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Synapse-specific stabilization of plasticity processes: The synaptic tagging and capture hypothesis revisited ten years later

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Running title: The STC hypothesis revisited

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Abstract

A decade ago, the synaptic tagging hypothesis was proposed to explain how newly synthesized plasticity products can be specifically targeted to active synapses. A growing number of studies have validated the seminal findings that gave rise to this model, as well as contributed to unveil and expand the range of mechanisms underlying late-associativity and neuronal computation. Here, we will review what it was learnt during this past decade regarding the cellular and molecular mechanisms underlying synaptic tagging and synaptic capture. The accumulated experimental evidence has widened the theoretical framework set by the synaptic tagging and capture (STC) model and introduced concepts that were originally considered part of alternative models for explaining synapse-specific LTP. As a result, we believe that the STC model, now improved and expanded with these new ideas and concepts, still represents the most compelling hypothesis to explain late-associativity in synapse-specific plasticity processes. We will also discuss the impact of this model in our view of the integrative capability of neurons and associative learning.

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Keywords: synaptic plasticity; LTP; associative learning; neuronal computation; synaptic tagging; synaptic capture; metaplasticity

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1. Description of the biological problem and first evaluation of the hypothesis

"Because protein synthesis occurs mainly in the cell body, whereas LTP is input specific, the question arises of how the synapse specificity of late LTP is achieved without elaborate intracellular protein trafficking" (Frey and Morris, 1997).

Current models to explain how memories are stored rely on stable changes in synaptic weight that modify the activity of specific neuronal circuits. However, the formation of stable memory can be blocked by inhibiting transcription or translation (Barondes and Jarvik, 1964; Flexner *et al.*, 1962; Flexner *et al.*, 1965). The requirement of *de novo* gene expression and, consequently, the likely participation of the cell nucleus in long-lasting forms of synaptic plasticity impose a critical requirement for any model trying to explain learning-related plasticity: There must be mechanisms that restrict the action of newly transcribed and/or translated gene products to recently active synapses but not to others. To address this biological problem, it was suggested that the persistence of the changes in synaptic strength is mediated by the generation of a transient local synaptic tag at recently activated synapses and by the production of plasticity-related proteins (PRPs) that can be used or *captured* only at those synapses marked by the tag. This idea, proposed now ten years ago (Frey and Morris, 1997; Martin *et al.*, 1997), was originally referred to us as the synaptic tagging hypothesis, but was later described as the synaptic tagging and capture (STC) hypothesis, a terminology introduced by Kelleher and Tonegawa (Kelleher *et al.*, 2004b) that we also prefer because it emphasizes the existence of two distinct phases in the consolidation of synaptic changes and so facilitates the dissection of the molecular events underlying such changes. Seminal studies in the rat hippocampus (Frey and

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Morris, 1997) and in cultured *Aplysia* neurons (Martin *et al.*, 1997) provided the first experimental evidence supporting the TSC model.

Long-term potentiation (LTP) is the prevalent cellular model for encoding memories in the brain (Martin *et al.*, 2000b). In LTP, as in memory storage, it is possible to distinguish, at least, two stages of storage: there is an early, protein synthesis-independent, short-term stage (E-LTP), which lasts minutes, and a later, long-term stage (L-LTP), which lasts much longer and shares with long-term memory (LTM) the requirement for the synthesis of new mRNA and protein (Frey *et al.*, 1988; Nguyen *et al.*, 1994). In the Schaffer collateral pathway of the rodent hippocampus, high frequency stimulation (HFS), such as a standard 100 Hz tetanus train of 1 sec duration, produces a non-saturating short-lasting LTP, whereas repeated tetanic stimulation elicits L-LTP. Frey and Morris demonstrated that L-LTP could be induced in synapses receiving subthreshold stimulation (i.e. one single train of HFS), which normally only elicits E-LTP, when it was preceded by the stimulation of another set of synapses using suprathreshold stimulation (i.e. three or four trains of HFS) (Figure 1A). They referred to this phenomenon as synaptic capture of L-LTP (Frey and Morris, 1997). A central idea for this model is that the gene products synthesized in response to the induction of the late phase LTP can also be available to other synapses, synapses which must be activated in order to utilize these new gene products. Frey and Morris referred to this local activation process as synaptic tagging. They tested this idea using the inhibitor of protein synthesis anisomycin and observed that the phenomenon of capture of late phase LTP, contrary to regular L-LTP, was protein synthesis-independent (Frey and Morris, 1997).

In parallel, work in branch-specific facilitation in *Aplysia* neurons revealed indications of the existence of synaptic marking (term originally used in this organism to refer to tagging) and

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capture in invertebrates, suggesting that these processes may be highly conserved through evolution. In sensory-motor neuronal cultures, a simplified model system to study sensitization in *Aplysia*, a single application of serotonin (5-HT) produces a short-term change in synaptic effectiveness (short-term facilitation or STF), whereas repeated and spaced applications produce changes in synaptic strength that can last for more than a week (long-term facilitation or LTF) (Montarolo *et al.*, 1986). Martin and colleagues found that a single pulse of 5-HT given to one branch produced LTF when preceded by five pulses of serotonin in the opposite branch (Martin *et al.*, 1997). Similarly to the findings in the rat hippocampus, this persistent form of facilitation elicited by one-pulse was independent of protein synthesis suggesting that it relied in the wave of gene expression previously induced by the strong stimulation (Figure 1B).

The STC hypothesis provided a compelling explanation for these experimental observations. However, many critical questions regarding STC mechanisms and their physiological role remained unanswered. Are these mechanisms conserved among different organisms and neural types? What is the spatial range and duration of the tag? What are the molecular and cellular mechanisms underlying synaptic tagging and synaptic capture? What gene products are captured in active synapses? What is the physiological relevance of STC for information encoding and memory? Many of these questions are still open. We will try to address them in the following sections.

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2. Refinement and consolidation of the model

2.1 A theoretical framework for late-associativity processes

The STC hypothesis introduced two important new perspectives on LTP studies. First, electrophysiologists had traditionally interpreted the associative properties of LTP and synaptic

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integration in terms of the interaction of two or more inputs over a time scale of milliseconds or seconds mediated by the coincidence detection mechanisms of N-methyl-D-aspartate receptors (NMDAR) and transient changes in Ca^{2+} (Martin *et al.*, 2000b). The STC model added a second form of associativity in which heterosynaptic interactions can take place over a much longer time scale (Figure 2A), what has been called late-associativity (Reymann and Frey, 2007). Based on this model, the neuronal response to a particular stimulus would depend on the previous history of activation of the neuron not only in a range of milliseconds, but over minutes or even hours (Frey and Morris, 1998a). In consequence, the persistence of LTP can be variable and is modulated by the previous history of activation of the entire neuron.

Second, the STC hypothesis has also changed our perception of the sequence of events required to achieve long lasting changes in synaptic strength. The most commonly accepted model to explain LTP consolidation (or LTF in *Aplysia*) defines three critical steps required to produce persistent changes in synaptic strength: (1) activation of second messenger cascades at stimulated synapses and induction of transient changes in synaptic transmission, (2) activity-triggered gene expression (with this term we refer to both transcription and translation-dependent mechanisms), as a late consequence of the increase in second messenger signaling, and (3) stabilization of the transient synaptic reinforcement as result of the functional incorporation of newly synthesized gene products to active synapses. The STC hypothesis and some of the key experiments inspired by it have changed the perception of the events required to achieve the late phase of LTF or LTP from sequential (synaptic activation/activity-dependent synaptic tagging → gene expression → synaptic capture/LTP consolidation) to additive (synaptic tag + gene expression = synaptic capture). It is therefore possible to obtain L-LTP even if the synaptic tag and the burst of gene expression have been independently initiated by different sort of stimuli.

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Considering this view, synaptic capture could be classified as homosynaptic, when synaptic capture takes place in the same set of synapses whose stimulation triggered the burst of gene expression (i.e., the regular transcription and translation-dependent late phase LTP, Figure 2C), and heterosynaptic, when it takes place in synapses different of those that received the stimulation that triggered gene expression (Figure 2D). The study of heterosynaptic capture is particularly relevant given its attractive implications in associative learning and memory processes. It should be noted, however, that the term *heterosynaptic* has been misleadingly used in the STC literature to define two inputs that arrive onto a neuron. In some cases it referred to axons that form two sets of glutamatergic or even GABAergic synapses, but frequently it was use to refer to the convergence of glutamatergic and dopaminergic, cholinergic, adrenergic or serotonergic inputs. Whereas the former forms synapses the seconds do not form synapses onto neurons and act like a neuromodulators. In order to avoid confusion, we will use the term *heterosynaptic* only when it describes two sets of synapses, as in our description of *heterosynaptic capture* above, and the term *neuromodulation* to refer to neurotransmitter inputs that activate metabotrophic receptors and do not form typical synapses with pre and postsynaptic structures.

Heterosynaptic capture is particularly accessible and has been thoughtfully investigated in the highly organized laminar structure of the mammalian hippocampus through two-pathway experiments, in which changes in synaptic strength are simultaneously assessed in two independent synaptic inputs to the same neuronal population in the CA1 region. Its study in the Schaffer collateral pathway has enabled to address questions such as the duration and nature of the tagging process that would be very difficult to approach in other systems. Experiments on synaptic capture carried out by different groups during the last decade addressed this and other

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related questions and tried to dissect the cellular and molecular events that underlie synapse-specific consolidation of plasticity changes. Thus, elegant electrophysiology experiments in the rodent hippocampus and in *Aplysia* cultured neurons have dissected synaptic tagging and activity-driven gene expression, showing that, as inferred by the STC model, tags in different inputs can be set independently and prior to the induction of gene expression (Figure 2E) (Casadio *et al.*, 1999; Frey and Morris, 1998b). Whereas other experiments demonstrated that transcriptional activation can prime LTP consolidation in the absence of synaptic tagging (Figure 2F) (Barco *et al.*, 2002; Casadio *et al.*, 1999; Dudek and Fields, 2002).

In the next sections, we will discuss recent developments that have contributed to refine and consolidate the STC hypothesis, and we will outline those aspect of the hypothesis that still require additional support. Although we will describe mainly STC experiments performed on the Schaffer collateral pathway of the hippocampus of mice and rats, because this has become the prevalent model for these studies, we will also discuss results obtained in cultured *Aplysia* neurons and other model systems, which reveal the prevalence and universality of the cellular and molecular mechanisms underlying synapse-specific consolidation of LTP (see also other comments on the hypothesis in recent reviews by (Govindarajan *et al.*, 2006; Morris, 2006; Reymann and Frey, 2007).

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2.2 Synaptic capture symmetry and the importance of weak-before-strong experiments

An alternative explanation to the original findings by Frey and Morris (1997) and Martin et al. (1997), and also to the reduced threshold for L-LTP observed in other paradigms that will be discussed later in this review, is that the persistence of LTP at a given synapse is determined exclusively at the time of induction and controlled by both the strength of the stimulus and the

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presence of certain Plasticity Related Proteins (PRPs) at the postsynaptic terminal. Strong tetanization, somatic action potential and other stimuli could result in the synthesis and diffuse distribution of these factors to the terminal, which in turn would lower the threshold for reaching the late phase of LTP and explain the results without the need of a local tag. Frey and Morris first discussed this idea as result of a personal communication by Roberto Malinow, and referred to it as the "sensitization" (Frey and Morris, 1998a) or the "plasticity-factors" (Frey and Morris, 1998b) hypothesis.

To address this issue Frey and Morris complemented their seminal study carrying out experiments that showed the symmetry of the synaptic capture process by delivering the weak tetanus before the repeated tetanization in the second pathway (Figure 2E). Their results demonstrated that capture of L-LTP in the pathway that received the weakest stimulation was possible both when tagging occurred before and after inducing L-LTP in the second pathway (Frey and Morris, 1998b). The process of synaptic capture is therefore symmetrical, and equivalent results are observed if the stronger stimulation preceded the weaker stimulation or vice versa. This observation can be explained considering that a physical change (the "tag") occurred in stimulated synapses after the weak tetanization and persisted for at least one hour. However, the sensitization model cannot explain "retroactive" effects of the repeated tetanization and only predicts changes in future synaptic responses.

These critical experiments were later independently replicated by Frey's (Sajikumar and Frey, 2004a) and Morris's (O'Carroll and Morris, 2004) groups, and by other labs working both in the mammalian hippocampus (Young and Nguyen, 2005) and in *Aplysia* neuronal cultures (Casadio *et al.*, 1999). They arguably represent the strongest evidence supporting the STC model.

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2.3 Tags duration and resetting

The coexistence of synaptic tags and waves of plasticity-related gene expression defines a time window during which a normally transient form of plasticity can become permanent (Figure 2A). It has been proposed that both synaptic tags and the expression of new PRPs are short-lived and last few hours. The weak-before-strong experiments in the Schaffer collateral pathway described above have allowed to assess the duration of the tag in hippocampal synapses. The capture of L-LTP by preceding subthreshold stimulation in one pathway was possible if the weak stimulation was delivered 5 minutes or one hour before inducing L-LTP in the second pathway, but not when L-LTP was delayed 2 or 4 hours. This suggests that the tag set by the weak stimulation last between 1-2 hours (Frey and Morris, 1998b). Also, synaptic capture was possible for 2-3 hours after the strong stimulation indicating that this is the time window for enhanced gene expression. However, all these experiments were carried out at not physiological temperature and the duration of the tag and the burst of gene expression could therefore be different *in vivo*, at 37°C. Similarly, experiments in cultured *Aplysia* neurons also demonstrated that capture of LTF was possible within a discrete time window from 1-2 hours before to 1-4 hours after the induction of LTF in the opposite branch by five pulses of serotonin (Casadio *et al.*, 1999).

It should be noted, however, that the duration of this coincidence window is likely subject of regulatory mechanisms that can accelerate or delay the turnover of synaptic tags and PRPs. For example, it has been suggested that low frequency stimulation (LFS) can prevent the formation of the synaptic tag, although does not affect L-LTP-associated gene expression (Young and Nguyen, 2005). Depotentiating stimuli have no effect on already captured L-LTP (Barco *et al.*, 2002; Young and Nguyen, 2005), but they do eliminate synaptic tags within 5-10

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min after their setting (Sajikumar and Frey, 2004b). This effect could be mediated by protein phosphatase proteins that counteract the action of the kinase activities involved in setting the tag (Barco *et al.*, 2002). Surprisingly, experiments by Young and Nguyen have shown that LFS triggers this inhibitory effect when applied either homosynaptically or heterosynaptically, or even when applied across dendritic compartments, suggesting that regulation of local tags can depend on LFS-mediated effects that are cell-wide but independent of somatic gene expression. Indeed, their results seem to indicate that LFS produced a transient synaptic depression of fEPSP that extended beyond the pathway receiving the stimulation and affected likely the whole neuron. This transient depression somehow prevents the formation of new synaptic tags for a given time window, but does not interfere with the burst of gene expression in the cell nucleus (Young and Nguyen, 2005). The cellular and molecular mechanisms underlying this cell-wide effect of LFS remain undetermined.

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2.4 Tags for different occasions

LTP and LTD tags: The STC model was originally described based on evidence obtained in hippocampal L-LTP and *Aplysia* LTF, however, synaptic capture has been later also observed in protein synthesis-dependent late long-term depression (L-LTD, Figure 2H) (Kauderer and Kandel, 2000; Navakkode *et al.*, 2005; Sajikumar and Frey, 2004a; Sajikumar *et al.*, 2005). As described for L-LTP, a strong low-frequency stimulation protocol (such as 900 bursts, consisting in 3 stimuli at 20Hz, delivered at 1 Hz) administered to a synaptic input induce a late phase of LTD that can be captured by a subthreshold low frequency stimulus delivered to a second synaptic input in the same population of neurons (Figure 2I). Inhibitors of transcription and translation, such as actinomycin D and anisomycin, did not block the capture of late phase LTD

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although interfered with L-LTD formation, suggesting that persistence of LTD in the second input depended on PRPs produced in response to stimulation in the first input. Recently, Sajikumar and colleagues suggested the extracellular signal-regulated kinase 1/2 (ERK) as a putative LTD-specific tag (Sajikumar *et al.*, 2007a), whereas other kinases activities such as calcium/calmodulin-dependent protein kinase II (CamKII), protein kinase A (PKA) or protein kinase Mζ (PKMζ) have been proposed to participate in synaptic tagging for LTP (Barco *et al.*, 2002; Sajikumar *et al.*, 2007a; Sajikumar *et al.*, 2005; Young *et al.*, 2006). Therefore, synaptic tags for LTP and LTD seem to rely on different molecular mechanisms. It should be however noted, that the approach used in these studies, based on the use of inhibitors more or less specific for some given kinases, may lack the required level of specificity to properly dissect the molecular activities underlying the tagging process.

Synaptic and compartment-specific tagging: The pyramidal neurons in the mammalian hippocampus have both apical and basal dendrites, which define two layers in the CA1 subfield: the *stratum radiatum* and the *stratum oriens*. In a recent study, we investigated whether capture of L-LTP, previously described only within the apical dendritic compartment, could also occur within the basilar dendritic compartment and, if so, whether capture could be accomplished from one dendritic compartment to the other (Alarcon *et al.*, 2006). We observed capture of L-LTP within the basilar dendritic compartment and found that the tagging signal mediating synaptic capture appeared to be the same in both dendritic compartments (1xHFS). However, capture across compartments, between the apical and basilar dendrites, required a stronger triggering stimulation than capture within a compartment. The typical stimulation protocol (1xHFS) that usually evokes capture of L-LTP within the same dendritic compartment (basilar or apical) failed

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to do so across dendritic compartments. Instead, a stronger stimulation or marking signal (2xHFS) was needed to evoke capture of L-LTP across dendritic compartments (Alarcon *et al.*, 2006) (Figure 2J).

What stimulus intensity-dependent mechanism sets the limit between synaptic and compartment-specific tagging? In recent studies Raymond and Redman described and characterized three different types of LTP. First, a relatively short-lasting form of LTP (~1 hr) that required activation of ryanodine receptors (RyRs) and mediated a release of intracellular Ca2+ that only propagated to the stimulated spines. A second, more enduring type of LTP (~2 hrs) that required inositol-3-phosphate receptor (IP_3Rs) activation, in which Ca2+ propagation extended to neighboring dendritic branches. Finally, a third and persistent type of LTP (> 4hrs) that was dependent on L-type voltage-dependent calcium channel (VDCC) activation, and mediated a large calcium influx affecting the entire somatodendritic area, including the cell nucleus (Raymond and Redman, 2002, 2006). It is, therefore, tempting to suggest that the same mechanisms underlying the induction and differential Ca^{2+} propagation of these three types of LTP correspond to mechanisms that set the spatial limit of the tagging signal (from synaptic to modular/clustered/compartmentalized to cell-wide) within a neuron. Structural and molecular constrains in the postsynaptic neurons, such as the GABAergic and neuromodulatory tone imposed onto CA1 pyramidal neurons can also importantly contribute to regulate compartmentalization.

Sajikumar et al. (2007a) have recently confirmed our results on capture across synaptic compartments and identified specific kinase activities differentially involved in synaptic tagging in the *stratum oriens* and the *stratum radiatum*. Whereas calcium/calmodulin-dependent protein kinase II is required to set LTP-specific tags in apical dendrites, the sum of PKA and PKMζ

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activities would set LTP-specific tags in basal dendrites. It should be noted, however, that previous studies pointed out a role for PKA also in tagging apical dendrites (Barco *et al.*, 2002; Young *et al.*, 2006).

2.5 Competitive maintenance

Fonseca and colleagues have recently described an interesting phenomenon referred to as competitive maintenance, which offers further support for the synaptic tagging hypothesis and shows that late-associativity is not always cooperative. In fact, under certain circumstances it can be a competitive process. Thus, under regimes of reduced protein synthesis, the induction of L-LTP in a given input occurs at the expense of the maintenance of prior potentation in an independent input (Fonseca *et al.*, 2004) (Figure 2K). These findings extend the synaptic capture model in two important ways: (1) Newly produced plasticity related proteins (PRPs) are shared among synapses whose activity may have not contribute to their induction, such as observed in two-pathway synaptic capture experiments. However, under circumstances of reduced availability synaptic competition will define which synapses take those PRPs. Both the competition and the sharing of PRPs would depend on the formation, duration and strength of synaptic tags. (2) Synaptic capture, at least under some given conditions, may not be an irreversible process, the competitive maintenance model suggest a dynamic scenario in which PRPs can move from previously stabilized synapses to neighboring ones. Interestingly, competitive L-LTP maintenance, as synaptic capture, is largely restricted to specific dendritic compartments.

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2.6 Cross-tagging and cross-capture

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Recent work by Sajikumar and colleagues has revealed another intriguing new feature of late-associativity in the mammalian hipocampus (Sajikumar and Frey, 2004a). They found that the consolidation of either LTP or LTD (capture of LTP or LTD) was facilitated in response to the previous induction of the late phase of the opposite form of synaptic plasticity in a separate population of synapses in the same neurons (Figure 2L), a paradoxical phenomenon they originally referred to as "cross-tagging" (Sajikumar and Frey, 2004a), although it has been later renamed as "cross-capture" by other authors (Govindarajan *et al.*, 2006; Morris, 2006), a term we also consider more appropriate. In this case, the situation seems to be radically different to that described in *Aplysia* cultured neurons, in which LTD overrides, instead of facilitate, LTF (Guan *et al.*, 2002). The striking result in the Schaffer collateral pathway suggests that a common genetic program is activated in CA1 pyramidal neurons by both L-LTP and L-LTD triggering stimuli, whereas differential tags set at the synapses would determine whether that genetic program led to the persistence of LTD or LTP in a given synapse. One biologically relevant advantage of this associativity between L-LTP and L-LTD is that bidirectional changes in synaptic weight, which are thought to underlie memory encoding, can be stabilized simultaneously.

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2.7 Cell-wide facilitation, modulation of intrinsic excitability, and other forms of late-associativity

A number of stimuli can reduce the threshold for L-LTP in the entire neuron in the absence of synaptic activity, a phenomenon that has been referred to as L-LTP facilitation or LTP reinforcement. Thus, Dudek and Fields have proposed that somatic action potential could activate gene expression and enable the cell-wide capture of L-LTP (Dudek and Fields, 2002).

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Also, neuromodulatory input, besides its role on modulation of synaptic processes, can play a major role in the activation of specific cascades of gene expression and facilitate synaptic plasticity changes in a cell-wide manner (Berke and Hyman, 2000; Reymann and Frey, 2007). Similar facilitatory effects have been also observed after enhancing CREB-dependent gene expression (Alarcon *et al.*, 2006; Barco *et al.*, 2002; Marie *et al.*, 2005). Although all these facilitatory effects can be, and have been, interpreted in terms of enhancing synaptic capture, the facilitation observed might also rely, as discussed in section 2.2, on sensitization mechanisms. One could consider at least two possible independent and converging mechanisms to facilitate L-LTP formation: (1) the reduction of the threshold for L-LTP induction (sensitization), and (2) the synaptic capture of PRPs that enables the immediate consolidation of the synaptic change. Even the facilitation underlying heterosynaptic capture in two pathway experiments might partially depend on these two components. This could explain why the captured LTP in strong-before-weak stimulation protocols is generally more robust than in weak-before-strong experiments (Frey and Morris, 1998a).

An example of these possible converging effects can be found in the reported effects of aminergic stimulation in two pathway experiments. The hippocampus receives dopaminergic, noradrenergic and cholinergic inputs that can modulate LTP induction and consolidation (Frey *et al.*, 1991; Frey *et al.*, 1990; Gelinas and Nguyen, 2005; Hu *et al.*, 2007), and play a role in synaptic capture of both L-LTD and L-LTP, likely by regulating the induction of PRPs and thereby priming the cells for synaptic capture (Navakkode *et al.*, 2007; Sajikumar and Frey, 2004a). However, ascending monoaminergic fibers may also facilitate hippocampal LTP induction and LTP-dependent learning processes in a global manner during arousal and attention by suppressing the slow afterhyperpolarization (sAHP) and increasing intrinsic excitability

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(Deng *et al.*, 2007; Fuenzalida *et al.*, 2007; Reymann and Frey, 2007; Zhang and Linden, 2003). Downstream of aminergic inputs, the prime candidates to regulate these changes in intrinsic excitability largely overlap with those thought to regulate LTP and synaptic capture. These include adenylyl cyclase and a number of kinases involved in synaptic tagging, such as PKA, PKC, CamKII and MAPK (Adams *et al.*, 2000; Anderson *et al.*, 2000; Zhang and Linden, 2003). In particular, the suppression of sAHP in CA1 pyramidal neurons appears to be mainly mediated by the level of the second messenger cAMP and the candidate kinase for synaptic tagging PKA (Haug and Storm, 2000; Pedarzani and Storm, 1993, 1995). Furthermore, recent evidence suggests that CREB activity, which is known to be downstream of such inputs (Berke and Hyman, 2000), might mediate sustained changes in intrinsic excitability (Dong *et al.*, 2006; Han *et al.*, 2006; Lopez de Armentia *et al.*, 2007). It is worth noting that intrinsic plasticity can also modulate dendritic voltage-sensitive channels in circumscribed dendritic modules and thereby contributing to compartment-restricted metaplasticity (Frick *et al.*, 2004; Johnston *et al.*, 2003; Zhang and Linden, 2003). The modulation of intrinsic excitability and consequent reduction in the threshold for L-LTP cannot, however, explain by itself the resistance of L-LTP to protein and RNA synthesis inhibitors observed in synaptic capture experiments, or the capture of L-LTP using the weak-before-strong protocol. We will further discuss the contribution of neuromodulatory inputs to STC events in the context of behavioral reinforcement of LTP and associative learning in a separate section.

Finally, it should be noted that not all forms of L-LTP are necessarily susceptible to capture. The experiments of Huang and Kandel in a long-lasting form of LTP induced by theta frequency stimulation (theta L-LTP) showed that some relatively enduring forms of LTP do not require nuclear participation and might rely exclusively on local protein synthesis. Therefore,

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they would not be accessible to synaptic capture (Huang and Kandel, 2005). In other cases, synaptic capture of L-LTP could occur in the absence of transcriptional activation and depends only on activity-dependent stimulation of somatic protein translation. This could suffice, as long as the generated protein products would become accessible to nearby synapses and support a synaptic capture model (Sajikumar *et al.*, 2007b).

3. Dissecting synaptic capture: Molecular mechanisms

3.1 The tagging process

As pointed out in previous sections, studies in *Aplysia* and rodents have revealed a diversity of candidate molecules for setting the synaptic tag. In fact, most likely, tagging may recruit an array of synaptic modifications, rather than being determined by a single candidate molecule or event. From a theoretical point of view, (Martin and Kosik, 2002) proposed that any candidate mechanism for synaptic tagging should fulfill three basic criteria: (1) It must be local, ideally spatially restricted to a single synapse, (2) It must be transient and (3) It should be able to interact with the newly synthesized gene products, either mRNAs or proteins. In addition, it has been suggested that a particularly economical arrangement from the biological point of view would be one in which the cascade responsible for mediating E-LTP would also contribute to tag the synapse and participate in sequestering the PRPs (Frey and Morris, 1998a). These general criteria are, in principle, valid for any kind of tagging mechanism, from mollusk to mammals. A number of molecular processes meeting these criteria have been suggested to participate in the setting of synaptic tags (Figure 3):

a. Activation of protein kinases: As mentioned before, several studies have proposed diverse protein kinase activities and the phosphorylation of specific synaptic substrates as suitable

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candidate mechanisms for the tag (Barco *et al.*, 2002; Casadio *et al.*, 1999; Sajikumar *et al.*, 2007a; Sajikumar *et al.*, 2005; Young *et al.*, 2006). Activity regulated kinases meet all the criteria for a synaptic tag: their activity can be spatially restricted by membrane anchoring, the half-life of protein phosphorylation is consistent with the temporal span of tag activity (few hours), and they can interact with a variety of substrates and modulate E-LTP. These criteria together with the evidence accumulated by different research groups have pointed to activation of PKA as the strongest candidate for synaptic tagging. To summarize this evidence: (1) The inhibition of phosphodiesterases (PDE) by rolipram and subsequent induction of rolipram-reinforced LTP in one synaptic input transformed E-LTP into L-LTP in a second independent input of the same neuronal population (Navakkode *et al.*, 2004), suggesting that the synaptic tag may depend on the second messenger cAMP. Indeed, it has been proposed that the inhibitory effect of LFS on synaptic tagging relies on cell-wide dampening of cAMP (Young *et al.*, 2006). (2) Pharmacological activation of the cAMP/PKA pathway produces a synaptic tag capable of capturing L-LTP expression (Navakkode *et al.*, 2004; Young *et al.*, 2006), whereas the inhibition of PKA by KT5720 blocked synaptic capture both in the hippocampus and in *Aplysia* neuronal cultures (Barco *et al.*, 2002; Casadio *et al.*, 1999; Young *et al.*, 2006). (3) The pharmacological inhibition of PKA anchoring also blocked the late phase LTP and synaptic capture, suggesting that spatial compartmentalization of PKA signaling via binding to A kinase-anchoring proteins (AKAPs) is critical in these processes (Huang *et al.*, 2006; Nie *et al.*, 2007). (4) This pharmacological evidence received further support from experiments with transgenic mice that have genetically reduced PKA activity and display impaired synaptic capture of L-LTP (Young *et al.*, 2006).

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However, as we discussed above, PKA is not the only kinase activity implicated in tagging. Pharmacological experiments indicate that the atypical protein kinase C known as protein kinase Mζ (PKM-ζ) may be necessary and sufficient for the maintenance of L-LTP in the hippocampus (Ling *et al.*, 2002; Pastalkova *et al.*, 2006) and can also be involved in synaptic tagging (Sajikumar *et al.*, 2005). More recently, it has been proposed that CaMKII could also participate in setting synaptic tags for L-LTP capture, whereas MAPKs would play an equivalent role for L-LTD (Sajikumar *et al.*, 2007a).

b. Regulation of local protein synthesis: According to the original experiments by Frey and Morris (1997), the capture of L-LTP was completely independent of protein synthesis, suggesting that the burst of transcription is immediately followed by somatic protein synthesis. However, based in the currently well established role of local protein synthesis in LTP (Sutton and Schuman, 2006) and the significant effect of protein synthesis inhibitors observed in more recent late-associativity studies both in the mouse hippocampus (Alarcon *et al.*, 2006; Barco *et al.*, 2002) and *Aplysia* neurons (Casadio *et al.*, 1999), it seems reasonable to consider the contribution of protein synthesis-dependent mechanisms in synaptic capture of L-LTP.

The local activation of protein synthesis, like the stimulus-driven activation of kinases, meets the two most critical requirements for a synaptic tag: is spatially restricted and can be transiently activated. Indeed, electron and confocal microscopy experiments indicate that local protein synthesis could be even restricted to individual synaptic spines (Aakalu *et al.*, 2001; Ostroff *et al.*, 2002). Furthermore, the transient activation of local protein synthesis is a particularly well-suited mechanism for coupling tagging with *de novo* transcription at the cell nucleus. Experiments with protein inhibitors or isolated dendrites, both in *Aplysia* and hippocampal slices, support a critical role for dendritic protein synthesis in the late-phase of LTP

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(Abraham and Williams, 2007; Cracco *et al.*, 2005; Martin *et al.*, 2000a; Martin *et al.*, 1997; Vickers *et al.*, 2005).

Different processes involved in the local activation of protein synthesis can be regulated by synaptic activation and serve as substrate for synaptic tagging, including the phosphorylation of translation factors by activity-dependent kinases, the translocation of polyribosomes into the dendritic spine, the recruitment of IRES-driven translation, and the activation of the translation of dormant mRNAs located at synaptic terminals through the elongation of their poly-A tail by neuronal cytoplasmic polyadenylation element binding protein (CPEB) (see recent reviews by (Klann and Dever, 2004; Richter, 2001; Sutton and Schuman, 2006). For example, the translation initiation factor eIF2a, which is required for the formation of the cap-structure necessary for the assembly of the ribosomal machinery, and its kinase GCN2 have been shown to regulate the threshold for L-LTP expression and memory consolidation (Costa-Mattioli *et al.*, 2005; Costa-Mattioli *et al.*, 2007). The mammalian target of rapamycin (mTOR) pathway (Hay and Sonenberg, 2004), which was first implicated in plasticity and memory by studies on LTF in *Aplysia* (Casadio *et al.*, 1999), also represents an interesting target for local protein synthesis regulation. Thus, the phosphorylation of 4E-BP1 and S6 in neurons is regulated by the ERK pathway and has been shown to play a major role in LTP (Kelleher *et al.*, 2004a). Other inhibitory eIF4E-binding proteins, such as 4E-BP2, can also play a critical role in the translational control of L-LTP and L-LTD (Banko *et al.*, 2006; Banko *et al.*, 2005).

Si and Kandel recently proposed an interesting new mechanism for tagging active synapses in *Aplysia* sensory neurons (Si *et al.*, 2003b). *Aplysia* CPEB (ApCPEB) has prion-like properties, i.e. it can switch between two conformational states, one active and another inactive, having the active one the capability to transform neighboring inactive molecules to the active

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state. In naive synapses, the basal level of ApCPEB expression is low and its state is inactive or even repressive, however, after synaptic activation there is an increase in the amount of ApCPEB and, if a given threshold is reached, ApCPEB may pass to a prion-like state, which is more active and lacks the inhibitory function of the basal state (Si *et al.*, 2003a). Once the prion state is established at activated synapses, dormant mRNAs, made in the cell body and distributed cell-wide, would be translated only at activated synapses. Since the active state of ApCPEB can self-perpetuate, this model could not only provide a mechanism for transient tagging, but also for the persistence of long-term memory storage (Si *et al.*, 2003b). Interestingly, this hypothesis has recently received further support from studies in *Drosophila* showing that the CPEB protein Orb2 is acutely required for long-term courtship memory (Keleman *et al.*, 2007). Four CPEB isoforms are found in the mouse (Theis *et al.*, 2003). One of which, CPEB-1, has been detected at postsynaptic sites of hippocampal neurons and participates in local NMDAR-dependent polyadenylation of αCamKII and other dendritic mRNAs (Huang *et al.*, 2003; Huang *et al.*, 2002; Wells *et al.*, 2001; Wu *et al.*, 1998). Experiments in CPEB-1 deficient mice revealed that synaptic capture of L-LTP was partially affected in those mice (Alarcon *et al.*, 2004). Furthermore, another mammalian CPEB isoform, CPEB-3, is structurally similar to neuronal *Ap*CPEB and is expressed in hippocampal neurons in response to the neurotransmitter dopamine (Theis *et al.*, 2003). This raises the possibility that activity-dependent regulation of mouse CPEB-3, similarly to serotonin-mediated regulation of CPEB activity in *Aplysia*, could act as a synaptic tag in mammalian synapses.

c. Release of neurotrophins: The neurotrophin brain derived neurotrophic factor (BDNF), in addition to its possible role in consolidation of LTP, was also recently implicated in synaptic tagging. The ablation of the neurotrophin BDNF in the entire mouse forebrain (forebrain

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restricted BDNF knockout mouse) produced a large deficit in capture of LTP assayed in the Schaffer collateral to CA1 synapses of the mouse hippocampus. Interestingly, the defect in capture of LTP was more pronounced in these mutant mice than in another strain in which the ablation of BDNF was restricted only to CA1 pyramidal cells (Barco *et al.*, 2005). Based on these results we proposed that the presynaptic release of BDNF into the synaptic cleft after tetanic stimulation may participate in the post-synaptic tagging of the synapse through activation of TrkB receptors (Barco *et al.*, 2005), perhaps by promoting local protein synthesis (Aakalu *et al.*, 2001; Purcell *et al.*, 2003). This idea, which could be indeed considered a specific mechanisms of the general view of regulation of local protein synthesis as substrate for synaptic tagging proposed above, has been further developed by Lu and colleagues in a recent review (Lu *et al.*, 2007).

d. Altered membrane expression of ionotropic receptors: The insertion and exchange of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) subunits has been suggested as a possible form of synaptic tagging (Carroll and Malenka, 2000; Frey and Morris, 1998b). Excitatory glutamatergic synapses whose postsynaptic membrane contains NMDARs but no AMPARs are frequently refereed as silent synapses (Malinow *et al.*, 2000; Malinow and Malenka, 2002). The activation of silent synapses by insertion of AMPARs into the postsynaptic membrane could therefore participate in both, the expression of LTP and the tagging of the synapse. Interestingly, the expression of a constitutively active CREB protein facilitates the late phase of LTP in a protein synthesis-independent manner that resembled the synaptic capture of L-LTP (Barco *et al.*, 2002), an effect that has been later linked to the overexpression of NMDAR and the presence of a larger number of silent synapses (Marie *et al.*, 2005). According to this view, tagging of the synapse by subthreshold tetanic stimulation would cause an enhanced silent

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synapses conversion and, in consequence, could explain the observed facilitation of L-LTP (Marie *et al.*, 2005). In addition, alterations in the subunits composition of AMPAR may represent an alternative mechanism for synaptic tagging. A recent report showed that the increase in Ca^{2+} levels observed at stimulated synapses following LTP induction coincided with the transient insertion (for about 25 min) of Ca^{2+}-permeant GluR2-lacking AMPA receptor channels into the synaptic membrane (Plant *et al.*, 2006). The authors suggested that the transient incorporation of this type of AMPA receptor could contribute to the activation and temporal nature of the synaptic tag.

Given the loose definition of synaptic tag and the open theoretical framework provided by the synaptic capture model, many other biological processes have been also proposed to participate in synaptic tagging. Thus, local protein degradation can work coordinately with local protein synthesis to change the molecular composition of active synapses and serve as a synaptic tag (Martin and Kosik, 2002). Indeed, the activation of ubiquitin-mediated protein degradation has been shown to be important for both L-LTP in rodents (Fonseca *et al.*, 2006) and LTF in *Aplysia* (Hegde *et al.*, 1997). Also, diverse studies in *Aplysia*, *Drosophila* and mammals indicate that synaptic activity regulates the internalization of adhesion molecules, such as fasciclin II, NCAM or β-catenin, at active synapses, what has been also proposed as a putative mechanism for synaptic tagging (Martin and Kosik, 2002). The specific role of these molecular processes in synaptic tagging has not been investigated, and no experimental evidence supports at the moment the relevance of these mechanisms.

In addition to these molecular events, it has been proposed that structural changes such as widening of the synaptic spine neck might participate in tagging the synapses by facilitating the capture of newly synthesized mRNAs or proteins (Frey and Morris, 1998a; Luo, 2002; Sanchez

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et al., 2000). Indeed, a recent study has shown that the same stimulation protocol used to induce synaptic tagging (1 train of HFS) produces a transient (< 20 min) expansion of synaptically connected dendritic spines (Lang *et al.*, 2004). Stronger synaptic stimulation (repetitive spaced tetani) can trigger more long-lasting remodeling of the actin network at both pre- and postsynaptic sites (Colicos *et al.*, 2001). However, the specific role of these processes in synaptic tagging in the hippocampus has not been yet explored.

Finally, we would like to point out that although we have conceptualized the synaptic tag in CA1 hippocampal neurons as a postsynaptically localized protein or process whose function is to sequester activity-induced gene products, it has been suggested that there may also be tags at presynaptic sites. In fact, synaptic tagging in *Aplysia* sensory neurons seems to rely exclusively in presynaptic mechanisms in the sensory neuron, whereas the motor neuron plays a relatively passive role (Martin *et al.*, 1997). Routtenberg speculated that even in the hippocampus, synapses could be tagged by the action of presynaptic proteins (Routtenberg, 1999). In this model, only presynaptic terminals that had been tagged would demonstrate potentiated release of neurotransmitter in response to a retrograde messenger released from the postsynaptic compartment (for example, in response to activity-induced BDNF released by the postsynaptic neuron).

All these mechanisms are obviously not exclusive. On the contrary, some of these processes are clearly related: the activities of the kinases involved in tagging are also known to regulate local translation, trafficking and changes in the cytoskeleton, the neurotrophin BDNF is known to regulate local protein synthesis, etc. It seems likely that several activity-triggered molecular modifications with their particular time course, spatial range and regulatory networks can set local traces at active synapses and participate in tagging. Modeling studies on the

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activation of the synaptic tag, both in *Aplysia* LTF (Song *et al.*, 2006) and hippocampal LTP (Smolen *et al.*, 2006), support the idea that a given activity threshold should be reached in order to activate the tag. The existence of these different types of tags provides more possibilities for late-associativity and regulation, and significantly increases the computational capabilities of neurons.

3.2 Activity-dependent gene expression

Although transcription has been shown to be critical for some long-lasting forms of LTP (Barco *et al.*, 2002; Costa-Mattioli *et al.*, 2005; Frey *et al.*, 1996; Huang and Kandel, 2005; Levenson *et al.*, 2004; Nguyen and Kandel, 1996; Vickers *et al.*, 2005) and long-term memory (Barondes and Jarvik, 1964; Kandel, 2001), there are also some long-lasting forms of LTP that apparently do not require transcription (Huang and Kandel, 2005; Otani *et al.*, 1989). As a consequence, there is nowadays discussion regarding the relevance of transcription-dependent gene expression as a universal requirement for late phase LTP and the relative contribution of transcription-dependent *de novo* gene expression versus gene expression mediated exclusively through activation of somatic and/or dendritic translation of preexistent mRNAs (Reymann and Frey, 2007). The toxicity and irreversible mechanism of action of actinomycin D, the inhibitor of transcription more frequently used in LTP and behavioral studies, limited the time window susceptible of analysis (<12h) and contributed to this controversy. Although, pharmacological experiments make difficult to conclude whether the requirement of transcription for the long-term maintenance of LTP is attributable only to the refill housekeeping proteins, more precise and elegant experiments using highly specific blockers of transcriptional activity, such as antisense and decoy oligonucleotides or dominant negative variants of transcription factors, as well as

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experiments in different mouse strains with impaired activity-dependent gene expression, strongly support a role for transcription beyond the mere maintenance of neuronal integrity.

Although we do not have yet a comprehensible list of the genes that are induced or repressed by L-LTP-inducing synaptic stimulation, the work of many groups in the last decade has identified several gene expression cascades that appear to be especially relevant for the consolidation of changes in synaptic strength and memory. In particular, the CREB family of transcription factors has been identified as a major regulator of activity-dependent gene expression in neurons (Barco *et al.*, 2007; Barco and Kandel, 2005; Lonze and Ginty, 2002), although some controversy remains regarding the role of specific family members and CREB isoforms in hippocampal L-LTP and memory (Balschun *et al.*, 2003). Studies on *Aplysia* neurons and the mouse hippocampus have revealed a direct role of CREB-dependent gene expression during synaptic capture. Kandel and co-workers demonstrated that repeated application of the neurotransmitter serotonin to one neuronal branch caused CREB activation in the cell nucleus, branch-specific long-term facilitation (LTF) and the growth of new synaptic connections in the stimulated branch (Martin *et al.*, 1997). Moreover, they found that the mere injection of active phospho-CREB when paired with a single pulse of 5-HT in one of the branches, which in normal conditions only produced short-term facilitation (STF) and no varicosity formation, produced LTF and increased the number of varicosities (Casadio *et al.*, 1999). Based on these data, the authors proposed that CREB activity might provide the building blocks necessary for the formation of new synaptic connections whereas the single pulse of 5-HT tagged the branch would enable the capture of these gene products (Casadio *et al.*, 1999). Similarly, the expression of a constitutively active variant of CREB, VP16-CREB, in CA1 hippocampal neurons of transgenic mice enhanced CRE-driven gene expression and reduced the threshold for eliciting a

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persistent late phase of LTP in the Schaffer collateral pathway (Barco *et al.*, 2002). The pharmacological characterization of this form of facilitated L-LTP suggested that VP16-CREB activity could lead to a cell-wide priming for LTP by seeding the synaptic terminals with proteins and mRNAs required for the stabilization of L-LTP (Barco *et al.*, 2002). Transcriptional profiling of this transgenic line has later identified the CREB-downstream gene *bdnf* as the most relevant effector molecule contributing to its enhanced LTP phenotype (Barco *et al.*, 2005).

However, CREB-dependent gene expression is not the only genetic program involved in late phase LTP. The activity of other transcription factors, such as SRF, c-fos, egr1 or NF-κβ (Albensi and Mattson, 2000; Izquierdo and Cammarota, 2004; Ramanan *et al.*, 2005; Tischmeyer and Grimm, 1999) can also promote *de novo* gene expression and support long-lasting changes in synaptic plasticity. The expression of some of these TFs, such as c-fos, egr1 or C/EBPβ, is itself induced during LTP formation and their activity can trigger a second wave of gene expression that may also be important for LTP consolidation. The genes downstream of these transcription factors are also likely to interact with synaptic tags and can be specifically recruited to active synapses. The interaction of these two or more waves of gene expression with tags of different duration may provide great capability for signaling integration.

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3.3 Cellular distribution of new plasticity products

According to the original hypothesis for synaptic tagging, the burst of transcription is followed by somatic protein synthesis, the wide spread distribution of PRPs throughout the cell and their functional incorporation to activity-tagged synapses. Alternatively, for some plasticity related genes, transcription can be coupled to activation of sorting machinery for mRNAs, their cell-wide distribution and local translation in tagged synapses. However, new evidence suggests a

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differential distribution of gene products from the soma to basal and apical dendrites (Alarcon *et al.*, 2006; Fonseca *et al.*, 2004; Sajikumar *et al.*, 2007a; Sajikumar *et al.*, 2007b). In consequence, gene products induced by signals that reach the nucleus seem to be primarily distributed back into the same dendritic compartment where the activation cascade was initiated. This does not mean that under certain circumstances there could not be a real cell-wide distribution of gene products. For instance, stimulation of the alveus, which contains the axons from CA1 pyramidal neurons, produces antidromic (back-propagating) action potentials that activate transcription at CA1 neurons nuclei and reduces the threshold for L-LTP, likely by priming the synapses for synaptic capture (Dudek and Fields, 2002). After antidromic stimulation, capture of L-LTP could be observed both in basilar and in apical dendrites (Alarcon *et al.*, 2006), hence supporting the idea of a cell-wide distribution of gene products. Enhanced CREB-mediated gene expression initiated in the absence of synaptic activity after phospho-CREB injection in *Aplysia* neurons (Casadio *et al.*, 1999) or expression of the constitutively active protein VP16-CREB in the hippocampus of transgenic mice can also lead to cell-wide distribution of PRPs (Alarcon *et al.*, 2006).

The distinction between cell-wide and compartment-restricted distribution of gene products likely depends on the strength and location of the initial stimulation (Alarcon *et al.*, 2006; Sajikumar *et al.*, 2007b). In hippocampal CA1 neurons, antidromic stimulation triggers transcription and promotes the diffuse distribution of mRNAs and proteins to all dendrites. In contrast, orthodromic activation originated via dendritic signals might only activate the sorting machinery in the dendritic compartment that received the stimulus. In support of this view, both the mRNA and the protein encoded by the immediate-early gene *Arc* (activity-regulated cytoskeleton-associated protein), which is critically involved in memory encoding (Tzingounis

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and Nicoll, 2006), accumulate selectively in previously activated dendritic domains of the molecular layer at the dentate gyrus, suggesting that after synaptic activation the mRNA molecules entering the dendrites are actively accumulated near active synapses and depleted from nonactivated regions (Steward *et al.*, 1998). Similarly, synaptic stimulation in *Aplysia* neurons strongly enhanced the transport of Ap-eEF1A mRNAs to axonal processes, while its transcripts accumulated around the nucleus when the stimulation was restricted to the cell body (Giustetto *et al.*, 2003). Conceivably, activity-mediated tagging of cytoskeleton structures by PKA or another activity-dependent kinase might enhance the transport of mRNAs and proteins to dendrites containing those synapses recently stimulated (Kotz and McNiven, 1994; Luo, 2002; Rodionov *et al.*, 2003; Sanchez *et al.*, 2000). Interestingly, recent studies in cultured neurons have demonstrated that post-Golgi membrane trafficking is polarized toward specific dendrites and small Golgi outposts partition selectively into longer dendrites and concentrate at branchpoints (Horton *et al.*, 2005).

3.4 The capture process

Synaptic capture results from the interaction between *de novo* gene products and synaptic tags. But, how does this interaction take place? What we refer to as synaptic capture depends to a great extend on what we consider to be the synaptic tag. Based on this, we can consider two main mechanisms for synaptic capture:

1. Recruitment and assembly of new protein complexes: This is conceptually the simplest form of synaptic capture. It corresponds to the original conception of the process in which proteins or vesicles loaded with protein are literally captured by active protein complexes located at postsynaptic sites. In this model, the activation of kinase activities appears as a likely candidate

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for tagging: the phosphorylation of these PRPs and their consequent functional incorporation to active synapses would represent the act of capture. Although very few studies have focused so far in the molecular mechanism for synaptic capture, Ling et al. examined in a recent report the mechanism by which PKMζ mediates LTP and increases synaptic transmission (Ling *et al.*, 2006). They found that postsynaptic PKMζ, whose activation has been postulated as a synaptic tag in apical dendrites (Sajikumar et al., 2005), potentiated the amplitude of AMPA receptor-mediated miniature EPSCs (mEPSCs) and doubled the number of functional postsynaptic AMPA receptor channels, suggesting that this kinase could regulate the functional incorporation of AMPA receptors into tagged synapses in order to maintain LTP.

2. Local protein synthesis: Not only proteins, but also mRNAS can be captured. In this case, capture can simply consist in the local translation of plasticity-related mRNAs. Although the conventional view of the STC model was limited to the capture of PRPs, recent studies have shown that synaptic activity can modulate general somatic or dendritic protein synthesis (Abraham and Williams, 2007; Kelleher *et al.*, 2004b), therefore, an increase in input-specific localized translation might be viewed as a parallel or supplementary mechanism for synapse stabilization. The molecular machinery for local translation would be active or enhanced only in previously activated synapses as a consequence of tagging (phosphorylation of translation factors, reorganization of the cytoskeleton and polyribosomes, etc). What mRNAs could be captured by tagged synapses? We do not have yet a definitive answer to this question, but input-specific stimulation delivered to the apical dendrites has been shown to trigger the translation of microtubule associated protein 2 (MAP2) and CaMKII mRNAs localized in that dendritic compartment (Huang *et al.*, 2005; Ouyang *et al.*, 1997; Ouyang *et al.*, 1999; Steward and Halpain, 1999). Much effort has been also put on the identification of the mRNA cargo that

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travels to synapses (Kiebler and Bassell, 2006). Experiments in neuronal cultures and mouse brain extracts have allowed to isolate large RNAase-sensitive granules and identified a number of dendritic proteins and mRNAs, such as those encoding for CamKIIα and Arc, associated to the motor protein kinesin (Kanai *et al.*, 2004). Along with the identification of the mRNA cargo at dendrites, the focus is now in understanding how the expression of this cargo is modulated by synaptic activity. It would not be surprising -at least to us- to find that the translation of most of the mRNAs that are the usual constituents of synapses (e.g. PSD-95 associated proteins) could be enhanced in tagged synapses. In a recent review, Klann and Sweatt developed the interesting idea that long-lasting effects in synaptic efficacy could depend on localized changes in translation efficacy (i.e. an altered rate of ongoing or "housekeeping" translation at particular synapses) (Klann and Sweatt, 2007), in contraposition to the role of *de novo* translation of specific mRNAs. These changes in the translation rate could occur within functional dendritic compartments and thereby contribute to compartmentization of plasticity, as observed in STC experiments. Advances on this model will not only improve our understanding of the processes leading to synaptic stabilization, but will also help to clarify the relative contribution of general protein synthesis versus translation of specific mRNAs.

As discussed for synaptic tags, these two mechanisms for capture are not exclusive. Indeed, the differential effects of transcription and translation blockers in synaptic capture (Alarcon *et al.*, 2006; Barco *et al.*, 2002) suggest the co-existence of protein-synthesis dependent and a protein synthesis independent component in capture of L-LTP. Unfortunately, although there is strong justification to believe that PRPs can be physically captured at tagged synapses, no direct observation of a tag-mediated capture of activity synthesized plasticity factors has been presented simultaneously to electrophysiological recordings showing LTP. The constant

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improvement of imaging techniques might allow in the near future visualizing in real time the distribution of plasticity-related gene products and their capture in previously activated synapses also in the rodent hippocampus. As a first step in that direction, *in vivo* imaging of the neuromuscular junction of the fly have revealed that transport vesicles transit continuously through resting terminals, but they seem to be subject of activity-dependent synaptic capture (Shakiryanova *et al.*, 2006).

4. Synaptic tagging and neuronal computation

The STC hypothesis presents attractive mechanisms for the integration of separate afferent inputs arriving at the same neuron in a period of time that scales from minutes to hours, well beyond the few milliseconds contemplated in purely electrophysiological models for LTP associativity and synaptic integration (Magee, 2000; Magee and Johnston, 2005).

Neurons in the CA1 area of the hippocampus receive inputs from different brain areas containing spatial, relational and other relevant forms of information that need to be integrated for proper encoding of memory traces (Amaral and Witter, 1989; Deuchars and Thomson, 1996; Dolleman-Van Der Weel and Witter, 1996; Ishizuka *et al.*, 1990; Pikkarainen *et al.*, 1999). Basilar dendrites receive information from the contralateral hippocampus, whereas apical dendrites mostly receive ipsilateral afferents from neighboring CA3 neurons. Moreover, physiological evidence suggests that the proximal and distal apical dendrites, although part of the same structure, function as two distinct compartments (Jarsky *et al.*, 2005; Nicholson *et al.*, 2006): whereas Schaffer-collateral fibers from neurons in area CA3 navigate the *stratum radiatum* and largely synapse onto the proximal apical dendrites, neurons from layer III of the entorhinal cortex send axons via the lateral perforant pathway connecting onto distal apical

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dendrites in the *stratum lacunosum-moleculare* (SLM). As described in a previous section, capture of L-LTP can occur within both the basilar and the apical dendritic compartment in response to the same tagging signal. However, capture across compartments required a stronger stimulation (Alarcon *et al.*, 2006; Sajikumar *et al.*, 2007b), which could be needed to set a compartmental tag that overcame the structural or molecular constrains that restricted capture across compartments, making the new gene products available in the different dendritic compartments within a neuron (Fonseca *et al.*, 2004; Sajikumar *et al.*, 2007a; Sajikumar *et al.*, 2007b; Young and Nguyen, 2005). The different stimulation thresholds gating the interaction between synaptic inputs during the regimes of cell-wide facilitation, compartment-restricted plasticity and synaptic capture can enable a neuron to differentially integrate information arriving from different brain areas into its distinct functional compartments as a function of the strength of the stimuli and their temporal proximity. As a consequence, the interaction between two or more forms of synaptic plasticity within the same or different "functional compartments" could regulate the prevalence or dismissal of certain forms of synaptic plasticity.

In a recent review article, Tonegawa's group developed the "clustered plasticity hypothesis" to explain memory encoding at the single neuron level (Govindarajan *et al.*, 2006). This model, contrary to previous computational models that relied on associational LTP and LTD as the predominant mechanism for memory formation, added local enhancement of protein synthesis, STC to explain the formation of long-term memory engrams through bidirectional synaptic weight changes among synapses within or across dendritic branches. They proposed that "clustered plasticity" occurs only within functional, independent neuronal compartments, which have independent regulation of local translation and distribution of plasticity products. The distance and morphological and structural restrictions would negatively influence the

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spreading of plasticity products from one branch where synapses have been activated/tagged to another, contributing to compartmentalization and increasing the computational capability of the neuron. This model predicts more efficient action potential firing during recall compared with conventional dispersed plasticity model and makes several testable predictions. The current development of imaging technologies and refinement of electrophysiological recordings will soon allow assessing some of these predictions and would support the validity of the synaptic capture and clustered plasticity models.

5. Synaptic tagging and associative learning

Neurons in the CA1 area of the hippocampus form part of circuits that underlie important forms of associative learning (Martin *et al.*, 2000b; Rolls and Kesner, 2006). Despise the recent progresses, we still do not know whether synaptic tagging occurs *in vivo* during learning and, if so, what are the consequences in memory formation. Is it possible to form a persistent memory of an experience that ordinarily would have produced only a short-lasting memory just by coupling that experience to another event that caused the activation of de novo gene expression in the same neuronal population? Can synaptic tagging and synaptic capture events underlie the formation of flashbulb memories? Could STC events underlie the difference between normal association of memories of our every day experiences and forever bonded post-traumatic stress disorder memories?

Richard Morris proposed in a recent review some behavioral experiments aimed to tackle these questions (Morris, 2006). Unpublished results by his group suggested that the burst of gene expression associated to exploratory activity and manifested in the induction of immediate early genes could prime memory formation. Thus, rats exposed to novelty exploration formed more

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persistent spatial memory in a event arena in which they were trained in a "one-shot" memory task than naïve animals not exposed to novelty exploration (Morris, 2006). Independent, but equivalent behavioral tagging experiments have been recently presented by Moncada and Viola using weak inhibitory avoidance (IA) training as tag and again open field exploration as the event triggering the burst of gene expression (Moncada and Viola, 2007). They found that a weak IA training, which induces short- but not long-term memory (LTM), could be consolidated into LTM by exploration to a novel environment occurring close in time, either before or immediately after, to the training session. The behavioral results therefore resemble the symmetry observed in weak-before-strong two pathway experiments in the Schaffer collateral pathway. Interestingly, the behavioral tagging experiment by Moncada and Viola also showed that the memory-promoting effect caused by novelty depended on activation of dopamine D1/D5 receptors and required newly synthesized proteins in the dorsal hippocampus. These results suggest that the exploration of a novel environment provided the PRPs required to stabilize the inhibitory avoidance memory trace, whereas the weak inhibitory avoidance training protocol would provide the tags also necessary for its stabilization. Other behavioral tagging experiments involving taste memory or contextual fear conditioning are also in progress (Morris, 2006).

As a new step to evaluate the relevance of synaptic capture events in associative learning, it would be very relevant to investigate whether the same late-associativity processes detected in acute hippocampal slices and cultured neurons can also be observed in the hippocampus of intact, freely moving animals. Recent experiments by Frey's group pointed in that direction. They developed a technique that can potentially allow the stimulation *in vivo* of two separate synaptic inputs, contralateral and ipsilateral, to the same CA1 neurons and the induction of distinct forms of LTP, shorter or longer-lasting (Hassan *et al.*, 2006). Unfortunately, they did not

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provide conclusive evidence supporting that the contralateral and ipsilateral inputs to CA1 represented really independent pathways.

Another aspect of synaptic tagging that should be investigated *in vivo* refers to the pharmacological or physiological activation of neuromodulatory afferents prior to a learning experience (Morris, 2006). Neurons in the CA1 area of the hippocampus receive strong neuromodulatory innervation, which has been involved in a range of behavioral processes, from novelty detection to arousal and contextual habituation in the mouse. The participation of dopaminergic input in STC (Sajikumar and Frey, 2004a) may explain why neuromodulatory events with an emotional component, such as stress, pain or pleasure may lead to flashbulb memories (Ahmed *et al.*, 2006; Hu *et al.*, 2007; Sajikumar *et al.*, 2007b; Seidenbecher *et al.*, 1997). In fact, experiments analyzing LTP reinforcement in DG by exploratory behavior has been proposed as a first evidence for STC events during behavioral process (Reymann and Frey, 2007). Recent results by the Frey's laboratory suggest that behavioral LTP-reinforcement could be mediated by novelty-triggered noradrenergic activity and rely on the synaptic capture of PRPs synthesized in response to the behavioral experience (Korz and Frey, 2007; Straube *et al.*, 2003a). However, these *in vivo* experiments failed to provide a compelling direct evidence for the tagging process, which as discussed in section 2.2 requires of weak-before-strong experiments that dissect synaptic tagging from synaptic capture, and therefore could not distinguish between sensitization and STC mechanisms. Weak evidence supporting the existence of STC events during behavioral reinforcement of DG-LTP could be found in a seminal article by Seidenbecher *et al.* showing that a behavioral experience (drinking water after deprivation) presented after weak tetanic stimulation slightly prolonged *unsaturated* LTP. Unfortunately, the observed increase, barely significant, is insufficient to conclude that a short-lasting form of LTP

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was transformed in L-LTP (Seidenbecher *et al.*, 1995). Indeed, studies using a more conventional behavioral paradigm, exploration of a novel environment, would suggest that the occurrence of this behavioral experience after LTP induction impairs rather than enhances the stability of LTP in DG (Abraham *et al.*, 2002; Xu *et al.*, 1998). More recent and detailed experiments by Frey's laboratory testing whether exploration of a novel environment after the tetanus can lead to LTP reinforcement (symmetry of capture experiments) provided mixed results: whereas L-LTP was impaired by unrestricted exploration, exploration only during one minute starting two minutes after LTP induction produced LTP prolongation (Straube *et al.*, 2003b). Based on these results is difficult to conclude whether behavioral LTP reinforcement depends on STC rather than on sensitization mechanisms. It is well known that DG granular neurons have a prominent sAHP and that this current is block by adrenergic and dopaminergic activation. Several studies have also showed that the reduction of the AHP facilitates synaptic plasticity phenomena (Cohen *et al.*, 1999; Fuenzalida *et al.*, 2007; Haug and Storm, 2000; Sah and Bekkers, 1996). Therefore, a reduction of the sAHP produced by β-adrenergic metabotropic receptors in granular neurons can reduce the threshold required to achieve L-LTP in glutamatergic inputs and contribute to behavioral LTP-reinforcement. Interestingly, in a recent study, Sajikumar et al. (2007b) speculated that whereas cognitive and information-processing behaviors may induce compartment-restricted plasticity, more stressful life-threatening experiences would cause a cell-wide upregulation of PRPs through activation of transcription at the cell nucleus. This graded response could be largely mediated by the differential activation of neuromodulatory inputs and depend on independent effects of neuromodulation in synaptic and intrinsic neural plasticity (Zhang and Linden, 2003).

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6. Concluding remarks

We have discussed the most important advances towards understanding late-associativity during neuronal integration and outlined some of the important challenges ahead. Based on all these findings, can we consider that the original synaptic tagging hypothesis has been proved? Can other models explain the experimental evidence?

Frey and Morris originally considered four alternative hypothesis to explain synapse-specific L-LTP (Frey and Morris, 1998a): (1) The "mail" hypothesis involving complicate intracellular protein trafficking that would target the newly synthesized PRPs uniquely to the synapses that triggered their expression; (2) the "local protein synthesis" hypothesis that asserts that the machinery for local protein translation is active only nearby activated synapses; (3) the "sensitization" hypothesis discussed in previous sections; and (4) the "synaptic tagging" hypothesis. As we have seen, the accumulated experimental evidence reviewed here has widened the theoretical framework set by the synaptic tagging model and introduced concepts that were part of these alternative models for synapse-specific LTP. Thus, the concept of clustered and compartment-specific plasticity (see section 2.2) brings the echoes of the "mail" hypothesis. The existence of compartment-specific tags would significantly increase the efficacy of synaptic tags, without involving complicated pathways of intracellular trafficking, because the percentage of newly synthesized mRNAs and PRPs that would be degraded without use would be significantly reduced. These concepts together with the new findings highlighting the relevance of local protein synthesis in plasticity process have also diluted the thin conceptual line that separated the "local protein synthesis" and the "synaptic tagging" hypotheses. The differential effects of protein synthesis and transcription inhibitors may reflect that existence of two kind of plasticity related gene products to be captured, proteins and dendrite-targeted mRNAs. Finally, it is now

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also clear that sensitization mechanisms exist and can importantly contribute to late-associativity. Although these mechanisms cannot explain all the experimental data resulting from the late-associativity studies carried out during the last decade, a significant percentage of these results could be partially explained considering the contribution of such mechanisms.

We think that the STC model, now improved and expanded with new ideas and concepts, still represents the most compelling hypothesis to explain late-associativity in synapse-specific plasticity processes, expanding beyond the range of NMDAR-dependent associativity, the possibilities of integration and competition of synaptic inputs. This model clearly overcomes the limitations for information processing and encoding associated to alternative models to explain late-associativity based in sensitization mechanisms. We fully expect that more details of the molecular mechanisms that underlie memory consolidation will come to light in the next years. Maybe, ten years was too short time to fully validate the synaptic tagging hypothesis, but at least we can confidently say that this model has paved the way for important developments and new ideas, such as cross-capture, clustered/compartmentalized plasticity or competitive maintenance, providing a compelling theoretical framework to interpret late-associativity and its impact on the computational capability of neurons.

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FIGURE LEGENDS

Figure 1: First evidence for synaptic tagging and capture in mollusks and rodents: A. Synaptic tagging in the rodent hippocampus (adapted from (Frey and Morris, 1997)): Left panel: In a hippocampal slice, two stimulating electrodes are used to stimulate two independent pathways, S1 and S2, that project to the same neuronal population in area CA1. Two recording electrodes are used to measure field potentials and population spikes. Right panel: A single train of high-frequency stimulation to one of the pathways produces a form of potentiation that decays after 1.5 hours (E-LTP, not shown). By contrast, several trains produce potentation that persists for more than 10 hours and is sensitive to inhibitors of transcription and translation (L-LTP). If a single train is given to S2 either before (not shown, (Frey and Morris, 1998b)) or after (right panel) repeated tetanic stimuli are applied to S1, persistent LTP occurs in both pathways. This indicates that the single tetanus produces a synaptic tag that can 'capture' the products of gene expression that are induced by the three trains. **B.** Synaptic tagging in *Aplysia* neurons (adapted from (Casadio *et al.*, 1999; Martin *et al.*, 1997)): Left panel: Photomicrograph of a single, bifurcated sensory neuron making synaptic contact with two spatially separated motor neurons. A perfusion pipette is used to deliver puffs of serotonin (5-HT) locally to the connection made onto the motor neurons. Right panel: Five puffs, but not a single puff, of serotonin produce branch-specific long-term facilitation (LTF). The LTF produced by five puffs of serotonin can be *captured* by the opposite branch if a single pulse of serotonin is given within a discrete time window with respect to the five puffs. This form of facilitation could not be blocked by the protein synthesis inhibitor anisomycin.

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Figure 2: The interaction between synaptic tags and activity-dependent gene expression results in different forms of late-associativity. A. The interaction between synaptic tags and activity-dependent gene expression defines a window for synapse-specific LTP consolidation: The red bell curve represents the burst or wave of gene expression, whereas the orange peak curves represent the tagging of the synapse. Tags decline after some time, however, if the burst of gene expression and the tag coincide in space and time, a persistent local potentiation (thick orange line) is produced (scheme modified from Morris, 2006). **B-L.** The cartoons depict the cellular processes underlying E-LTP (**B**), L-LTP (**C**), synaptic capture of L-LTP using a strong-before-weak stimulation protocol (**D**), synaptic capture of L-LTP using a weak-before-strong stimulation protocol (**E**), cell-wide facilitation induced by antidromic stimulation in absence of synaptic stimulation followed by synaptic capture of L-LTP both in basal and apical dendrites (**F**), E-LTD (**G**), L-LTD (**H**), synaptic capture of L-LTD (**I**), transcompartmental capture (**J**), competitive LTP maintenance (**K**), and cross-capture of L-LTP and L-LTD (**L**). High frequency stimulation (HFS), such as one train at 100Hz (red ray), elicits E-LTP (**B**) and sets a local synaptic tag for LTP capture (orange semicircle). Several trains of 100Hz stimulation trigger a burst of gene expression (red nucleus), the interaction of this wave of gene expression with local synaptic tags enables the consolidation of synapse specific LTP (red/orange yin yang), this can take place either homosynaptically (**C**, L-LTP) or heterosynaptically (**D** and **E**, synaptic capture of L-LTP). Panels E and F show that using specific stimulation protocols it is possible to independently investigate the requirements and time curse of the synaptic tag (**E**) and the burst of gene expression (**F**). Similarly, low frequency stimulation (LFS), such as a train of 15 min at 1Hz (green ray), elicits E-LTD (**G**) and sets a local synaptic tag for LTD capture (light green

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semicircle). Repeated LFS stimulation triggers a burst of gene expression (H, dark green nucleus). The interaction of this wave of gene expression with local synaptic tags enables the consolidation of synapse specific LTD (dark/light green yin yang), as in the case of LTP, this capture can be homosynaptic (**H**, L-LTD) or heterosynaptic (**I**, synaptic capture of L-LTD). Recent experiments revealed new forms of interaction between synaptic tags and activity-dependent gene expression: **J.** Compartment-specific marking and transcompartmental capture of L-LTP in CA1 pyramidal neurons, whereas 1x100Hz stimulation elicits E-LTP and sets a local synaptic tag, 2x100Hz stimulation would set a broader mark (compartment-specific marking in pink). **K.** Under a regime of low protein synthesis the interaction between synaptic tags and the wave of activity-induced gene expression results in the competence between recently activated synapses. As a consequence of this competition, there is a decline of potentiation in the pathway in which LTP was first induced, this phenomenon has been referred to as LTP competitive maintenance. **L.** Cross-capture: Previous panels in this figure represented the burst of gene expression triggered by repeated HFS in red and the burst of gene expression triggered by repeated LFS in green. The experiments by Sajikumar and colleagues suggest a different scenario that requires a reconsideration of the traditional view of L-LTP and L-LTD: Both HFS and LFS seems to induce the same set of genes (yellow nucleus). Therefore, the different outputs of these protocols of stimulation rely in the tagging signal they elicit, rather than in the genes they induce. Either the activated genes encode for proteins required for the consolidation of LTP (red/orange yin yang) and the consolidation of LTD (dark/light green yin yang), or alternatively, they encode for proteins that participate in both processes (insets: yellow/orange yin yang for consolidated LTP and yellow/green yin yang for consolidated LTD).

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Figure 3: Molecular networks underlying synaptic tagging: Cartoon depicting the various molecular mechanisms for synaptic tagging proposed in *Aplysia* sensory neurons or rodent CA1 pyramidal neurons: (1) activation of protein kinases, (2) activation of local protein synthesis, (3) release of neurotrophic factors, (4) changes in ion channels, (5) local degradation of protein, (6) internalization of adhesion molecules, and (7) structural changes. Synaptic capture might result from the interaction of these mechanisms with the burst of gene expression achieved through the activation of transcription and/or translation. Molecular details are discussed in the text.

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Fig 1

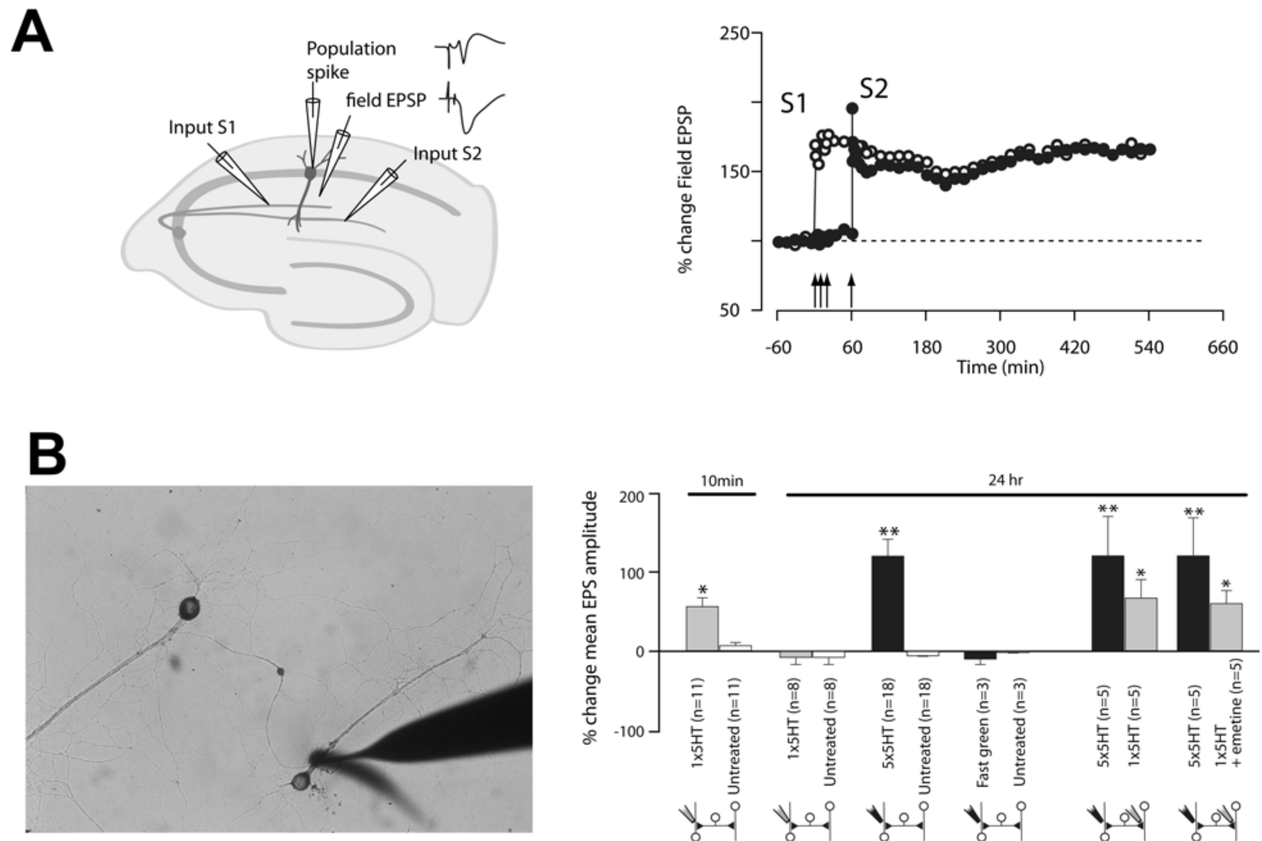

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Fig 2

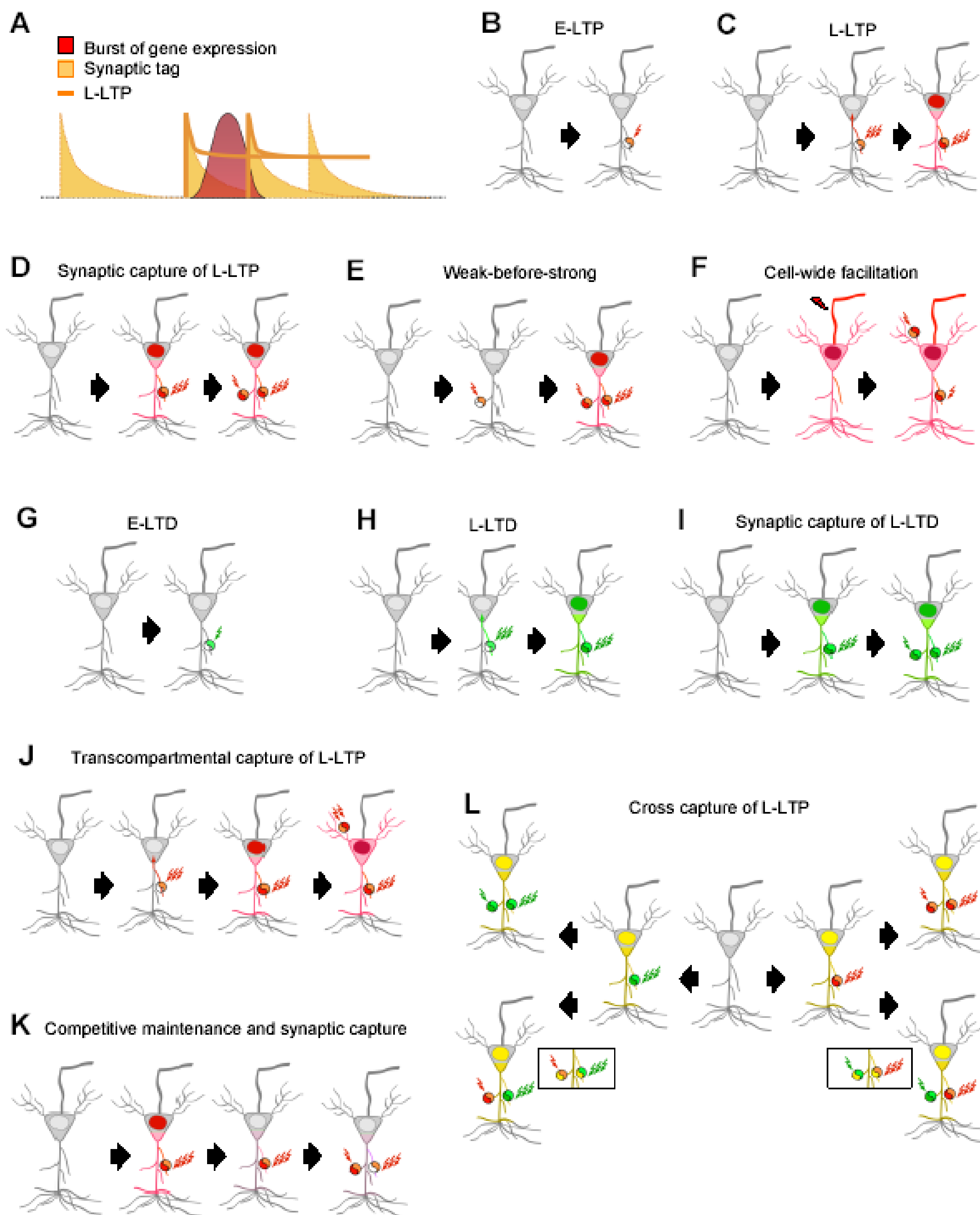

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Fig 3