicate that similar cellular processes have been selected during the evolution of phylogenetically remote animals with complex forms of learning. This, in turn, suggests that LTP emerges from mechanisms important for complex learning processes, strengthening the association between LTP and learning and memory, still a controversial issue (Martin et al. 2000).

As in different parts of the hippocampus, there appear to be two different types of mechanisms for LTP-induction in the octopus VL; one type appears to depend on the postsynaptic response, while the other does not require a strong postsynaptic response for induction. This latter form may be mediated entirely within the presynaptic domain or postsynaptically by nonionotropic-glutamatergic transmission, by a different transmitter or even by some low threshold mechanisms which may be activated by a residual unblocked glutamatergic postsynaptic response.

At least one form of LTP induction in the octopus has properties similar to the NMDA-dependent associative LTP in hippocampal synapses (Malenka and Nicoll 1999) and at least some of the connections in the VL show some of the basic properties of Hebbian type synaptic plasticity. In this context, our failure to demonstrate an NMDA-like component in the synaptic field potential or its involvement in LTP induction is puzzling. It suggests the evolution of a non-NMDA mechanism for associative synaptic plasticity in the octopus. The finding is all the more puzzling as *Aplysia* shows NMDA-like currents (although APV insensitive, Dale and Kandel 1993) and APV sensitive homosynaptic plasticity (Lin and Glanzman 1994; Murphy and Glamzman 1999). It is unlikely that species-specific pharmacology prevented the observation of NMDA-like effects, since APV and MK-801 sensitive L-glutamate/NMDA-induced currents have been recorded in squid chromatophore muscle cells (Lima et al. 2003). This point must be clarified by searching directly for the presence of NMDA-like current and receptors and testing whether other processes, such as voltage-dependent Ca^{2+} current (Grove and Teyler 1990; Anikstejn and Ben-Ari 1991), parallel the NMDA mechanisms in LTP induction.

The robust and unique LTP-associated changes in the dynamic properties of fPSPs (Fig. 3) strongly suggest that LTP in the VL involves presynaptic modification. This makes the postsynaptically induced LTP mechanism in the octopus VL more similar to that suggested by Yeckel et al. (1999) and Contractor et al. (2002) for the mammalian mossy fiber synapses (but see Kakegawa et al. 2002 and Mellor and Nicoll 2001) because its expression is presynaptic even though it is postsynaptically induced, most likely by an APV insensitive mechanism.

The octopus is much more advanced than molluscs like *Aplysia*, in which synaptic plasticity has been extensively characterized. Therefore it is interesting to compare the plasticity mechanisms evolved in the VL to those present in *Aplysia*. Serotonin, a powerful heterosynaptic neuromodulator of both short- and long-term plasticity in *Aplysia* (Kandel 2001) is not directly involved in octopus LTP. Thus a homosynaptic glutamatergic mechanism may have evolved as the mechanism most suitable for an associative learning network. In *Aplysia*, a fundamentally similar process (albeit APV sensitive) is thought to be involved in development (Conrad et al. 1999) and to contribute partially to a simple form of associative learning (Bailey et al., 2000; Lin and Glanzman 1994; Murphy and Glanzman 1997, 1999). This mechanism thus probably arose early in mollusc evolution and was selected and conserved in a brain area evolving to mediate learning within complex cognitive behaviors.

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REFERENCES

Anikstejn L and Ben-Ari Y. Novel form of long-term potentiation produced by a K^+ channel blocker in the hippocampus. *Nature* 349: 67–69, 1991.

Bailey CH, Giustetto M, Huang YY, Hawkins R, and Kandel ER. Is hetrosynaptic modulation essential for stabilizing Hebbian plasticity and memory? *Nature Rev Neurosci* 1: 11–20, 2000.

Boycott BB and Young JZ. A memory system in *Octopus vulgaris* Lamarck. *Proc Roy Soc Lond B* 143: 449–480, 1955.

Conrad P, Wu F, and Schacher S. Changes in functional glutamate receptors on a postsynaptic neuron accompany formation and maturation of an identified synapse. *J Neurobiol* 39: 237–248, 1999.

Contractor A, Rogers C, Maron C, Henkemeyer M, Swanson GT, and Heinemann SF. Trans-synaptic Eph receptor-ephrin signaling in hippocampal mossy fiber LTP. *Science* 296: 1864–1869, 2002.

Dale N and Kandel E. L-glutamate may be the fast excitatory transmitter of Aplysia sensory neurons. *Proc Natl Acad Sci USA* 90: 7163–7167, 1993.

Fiorito G and Chichery R. Lesions of the vertical lobe impair visual discrimination learning by observation in *Octopus vulgaris. Neurosci Lett* 192: 117–120, 1995.

Fiorito G and Scotto PX. Observational learning in *Octopus vulgaris. Science* 256: 545–547, 1992.

Gray EG. The fine structure of the vertical lobe of *octopus* brain. *Phil Trans Roy Soc Lond B* 379: 379–394, 1970.

Grover LM and Teyler TJ. Two components of long-term potentiation induced by different patterns of afferent activation. *Nature* 347: 477–479, 1990.

Hanlon RT and Messenger JB. *Cephalopod Behaviour*. Cambridge: Cambridge University Press, 1996.

Kakegawa W, Yamada N, Iino M, Kameyama K, Umeda T, Tsuzuki K, and Ozawa S. Postsynaptic expression of a new calcium pathway in hippocampal CA3 neurons and its influence on mossy fiber long-term potentiation. *J Neurosci* 22: 4312–4320, 2002.

Kandel E. The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 294: 1030–1038, 2001.

Lima PA, Nardi G, and Brown ER. AMPA/kainate and NMDA-like glutamate receptors at the chromatophore neuromuscular junction of the squid: role in synaptic transmission and skin patterning. *Eur J Neurosci* 17: 507–516, 2003.

Lin XY and Glanzman DL. Hebbian induction of long-term potentiation of *Aplysia* sensorimotor synapses: partial requirement for activation of an NMDA-related receptor. *Proc Roy Soc Lond B* 255: 215–221, 1994.

Malenka RC and Nicholl RA. Long-term potentiation—a decade of progress? *Science* 285: 1870–1874, 1999.

Martin SJ, Grimwood PD, and Morris RG. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci* 23: 649–711, 2000.

Matzner H, Gutfreund Y, and Hochner B. Neuromuscular system of the flexible arm of the octopus: physiological characterization. *J Neurophysiol* 83: 1315–1328, 2000.

Messenger JB. Neurotransmitters of cephalopods. *Invertebrate Neurosci* 2: 95–114, 1996.

Mellor J and Nicoll RA. Hippocampal mossy fiber LTP is independent of postsynaptic calcium. *Nature Neurosci* 4: 125–126, 2001.

Murphy GG and Glanzman DL. Mediation of classical conditioning in *Aplysia californica* by LTP of sensorimotor synapses. *Science* 278: 467–471, 1997.

Murphy GG and Glanzman DL. Cellular analog of differential classical conditioning in *Aplysia*: disruption by the NMDA receptor antagonist DL-2-amino-5-phosphonovalerate. *J Neurosci* 19: 10595–10602, 1999.

Packard A. Cephalopods and fish: the limits of convergence. *Biol Rev* 47: 241–307, 1972.

Rothman S. Noncompetitive *N*-methyl-D-aspartate antagonists affect multiple ionic currents. *J Pharmacol Exp Ther* 246: 137–142, 1988.

Sanders GD. The Cephalopods. In: *Invertebrate Learning*, edited by Corning WC, Dyal JA, and Willows AOD. New York: Plenum Press, 1975, p. 1–101.

Wells MJ. *Octopus.* London: Chapman and Hall, 1978.

Williamson R and Budelmann BU. Convergent inputs to octopus oculomotor neurones demonstrated in a brain slice preparation. *Neurosci Lett* 121: 215–218, 1991.

Yeckel MF, Kapur A, and Johnston D. Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism. *Nature Neurosci* 2: 625–633, 1999.

Young JZ. *The Anatomy of the Nervous System of Octopus vulgaris.* Oxford: Oxford University Press, 1971.

Young JZ. Multiple matrices in the memory system of octopus. In: *Cephalopod Neurobiology*, edited by Abbott JN, Williamson R, and Maddock L. Oxford: Oxford University Press, 1995, p. 431–443.

Zalutsky RA and Nicoll RA. Comparison of two forms of long-term potentiation in single hippocampal neuron. *Science* 248: 1619–1624, 1990.