Synaptic capture-mediated long-lasting long-term potentiation is strongly dependent on mRNA translation

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In the CA1 region of mice hippocampal slices, a strong tetanic stimulation of an input pathway triggers a long-lasting long-term potentiation (L-LTP), which requires protein synthesis for the development of its late phase. A weak tetanic stimulation of one pathway, which is incapable of triggering protein synthesis on its own, can nonetheless induce L-LTP if it is preceded by a strong stimulation of another pathway (synaptic capture-mediated L-LTP). We found that anisomycin (25 μM), a translational inhibitor, impaired the strong stimulation-induced L-LTP more severely when the drug was applied during the whole experiment than when delivered only around the induction period. Taking advantage of this phenomenon, we showed that the synaptic capture-mediated L-LTP was strongly dependent on mRNA translation. *NeuroReport* **20:1572–1576 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

One of the most studied types of synaptic plasticity is the long-term potentiation (LTP) elicited in the CA1 region of hippocampal slices by stimulation of the Schaffer collaterals. A single train of high-frequency stimulation (100 Hz, 1 s) triggers a relatively short-lasting LTP (1–3 h) that relies only on posttranslational modifications and trafficking of proteins. By contrast, three or four stimulation trains (5–10 min apart) trigger an LTP that lasts more than 4 h (long-lasting LTP or L-LTP) and which requires the synthesis of new proteins for the development of its late phase [1–3]. Both short lasting and L-LTP are input-selective, that is, they are restricted to the activated synapses [4,5].

The input selectivity of L-LTP raises the question of how the products of gene expression are dispatched selectively to the synapses submitted earlier to the trains of high-frequency stimulation. In 1997, Frey and Morris [6] proposed a hypothesis, 'synaptic tagging', which could solve that problem. They observed that, when an L-LTP had been induced in one pathway, the long lasting aspect of that L-LTP could be 'captured' by another pathway submitted only to a single train, a stimulation which would normally induce only a short lasting LTP. To explain this phenomenon, Frey and Morris have suggested that a single train, which is not strong enough to induce gene expression, induces a 'synaptic tagging' that can capture the products of the gene expression triggered by three or four trains in another pathway.

This theory predicts that the development of the late phase of the LTP mediated by synaptic capture should be strongly dependent on protein synthesis. Surprisingly, anisomycin (Sigma, St. Louis, Missouri, USA), an inhibitor of translation, was reported to inhibit this phenomenon only moderately [7].

Here, we found that anisomycin was more effective in inhibiting the late phase of the L-LTP triggered by four trains when applied during the whole experiment rather than only around LTP induction, as is often done. Taking advantage of this observation, we show here that the synaptic-capture mediated L-LTP is, as predicted by Frey and Morris' theory, strongly dependent on protein synthesis.

Methods

Slice preparation

Male C57BL/6 mice, aged 6–10 weeks (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. Transverse hippocampal slices (400-μm thickness) were prepared as described by Nguyen and Kandel (1997) [8]. The hippocampus was isolated and sliced with a McIlwain chopper. Slices were perfused with artificial cerebro-spinal fluid (ACSF) of the following composition: 124 mM NaCl, 5 mM KCl, 26 mM $NaHCO_3$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, and 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 this recovery period all the recordings were made in an interface chamber (FST, Vancouver, Canada) at 28°C. In mice, this temperature was indeed found to be optimal to observe an L-LTP in hippocampal slices. The rate of flow of the perfused liquid was 1 ml/min.

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Electrophysiological recordings

Schaffer collaterals were stimulated with 0.08-ms pulses using bipolar nickel–chromium electrodes. Extracellular field excitatory postsynaptic potentials (fEPSPs) 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 collaterals once per minute at this test stimulation intensity. Slices that showed maximal fEPSPs of less than 2 mV were rejected. LTP was induced after recording a stable 30 min baseline of fEPSPs. LTP was induced electrically by applying one or four 1-second trains (100 Hz, at test strength) 5 min apart and monitored for a period of 4 h after the end of application of the trains, in the presence or absence of anisomycin, a protein-synthesis inhibitor. In certain experiments, two bipolar nickel–chromium stimulating electrodes were placed on each side of the recording electrode to evoke fEPSPs in two independent pathways. In these cases, we always checked for pathway independence by applying two pulses with a 50-ms interval to the two pathways and verifying the absence of paired-pulse facilitation.

Drug treatment

Anisomycin was dissolved in dimethyl sulfoxide and diluted down to reach a final concentration of 25 μM (in 0.1% dimethyl sulfoxide).

Data analysis

Stimulation, data acquisition, and analysis were performed using the WinLTP program (*www.winltp.com*) [9]. For each slice, the fEPSP slopes were normalized against the average slope over the 30 min before LTP induction. In all the experiments of this work, the late phase of L-LTP was assessed by comparing the mean slopes (± SEM) of the fEPSP measured at the end of the experiments. One-way analysis of variance (ANOVA) and Student's *t*-test were used for data analysis.

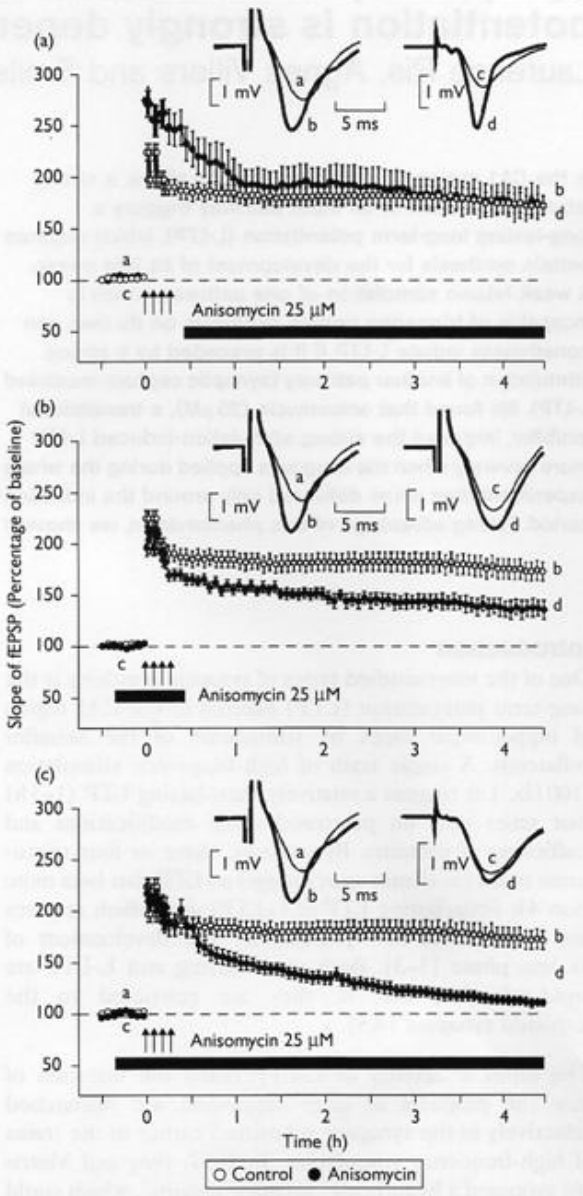
Results

Inhibition of translation during different time windows

We first compared the influence of anisomycin, an inhibitor of translation, (at a concentration of 25 μM) on the late phase of the L-LTP induced by the application of four high-frequency trains (strong stimulation) when this drug was applied either (i) after the delivery of the trains, or (ii) before and during the induction period, or (iii) throughout the experiment.

When the addition of anisomycin started 10 min after the trains, the fEPSP slopes measured at the end of the experiments were not different whether the drug was applied or not (174 ± 15%, $n = 15$, in presence vs. 169 ± 8%, $n = 15$, in absence of the drug, $P = 0.99$) (Fig. 1a). In contrast, when anisomycin was applied

Fig. 1



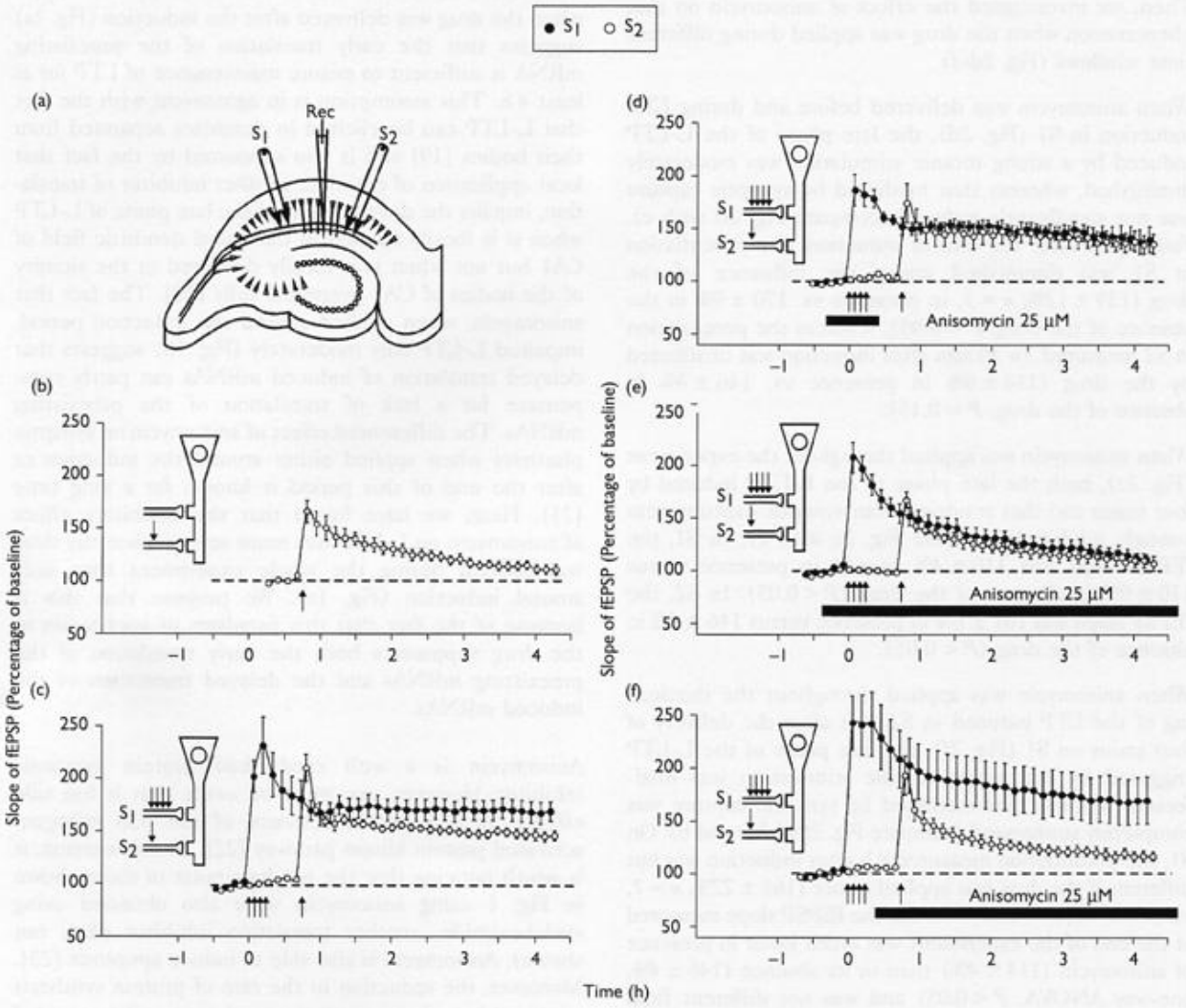
Impairment of the late phase of long-lasting long-term potentiation (L-LTP) by anisomycin, a translation inhibitor, in function of the period of application of the drug. In each part of the figure, LTP was induced by four trains of high-frequency stimulation (100 Hz, 1 s) 5 min apart (symbolized by four arrows) in absence or in presence of anisomycin and the corresponding time courses of the field excitatory postsynaptic potential (fEPSP, empty circles in absence and filled circles in presence of the drug) are displayed and compared. (a) Anisomycin was delivered starting 10 min after the four trains. The late phase of L-LTP was not different in absence or in presence of the drug. (b) Anisomycin was applied 20 min before, during, and 10 min after the four trains. The late phase of L-LTP (from 3 to 4 h after induction) was smaller in presence than in absence of the drug. (c) Anisomycin was present throughout the experiment. The late phase of L-LTP was smaller in presence of the drug than in its absence. The late phase of L-LTP was smaller when anisomycin was present during the whole experiment than when it was present only before and during induction (see b).

20 min before, during, and 10 min after induction, it partially blocked the expression of the late phase of L-LTP. Four hours after induction, the slope of the fEPSP was lower in presence ($135 \pm 8\%$, $n = 16$) than in absence of the drug ($169 \pm 8\%$, $P < 0.05$) (Fig. 1b). Interestingly, when anisomycin was perfused from 20 min before the induction period till the end of the experiment, the inhibitory effect of the drug on the generation of the late phase of L-LTP was more severe than that observed when the drug was added only around the induction period. The fEPSP slope measured 4 h after the end of induction was smaller in the first case ($108 \pm 3\%$, $n = 12$, Fig. 1c) than in the second case ($135 \pm 8\%$, $P < 0.05$, Fig. 1b).

Effect of anisomycin on synaptic capture

We next reproduced the result by Frey and Morris (1997) [6] (Fig. 2a–c). When a single train (weak tetanic stimulation) was applied to one pathway in isolation, it triggered an LTP that was not sustained (Fig. 2b). The fEPSP slope observed 3 h 30 min after induction was

Fig. 2



Effect of anisomycin on synaptic capture in function of the period of application of the drug. (a) Sketch of the experimental paradigm. Two independent input pathways are stimulated by two different electrodes (S1 and S2). The recording site of the field excitatory postsynaptic potential (fEPSP) is shown. Except in (b), S1 was stimulated by four 100-Hz trains, whereas S2 was submitted to a single 100-Hz train 45 min later. (b) A single train applied in isolation elicited a long-term potentiation (LTP) which is not sustained. (c) When a single train applied on S2 pathway was preceded by a strong stimulation of S1 pathway, it triggered a long-lasting-LTP by synaptic capture. (d) Synaptic capture was not affected when anisomycin was applied only around and during the period of application of four trains on S1 pathway. (e) When anisomycin was present during the whole experiment, the late phases of the L-LTP induced by both the strong stimulation (S1) and synaptic capture were suppressed. (f) When anisomycin was applied during the monitoring of the LTP elicited in S2, the late phase of the L-LTP resulting from synaptic capture was completely suppressed.

$109 \pm 5\%$ ($n = 9$). By contrast, when the delivery of a single train on one pathway (S2) was preceded by the stimulation of another pathway (S1) by four trains 45 min earlier; the situation was completely different (Fig. 2c). 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. 2c). In S1, the fEPSP slope measured at the end of the experiment was $170 \pm 9\%$ ($n = 9$) in S1 and $146 \pm 4\%$ in S2. In S1, L-LTP was triggered by the strong synaptic activation of that pathway; in S2, it resulted from the synaptic capture of a process initiated in another pathway.

Then, we investigated the effect of anisomycin on that phenomenon when the drug was applied during different time windows (Fig. 2d–f).

When anisomycin was delivered before and during LTP induction in S1 (Fig. 2d), the late phase of the L-LTP induced by a strong tetanic stimulation was moderately diminished, whereas that mediated by synaptic capture was not significantly reduced (compare Fig. 2d with c). Four hours after the end of induction, the potentiation in S1 was diminished under the influence of the drug ($139 \pm 12\%$, $n = 7$, in presence vs. $170 \pm 9\%$ in the absence of the drug, $P < 0.05$), whereas the potentiation in S2 measured 3 h 30 min after induction was unaffected by the drug ($134 \pm 8\%$ in presence vs. $146 \pm 4\%$ in absence of the drug, $P = 0.15$).

When anisomycin was applied throughout the experiment (Fig. 2e), both the late phase of the L-LTP induced by four trains and that resulting from synaptic capture were strongly inhibited (compare Fig. 2e with c). In S1, the fEPSP slope was $110 \pm 4\%$ ($n = 6$) in presence versus $170 \pm 9\%$ in absence of the drug ($P < 0.05$). In S2, the fEPSP slope was $107 \pm 6\%$ in presence versus $146 \pm 4\%$ in absence of the drug ($P < 0.05$).

When anisomycin was applied throughout the monitoring of the LTP induced in S2, but after the delivery of four trains on S1 (Fig. 2f), the late phase of the L-LTP triggered by the strong tetanic stimulation was unaffected, whereas that mediated by synaptic capture was completely suppressed (compare Fig. 2f with c and b). On S1, the potentiation measured 4 h after induction was not different if the drug was applied or not ($168 \pm 22\%$, $n = 7$, vs. $170 \pm 9\%$, $P = 0.77$). On S2, the fEPSP slope measured at the end of the experiment was much lower in presence of anisomycin ($114 \pm 4\%$) than in its absence ($146 \pm 4\%$, one-way ANOVA, $P < 0.05$) and was not different from that observed when one pathway was stimulated by a single train in isolation and in absence of anisomycin (Fig. 2b, $109 \pm 5\%$, one-way ANOVA, $P < 0.05$).

Discussion

The development of the late phase of an L-LTP triggered by the application of multiple trains on an input pathway is dependent on *de novo* protein synthesis. This synthesis takes place in two rounds [10]. During a first round, translation of mRNAs that exist at the level of the activated synapses before the application of the inductive stimulus (preexisting mRNAs), is triggered by the strong tetanic stimulation [11–14]. During a second round, the same strong inductive stimulus also triggers gene transcription [3,15,16]. The resulting mRNAs (induced mRNAs) are then translated in the soma and later transported along the dendrites [17] or directly transported towards the synaptic spines where they are eventually translated [18].

The fact that L-LTP was not affected by anisomycin when the drug was delivered after the induction (Fig. 1a) suggests that the early translation of the preexisting mRNA is sufficient to ensure maintenance of LTP for at least 4 h. This assumption is in agreement with the fact that L-LTP can be elicited in dendrites separated from their bodies [19] and is also supported by the fact that local application of emetine, another inhibitor of translation, impairs the development of the late phase of L-LTP when it is focally applied in the apical dendritic field of CA1 but not when it is focally delivered in the vicinity of the bodies of CA1 pyramidal cells [20]. The fact that anisomycin, when applied around the induction period, impaired L-LTP only moderately (Fig. 1b) suggests that delayed translation of induced mRNAs can partly compensate for a lack of translation of the preexisting mRNAs. The differential effect of anisomycin on synaptic plasticity when applied either around the induction or after the end of this period is known for a long time [21]. Here, we have found that the inhibitory effect of anisomycin on L-LTP was more severe when the drug was applied during the whole experiment than only around induction (Fig. 1c). We propose that this is because of the fact that this paradigm of application of the drug suppresses both the early translation of the preexisting mRNAs and the delayed translation of the induced mRNAs.

Anisomycin is a well established protein synthesis inhibitor. However, one must be aware that it has side effects. It is a potent activator of the p38 mitogen-activated protein kinase pathway [22]. In this context, it is worth noticing that the results similar to those shown in Fig. 1 using anisomycin were also obtained using cycloheximide, another translation inhibitor (data not shown). Anisomycin is also able to induce apoptosis [23]. Moreover, the reduction in the rate of protein synthesis triggered by anisomycin (or any other cause) is coupled with a decrease in the degradation of long-lived proteins [24]. However, these two side effects are not relevant on the time scales used in our study.

An L-LTP can also be induced in a pathway by only a weak tetanic stimulation if this is preceded by a strong tetanic stimulation of another pathway (Frey and Morris'

paradigm, Fig. 2a–c), a phenomenon that is interpreted as follows [6,10,25]. As a result of the application of four tetanic trains, mRNAs essential for synaptic plasticity are synthesized (induced mRNAs). In addition, the activation of a synapse whether it is weak or strong creates a 'synaptic tag' that can capture the relevant macromolecules. These can be either proteins resulting from translation of induced mRNAs in the soma, or induced mRNAs that, once captured, are translated into locally retained proteins.

According to this point of view, the development of the late phase of the synaptic capture-mediated LTP should be dependent on translation of only the induced mRNAs. When anisomycin is delivered only during and around the application of a strong tetanic stimulation on the pathway stimulated first, it is expected to prevent early translation of preexisting mRNAs but not to suppress delayed generation and translation of induced mRNAs. Consequently, the late phase of the synaptic capture-mediated L-LTP should not be affected, a theoretical prediction confirmed by our results (Fig. 2d). By contrast, when translation of the induced mRNA is blocked by the application of anisomycin at least during the whole monitoring of LTP in the pathway S2 (Fig. 2e and f), the late phase of the synaptic capture-mediated L-LTP should be suppressed, a prediction also confirmed by our observations (Fig. 2e and d).

Furthermore, it is worth noticing that, whatever the time-window of application of anisomycin used, the effects of the drug on the L-LTP induced by four trains were the same, whether the four trains were applied in isolation or followed by one train on another pathway (compare Fig. 1a with Fig. 2f, Fig. 1b with Fig. 2d, and Fig. 1c with Fig. 2e).

Conclusion

(1) The late phase of the L-LTP induced by a strong tetanic stimulation is completely suppressed by anisomycin when it is applied throughout the experiment, allowing the drug to block translation of both the mRNA stored at synaptic sites before induction and the mRNA formed as a result of the induced gene expression.

(2) The late phase of the L-LTP mediated by synaptic capture (Frey and Morris' paradigm) is completely suppressed by anisomycin when this drug is applied before and after the application of the weak stimulation on the capturing pathway. This strongly suggests that this type of L-LTP relies on the translation of the mRNAs induced by the strong activation of the other pathway.

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