Late phase of L-LTP elicited in isolated CA1 dendrites cannot be transferred by synaptic capture

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In the CA1 region of mouse hippocampal slices, a strong tetanic stimulation triggers a long-lasting long-term potentiation (L-LTP), which requires transcription for the development of its late phase. Nevertheless, we were able to elicit such an L-LTP in CA1 dendrites separated from their somas provided that we restricted our investigations to isolated dendrites where a very robust early LTP was triggered. This particular type of L-LTP, which relied on translation of preexisting messenger RNAs – as it was blocked by anisomycin – could not be captured by another pathway activated only by a weak tetanic stimulation. This suggests that the plasticity-related proteins resulting from translation of messenger RNAs in dendrites cannot pass from the synaptic site where they were synthesized to another one. *NeuroReport* **21:210–215 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

In the CA1 region of hippocampal slices, stimulation of Schaffer collateral axons by delivering one or several trains of high-frequency stimulation induces an immediate and prolonged increase in synaptic strength called long-term potentiation (LTP). A single train triggers an early LTP that lasts less than 3 h and depends only on modification and translocation of preexisting proteins. Several trains (usually three or four) cause a long-lasting LTP (L-LTP) that persists more than 4 h and requires synthesis of new proteins [1,2]. It is well established that these proteins result from induced transcription. Actinomycin D, an inhibitor of transcription, has been repeatedly reported to block this form of L-LTP [3–5]. As the late phase of L-LTP, like its early phase, is input-selective, that is, it is restricted to the activated synapses, the products of gene transcription must be dispatched only to these activated synapses [6,7]. According to Frey and Morris' model, this is achieved thanks to the creation in each stimulated synapse of a 'tag' capable of capturing the products of the gene expression after their transport along dendrites [4,6,8]. Assuming that a single train of stimulation – which is incapable of triggering transcription on its own – is able to create a synaptic tag, Frey and Morris' theory makes it possible to understand that, when L-LTP has been induced by a three or four-train stimulation in one pathway, the long-lasting aspect of that L-LTP can be captured by another pathway submitted only to a single train – a stimulation which would normally induce only a short-lasting LTP [6]. It has recently been confirmed that the molecules captured by the weakly activated synapses are products of gene expression, as capture is suppressed by actinomycin D [9].

However, there are forms of L-LTP that are independent of induced transcription. Recently, Huang and Kandel [10] reported that a particular paradigm of stimulation (5 Hz for 30 s) induced an L-LTP whose late phase was dependent on protein synthesis but independent of transcription. This strongly indicates that translation of messenger RNAs (mRNAs) in dendrites can support on its own the late phase of certain forms of L-LTP.

In agreement with the fact that transcription is necessary for the development of the late phase of L-LTP induced by several trains of high-frequency stimulation, it has been reported that LTP triggered by such a pattern of stimulation in CA1 dendrites separated from their somas declined to baseline values after 3 h [11].

Here, we monitored for 8 h the LTP induced by a strong stimulation (4 × 100 Hz) in CA1 dendrites separated from their somas when a favoring condition was used: analysis was restricted to isolated dendrites where the potentiation was at least 150% 1 h after the end of induction. In such circumstances, we were able to elicit a long-lasting LTP whose late phase was protein synthesis-dependent. In this case the plasticity-related proteins must result from translation of preexisting mRNAs present in dendrites.

Taking advantage of our observation on isolated dendrites, we addressed the following question. Do the plasticity-related proteins resulting from translation of preexisting mRNAs in dendrites remain stuck in the synaptic region where they were synthesized or can they be captured by other synapses tagged by a weak stimulation?

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Methods

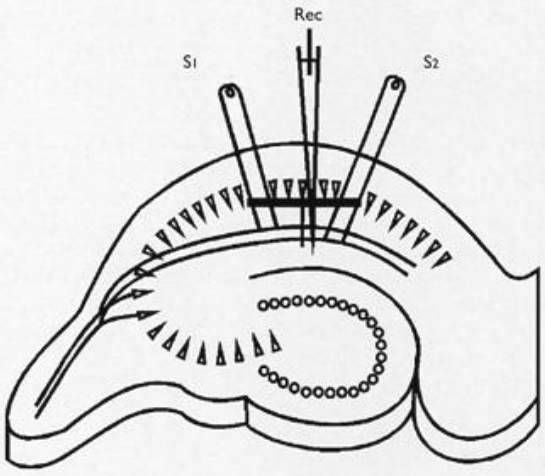
Preparation of slices

The experiments were performed on transverse hippocampal slices (400-µm thickness) prepared as described by Nguyen and Kandel [12]. Male C57BL/6 mice, aged 6–10 week (Charles River) were used for all the experiments, which were carried out in accordance with National Institutes of Health regulations for the care and use of animals in research and with local ethics committee guidelines. The hippocampus was isolated and sliced with a McIlwain chopper. Slices were perfused with artificial cerebrospinal fluid (ACSF) of the following composition: 124 mM NaCl, 5 mM KCl, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 10 mM glucose. The ACSF was aerated with 95% O_2 and 5% CO_2. Slices were allowed to recover at 28°C for 1.5 h in interface. After recovery, all the recordings were made in an interface chamber (FST, Vancouver, Canada) at 28°C. The rate of flow of the perfused liquid was 1 ml/min.

Electrophysiology

In all the experiments (in isolated dendrites as well as in control slices), two bipolar nickel–chromium stimulating electrodes were placed on each side of the recording electrode in order to evoke field excitatory post-synaptic potentials (fEPSPs) in two independent pathways (Fig. 1). The independence of these two pathways was ascertained by applying two pulses with a 50-ms interval to the two pathways and verifying the absence of paired-pulse facilitation. Extracellular fEPSPs (field excitatory postsynaptic potentials) were recorded with a glass microelectrode (2–5 MΩ, filled with ACSF) positioned in the stratum radiatum of area CA1. Stimulation intensity was adjusted to elicit fEPSP amplitudes that were around 40% of maximum size. Basal synaptic transmission was assessed by stimulating Schaffer collateral axons once every 15 min at this test stimulation intensity. Stimulation, data acquisition and analysis were performed using the WinLTP program (*www.winltp.com*) [13].

Fig. 1



Sketch of the 'isolated' CA1 dendrites preparation showing the two independent synaptic inputs S1 and S2 to the same neuronal population, the glass-recording electrode (Rec) and the position of the cut (black bar).

Separation of CA1 dendrites from their cell bodies

In slices where the apical dendrites of CA1 pyramidal neurons had to be separated from their cell bodies, an incision was made directly below the cell body layer using a razor blade under a dissecting microscope (Fig. 1). These slices were left to recover from the microlesion for a further hour and a half. The isolation of the CA1 dendrites from their cell bodies was confirmed electrophysiologically when stimulation of the Schaffer collateral axons failed to elicit population spikes in the cell body layer [10,14–16]. We also videotaped the making of the cut and checked off-line whether the severance of the dendrites from their somas was complete. After recording a stable 30 min baseline of fEPSPs, LTP was induced by applying one or four 1-s trains (100 Hz, at test strength) 5 min apart and was monitored for a period of 8 h after the end of application of the trains.

Results

We analyzed long-term synaptic plasticity in two types of slices: those where the dendrites were left intact (control slices) and those where the dendrites were separated from their cell bodies (isolated dendrites).

Long-lasting long-term potentiation in 'isolated' hippocampal CA1 dendrites

As far as isolated dendrites are concerned, we restricted our analysis only to slices where a robust early LTP could be induced, that is, when the potentiation was still at least 150% 1 h after the end of induction. We explored the possibility that L-LTP could develop in isolated dendrites, selected according to the criterion described above, on the basis of local, dendritic protein synthesis. In control slices (Fig. 2a) as well as in isolated dendrites (Fig. 2b), we triggered LTP by delivering four trains of tetanic stimulation to one pathway (S1) whereas another pathway (S2), not submitted to any tetanic stimulation, served as a control. While monitoring the fEPSP for 8 h, we observed a sustained L-LTP in isolated dendrites as well as in control slices. Eight hours after induction in control slices, the fEPSP slope in the stimulated pathway was 182.1 ± 9.3% of baseline ($n = 6$) (Fig. 2a). At the end of the experiment in isolated dendrites, the fEPSP slope was 155.0 ± 28.2% of baseline ($n = 6$) (Fig. 2b). So, we confirmed and extended the result of Vickers *et al.* [15] who used the same criterion for selecting the isolated dendrites for L-LTP analysis.

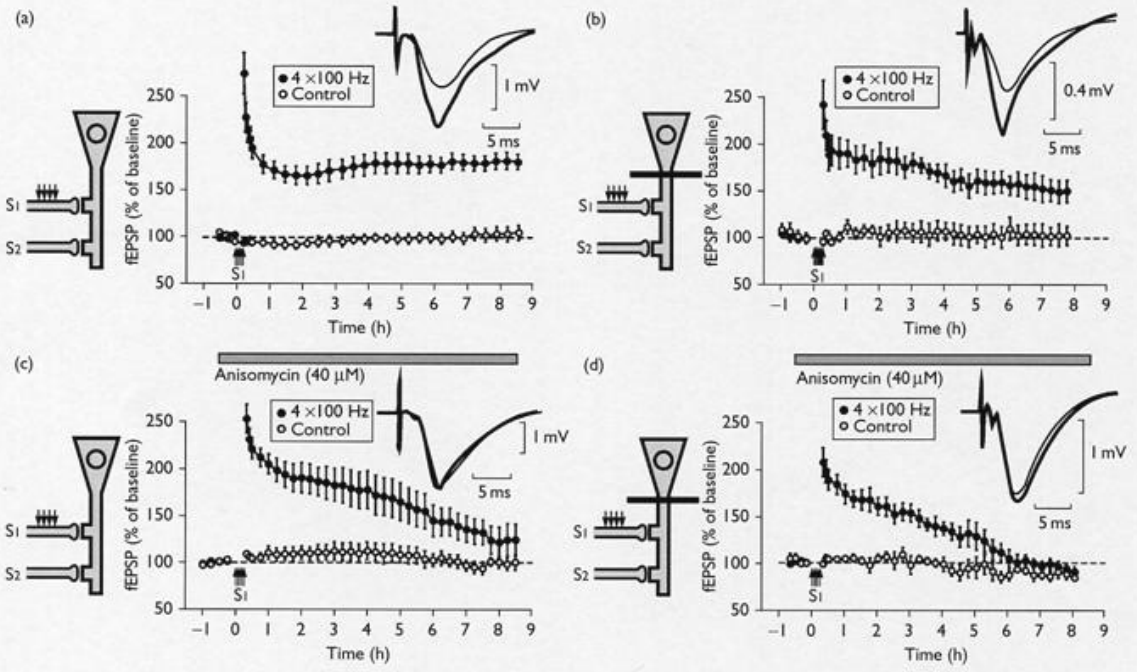
We next investigated whether the capability of synapses in 'selected' isolated dendrites to remain potentiated for such a long time was dependent or not on protein synthesis. On both types of slices, we applied anisomycin (40 μM), an inhibitor of translation, from 30 min before LTP induction till the end of the experiment. Under the influence of the drug, isolated dendrites, like control slices, showed decaying LTP. Eight hours after induction, the fEPSP slope was smaller in control slices treated with anisomycin (121.3 ± 16.2% of baseline, $n = 5$) than in drug-free control slices (Fig. 2c, t-test, $P < 0.05$). In isolated dendrites, the fEPSP slope measured at the end of the experiment was much smaller in presence of the drug (90.5 ± 12.3% of baseline, $n = 6$) than in its absence (Fig. 2d, t-test, $P < 0.001$).

Synaptic capture is not possible in 'isolated' hippocampal CA1 dendrites

On 'selected' isolated dendrites, we were able to elicit an L-LTP relying on new proteins synthesized by translation of preexisting mRNAs in dendrites. We next investigated whether, in this isolated dendrites preparation, the late phase of the L-LTP induced on one pathway by a strong stimulation (several trains) could be 'captured' by a pathway that is only weakly activated (one train), a phenomenon discovered by Frey and Morris [6] in slices with intact dendrites. We first reproduced this result in intact slices. When a single train (weak stimulation) was applied to one pathway in isolation, it triggered an LTP that was not sustained (Fig. 3a). The fEPSP slope observed 8 h after induction was 119.1 ± 9.2% ($n = 6$). By contrast, when the delivery of a single train on one pathway (S2) was preceded by the stimulation of another pathway (S1) by 4 trains (strong tetanic stimulation) 45 min earlier, the situation was completely different (Fig. 3b). In this case, the late phase of L-LTP fully developed not only in the pathway stimulated by four trains but also in the pathway submitted only to a single train (Fig. 3b). In S2, the fEPSP slope measured at the end of the experiment was 145.2 ± 7.0% ($n = 6$), a value larger than that observed when a single train was applied in isolation (Fig. 3b, t-test, $P < 0.05$). In S1, it was 160.9 ± 8.4% ($n = 6$).

Then, to investigate whether such a phenomenon – mediated by synaptic capture – could take place when dendrites were separated from their cell bodies, we

Fig. 2



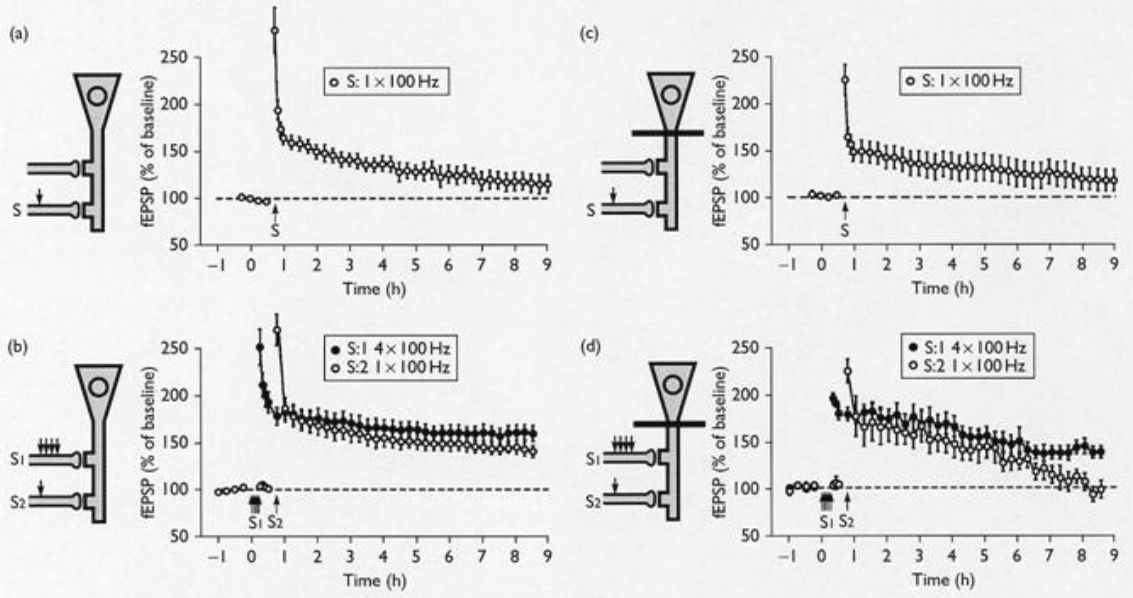
A protein synthesis-dependent, long-lasting long-term potentiation (LTP) can be elicited by a strong stimulation both in control slices and in isolated dendrites. In each block of the figure, S1 is the stimulated pathway whereas the S2 pathway serves as a control. (a and b) LTP-induced by 4 trains of tetanic stimulation 5 min apart in control slices (a) and in isolated dendrites (b). (c and d) Anisomycin (40 μM, Sigma) prevented the development of the late phase of long-lasting LTP both in control slices (c) and in isolated dendrites (d). Anisomycin was prepared as a concentrated stock solution in dimethyl sulfoxide and then diluted to 0.1% in artificial cerebrospinal fluid to its final concentration. (Insets) Representative potentials recorded immediately before (thin line) and 8 h after (thick line) tetanization.

applied the same stimulation paradigm to isolated dendrites (Fig. 3c and d). When a single train (weak stimulation) was delivered in isolation to one pathway in the isolated dendrites, it elicited an LTP that was not sustained. Eight hours after induction, the fEPSP slope was $117.3 \pm 25.9\%$ ($n = 5$). When the synaptic-capture stimulation paradigm was applied, as expected, the strong tetanic stimulation of pathway S1 went on to trigger an L-LTP but there was no development of a late phase of LTP mediated by synaptic capture in pathway S2. In S1, the fEPSP slope measured 8 h after induction was $137.3 \pm 6.5\%$ ($n = 7$). In S2, the fEPSP slope measured at the end of the experiment was as low as $97.1 \pm 25.0\%$ ($n = 7$), which was not statistically different from the value observed when one pathway was stimulated by a single train in isolation (compare Fig. 3c to Fig. 3d, *t*-test, $P = 0.20$).

Discussion

It is now well established that dendrites of hippocampal neurons are translationally competent. Ribosomes and many mRNAs (perhaps several hundreds) are present in them [17–19]. Moreover, using a dendritic protein synthesis reporter, Aakalu *et al.* [20] showed unequivocally that protein synthesis could be stimulated by brain-derived neurotrophic factor in isolated dendrites. Among the dendritic mRNAs, some, like that of CaMKII, are stored in the dendrites independently of any synaptic stimulation (preexisting mRNAs) [21]. Others, like that of Arc/Arg 3.1, are directed to the dendrites mainly in response to strong synaptic activation (induced mRNAs) [22]. Translation of preexisting mRNAs in dendrites, on one hand, has been proved to play a role in several types of synaptic plasticity. Kang and Schuman [14] showed that brain-derived neurotrophic factor could induce LTP that was blocked by protein synthesis inhibitors even when the dendrites were severed from their somas. A role for rapid dendritic protein synthesis was also demonstrated in mGluR-dependent LTD [23]. Finally, it is also possible to elicit an L-LTP in isolated CA1 dendrites by stimulating Schaffer collateral axons at 5 Hz for 30 s [10]. Translation of induced mRNAs, in contrast, has been demonstrated to play a role in other forms of synaptic plasticity [2,3]. In particular, the late phase of L-LTP triggered by a three- or four-train stimulation has been repeatedly shown to be blocked by actinomycin D, and thus dependent on induced transcription [3]. Here, we found that, in selected isolated CA1 dendrites where LTP was at least 150% 1 h after

Fig. 3



The late phase of the long-lasting long-term potentiation (L-LTP) induced by a strong stimulation of one pathway is accessible to another pathway by synaptic capture in intact slices but not in isolated dendrites. (a and b) In control slices, a single train, delivered in isolation on one pathway (S), elicited an LTP that was not sustained (a). By contrast, when a single train was applied on one pathway (S2) 45 min after another pathway (S1) had been submitted to four trains, it induced an L-LTP. Compare the temporal profile of the curves linking the circles in (a) and in (b). (c and d) In isolated dendrites, a single train did not induce an L-LTP whether applied in isolation (c) or after another pathway had received four trains of stimulation (d). The LTP triggered by a single train decayed in the same way in both cases. Compare the temporal profile of the curve linking the circles in (c) to that of the curve linking the circles in (d).

induction, it was possible to elicit an L-LTP whose late phase was dependent on protein synthesis (as it was blocked by anisomycin, a translation inhibitor) and independent of induced transcription (as it occurred in isolated dendrites). As far as the L-LTP triggered in intact slices by three or four trains of stimulation is concerned, the inhibitory effect of actinomycin D shows that it relies mainly on induced transcription, but our results suggest that translation of preexisting mRNAs might act as a helping factor.

Basically, the L-LTP triggered in the CA1 region by the application of three or four trains of high-frequency stimulation on the Schaffer collateral axons is dependent on transcription and is input-selective. According to the Frey and Morris model, a strong activation of one pathway triggers a signal routed to the nucleus to activate the transcription of a set of genes and, at the activated synapses, the making of a local 'tag' capable of capturing the products of gene transcription. According to this model too, a weak activation of another input is not sufficient to activate transcription but enough to create a tag. Two possibilities must be considered. On the one hand, the induced mRNAs could be translated at the level of the soma and the resulting proteins transported afterwards along the dendrites towards the synapses. In contrast, the induced mRNAs could be directed to the dendrites where they would be translated. The experiment by Bradshaw *et al.* [24] suggests that the latter possibility is the right one: the late phase of L-LTP was blocked when a translation inhibitor was focally injected in the CA1 dendritic region but not when it was injected in the somatic region [24]. The next question concerns the nature of the products captured by the tag. Is it the mRNA or the resulting protein? Our results suggest that it is the mRNA. Indeed, in our paradigm, when a protein synthesis-dependent L-LTP was elicited in isolated dendrites by a strong stimulation of one pathway, it could not be captured by another pathway submitted to a weak stimulus. This suggests that the plasticity-related proteins synthesized locally by translation of mRNAs cannot pass from one synaptic site to another. In other words, to secure input-selectivity, the tag has to capture the mRNA. Once the resulting protein is synthesized, it remains on site.

Conclusion

(1) In certain circumstances, it is possible to elicit an L-LTP in CA1 isolated dendrites, which suggests that the L-LTP triggered by a strong tetanic stimulation in intact slices might partly depend on translation of preexisting mRNAs.
(2) This particular L-LTP triggered in isolated dendrites by a strong tetanic stimulation of one pathway cannot be captured by another pathway activated by a weak stimulation. This strongly suggests that the proteins resulting from translation of mRNAs in dendrites remain stuck on the synaptic site where they were synthesized.

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