Synaptic tagging and memory trace

J. López-Rojas [a], W. Almaguer-Melián [b], J.A. Bergado-Rosado [b]

SYNAPTIC TAGGING AND MEMORY TRACE

Summary. Aim. *To present a panorama of the main features and possible identity of the synaptic tag, such as to discuss some of its functional implications.* Development. *Long-term potentiation (LTP) constitutes a very attractive synaptic/cellular memory model. LTP, like memory, can manifest itself early (essentially depending on the modification of pre-existing proteins at synapse) and late (depending on new protein synthesis). As LTP is a highly specific phenomenon, a dilemma arises: how can the proteins, required to plastic change stabilization, that are synthesized at the soma of a neuron containing thousands of synaptic contacts –all depending of the same nucleus– go to the appropriate synapses? In this review, we present some of the models that intend to explain this question, making emphasis on synaptic tagging hypothesis. Some of the main findings that have contributed to tagging hypothesis are exposed. The local protein synthesis and the activation of protein kinases are analyzed as candidates to be the synaptic tag. Additionally, some of the functional implications of synaptic tagging are discussed.* Conclusions. *The synaptic tagging hypothesis offers a very flexible and reasonable solution to the specificity of long-lasting synaptic changes. Although some of the tagging features are known, the synaptic tag identity has not yet been elucidated. It seems that there is not a unique synaptic tag, but there are rather multiple molecular synaptic tags involved. Each of them might function as a synaptic tag under particular circumstances. Each might be differentially recruited by specific stimuli and mediate plasticity over different time domains. [REV NEUROL 2007; 45: 607-14]*

Key words. *LTP. Memory. Protein kinases. Protein synthesis. Synapse. Synaptic tagging.*

SYNAPSE AND MEMORY

For a long time up to the present, due to the significance of learning and memory especially for their importance in adaptation, the man have been motivated to understand their physiological mechanisms, and how to modulate them for his benefit. But, it is not until the second half of the 20th century that technological advances and most precise knowledge of the Nervous System, have allowed us to begin to discern on the possible mechanisms underlying these complex phenomena.

Since there are no noticeable changes in the number of neurons that can explain the amount of information stored during a lifetime, therefore the synapse has been a good candidate to mnemonic substrate [1]. Synapse is a highly specialized type of cellular junction. It constitutes the principal bridge to the flow of information from one neuron to the other, thus allowing all the parts of the System to interact functionally [2]. The importance of synapse in storing information has been postulated since the times of Ramón y Cajal and more recently in Hebb and Matthies' works [3,4]. In this sense, it is indispensable to point out the significant contribution made by Bliss, Lomo et al who gave the first detailed description of the long-term potentiation (LTP). LTP consist in a sustained increment of synaptic efficacy after afferent path stimulation by high frequency electrical stimulus [5,6]. Input specificity, associativity, rapid induction and prolonged duration, are some of the properties that render LTP as a very attractive memory model [7,8].

The specificity conflict

LTP, like memory, can manifest itself early (early-LTP, E-LTP, duration < 4 h) and late (late-LTP, L-LTP, duration > 4 h). E-LTP is essentially dependent of modification of pre-existing proteins at synapse [9], whereas L-LTP depends, *in vitro* and *in vivo*, of the synthesis of new proteins [10,11] and RNA [12].

The soma has been traditionally considered as the main site in the macromolecular synthesis of the neuron. In fact, it has been reported that strong electrical stimulation of Schaffer collaterals to CA1 dendrites separated from their somas, only produces a transient LTP of 3 h approximately; but when the same stimulation pattern is applied to intact neurons an LTP of at least 8 h is induced [13].

LTP is a highly specific phenomenon, thus the produced change in synaptic efficacy is limited to synapses that received the stimulation [14]. This is consistent with the role that LTP-like changes are supposed to play in memory formation and it generates a high capacity for storing information. However, the dependence of the specific and long-lasting synaptic changes on the neuron soma presents a dilemma: how can the proteins required to plastic change stabilization, that are synthesized at the cellular soma of a neuron that contains thousands of synaptic contacts –all depending of the same nucleus– go to the appropriate synapses? [7,15].

Existing hypotheses intended to explain the specificity of long-lasting synaptic changes

Theoretically, at least, it is possible to establish four hypotheses to explain this question: the 'mail' hypothesis, the 'local synthesis' hypothesis, the 'sensitization' hypothesis and the 'synaptic tagging' hypothesis [7] (Fig. 1)

The mail hypothesis attempts to explain the specificity of L-LTP associated synaptic changes with elaborated intracellular

Accepted: 23.03.07.

[a] Experimental Neurology Department. Neurology and Neurosurgery Institute (INN). [b] Experimental Neurophysiology Department. International Center for Neurological Restoration (CIREN). Havana, Cuba.

Corresponding author: Dr. Jeffrey López Rojas. Departamento de Neurología Experimental. Instituto de Neurología y Neurocirugía. Calle 29, n.º 139, esq. D. Vedado, Plaza. 10400 La Habana (Cuba). E-mail: erojas@infomed.sld.cu

Acknowledgments. We thank Martha Cristofol for language assistance.

Versión española disponible en www.neurologia.com

macromolecules traffic: proteins recently synthesized, are given a 'synaptic address' that leads it exclusively to the afferents responsible for their induction. However it seems unlikely that this hypothesis function on a cell that, in the case of a typical CA1 neuron, might have more than 10,000 synaptic contacts [7,16] (Fig. 1a).

The local synthesis hypothesis essentially proposes that specificity is a result of that synapses, once activated, are able to synthesize and locally use the necessary proteins to the consolidation of the synaptic change [16] (Fig. 1b).

On the other hand, synaptic tagging hypothesis, established by Frey and Morris in 1997, proposes that selectivity is the result of setting at stimulated synapses of a local synaptic tag, which is able to capture the plasticity-related proteins (to which we will refer later as plasticity factors, and not only related to proteins), those that according to the model are (once synthesized) diffusely distributed to dendrites (Fig. 1d).

Using rat hippocampal (CA1) slices *in vitro*, with the possibility to study two independent synaptic afferents (S1 and S2) to the same neuronal population, Frey and Morris obtained the following results in their first studies about tagging:

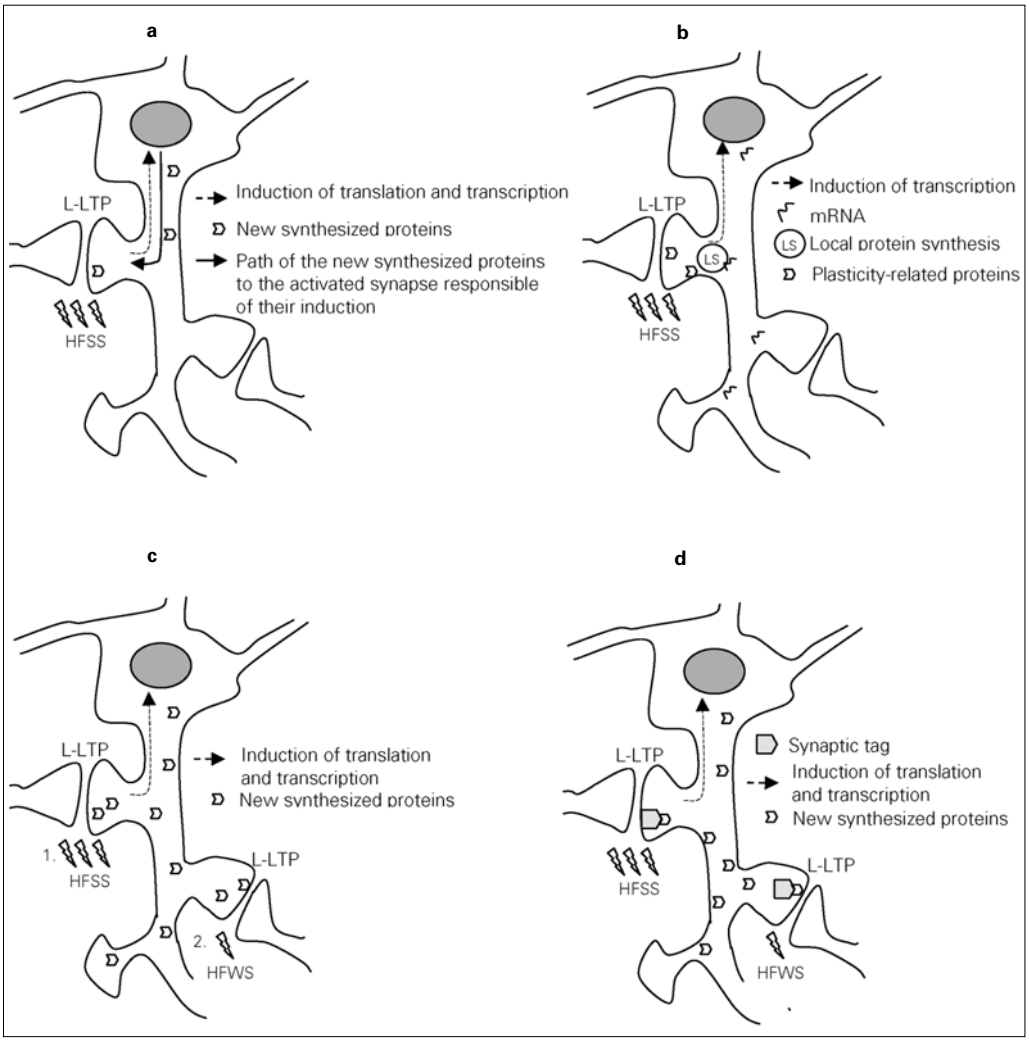

Figure 1. Existing hypotheses intended to explain the specificity in long lasting synaptic changes. a) Mail hypothesis: the long-term plastic change initiated at a synaptic afferent generates a signal that travels to the nucleus promoting transcription and translation, and marking the path to follow for the new synthesized proteins; b) Local synthesis hypothesis: the machinery necessary to the protein synthesis is present at dendrites and it is activated by synaptic stimulation. The proteins locally synthesized are responsible of the long-lasting synaptic change; c) Sensitization hypothesis: plasticity factors are widely distributed to every synapse of the cell. These would have the effect of altering the threshold at which synaptic activation (the Ca^{2+} influx, for example) give rise to long-lasting changes. When few plasticity factors are available, a high threshold prevails, and a weak tetanus induces only transient changes; but when many plasticity factors are available, the threshold diminishes and it is much easier to L-LTP to be induced; d) Synaptic tagging hypothesis: the synthesized proteins at neuronal soma, or locally, are diffusely distributed throughout the dendritic tree, being captured, and used, only by the 'tagged' synapses. HFSS: high frequency strong stimulation; HFWS: high frequency weak stimulation.

S1 was tetanized with a strong tetanus to induce L-LTP and 35 minutes later anisomycin (a protein synthesis inhibitor) was added to the test chamber. Blocking protein synthesis at this time had no appreciable influences on LTP at this afferent. Then, 25 minutes later, with protein synthesis arrested, S2 was tetanized with a strong tetanus similar to the one applied to S1. An L-LTP, analogous to the S1 L-LTP, was established at S2. This was a surprising finding since, as it was referred before, L-LTP induction requires protein synthesis and this S2 L-LTP was induced in the presence of anisomycin. This result is incompatible with mail and local synthesis hypotheses (about this last hypothesis it will be discussed later) and it was interpreted by the authors as an expression of synaptic tagging: a tag setting at S2 as a consequence of the synaptic activity (strong tetanus), allowed to capture the previously induced proteins (Fig. 2a).

In the next experiment, tetanus was applied to S1 in the presence of anisomycin. Later, the anisomycin was washed out and then 3 hours after S1 tetanus, a strong tetanus was applied to S2. An L-LTP was obtained at S2 and only an E-LTP at S1. This result suggests that tagging is established only transiently with a duration inferior to 4 hours, because proteins induced from S2 tetanus did not support S1 LTP stabilization (Fig. 2b).

Finally, S1 was tetanized and 1 hour later a weak tetanus (capable by itself of producing only an E-LTP) was applied to S2. An L-LTP was obtained in both afferents. This experiment, together with the one illustrated in figure 2a, point out that as

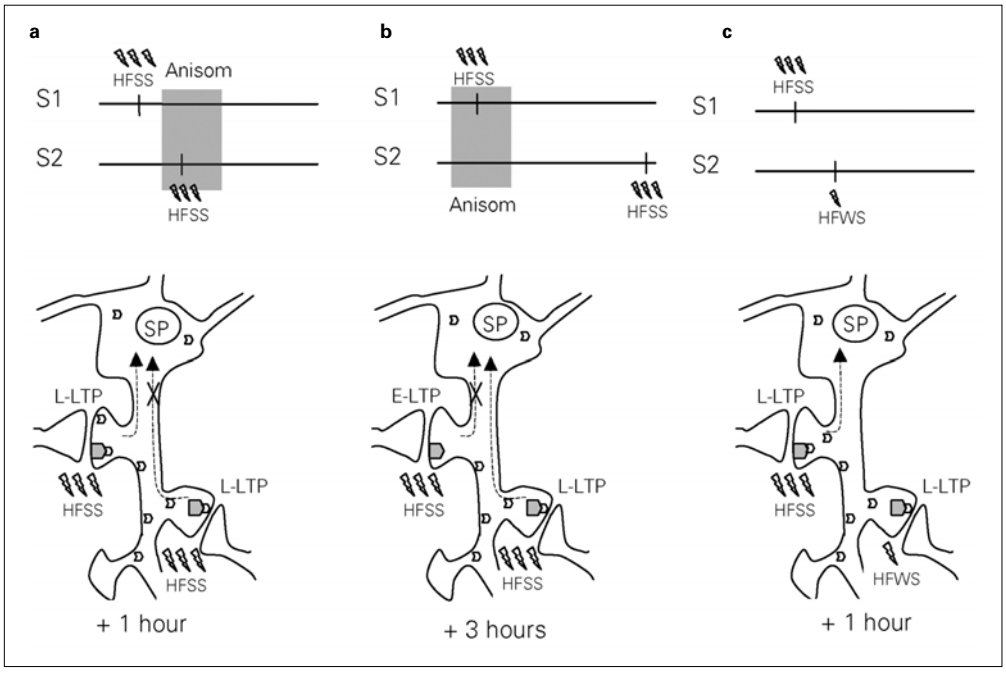

Figure 2. Scheme of main results obtained by Frey and Morris in their pioneering experiments about synaptic tagging, and their interpretation: a) Blocking the protein synthesis during the application of a strong tetanus to S2, with 1 hour inter-tetanus, do not prevent L-LTP at this afferent; b) Associating two strong tetanus, with 3 hours inter-tetanus, and inhibiting protein synthesis during the application of the first tetanus (to S1), it only produces an E-LTP at S1; c) A weak tetanus applied to S2 1 hour after having applied a strong tetanus to S1 allows an L-LTP at this afferent. The used symbols are similar to the symbols from figure 1. HFSS: high frequency strong stimulation; HFWS: high frequency weak stimulation; SP: synthesis of proteins.

much a strong (able to induce L-LTP) as a weak tetanus (able to induce an E-LTP) can set a tag at synaptic sites that receive the stimulation [14] (Fig. 2c).

Although the results described up to this point are interpretable under tagging hypothesis, it is interesting to note that sensitization hypothesis is able too to explain what occurred. Nevertheless, later experiments showed tagging as the correct hypothesis [17]. According to sensitization, the association of one weak tetanus with a strong one, in this order, should not consolidate the E-LTP induced by the weak tetanus; however the tagging hypothesis predicts that stabilization of an E-LTP into L-LTP will be a function of the intersection of: 1) the decay-time course of the synaptic tag and 2) the intracellular dynamics of the synthesis and distribution of plasticity factors, being irrelevant the order of occurrence of these events [17].

Since this new relation described extends to hours the temporal window in which an afferent can exert influences on the others, it was named late associativity [7].

With these experiments, Frey and Morris were considered as the first investigators who brought direct evidences and concretely hypothesized on synaptic tagging. However, Matthies in the 1970's had already sketched the tagging idea [1]. Additionally, before Frey's experiments, Sossin had published an article doing a detailed analysis of possible mechanisms guaranteeing specificity in long-term synaptic changes, concluding in his reasoning that these changes require of synaptic tags able to identify activated synapses. He discusses what he denominated the 'activation model': plasticity factors are widely distributed in the neuron, but its activation, and consequently its action, is limited only to specific synapses. According to this idea Sossin proposes some examples:

- If the plasticity factors were mRNA, then translation could be differentially regulated at each synapse depending on its activation status.
- If the plasticity factors were proteins these could require phosphorylation or proteolysis for activation, then the proteins could be transported inactive and only be processed in synapses possessing active kinases or proteases necessary for protein processing.

Additionally, Sossin thought about the tag as a drain of plasticity factors: tag could be a molecule, or a set of them, present at activated synapses and able to interact specifically with another molecule, or a set of them, present in certain vesicles in charge of transporting and targeting of plasticity factors [18].

LTD and synaptic tagging

The long-term depression (LTD) is similar to LTP in many senses: both depend on the activation of NMDA receptor during their induction [19,20], both have similar time courses and depend on protein synthesis for the establishment of long-lasting maintenance [10,21] and both are considered as cellular correlates of learning and memory [22]. Additionally, in LTD, in a similar way as it occurred in LTP, late associativity and synaptic tagging are observed [23].

'Cross-tagging'

Recently it has been reported that there is a late associative relation for LTP and LTD called cross-tagging. Cross-tagging describes the capability of L-LTP/L-LTD in one synaptic input to transform its opposite, protein synthesis independent E-LTD/E-LTP in an independent synaptic input, into its long lasting form [23].

This raises the following question: are the same proteins used for L-LTP and L-LTD or some of them are used differentially for each particular process? Obtained results point out to both alternatives. Thus, it has been reported that protein kinase Mζ is necessary for L-LTP, but not for L-LTD, maintenance [24], although it has been suggested that others, like phosphodiesterase 4B3, can be used indistinctly for both processes [25,26].

Time-decay of synaptic tagging

Since their first report Frey and Morris had shown that, in hippocampal slices at 32 ºC, synaptic tag decays between 3 and 4 hours after tetanus that originates it [14]. In later experiments, they complemented this result showing that the tag begins to decay after 1 hour, disappearing between the 2 and 4 hours of be-

ing established [17]. Studies in simpler models have thrown similar results [15] and for LTD it has been shown that tag decays before 2 hours of being induced [23].

But, is tag decay only time-dependent? Sajikumar et al [27] were the first researchers supporting the idea that tag is regulated in an activity-dependent manner in addition to its known time dependency. They showed that homosynaptic low frequency stimulation (LFS) shortly (5 minutes) after weak tetanus resets the tag. Later it was described too that homosynaptic or heterosynaptic LFS 10 minutes before a tetanus resets the tag [28].

Competing for plasticity factors

In 2004, very interesting experiments were published on hippocampal (CA1) slices. An associative L-LTP was induced in two independent inputs of a neuronal population and after 4 hours, anisomycin was added to the test chamber. Later, one of the afferents (V1) was tetanized for second time, while the protein synthesis was arrested. It was observed that the additional potentiation on this input (V1) occurs at the expense of maintenance of prior potentiation in the other input (V2, no re-tetanized). This was interpreted by the authors as an expression of a competence between synapses for plasticity factors -phenomenon they called 'competitive maintenance'. Additionally, they showed that a stronger reactivation stimulus provoked a stronger competence [29].

These results suggest that synapses need a continuous replacement of plasticity factors (or binding of factors to synaptic sites is reversible), that synaptic tags can be maintained for a time longer than 4 hours provided that plasticity factors are available, that more or less tag (or tag affinity for plasticity factors) is set in dependence of the strength of tetanus and that synapses compete for plasticity factors in a regime of reduced protein synthesis [30].

General features of synaptic tagging

The results exposed up to this point, allow us to make some generalizations concerning synaptic tag:

- After a tetanus able to induce an E- or L-LTP (E- or L-LTD) a synaptic tag is established, supposedly in an immediate way [17,27].
- According to the strength of tetanus it seems that more or less tag (or tag affinity for plasticity factors) is set [29].
- A different synaptic tag is set as a consequence of LTP or LTD [23,31].
- Synaptic tagging is independent of protein synthesis [14,17].
- Synaptic tag is able to identify and capture a specific subset of proteins induced as a consequence of L-LTP or L-LTD, but beyond that, the tag-macromolecule relationship is promiscuous. A tag at one synapse can hijack proteins synthesized in response to activity at another synapse [7,14].
- The tag setting probably involves a transient process, such as protein phosphorylation, since it has a limited time course of less than 4 hours in rat hippocampal slices at 32 ºC [14,17]. Nevertheless, there are evidences that suggest that the presence of plasticity factors can prolong the time course of the tag [29].
- Tag decays is not only time dependent, but also activity dependent [27,28].

Although all we know about synaptic tagging, tag identity is yet an unresolved question interesting many researchers.

IDENTITY OF THE SYNAPTIC TAG

What is the molecular identity of the synaptic tag?

Evidences about the existence of synaptic tag have led to intense efforts directed to discover its identity. Many candidates have been proposed including protein kinases, changes in adhesion molecules at the synapse, alterations in cytoskeleton, activation or traffic of channels and local protein synthesis [32].

Local protein synthesis

Traditionally, it has been thought that the cellular soma represents the main site of macromolecular synthesis at a neuron and that synapses depend on this synthesis for their performance. As mentioned before, there are experiments that support this idea, however, in the last years new experiments have appeared suggesting that the local processes have a non depreciable role in plastic changes [33-35].

The ability of isolated synaptic fractions to support de novo protein synthesis was first reported 30 years ago approximately [36,37]. Recently, the presence of many components of the translational machinery has been corroborated, by means of immunocytochemical analyses, at the post-synaptic vicinity [38, 39] and a great number of mRNAs has been described at dendrites [40].

Even more, it has been shown that synaptic stimulation induces an increase in local protein synthesis [41,42]. Experimental evidence point out that synaptic activity can activate some components of the protein synthesis machinery by the phosphorylation of specific translation factors [43,44]. In addition, it is interesting to note that the percentage of spines containing polyribosomes increases notably after tetanic stimulation [45] and that with depolarization a local release of mRNAs to the active polysomes can occur from the RNA granules, where they are under translational arrest [46]. Besides, there are evidences of a regulated transport of mRNA synthesized *de novo* to active synapses, as in the case of the mRNA *Arc* [47].

Numerous theories have derived from local protein synthesis aimed to explain the specificity in long-term synaptic changes and late associativity. The most traditional theory involves local synthesis and limited use of plasticity related protein in activated synapses. However, this hypothesis cannot really explain the late associativity phenomenon. But Biltzer et al [48] have an interesting hypothesis. They propose that synaptic tagging is related with the release of mRNAs from the granules, where the mRNAs are in a translational arrested state, at specific dendritic sites. According to the model, the mRNA released would include, in addition to the mRNA related to the plastic change, transcripts codifying for a rate-limiting or activating component of the translational machinery; although all these mRNAs will be untranslated, and therefore the induced LTP will be transient (E-LTP), if the protein synthesis is not recruited in an adequate temporal window. Thus, late associativity could be explained on the basis that a strong tetanus unravels nearby RNA granules, but additionally it causes a local boost in translation (whereas a weak tetanus only unravels granules). The translation of recently released mRNAs at these strong stimulated synapses would provoke an additional increment in the protein synthesis rate, due to the presence of regulatory proteins. These regulatory proteins could in turn diffuse out of the spine to be transported to other regions of the dendrite being captured by some weakly stimulated synapses. At these synapses the regulatory proteins could induce the synthesis of the released mRNAs, if these

events coexist in an adequate temporal window. It is proposed that depending on the mRNAs that have been made available as response to stimulation, either strengthening or weakening of synapses could result [48].

Although the proposed model fits with the general conception that synaptic tagging does not require protein synthesis, anyway this model is in conflict with Frey's experiments; since it is necessary the protein synthesis to occur for the weak synapses to be consolidated and Frey showed in his late associativity experiments that weakly stimulated synapses are able to be consolidated even in the presence of anisomycin applied up to 1 hour after the weak tetanus (having demonstrated previously that anisomycin applied 35 minutes after a strong tetanus does not impair the L-LTP at the stimulated afferents). Nevertheless, it is interesting to note that according to Blitzer local protein synthesis is directly activated at strongly stimulated synapses, but indirectly at weakly stimulated, a reason why time limits for the protein dependence have not to been necessarily similar for both. It would be interesting to prove if inhibiting protein synthesis during all the time that the tag remains, it is possible for the weakly stimulated synapses to capture the proteins.

On the other hand, with respect to the local protein synthesis, experiments by Casadio et al have insinuated that this could function as a tag for 'late stabilization' of synaptic changes [49]. Therefore, it might be that local translation could be necessary too as synaptic tag in hippocampal neurons, if LTP were examined over more extended time periods [15].

In spite of accumulated evidence and proposed models, the relation synaptic tagging-local translation needs further investigations for its clarification.

Protein kinases. PKA. CAMK

The protein kinases have been linked to synaptic tagging, even before Frey and Morris showed the authenticity of the synaptic tag hypothesis [1,18]. Protein kinases activation can constitute a mechanism allowing synapses to 'remember' the previous synaptic activity in a spatially restricted and reversible manner, which are requisites to be fulfilled by any candidate to synaptic tag [32].

Although several studies have demonstrated a requirement for PKA in the expression of L-LTP, involvement of PKA in E-LTP is less clear [50]. Special importance has been attributed to the CREB (cyclic AMP responsible element binding protein)-mediated gene expression for E-LTP consolidation in L-LTP as result of PKA activation [51].

Recently, Barco et al [52] showed a new feature for the PKA (suggested before by Casadio et al [49]): it is a critical element in synaptic tagging. These findings were later corroborated by Young et al. They had previously showed that LFS inhibit synaptic tagging [28] and in a recent article Young suggests that this LFS action is due to an interference with the AMPc/PKA signaling pathway. Likewise, using pharmacological, electrophysiological and genetic tools, Young proved that PKA is related to synaptic tagging [53]. Further experiments have extended these results, showing that inhibiting pharmacologically the anchoring of PKA to AKAPs (A-kinase anchoring proteins) impairs the expression of L-LTP and suppresses late-associativity in hippocampal slices. However, the inhibitor used in this experiment blocks the interaction of the PKA with most AKAPs, so it will be necessary to perform assays with a major specificity to elucidated the identity of the AKAPs involved with this phenomenon [54].

Numerous AKAPs have been related with PKA anchoring to synaptic proteins like the NMDA and AMPA receptors, whose function in plastic events is crucial [55,56]. Thus, the anchoring of PKA to specific microdomains is a process whose study could bring us a better knowledge of plastic changes and synaptic tagging.

The CAMKII is a major member of the post-synaptic density (PSD) and represents 1-2% of total proteins in the brain [57]. A protagonist role for CAMKII has recently been shown in LTP tagging, but not in LTD tagging, whereas MAPK seems to be necessary to setting of LTD, but not LTP, tagging [31].

The CAMKII is necessary for LTP induction and it has been proposed too to mediate more late effects [57], but nevertheless its role in the maintenance of LTP is not so clear [58]. After NMDA receptor activation and consequent Ca^{2+} influx, CAMKII translocates to the PSD of stimulated synapses, where it associates with the NMDA receptors [59]. After the CAMKII binds to NMDA, it remains active even after the dissociation of Ca^{2+}/calmodulin [60]. Once active, the CAMKII induces enzymatic and structural processes at PSD, thus incrementing the conductance and the number of AMPA receptors [57], changes associated to an enhanced synaptic transmission that underlie the process of LTP induction. Recently, Hudmon proposed a novel mechanism for the targeting of CAMKII to PSD after neuronal activation: the self-association. He says that CAMKII may form a scaffold that, in combination with other synaptic proteins, recruits and localizes additional proteins to the PSD [61].

SYNAPTIC TAGGING *IN VIVO*

All results about synaptic tagging cited up to this point, have been performed *in vitro*. Although this kind of experiments bears a lot of technical advantages, we should not forget that these are carried out under artificial conditions and to avoid the manifestation of possible artifacts it is necessary to search for similar phenomena in the intact animal *in vivo*. Recently Hassan et al [62] presented a technique, which now allows activating two independent synaptic inputs to the same neuronal population in freely moving rats. This is a pre-requisite for studying synaptic tagging *in vivo* in a similar way as it has been studied *in vitro*.

However, an indication that synaptic tagging might occur in the intact organism has already become from experiments investigating the influence of emotional/motivational stimulus on LTP. Thus, E-LTP can be reinforced or extended if, in an appropriate time window of about 30 minutes after tetanization, water is made available to water-deprived rats [63,64]. In our lab we have found that behavioral reinforcement is protein synthesis dependent [65], that it is mediated by the basolateral amygdala [66] (whose stimulation mimics the behavioral reinforcement [67]), that it is impaired by subcortical deafferentation of the hippocampus [68] and that applying norepinephrine intraventricularly E-LTP is reinforced in a similar way as it was done with him [69].

Although all these results there are not direct proofs of synaptic tagging hypothesis, constitute positive evidences in their favor, since they can be logically interpretable under this. Thus, a weak tetanus would be able to induce the setting of the synaptic tag at stimulated synapses. Then, the protein synthesis induced by the reinforcement stimulus (to make water available to water-deprived animals, to stimulate the basolateral amyg-

dala, to apply norepinephrine intraventricularly) would allow to extend the induced LTP for more than 4 hours at stimulated, and tagged, synapses.

It is interesting to note that these studies *in vivo* about LTP reinforcement suggest that the tag decays in less than 1 hour [63,67], a result that agrees with the obtained one in *in vitro* conditions, taking into account that slices are incubated at 32 ºC, whereas temperature at intact animals is 38 ºC approximately (a reason for which it is logic to expect a faster decay at intact animals).

At this point, it is necessary to mention the results of recent investigations supporting the sustained idea that as the result of learning and memory (at least for some kinds of these) are induced at hippocampus LTP-like changes [70-72]. At this 'naturally' induced processes, could operate synaptic tagging in a similar way as it does in 'artificially' induced LTP. On the other hand, although the participation of LTP is discussed in other structures associated to memory process [73], the general mechanism of receptor activation, activation of protein kinases and *de novo* synthesis of the proteins necessary to make the plastic change lasting seems to be universal [74]. Thus, the destiny of recent synthesized proteins could be determined by some of the variants of synaptic tagging.

SOME FUNCTIONAL IMPLICATIONS OF SYNAPTIC TAGGING

The synaptic tagging hypothesis could help us to explain why inconsequent events, or events often remembered transiently, are better remembered when they occur temporally near to someone with a strong motivational/emotional content [17]. However, this is counteractive, since spurious associations can occur: weak stimulus could indiscriminately stabilize at expense of stronger stimulus. This problem is accentuated if we take into account that this spread of stabilization can occur both temporally (weak stimulus needs not to be closely associated in time with strong activation) and spatially (the weak input needs not to be located close to the strong input); so this promiscuity would seem counterproductive for a memory storage device [30]. Nevertheless, it is worthy to point out that, besides described relations of cooperation and association, there is a competence for plasticity factors between activated synapses in conditions of reduced protein synthesis [29]; competence that, together with homo and heterosynaptic modulations of activity, would be some of the control mechanisms used by neurons to efficiently integrate their afferents.

CONCLUSIONS

The synaptic tagging hypothesis offers a very flexible and reasonable solution to the specificity of long-lasting plastic changes and constitutes, up to the present, the most accepted and spread hypothesis. As discussed before, there are numerous candidates to be the synaptic tag. However, it seems there is not a unique synaptic tag, but rather multiple molecular synaptic tags exist. Each of them might function as a synaptic tag under particular circumstances, and they might be differentially recruited by specific stimulus and mediate plasticity over different time domains [32].

The knowledge that we achieve in relation to synaptic tagging and its regulation would be of great theoretical importance and practical utility, not only concerning to memory-related processes, but also in function recovery after lesions [75]. If we know thoroughly the mechanism involved in synaptic tagging, we could extend plastic changes of interest by associating it efficiently with motivational/emotional stimuli or through specific manipulations, we could delete the trace of those events whose preservation will be harmful to the subject.

REFERENCES

1. Matthies H. Neurobiological aspects of learning and memory. Annu Rev Psychol 1989; 40: 381-404.
2. Kandel ER, Siegelbaum SA. Overview of synaptic transmission. In Kandel ER, Schwartz JH, Jessell TM, eds. Principles of neural science. New York: McGraw-Hill, Health Professions Division; 2000. p. 175-86.
3. Hebb DO. The organization of behaviour. New York: Wiley & Sons; 1949.
4. Matthies H. Cellular mechanisms of learning processes and the shaping of memory. Z Psychol 1976; 184: 308-28.
5. Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 1973; 232: 331-56.
6. Bliss TV, Gardner-Medwin AR. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol (Lond) 1973; 232: 357-74.
7. Frey U, Morris RGM. Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. Trends Neurosci 1998; 21: 181-8.
8. Almaguer-Melián W, Bergado-Rosado JA. Interacciones entre el hipocampo y la amígdala en procesos de plasticidad sináptica. Una clave para entender las relaciones entre motivación y memoria. Rev Neurol 2002; 35: 586-93.
9. Goelet P, Castellucci VF, Schacher S, Kandel ER. The long and the short of long-term memory –a molecular framework. Nature 1986; 322: 419-22.
10. Krug M, Lössner B, Ott T. Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. Brain Res Bull 1984; 13: 39-42.
11. Frey U, Krug M, Reymann KG, Matthies H. Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. Brain Res 1988; 452: 57-65.
12. Frey U, Frey S, Schollmeier F, Krug M. Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons *in vivo* and *in vitro*. J Physiol 1996; 490: 703-11.
13. Frey U, Krug M, Brödemann R, Reymann K, Matthies H. Long-term potentiation induced in dendrites separated from rat's CA1 pyramidal somata does not establish a late phase. Neurosci Lett 1989; 97: 135-9.
14. Frey U, Morris RGM. Synaptic tagging and long-term potentiation. Nature 1997; 385: 533-6.
15. Martin KC. Synaptic tagging during synapse-specific long-term facilitation of aplysia sensory-motor neurons. Neurobiol Learn Mem 2002; 78: 489-97.
16. Schuman EM. Synapse specificity and long-term information storage. Neuron 1997; 18: 339-42.
17. Frey U, Morris RGM. Weak before strong: dissociating synaptic tagging and plasticity-factor accounts of late-LTP. Neuropharmacology 1998; 37: 545-52.
18. Sossin WS. Mechanisms for the generation of synapse specificity in long-term memory: the implications of a recruitment for transcription. TINS 1996; 19: 215-8.
19. Bear MF, Malenka RC. Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 1994; 4: 389-99.
20. Hrabetova S, Sacktor TC. Long-term potentiation and long-term depression are induced through pharmacologically distinct NMDA receptors. Neurosci Lett 1997; 226: 107-10.
21. Sajikumar S, Frey JU. Anisomycin inhibits the late maintenance of long-term depression in rat hippocampal slices in vitro. Neurosci Lett 2003; 338: 147-50.
22. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004; 44: 5-21.
23. Sajikumar S, Frey JU. Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. Neurobiol Learn Mem 2004; 82: 12-25.
24. Sajikumar S, Navakkode S, Sacktor TC, Frey JU. Synaptic tagging and

cross-tagging: the role of protein kinase Mzeta in maintaining long-term potentiation but not long-term depression. J Neurosci 2005; 25: 5750-6.
25. Ahmed T, Frey JU. Expression of the specific type IV phosphodiesterase gene PDE4B3 during different phases of long-term potentiation in single hippocampal slices of rats in vitro. Neuroscience 2003; 117: 627-38.
26. Navakkode S, Sajikumar S, Frey JU. The type IV-specific phosphodiesterase inhibitor rolipram and its effect on hippocampal long-term potentiation and synaptic tagging. J Neurosci 2004; 24: 7740-4.
27. Sajikumar S, Frey JU. Resetting of 'synaptic tags' is time- and activity-dependent in rat hippocampal CA1 in vitro. Neuroscience 2004; 129: 503-7.
28. Young JZ, Nguyen PV. Homosynaptic and heterosynaptic inhibition of synaptic tagging and capture of long-term potentiation by previous synaptic activity. J Neurosci 2005; 25: 7221-31.
29. Fonseca R, Nägerl UV, Morris RG, Bonhoeffer T. Competing for memory: hippocampal LTP under regimes of reduced protein synthesis. Neuron 2004; 44: 1011-20.
30. Turrigiano G. A competitive game of synaptic tag. Neuron 2004; 44: 903-4.
31. Reymann KG, Frey JU. The late maintenance of hippocampal LTP: requeriments, phases, 'synaptic tagging', 'late-associativity' and implications. Neuropharmacology 2007; 42: 24-40.
32. Martin KC, Kosik KS. Synaptic tagging –who's it? Nat Rev Neurosci 2002; 3: 813-20.
33. Vickers CA, Dickson KS, Wyllie DJ. Induction and maintenance of late-phase long-term potentiation in isolated dendrites of rat hippocampal CA1 pyramidal neurones. J Physiol 2005; 568: 803-13.
34. Cracco JB, Serrano P, Moskowitz SI, Bergold PJ, Sacktor TC. Protein synthesis-dependent LTP in isolated dendrites of CA1 pyramidal cells. Hippocampus 2005; 15: 551-6.
35. Bradshaw KD, Emptage NJ, Bliss TV. A role for dendritic protein synthesis in hippocampal late LTP. Eur J Neurosci 2003; 18: 3150-2.
36. Autilio LA, Appel SH, Pettis P, Gambetti PL. Biochemical studies of synapses in vitro. I. Protein synthesis. Biochemistry 1968; 7: 2615-22.
37. Morgan IG, Austin L. Synaptosomal protein synthesis in a cell-free system. J Neurochem 1968; 15: 41-51.
38. Tiedge H, Brosius J. Translational machinery in dendrites of hippocampal neurons in culture. J Neurosci 1996; 16: 7171-81.
39. Pierce JP, Van Leyen K, McCarthy JB. Translocation machinery for synthesis of integral membrane and secretory proteins in dendritic spines. Nat Neurosci 2000; 3: 311-3.
40. Zhong J, Zhang T, Bloch LM. Dendritic mRNAs encode diversified functionalities in hippocampal pyramidal neurons. BMC Neurosci 2006; 7: 17.
41. Ouyang Y, Rosenstein A, Kreiman G, Schuman EM, Kennedy MB. Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. J Neurosci 1999; 19: 7823-33.
42. Tsokas P, Grace EA, Chan P, Ma T, Sealfon SC, Iyengar R, et al. Local protein synthesis mediates a rapid increase in dendritic elongation factor 1A after induction of late long-term potentiation. J Neurosci 2005; 25: 5833-43.
43. Khan A, Pepio AM, Sossin WS. Serotonin activates S6 kinase in a rapamycin-sensitive manner in *Aplysia* synaptosomes. J Neurosci 2001; 21: 382-91.
44. Huang YS, Jung MY, Sarkissian M, Richter JD. N-methyl-D-aspartate receptor signaling results in Aurora kinase-catalyzed CPEB phosphorylation and alpha CaMKII mRNA polyadenylation at synapses. EMBO J 2002; 21: 2139-48.
45. Ostroff L, Fiala J, Allwardt B, Harris K. Polyribosomes Redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. Neuron 2002; 35: 535-45.
46. Krichevsky AM, Kosik KS. Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. Neuron 2001; 32: 683-96.
47. Steward O, Worley P. Local synthesis of proteins at synaptic sites on dendrites: role in synaptic plasticity and memory consolidation? Neurobiol Learn Mem 2002; 78: 508-27.
48. Blitzer RD, Iyengar R, Landau EM. Postsynaptic signaling networks: cellular cogwheels underlying long-term plasticity. Biol Psychiatry 2005; 57: 113-9.
49. Casadio A, Martin KC, Giustetto M, Zhu H, Chen M, Bartsch D, et al. A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. Cell 1999; 99: 221-37.
50. Nguyen PV, Woo NH. Regulation of hippocampal synaptic plasticity by cyclic AMP-dependent protein kinases. Prog Neurobiol 2003; 71: 401-37.
51. Impey S, Mark M, Villacres EC, Poser S, Chavkin C, Storm DR. Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron 1996; 16: 973-82.
52. Barco A, Alarcón JM, Kandel ER. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell 2002; 108: 689-703.
53. Young JZ, Isiegas C, Abel T, Nguyen PV. Metaplasticity of the late-phase of long-term potentiation: a critical role for protein kinase A in synaptic tagging. Eur J Neurosci 2006; 23: 1784-94.
54. Huang T, McDonough CB, Abel T. Compartmentalized PKA signaling events are required for synaptic tagging and capture during hippocampal late-phase long-term potentiation. Eur J Cell Biol 2006; 85: 635-42.
55. Westphal RS, Tavalin SJ, Lin JW, Alto NM, Fraser ID, Langeberg LK, et al. Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. Science 1999; 285: 93-6.
56. Colledge M, Dean RA, Scott GK, Langeberg LK, Huganir RL, Scott JD. Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. Neuron 2000; 27: 107-19.
57. Lisman J, Schulman H, Cline H. The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev Neurosci 2002; 3: 175-90.
58. Otmakhov N, Griffith LC, Lisman JE. Postsynaptic inhibitors of calcium/calmodulin-dependent protein kinase type II block induction but not maintenance of pairing-induced long-term potentiation. J Neurosci 1997; 17: 5357-65.
59. Leonard AS, Lim IA, Hemsworth DE, Horne MC, Hell JW. Calcium/calmodulin-dependent protein kinase II is associated with the N- methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 1999; 96: 3239-44.
60. Bayer KU, De Koninck P, Leonard AS, Hell JW, Schulman H. Interaction with the NMDA receptor locksCaMKII in an active conformation. Nature 2001; 411: 801-5.
61. Hudmon A, Lebel E, Roy H, Sik A, Schulman H, Waxham MN, et al. A mechanism for Ca^{2+}/calmodulin-dependent protein kinase II clustering at synaptic and nonsynaptic sites based on self-association. J Neurosci 2005; 25: 6971-83.
62. Hassan H, Frey S, Frey JU. Search for a two-input model for future investigations of 'synaptic tagging' in freely moving animals in vivo. J Neurosci Methods 2006; 152: 220-8.
63. Seidenbecher T, Reymann KG, Balschun D. A post-tetanic time window for the reinforcement of long-term potentiation by appetitive and aversive stimuli. Proc Natl Acad Sci U S A 1997; 94: 1494-9.
64. Seidenbecher T, Balschun D, Reymann KG. Drinking after water deprivation prolongs 'unsaturated' LTP in the dentate gyrus of rats. Physiol Behav 1995; 57: 1001-4.
65. Bergado JA, Almaguer-Melián W, Kostenko S, Frey S, Frey JU. Behavioral reinforcement of long-term potentiation in rat dentate gyrus in vivo is protein synthesis-dependent. Neurosci Lett 2003; 351: 56-8.
66. Almaguer-Melián W, Martínez-Martí L, Frey JU, Bergado JA. The amygdala is part of the behavioral reinforcement system modulating long-term potentiation in rat hippocampus. Neuroscience 2003; 119: 319-22.
67. Frey S, Bergado JA, Seidenbecher T, Pape HC, Frey JU. Reinforcement of early-LTP in dentate gyrus by stimulation of the basolateral amygdala: heterosynaptic induction of late-LTP. J Neurosci 2001; 21: 3697-703.
68. Almaguer-Melián W, Rosillo JC, Frey JU, Bergado JA. Subcortical deafferentation impairs behavioral reinforcement of long-term potentiation in the dentate gyrus of freely moving rats. Neuroscience 2006; 138: 1083-8.
69. Almaguer-Melián W, Rojas-Reyes Y, Alvaré A, Rosillo JC, Frey JU, Bergado JA. Long-term potentiation in the dentate gyrus in freely moving rats is reinforced by intraventricular application of norepinephrine, but not oxotremorine. Neurobiol Learn Mem 2005; 83: 72-8.
70. Gruart A, Muñoz MD, Delgado-García JM. Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. J Neurosci 2006; 26: 1077-87.
71. Whitlock JR, Heynen AJ, Shuler MG, Bear MF. Learning induces long-term potentiation in the hippocampus. Science 2006; 313: 1093-7.
72. Pastalkova E, Serrano PPB, Wallace E, Fenton AA, Sacktor TC. Storage of spatial information by the maintenance mechanism of LTP. Science 2006; 313: 1141-4.
73. Izquierdo I, Bevilaquua LR, Rossato JI, Bonini JS, Medina JH, Cammarota M. Different molecular cascades in different sites of the brain control memory consolidation. Trends Neurosci 2007; 29: 496-505.
74. Bergado-Rosado JA, Almaguer-Melián W. Mecanismos celulares de la neuroplasticidad. Rev Neurol 2000; 31: 1074-95.
75. Hernández-Muela S, Mulas F, Mattos L. Plasticidad neuronal funcional. Rev Neurol 2004; 38 (Supl 1): S58-68.

LA 'MARCA SINÁPTICA' Y LA HUELLA DE LA MEMORIA

Resumen. Objetivo. *Presentar una visión de las principales características y posible identidad de la marca sináptica, así como discutir algunas de sus implicaciones funcionales.* Desarrollo. *La potenciación sináptica a largo plazo, dadas sus características, se ha impuesto como un modelo sinapticocelular de memoria muy atractivo. De modo similar a la memoria, puede manifestarse como temprana (dependiente fundamentalmente de la modificación de proteínas preexistentes en la sinapsis) o tardía (dependiente de la síntesis de nuevas proteínas). Debido a que la potenciación sináptica a largo plazo es un fenómeno altamente específico, surge un dilema: ¿cómo llegan a las sinapsis apropiadas las proteínas requeridas para la estabilización del cambio plástico en una neurona que normalmente posee miles de contactos sinápticos, todos dependientes del mismo núcleo? En este trabajo se presentan algunos de los modelos que aportan posibles soluciones a este interrogante, haciendo énfasis en la hipótesis del marcaje sináptico. Se exponen los principales hallazgos que han ido conformando esta hipótesis y se analiza la síntesis local y la activación de proteincinasas como posibles candidatos de ser la marca sináptica. Adicionalmente, se discuten algunas implicaciones funcionales del marcaje sináptico.* Conclusiones. *La hipótesis de la marca sináptica ofrece una explicación muy flexible y razonable acerca de la especificidad del cambio sináptico duradero. Aunque se conocen algunas de sus características, la identidad de la marca no se ha dilucidado aún. Al parecer, existen múltiples marcas que, al ser reclutadas por estímulos específicos, median los efectos plásticos en diferentes dominios temporales. [REV NEUROL 2007; 45: 607-14]*
Palabras clave. *LTP. Marca sináptica. Memoria. Proteincinasa. Sinapsis. Síntesis de proteínas.*