Sreedharan Sajikumar *Editor*

Synaptic Tagging and Capture

From Synapses to Behavior

Springer

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Preface

Synaptic tagging was first proposed by Frey and Morris in 1997 and is now widely referred to as synaptic tagging and capture (STC). STC provides a conceptual basis for how short-term memory is transformed into long-term memory in a time-dependent fashion. The idea of publishing a book on STC was conceptualized during the Eighth Federation of European Neuroscience Societies (FENS) meeting held on July 14–18, 2012, in Barcelona, Spain. We had a symposium on tagging covering the latest developments in the field, chaired by Richard Morris. The focus of this book is on STC and it is intended to inform the reader about the current understanding of STC, 17 years after its first discovery. The book provides a unique opportunity for beginners in the field to have a deeper understanding of and to navigate the complexities of long-term associative memory mechanisms. The book is divided into two parts: the first part provides a detailed picture about the cellular aspects of STC while the second part deals with metaplastic and behavioural views. Therefore, as a whole, a wide-ranging view of STC from synapses to behaviour is encapsulated.

Writing a book chapter is not an easy task especially in a scientific world dominated by *Impact Factors*. I would like to warmly thank the authors for their willingness and commitment to contribute to this book as their efforts will contribute significantly to the excellent intellectual discourse in this exciting area of memory research. I am extremely fortunate to have short introductions from Prof. Richard Morris and Prof. Wickliffe Abraham for this book found in parts 1 and 2, respectively.

At the time of writing, I realized that more than a year has passed since I started this book project. I appreciate the patience of many of the authors. I would like to thank the Neuroscience section editor, Simina Calin, and Development Editor, Portia F. Wong, for their patience and guidance during this project, and all of the editorial staff at Springer, New York, for their patience and professionalism. In addition, the great support that I received during my career and for this project and from

the pioneers in the field is acknowledged here: Prof. Julietta U. Frey, Prof. Martin Korte, Prof. Wickliffe Abraham, Prof. Richard Morris, and Prof. Todd Sacktor.

It has been a rewarding professional and personal experience for me and I hope that all the readers will find something valuable in this book.

Singapore Sreedharan Sajikumar

Part I: Introduction

The concept of 'synaptic tagging and capture' (STC) that is the subject of this book arose in the course of experimental studies of protein synthesis-dependent long-term potentiation (LTP) during the mid-1990s. The ramifications of the original idea, and the way it has developed through the creative research of numerous scientists around the world, have now extended well beyond the immediate aims of the original research. As this timely book amply testifies, the concept of STC is leading us into new realms of thinking about synaptic plasticity, dendritic integration within neurons, and the neural basis of learning and memory.

Having conducted research in the late 1980s revealing the role of the *N-methyl-d*-aspartate receptor in memory encoding for spatial information (Morris et al. 1986), I became interested in the role of activity-dependent synaptic plasticity in learning and memory. Collingridge et al. (1983) had established the necessity of NMDA receptor activation for the induction of LTP, a physiological phenomenon with intriguing properties that might be relevant for memory. This led in turn to reflecting on the possibility that the temporal persistence of LTP might be one determinant (though not the only one) of the persistence of memory itself-an idea that Barnes (1979) was already developing in the studies of ageing. This line of thinking led me to the important work of the Magdeburg group led originally by Hans-Juergen Matthies who were early advocates of the idea that long-term plasticity and long-term memory both require protein synthesis. I read several of the groups' papers but found myself puzzled by something left out in their arguments. If the relevant protein synthesis were to be somatic, how do the synthesized proteins find their way to the correct synapses? That is, how do they target the very synapses that have recently been subject to the early or post-translational form of LTP? It occurred to me that the 'plasticity related proteins' (PRPs) that were synthesized in response to strong patterns of tetanic stimulation in LTP experiments, or to strong natural stimulation during learning, might have the potential of being shared around the dendrites of the neuron but only used as required.

I visited Magdeburg in 1995 and discussed this question with Uwe Frey (later, Julietta Frey) who immediately grasped its significance. I put to her the prediction that, in an LTP experiment, a second independent pathway tetanized in the presence

of anisomycin might still show protein synthesis-dependent late-LTP if an independent pathway had been tetanized shortly beforehand. I thought about our discussion in the weeks and months that followed but, unknown to me, Julietta began experiments herself and found this prediction to be upheld. She came to Edinburgh to give a talk and shared with me the by then quite extensive set of data that she had collected. It was a magical moment. We discussed some other ideas for experiments together, but all the credit for the original experimental work rests entirely with her. We prepared the final version of the manuscript together in one of the great coffee shops of the former East-German world, beside the Opera House on Unter den Linden in Berlin, and then tried our luck at *Nature*. The refereeing was fair but tough, as it generally is, and one of the experiments in the final paper was actually suggested by one of the referees. The paper was published (Frey and Morris 1997) and we both felt the paradox that protein synthesis-dependent LTP could actually be induced during the inhibition of protein synthesis was an observation that could tell us something important about neural function.

But what was it telling us and what did it mean? Frey and I argued, in a strictly abstract and conceptual manner, that the induction of LTP above a certain minimum threshold would, in addition to increasing synaptic strength temporarily, set a 'tag' that had the function of sequestering or 'capturing' plasticity proteins (Frey and Morris 1997). Thus, the tag was set locally, reflected the input specificity of LTP, and was induced by a post-translational mechanism. However, given the presence of this tag, the synthesis and distribution of PRPs could afford to be more global. Synthesis could perhaps be somatic, but with PRPs distributed all over the neuron even though they would only be captured at sites that had recently undergone potentiation. Only later was the concept of dendritic protein synthesis really developed.

With the exception of a few labs, including Eric Kandel's group at Columbia who conducted similar studies in Aplysia neurons in culture (Martin et al. 1997) and that of Doug Fields and Serena Dudek at NIH (Dudek and Fields 2002), there was little initial interest in STC. Frey's group pressed on, and in a series of brilliant studies, many conducted in Magdeburg by the editor of this book, Sreedharan Sajikumar, further properties of STC were identified. These include the idea that the tag can be reset by specific patterns of neural activity if applied very quickly (Sajikumar and Frey 2004b) and that the PRPs synthesized in response to strong LTP must overlap with or be identical to those synthesized in response to strong long-term depression (LTD). The latter is the phenomenon of cross-tagging (Sajikumar and Frey 2004a; Sajikumar et al. 2005). Sajikumar, Navakkode, and Frey went on to conduct important pharmacological studies that pointed to specific signal transduction pathways for tagging (CaM kinase) and others for the synthesis/distribution of PRPs (PKA), while my own group began studies in our new 'event arena' to see if we could create a behavioural analogue of STC. We knew that we could do this as early as 2004, but the full body of work took several years. This was collected into our study establishing that dopamine receptor-dependent novelty could help transform a decaying form of long-term spatial memory into one that persisted for at least 24 h (Wang et al. 2010). The Buenos Aires group of Viola submitted well before us using a similar novelty-enhancement approach but using inhibitory avoidance rather than spatial

memory (Moncada and Viola 2007) and I had the uncertain privilege of serving as an enthusiastic but 'scooped' referee of their manuscript! STC was therefore established worldwide, in the northern and southern hemispheres, as a concept based on a reliable and diverse set of findings using brain slice physiology, pharmacological and behavioural studies. Fittingly, the link between activity-dependent synaptic plasticity and memory was extended in an interesting new way into the domain of behavioural memory persistence.

Scientists are creative people and so, not surprisingly, when others turned to studying STC they opened up entirely new ideas, techniques, and experimental approaches than those which Frey and I had contemplated at the outset. That is surely part of the fun of science! One idea, due to Fonseca and Bonhoeffer, was that the heterosynaptic interactions that characterize STC may be competitive as well as synergistic and that this would be most likely in circumstances where the availability of PRPs is scarce (Fonseca et al. 2004). Govindarajan, Israely, and Tonegawa sought to establish that STC really did operate at the level of single spines, and in the process developed the concept of clustered plasticity based around ideas including local translation of dendritic mRNAs and more limited diffusion of PRPs than had been considered earlier (Govindarajan et al. 2011). These ideas complemented but also extended the earlier work from the Kandel's group suggesting more limited cross-capture between apical and basal dendrites (Alarcon et al. 2006), and his group was thinking about new ways to exploit molecular engineering techniques with respect to the PRP side of the equation (Barco et al. 2005). Nguyen offered ideas and data about the possible links between metaplasticity and STC (Young et al. 2006), and he and Abel kept up the reminders that cAMP-PKA must have a place in the story (Abel and Nguyen 2008). Yet more ideas tumbled out from others.

This book celebrates the developing concept and brings into a single volume the diverse body of recent research that has been conducted in relation to STC. Redondo and Morris present evidence that distinct CaM kinase pathways are involved in tagging and PRP synthesis. Alarcon summarizes his important and pioneering studies of neuronal compartmentalization. Fonseca introduces her concept of synaptic competition and explores the relevance of this idea in brain areas beyond the hippocampal formation. Ishikawa and Shiosaka are pioneers in novel thinking about the signal transduction pathways responsible for tag-setting, while Park and Abel on the one hand, and Blitzer on the other, discuss work on PKA anchoring and mTOR signalling. While Frey and Morris's initial (and incorrect) speculation about tagging was that it might be the temporary phosphorylation of a synapse-associated protein, Okada and Inokuchi open our eyes to entirely different possibilities that could include protein transport. Navakkode presents a summary of the now comprehensive data suggesting a role for dopaminergic activation in STC. Farris and Dudek contribute a reflective piece, backed by valuable data, that encourage us to think about the algorithmic logic of tagging: How can it help a neuron, but what price might the neuron sometimes have to pay for doing things in this heterosynaptic way. Together, these chapters set the scene for Part II of the book that is summarized separately by Cliff Abraham.

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I had the privilege of research funding in the form of a renewable Programme Grant from the UK Medical Research Council from 1987 to 2011. I am enormously grateful to them for their long-term funding through this major part of my career. I also wish to acknowledge and thank the various students and colleagues who worked with me on the STC projects. My research in this domain is now supported by the European Research Council.

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Part II: Introduction

The preceding section contains excellent reviews detailing the ever increasing understanding of the enigmatic mechanisms mediating synaptic tag and capture (STC). Although much remains to be discovered mechanistically, it is timely to consider the significance of this phenomenon from a wider perspective. Certainly from a synaptic plasticity point of view, the discovery of STC has revealed that there is a much greater range in the interval between events driving associative long-term change at synapses than previously realized. The fact that associative LTP (or LTD) can couple events spanning hours rather than seconds or minutes lends a power to the information processing capability of dendrites and synapses, albeit while also raising a risk that non-related events may become associated inadvertently. The question that now needs addressing is whether STC has any significance or relevance to learning and memory phenomena out there in the real world.

If we consider LTP as the archetypal synaptic plasticity phenomenon, we can reflect on the nature of the attention that has been given to answering the similarly important question of LTP, i.e., its behavioural relevance. Among the many cogent arguments arising a priori from its very interesting properties, such as rapid induction, input specificity, associativity, and persistence, it has been argued that if LTP serves as a memory mechanism, then like memory it should be a highly regulated function. This prediction has been repeatedly borne out through comparative studies of their regulation by factors such as ageing, stress hormones, monoamines, and cytokines. LTP (and LTD) is also highly sensitive to the state of the neurons and synapses as set by the history of prior activity, i.e., metaplasticity, a class of phenomena that has also been linked to memory regulation (Hulme et al. 2013). Moreover, memory and LTP can be disrupted in similar ways by disease, such as the amyloid-beta accumulation that is characteristic of Alzheimer's disease. These data strongly support LTP as a memory mechanism, although of course full assessment of the hypothesis requires assessing the effects on memory of specific manipulations of LTP mechanisms, another active area of research.

Returning to STC, researchers have begun to take a similar approach to thinking about its behavioural relevance. Although there are no known mechanisms specific to STC (and not LTP per se, for example) that can be specifically targeted to assess its contribution to memory, other questions like the ones raised above for LTP can be asked. Is it a regulated function? Is it susceptible to metaplasticity influences? Does it underpin behavioural learning? These questions are taken up by the authors of the next set of chapters.

Already in Chap. 8, Navakkode has described studies detailing the regulation of STC by the neuromodulatory neurotransmitter dopamine, which may thereby help further our understanding of how dopamine regulates long-term memory formation. The first three intriguing chapters in this next section then address connections between STC induction and metaplasticity. STC has been considered itself to represent a kind of metaplasticity, in that activity at one point in time that leads to PRP production can alter the persistence of plasticity induced elsewhere on the same neurons at a later time. In Chap. 10, Korte considers the multifunctional nature of brain-derived neurotrophic factor (BDNF) and its contributions to structural and functional plasticity. Of particular interest is the concept that BDNF may act to change the state of clusters of synapses in a way that influences future plasticity outcome, i.e., creating metaplasticity effects through STC mechanisms. This general theme is continued in Chap. 11 by Connor and Nguyen, who again link STC and metaplasticity conceptually, and discuss how this is an effective means of enhancing memory function in the brain. Interestingly, the broad associative window typically described for STC interactions is itself subject to additional metaplasticity-like regulation by prior activity. Thus, Sharma and Sajikumar (Chap. 12) present data showing that the temporal window of associativity can be greatly expanded by priming activity and discuss the implications of this for memory processing as well as the possibility that these mechanisms are disease targets.

Does STC in fact occur during behavioural learning to facilitate the association of temporally separated events, as suggested by authors of previous sections? Evidence has been slowly accumulating that weak memory traces can indeed be turned into persistent ones if a strong, presumably protein synthesis activating signal occurs within STC-relevant time windows. Moncada et al. (Chap. 14) review the evidence that such interactions occur and discuss the strong possibility that these associative interactions occur via STC mechanisms. This is an important and active area of research that depends to a great extent on further clarification of the nature of synaptic tags, and the critical PRPs, so that these molecules can be targeted in the behavioural experiments. Richter-Levin et al. (Chap. 13) look at these interactions from a different perspective, namely the key role that the amygdala plays in determining which weak memory traces are strengthened into persistent traces. Like everything to do with STC, the timing of amygdala activation is important, i.e., it is a critical factor in determining whether a weak trace located elsewhere in the limbic

system is strengthened or weakened. Thus, there is a considerable complexity of communication between brain areas in determining the fate of an initially stored trace. Determining the mechanisms mediating these complex interactions presents a significant challenge for future research.

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About the Editor

Sreedharan Sajikumar obtained his Ph.D in 2005 from the Leibniz Institute for Neurobiology (LIN), Otto-von-Guericke University, Magdeburg, Germany. He did his Ph.D research on the fundamental mechanisms of memory under the supervision of Professor Julietta U. Frey. During the course of his postdoctoral training in Prof Martin Korte's laboratory at the Technical University, Braunschweig, Germany, he investigated the role of metaplasticity on associative memory at the cellular level. His research on synaptic tagging and capture has uncovered many important mechanisms and molecules for the establishment of associative plasticity. He has a long-standing interest in the neural basis of long-term memory. Currently, he is an Assistant Professor at the Department of Physiology, Yong Loo Lin School of Medicine at the National University of Singapore. His work has been well supported over the years from prestigious funding bodies including the Alexander von Humboldt foundation, Germany, Deutsche Forschungsgemeinschaft, Germany, the National Medical Research Council, Singapore and the Ministry of Education in Singapore.

Part I
Cellular and Molecular Aspects of Synaptic Tagging and Capture

Chapter 1
Differential Role of CaMK in Synaptic Tagging and Capture

Roger L. Redondo and Richard G.M. Morris

Abstract Long-term potentiation (LTP) of synaptic connectivity is theorized to be a physiological correlate of memory formation. Changes in synaptic strength, as well as their maintenance, depend on a network of chemical interactions that occur both locally at the synapse and across the dendrites, axons, and nucleus of the neuron. The Calmodulin Kinase (CaMK) family can be divided into CaMKI/IV and CaMKII subfamilies among others, all with central roles in synaptic plasticity. The question that we address in this chapter is whether the necessary roles of particular CaM Kinases in LTP are restricted to the synthesis of plasticity-related products or to the local phosphorylation of synaptic proteins. We use analytically powerful three-pathway protocols and kinase-specific drugs to dissociate the distinct roles of the CaMK pathways in LTP.

Keywords Synaptic tagging • Long-term memory • Long-term potentiation • CaMKII • Immediate early genes • Hippocampus

1.1 Introduction

The synaptic tagging and capture (STC) hypothesis proposes two conditions for the maintenance of long-term changes in synaptic efficacy: (1) the local setting of tags at stimulated synapses, believed to be protein synthesis independent, and (2) the cell-wide or dendritic-domain-wide availability of plasticity-related products (PRPs) (Frey and Morris 1998a; Reymann and Frey 2007; Redondo and Morris 2011). These PRPs act on those synapses that are found in a tagged state. The signal transduction pathways responsible for the setting of the tag and the availability of

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PRPs are beginning to be elucidated. In the process of discovery of the molecular players involved in LTP, one can predict that a subset of them will have necessary roles specific to the setting of the tag but not for the availability of PRPs and vice versa. This chapter describes the techniques and the findings that resolved the existence of two dissociable molecular cascades within the CaM kinase pathways.

The CaMK family comprises the CaMKI/IV and CaMKII subfamilies among others. CaMKII, with multitude targets (Erondu and Kennedy 1985; Braun and Schulman 1995; Yamauchi 2005), has been shown with live imaging and within single dendritic spines to regulate the actin cytoskeleton by modifying a pool of actin and producing the spine expansion needed for the cellular consolidation of synaptic memories (Cingolani and Goda 2008; Hotulainen and Hoogenraad 2010; Bramham 2008; Okamoto et al. 2004, 2007; Honkura et al. 2008; Lin et al. 2005). These local actions of CaMKII around the plastic synapse suggest a role in tagging. The timing of CaMKII activity also supports a role in the tagging of synapses, as an autophosphorylated form of CaMKII remains active even after the calcium concentration returns to baseline levels (Yamauchi and Fujisawa 1985; Yoshimura and Yamauchi 1997). These factors make CaMKII a strong candidate to possess a role limited to tag-setting while being unnecessary for the availability of PRPs. This chapter describes the experiments and the results that confirmed such a role for CaMKII.

However, away from synapses, in the neuronal soma, another CaMK pathway is recruited by increases in intracellular calcium concentrations. CaMK Kinase (CaMKK) senses calcium entries and stimulates CaMKIV, which enters the nucleus and phosphorylates the Ca^{2+}/cAMP-response element-binding protein (CREB), engaging gene transcription (Bito et al. 1996; Ho et al. 2000; Kang et al. 2001). Is this CaMK pathway necessary for the transcription of PRPs? And if so, can its functions be dissociated from those of CaMKII? To assess this, STC experiments were used as tools for the identification of necessary roles of CaM Kinases (Redondo and Morris 2013) (Fig. 1.1).

1.2 STC Protocols as Tools to Elucidate the Role of Molecules in Synaptic Plasticity

The electrophysiological protocols employed for the study of STC can be used to pinpoint the role of candidate molecules in synaptic plasticity. These experiments rely on the reversible inhibition of the target molecule (i.e., CaMKII) during the induction of LTP. By restricting the timing of the effect of the inhibitor to the unique set of synapses recruited by a stimulation pathway, the heterosynaptic effect of stimulating another independent input can be tested. Following two logical steps, molecules can be assigned necessary roles in either the setting of tags or the availability of PRPs (Box 1).

To summarize, the first step involves the characterization of a reversible inhibitor of the candidate molecule, capable of interfering with the maintenance of LTP.

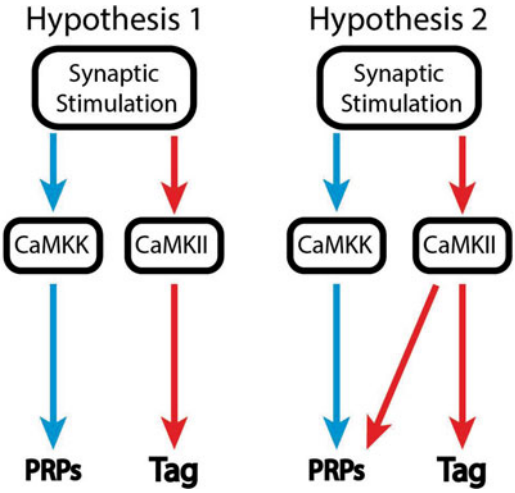


Fig. 1.1 Alternative hypotheses about the roles of the CaM Kinases in synaptic tagging and capture. From previous knowledge about the localization and the actions of CaM Kinases the following predictions arise: CaMKIV has a necessary role only in the availability of PRPs but not in the setting of the tag; CaMKII is necessary only for tag-setting or, alternatively, it is necessary for both tag-setting and the availability of PRPs. Derived from Journal of Neuroscience 2010 Vol. 30, 14:4981–4989

Such blocking of LTP, within the framework of the STC hypothesis, can be due to either the block of tagging, the lack of availability of PRPs, or both. After such inhibitor has been found, a second step, involving the use of the inhibitor during the induction of LTP in one set of synapses but not during the tetanization of another independent set of synapses, reveals the extent of the effect of such inhibition on LTP. Three possible outcomes are possible: the inhibitor specifically blocked the setting of the tag while allowing for the synthesis and availability of PRPs; the inhibitor prevented the PRPs from becoming available to the synapse but without impairing tag-setting; or the inhibitor had a general effect that blocked both tag-setting and PRP availability.

Box 1. The response of independent but convergent pathways (i.e., different synapses onto the same cell) to strong or weak tetanization can be used to discover the role of particular molecules in synaptic tagging and capture

The approach involves a series of logical steps: *Step 1*. Find a reversible inhibitor of late-long-term potentiation (L-LTP) (see the figure, top). Application of such a drug (indicated by the purple bar) during strong tetanization (shown by dark arrows) blocks L-LTP, as indicated by the schematic representation of

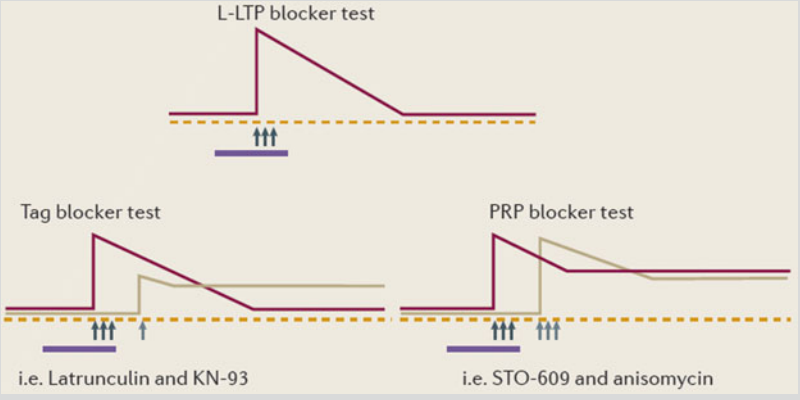
(continued)

Box 1. (continued)

normalized field excitatory postsynaptic potential slope. Several drugs can block L-LTP, but this raises the question of the mechanism of action: are these drugs acting on a tag-specific mechanism, are they specific to PRPs, or general blockers of PRPs and tags?

Step 2. The tag-blocker test. An analytical protocol can be used to determine one possible mechanism responsible for the block of L-LTP by the drug identified in Step 1. The key result is the fate of subsequent weak tetanization (light arrow) at an independent but convergent set of synapses after the drug present at the time of strong tetanization has been washed out. If the weak pathway is capable of maintaining L-LTP, the drug is a candidate tag-specific blocker. The synapses tagged by the weak stimulation have captured PRPs that were synthesized in response to the strong stimulation during application of the drug. These tag–PRP interactions on the weakly tetanized pathway stabilize L-LTP even though the strongly tetanized pathway fails to show L-LTP. This test can also be run with the weak tetanus delivered before application of the drug and the strong tetanus (Ramachandran and Frey 2009; Redondo et al. 2010).

Step 3. The PRP-blocker test. A different analytical protocol ("strong before strong") reveals that a drug that blocks L-LTP in a single-pathway study may be doing so by limiting PRPs. The key result in a two-pathway study is the outcome of the potentiation that is induced in the presence of the L-LTP blocker. If this pathway shows L-LTP, the drug is a likely to be a PRP-specific inhibitor. This is because synaptic tags that were created during application of the drug captured the PRPs that were synthesized in response to the later strong tetanization of the other pathway (Frey and Morris 1997; Redondo et al. 2010). Box derived from Nat Rev Neurosci 2011 Vol. 12, 1:17–30.



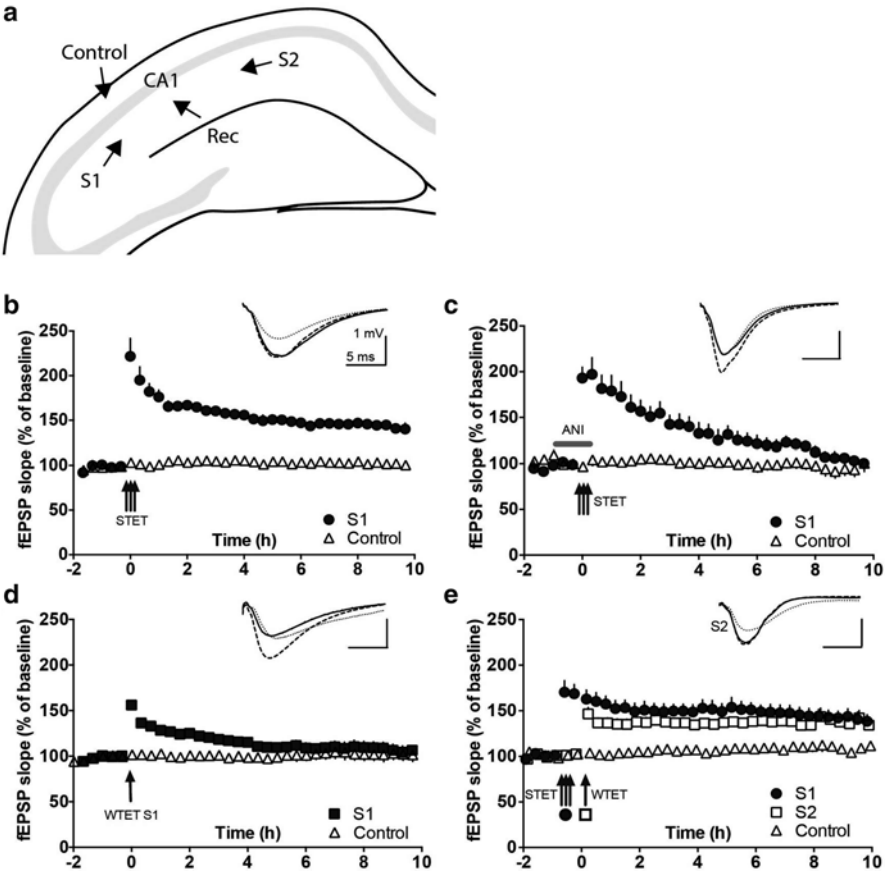


Fig. 1.2 Three-pathway synaptic tagging and capture in the stratum radiatum of CA1 pyramidal cells. (**a**) Strong tetanization (STET) produces late-LTP. Insets depict typical EPSP traces 30 min before (*dotted line*), 30 min after (*broken line*), and 10 h after (*full line*). (**b**) Anisomycin, a protein synthesis blocker, impairs the maintenance of late-LTP. (**c**) A weak tetanus (WTET) elicits early-LTP present at 2 h that returns to baseline strength after 3 h. (**d**) Early-LTP (*orange symbols*) is rescued into late-LTP when one set of synapses receives the weak tetanus 20 min after another set of synapses onto the same population of pyramidal cells has received a strong tetanus ($n=8$). Error bars indicate SEM. Derived from Journal of Neuroscience 2010 Vol. 30, 14:4981–4989

The development of the application of the "tag-blocker test" and "PRP-blocker test" protocols proved essential to identifying the role of CaMKII and CaMKK pathways in LTP.

In order to perform these experiments, a third nontetanized pathway to monitor the overall stability of the slice preparation was necessary (Fig. 1.2). This is due to the fact that after two independent pathways are tetanized, the lack of a third control pathway would leave the experimenter without control readout for changes in the overall excitability of the slice preparation. A detailed description of our experimental

settings and modifications to classical in vitro acute slice electrophysiology are described elsewhere (Redondo and Morris). Protein synthesis-dependent late-LTP, as well as early-LTP, could be obtained in this three-pathway setup (Fig. 1.2a–c). Using this system, we replicate the original observation (Frey and Morris 1998b) where a weakly tetanized pathway can express late-LTP due to the strong tetanization of an independent set of synapses onto the same neurons. The pairing of the two stimulation protocols produced a rescue of the weakly tetanized pathway into expressing late-LTP (Fig. 1.2d).

1.3 Roles of CaMKII and CaMKK in STC

With the use of reversible CaMK inhibitors at doses specific to either CaMKII (KN-93) or to CaMKIV (STO-609) (Fig. 1.3a, b), STC experiments (Box 1) were used to assess the necessary roles of the individual kinases. We used a concentration of the CaMK inhibitor KN-93 capable of inhibiting CaMKII autophosphorylation without disrupting the phosphorylation of CREB (1 μM). At this concentration KN-93 prevents the synapse-specific tag-setting while permitting PRPs to remain available to other synapses (Fig. 1.2c, d): Either in a "weak-before-strong" or a "strong-before-weak" protocol [tag-blocker experiments (Box 1)], a strongly tetanized pathway in the presence of KN-93 at 1 μM fails to show late-LTP. This is interpreted as that pathway lacking some of the elements necessary for the maintenance of LTP.

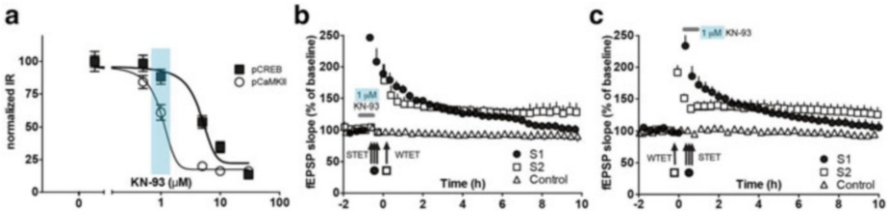


Fig. 1.3 Dose-related effects of KN-93 and STO-609 on phosphorylation of CaMKII and CREB. (**a**) Differential dose–responses of KN-93 on distinct CaMK pathways in culture neurons. *Top*, Effects of KN-93 on CaMKII autophosphorylation at Thr-286. Dissociated hippocampal cultures were treated with a series of concentrations of KN-93, stimulated with glutamate, and immunostained. Immunoreactivity for pCaMKII was quantified in dendritic spines and displayed as a function of KN-93 concentration. The ordinate represents basal (no stimulation, 0 %) to maximum (stimulated without inhibitors, 100 %) activities. (**b**) Effects of KN-93 on CREB phosphorylation at Ser133. Suppression of pCREB immunoreactivity in the neuronal nuclei was displayed. Note greater sensitivity of KN-93 for CaMKII. C-D, KN-93 dissects a role for CaMKII in a synapse-specific process necessary for late-LTP. (**c**) Strong tetanization (STET) in the presence of 1 **μ**M KN-93 (pathway S1) induces LTP that decays to baseline over 10 h (whereas an independent set of weakly tetanized (WTET) synapses (S2; *orange symbols*) successfully shows stable potentiation for 10 h after tetanus. (**d**) In a weak-before-strong protocol, early-LTP is still rescued to late-LTP although late-LTP fails to be maintained in those synapses tetanized in the presence of 1 **μ**M KN-93 Error bars indicate SEM. Symbols as in Fig. 1.2. Derived from Journal of Neuroscience 2010 Vol. 30, 14:4981–4989

However, the weakly tetanized pathway, onto the same cells but not under the influence of the CaMKII inhibitor during the induction of plasticity, shows late-LTP and therefore successfully makes use of all the elements required for LTP maintenance. Within the STC framework, the only way to explain such result is that the weakly tetanized pathway made use of PRPs available to it, PRPs that the weak tetanization cannot bring about by itself. The PRPs must then have come from the strongly tetanized pathway, which itself fails to make use of them in order to sustain LTP. If, as the "tag-blocker" protocols reveal, the PRPs are available, then the CaMKII inhibitor is specifically impairing the setting of a functional tag capable of capturing the PRPs. Other CaMK inhibitors, like KN-62, have also been shown to block tag-setting in similar ways (Sajikumar et al. 2007).

On the other hand, the CaMK Kinase inhibitor STO-609 present at the time of induction is capable of preventing the maintenance of LTP without affecting tag-setting processes (Fig. 1.4). This is evidenced by the two observations central to a

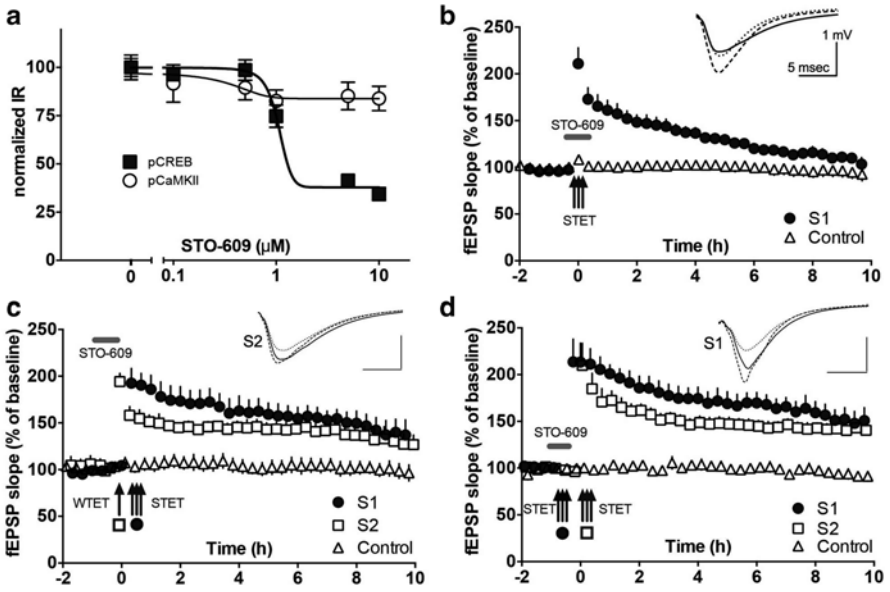


Fig. 1.4 STO-609 dissects a role for CaMKK in a cell-wide process necessary for late-LTP. (**a**, **b**) Differential dose–responses of STO-609 on distinct CaMK pathways in culture neurons. (**a**) Effects of STO-609 on CaMKII autophosphorylation at Thr286. (**b**) Effects of STO-609 on CREB phosphorylation at Ser133. (**c**) The CaMKK inhibitor STO-609 (5 **μ**M) present at the time of induction blocks late-LTP. (**d**) Weakly tetanized synapses show late-LTP when STO-609 is applied during the weak tetanus and then removed before strong stimulation is delivered to an independent pathway. (**e**) STO-609 has no effect on a weakly tetanized pathway S1. (**f**) In a strong-before-strong protocol, STO-609 given tetanization to S1 does not prevent that pathway showing late-LTP if, after drug washout, strong tetanization is delivered to a second S2 pathway. Error bars indicate SEM. Symbols as in Fig. 1.2. *STET* strong tetanization, *WTET* weak tetanization. Derived from Journal of Neuroscience 2010 Vol. 30, 14:4981–4989

PRP-blocking experiment. First, CaMKK inhibition does block late-LTP in a strongly tetanized pathway (Fig. 1.4c). Second, in an STC experiment, the mechanism by which CaMKK blocks late-LTP does not prevent the rescue of early-LTP into late-LTP when STO-609 is present during the delivery of weak tetanization (Fig. 1.4d) even though STO-609 has no enhancing effect on the LTP elicited by a weak tetanus per se (Fig. 1.4e). A similar lack of impairment of LTP is seen when a strongly tetanized pathway under the presence of STO-609, which would normally fail to sustain LTP (Fig. 1.4c), successfully shows late-LTP when an independent set of synapses is also strongly tetanized. This suggests that the tags in the pathways tetanized under the actions of STO-609 are capable of making use of available PRPs, introduced by the second strongly tetanized pathway. We can conclude that when STO-609 blocks LTP (Fig. 1.4c) it does so not by blocking tag-setting but by preventing the availability of PRPs. STO-609 therefore reveals a necessary role for CaMKK in the availability of PRPs.

Altogether, tag-blocker experiments with CaMKII inhibitors and PRP-blocker experiments with CaMKK inhibitors support dissociation between the roles of CaM Kinases in STC: CaMKII activity is necessary for tag-setting but not for the availability of PRPs while CaMKK function is required for the availability of PRPs but not for successful tagging. How the two elements (tag and PRPs) interact in order to sustain LTP remains unknown and escapes the power of the technology used in these experiments. As much as CaMKII activity is necessary for tag-setting, it would be erroneous to equate CaMKII, or any other necessary molecule, with the "tag." Many other molecules have been, and more will be, identified as necessary for tagging (Ramachandran and Frey 2009; Nagy et al. 2006) and we argue that it is counterproductive to refer to them as "the tag" (Redondo and Morris 2011). Instead, tagging molecules or molecules necessary for tagging is accurate.

Worth noting is the fact that inhibiting CaMKII allows for the expression of E-LTP, even though tagging is compromised (Redondo et al. 2010; Sajikumar et al. 2007). For example, as seen in Fig. 1.3c, d, the strongly tetanized pathway successfully sustains a potentiated state for many hours before the effects of inhibiting tagging are revealed. This contrasts with the impairments in LTP revealed in knock-out animals of CaMKII and by inactivation of the catalytic function of CaMKII (Redondo et al. 2010; Giese et al. 1998; Sanhueza et al. 2007). Somehow, the use of drugs like KN-93, which prevent CaMKII autophosphorylation, allows for the expression of E-LTP while blocking tagging. A similar phenomenon is observed with actin polymerization blockers like latrunculin (Ramachandran and Frey 2009). Altogether, the data points towards the necessity of a structural change at the synapse, on which the tag state relies, that is independent of the expression of LTP (Redondo and Morris 2011). As seen here, CaMKII autophosphorylation seems necessary for such structural change.

The experiments described in this chapter reveal distinct roles for two CaM kinase pathways in the maintenance of LTP (Fig. 1.1, Hypothesis 1).

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Chapter 2
Compartmentalization of Synaptic Tagging and Capture

Juan Marcos Alarcon

Abstract Testing of the synaptic tagging and capture (STC) hypothesis has produced remarkable work on the understanding of how a single neuron undergoes spatial and temporal encoding of information. Central to this work is the notion that STC processes can be compartment specific. Formed by activation of synaptic plasticity mechanisms and extending along confined dendritic domains, these compartments can work as the neuron's information integration units. Association or dismissal of incoming information would depend on the plasticity-driven functional state of the compartment. With multiple streams of neural activity arriving at distinct synapses of a neuron, compartmentalization emerges as a key strategy to organize this information and enhance the neuron's computing capability.

Keywords STC model • Synaptic plasticity • Compartmentalization • Neuronal integration • Information coding • Memory

2.1 Why Compartmentalization?

Information carried by distinct neural paths in the brain is integrated to generate a vast number of cognitive processes, including representations of thoughts, recollections, planning, and actions. A typical neuron in the brain receives thousands of synaptic afferents. These synaptic connections can convey relevant information from other neurons. Hence, a single neuron can potentially code thousands of streams of information, with each synapse acting as a unique integrative unit (Yuste 2013). This immense computing capability emerges as one of the most remarkable features of the neuron's anatomy.

In addition to single synapse computation, it is also suggested neurons integrate information within confined dendritic areas comprising large groups of synapses, referred to here as *compartments*. Compartmentalization, is the process by which

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the biochemical and biophysical properties of a particular dendritic region (compartment) are modified by plasticity mechanisms. Synaptic function within the compartment will be largely conditioned by the molecular setting imposed by these plasticity mechanisms. As a result, each compartment will process distinct streams of information arriving to its synapses according to the unique molecular environment of the compartment. Streams of information that arrive at distinct compartments will be encoded in terms of the particular molecular history experienced by each compartment (Sajikumar and Korte 2011b). The potential combinations of different streams of neural activity and different compartments highlight the computational capability of compartment-specific integration of information. If compartmentalization is a mechanism for processing information, then, two important questions arise: (1) how do compartments form? and (2) what functional purpose do they serve?

2.2 STC and Compartmentalization

Clues to a compartmentalized nature of the STC process came from the characterization of synaptic capture of long-term synaptic potentiation (LTP), a form of LTP associativity, between two separate synaptic inputs located within and across the two main dendritic projections of CA1 pyramidal neurons of the rodent hippocampus, basal and apical dendrites (Alarcon et al. 2006; Fonseca et al. 2004; Sajikumar et al. 2007a, b). Prior to this work, LTP associativity had been studied primarily between two separate sets of synapses within apical dendrites in the *stratum radiatum* of CA1 pyramidal neurons (Barco et al. 2002; Frey and Morris 1997, 1998). Nothing was known of the properties for STC at CA1 basal synapses. Synaptic capture of LTP between two sets of synapses within the basal dendrites occurred just as it did for apical synapses suggesting that LTP associativity processes are functionally similar in both types of synapses (Alarcon et al. 2006). However, LTP associativity, in the form of synaptic capture of LTP, did not occur when tested between synapses located in separate apical and basal dendrites (Alarcon et al. 2006). The failure of synaptic capture of LTP across the basal and apical dendrites led to a fundamental notion: LTP associativity, and perhaps all of the STC processes, can be compartment specific; that is, restricted within domains of the neuron's dendritic arbor. Further work supported this notion by demonstrating the associative properties between LTP and long-term synaptic depression (LTD) can also be compartmentalized (Pavlowsky and Alarcon 2012; Sajikumar et al. 2005).

These findings led to a revision of the original STC model. In its original form, the STC model posits that upon activation of transcription and somatic protein synthesis, plasticity products would be distributed throughout the cell and then productively incorporated into activity-tagged synapses. The notion of a compartmentalized STC process suggests that there could be activity-dependent mechanisms that restrict the availability of the plasticity products to confined dendritic domains; possibly through selective cellular sorting or local protein synthesis. Dendritic sorting

and local translation would allow plasticity products, such as mRNAs granules and proteins, to be used by particular groups of tagged synapses within a confined dendritic domain (Alarcon et al. 2006; Fonseca et al. 2004; Govindarajan et al. 2006; Pavlowsky and Alarcon 2012; Sajikumar and Frey 2004; Young and Nguyen 2005).

2.3 Making a Compartment

The formation of dendritic domain-restricted compartments is thought to depend on two main factors: (1) the activation of synaptic plasticity-associated mechanisms, and (2) the geometry of the dendrite (Alarcon et al. 2006; Govindarajan et al. 2006, 2011; Makino and Malinow 2011; Sajikumar et al. 2007a). Additionally, organelle architecture within dendrites can greatly impact the shape and function of a compartment (Cui-Wang et al. 2012; Makino and Malinow 2011).

Induction of synaptic plasticity largely depends on the activation of Ca^{2+}-mediated mechanisms (Colbran and Brown 2004; Fitzjohn and Collingridge 2002; Sjostrom and Nelson 2002; Zucker 1999). Not surprisingly, the Ca^{2+} signal turns out to be the primary modulator of synaptic plasticity-induced associative processes between separate synapses of a neuron (Abraham et al. 1994; Christie and Abraham 1994; Christie et al. 1995). Key to the associative properties of synaptic plasticity may be the control of Ca^{2+} propagation within the neuron (Nishiyama et al. 2000; Raymond and Redman 2002, 2006; Sajikumar et al. 2009). Induction of synaptic plasticity that activates ryanodine receptors (RyRs) releases intracellular Ca^{2+} within stimulated synaptic spines. A stronger synaptic stimulation that activates inositol-3-phosphate receptors (IP_3Rs) propagates Ca^{2+} within dendritic branches. A much stronger synaptic stimulation that leads to L-type voltage-dependent calcium channel (VDCC) activation mediates a large Ca^{2+} influx comprising the entire somatodendritic area, including the cell nucleus (Raymond and Redman 2002, 2006). Hence, synaptic activation propagates Ca^{2+} within a neuron in an activity-dependent manner. This mechanism may be a primary factor for establishing compartment size.

Upon synaptic activation, the Ca^{2+} signaling pathway is engaged with another second messenger path: the cAMP signaling pathway. Indeed, Ca^{2+} and cAMP signaling paths activate calcium/calmodulin-dependent protein kinase II and PKA activity, respectively-protein kinase activities known to be important for STC processes (Barco et al. 2002; Young et al. 2006). Importantly, these protein kinase activities can be spatially restricted by membrane anchoring and protein clustering. For instance, spatial restriction of cAMP/PKA signaling via binding to A kinase-anchoring proteins (AKAPs) may be critical for compartmentalization (Huang et al. 2006; Nie et al. 2007). Compartmentalized protein kinase activity might, in turn, restrict the distribution and access of plasticity products to specific dendritic regions forming a compartment (Horton et al. 2005; Li et al. 2014).

Synaptic stimulation can trigger long-lasting remodeling of the actin network at both pre and postsynaptic sites (Colicos et al. 2001). The activity-mediated recruitment of cytoskeleton structures by kinase activity might enhance the transport of

mRNAs and proteins to dendritic compartments containing stimulated synapses (Kotz and McNiven 1994; Luo 2002; Rodionov et al. 2003; Sanchez et al. 2000). Activity-dependent transport of RNA granules containing a number of plasticity proteins and mRNAs depends on the expression and activity of the motor protein kinesin (Kanai et al. 2004; Kiebler and Bassell 2006; Puthanveettil et al. 2008). For instance, the activity-regulated cytoskeleton-associated (Arc) protein and mRNA accumulates selectively in activated dendritic domains (Steward et al. 1998). Another example is the transport of *Aplysia* elongation factor 1A (Ap-eEF1A) mRNAs to neurites after synaptic stimulation (Giustetto et al. 2003).

2.4 Consolidating a Compartment

Because compartment formation is a process that depends on the induction of synaptic plasticity, synaptic plasticity and compartmentalization may also share common mechanisms for consolidation. The recruitment of new protein synthesis, which is already established for synaptic plasticity, is proposed to be the chief mechanism that underlies compartment consolidation (Alarcon et al. 2006; Pavlowsky and Alarcon 2012). Compartmentalization presumes that new synthesis of protein is localized to confined dendritic domains. Indeed, local protein synthesis can be spatially restricted by the capture, activation, and translation of dormant mRNAs (Aakalu et al. 2001; Klann and Dever 2004; Ostroff et al. 2002; Richter 2001; Sutton and Schuman 2006). Protein synthesis may be locally regulated by: translocation of ribosomes into particular dendritic branches and spines, localized activation of the mammalian target of rapamycin (mTOR) pathway, localized activation of translation factors, or localized presynaptic release of brain-derived neurotrophic factor (BDNF) (Banko et al. 2005, 2006, 2007; Casadio et al. 1999; Costa-Mattioli et al. 2005, 2007; Kelleher et al. 2004).

The consolidation of compartments capable of STC processing may require new synthesis of protein. However, the long-term maintenance of a compartment may just require adjusting the rate in translation efficacy (Klann and Dever 2004). After the initiation of new protein synthesis and compartment consolidation, each compartment could adjust and exhibit a functionally defined state of translation efficacy by regulating the rate of local protein synthesis and degradation (Fonseca et al. 2006; Martin and Kosik 2002). The existence of compartments might significantly increase the efficacy of active (tagged) synapses to store information by enhancing pathways of intracellular trafficking, efficiency of mRNA translation and protein capture that otherwise would be degraded without use.

2.5 Functional Compartmentalization

Compartment formation, consolidation, and maintenance are not seen as immutable, irreversible processes. The size of a compartment would primarily depend on the magnitude of synaptic activation (Alarcon et al. 2006; Sajikumar et al. 2007a)

and the morphology and excitability properties of dendritic branches (Makara et al. 2009). Hence, compartmentalization can be thought to be a function of (1) the activity of plasticity mechanisms recruited within a particular dendritic area (Alarcon et al. 2006), and (2) the temporal dynamics of these mechanisms (Pavlowsky and Alarcon 2012). Therefore, the magnitude of a compartment would be defined by the activity function of the recruited plasticity mechanisms. We could visualize this activity function (wave) with its apex at the point of maximal synaptic stimulation and then extending from this center, possibly in a Gaussian-like manner, across a particular dendritic area. Increases in synaptic activation would give rise to corresponding increases in the activity function/wave and, consequently, compartment size. Importantly, this notion suggests that compartment size and compartmentalization have no fixed boundaries.

In this context, a way to define a compartment would be through its category of functional properties. For instance, electrotonic changes triggered by synaptic activation can be spine specific, branch specific, or extend across multiple dendritic branches (Frick et al. 2004; Johnston et al. 2003; Zhang and Linden 2003). Synaptically driven signaling pathways can be localized via dendritic translation (Aakalu et al. 2001; Purcell et al. 2003) or surpass dendritic boundaries and extend as far as the nucleus (Kandel 2001). A functional compartment would be operationally defined as a particular region of the dendritic arbor with modified excitability and selective access to plasticity products where late-associative processing can occur (Alarcon et al. 2006; Fonseca et al. 2004; Govindarajan et al. 2006; Pavlowsky and Alarcon 2012; Sajikumar and Frey 2004; Young and Nguyen 2005).

2.6 Compartmental Computation

A neuron can be activated by distinct learning-associated information streams. During initial experience, such activation elicits synaptic plasticity at a subset of synapses, a phenomenon thought to be the cellular substrate of memory (Bliss and Collingridge 1993; Kandel 2001; Malenka and Bear 2004; Neves et al. 2008). One can imagine that learning can prompt the storage of different bits of information in multiple synapses of a neuron by means of synaptic plasticity. These bits of synaptic plasticity-encoded information must be correctly associated or segregated at the cellular level to properly contribute to the neuronal ensemble that constitutes the memory engram.

STC is a process that allows the interaction between multiple forms of synaptic plasticity induced at separate synapses within a single neuron. STC compartmentalization presents an attractive mechanism for the correct integration of separate streams of information arriving at the same neuron within time periods ranging from minutes to hours (Alarcon et al. 2006; Frey and Morris 1997, 1998; Sajikumar et al. 2007a; Young and Nguyen 2005). This dynamic process might be important for the association of relevant and dismissal of irrelevant information in a setting where information encoding is done over longer periods of time. This kind of temporal integration has the behavioral connotation that allows information experienced

distantly in time to be either associated or segregated; and it adds to the well-established mechanisms for cellular integration at the sub-second and seconds time frames by associativity models of synaptic integration (Magee 2000; Magee and Johnston 2005).

As previously described, the induction of plasticity-associated metabolic activity within dendrites can give rise to functional compartmentalization. These functional dendritic compartments may be key to the proper association and segregation of learning-associated information and its decoding (Alarcon et al. 2006; Fonseca et al. 2004; Govindarajan et al. 2006; Morris 2006; Reymann and Frey 2007; Sajikumar et al. 2007a; Young and Nguyen 2005). For instance, neurons in the CA1 area of the hippocampus form part of different ensembles and circuits that underlie behavior (Amaral and Witter 1989; Kramar and Lynch 2003). CA1 principal neurons receive inputs from different brain areas to morphologically defined dendritic domains which are potential substrates for compartmentalization: basal dendrites (within the *stratum oriens*) receive information from the contralateral hippocampus; proximal apical dendrites (within the *stratum radiatum*) receive ipsilateral afferents from neighboring CA3 neurons via Schaffer-collateral fibers; and distal apical dendrites (within the *stratum lacunosum-moleculare*) receive inputs from layer III of the entorhinal cortex via the temporo-ammonic pathway (Amaral and Witter 1989; Deuchars and Thomson 1996; Dolleman-Van Der Weel and Witter 1996; Ishizuka et al. 1990; Pikkarainen et al. 1999). The anatomical distinction between these dendritic domains is also functional as they differ on their biophysical properties (Arai et al. 1994; Cavus and Teyler 1998; Haley et al. 1996; Jarsky et al. 2005; Kawakami et al. 2003; Kloosterman et al. 2001; Kramar and Lynch 2003; Leung and Shen 1999; Nicholson et al. 2006). Inputs to these dendritic domains may contain spatial, relational, and other relevant forms of information that need to be integrated for proper encoding of memory traces. Compartment specificity might enable a neuron to compare information arriving into its distinct functional compartments from different brain areas and estimate its relevance.

A proposition for compartmentalization and memory encoding at the single neuron level is the "clustered plasticity" hypothesis (Govindarajan et al. 2006). The hypothesis posits that information encoding occurs within clusters of synapses located within particular dendritic branches. Physical distance, morphology and structural restrictions would constrain the spreading of plasticity products between clusters of synapses located at separate dendritic branches. Synapses within each cluster might be tagged by different plasticity-inducing stimuli, but only the strongest plasticity-dependent metabolic cascade would dominate the molecular environment of the cluster. Branch-specific local translation mechanisms would be key to this process (Govindarajan et al. 2011). This branch-specific protein synthesis-dependent homogenization of synaptic weights of a synaptic cluster would produce a more efficient action potential firing during recall compared with conventional dispersed plasticity models (Govindarajan et al. 2006, 2011).

Compartment-specific homogenization of synaptic weights is seen in the interaction between LTP and LTD, two opposite forms of synaptic plasticity (Han and Heinemann 2013; Pavlowsky and Alarcon 2012). Plasticity-induced protein synthesis

underlies the antagonistic interaction between LTP and LTD elicited at two synaptic inputs to the same dendritic region in CA1 principal neurons of the mouse hippocampus (Pavlowsky and Alarcon 2012). Interestingly, compartment-specific interactions between LTP and LTD can also be cooperative (Pavlowsky and Alarcon 2012; Sajikumar and Frey 2004). The type of interaction, antagonistic or cooperative, is regulated in a time-dependent fashion (Pavlowsky and Alarcon 2012). Antagonistic interactions, which disfavor the coexistence of LTP and LTD, occur when both forms of synaptic plasticity are induced within less than an hour from each other. Cooperative interactions between and coexistence of these opposite forms of synaptic plasticity begin to be seen after a longer time interval between inductions (Pavlowsky and Alarcon 2012).

How could the temporal control of compartment-specific interactions lead to proper information encoding? Interactions among different forms of synaptic plasticity may underline a form of competition by synapses and memories for access to retrieval resources (Diamond et al. 2005). Compartmental interference between learning-associated plastic events that occur within nearby time frames could provide a mechanism for disruption of unwanted information. Activity-dependent disruption of unwanted information seems to be a step necessary for the stabilization of a memory trace (Levy and Steward 1979; Martin and Morris 2002; Thomas et al. 1994; Villarreal et al. 2002; Xu et al. 1998). This mechanism is thought to prevent adding existent but irrelevant information to a memory experience. But this restriction may not be permanent. After a period of time, once the relevant plasticity-associated trace is consolidated, another one could be associated with it (Pavlowsky and Alarcon 2012).

2.7 Compartmental Encoding

In the hippocampus, encoding of information at specific compartments is mainly defined by the anatomy of hippocampal circuits (Amaral and Witter 1989; Kramar and Lynch 2003; Morris 2006). Changes in neural activity that modulate hippocampal oscillations (e.g., theta, gamma) (Atallah and Scanziani 2009) are suitable candidates to modulate the induction of synaptic plasticity in these synaptic paths (Colgin et al. 2009; Isomura et al. 2006). Indeed, changes in hippocampal oscillations do occur with learning (Bastiaansen et al. 2002; Jones and Wilson 2005; Montgomery and Buzsaki 2007). As induction of synaptic plasticity develops in various temporal fashions in multiple synapses, neurons could utilize compartmentalization mechanisms to integrate the information associated to these plastic events. Spatial and temporal interactions among plastic synapses of a neuron might enable the processing of information arriving from different brain areas into the neuron's distinct functional compartments and associate or segregate such information (Alarcon et al. 2006; Barco et al. 2002; Govindarajan et al. 2006; Sajikumar and Korte 2011a; Sajikumar et al. 2007a). Conceivably, the relationship between changes in input activity, hippocampal oscillations, and compartmentalization could

shape a subset of the neuron population to specifically encode information related to a given behavioral experience (Diba and Buzsaki 2008; Fenton et al. 2008; Geisler et al. 2010; O'Neill et al. 2008). These neurons could be part of a particular population ensemble that could generate particular output spike activity stamps (Broome et al. 2006; Dragoi et al. 2003; Marder and Buonomano 2003) that will impact the decoding of information in order to produce behaviorally relevant outputs (Benchenane et al. 2010; Lansink et al. 2009; Sirota et al. 2008).

2.8 Experience-Dependent Compartmentalization

Experimental and theoretical work has characterized the properties of plasticity-induced dendritic compartments. However, the emergence of a functional role for compartmentalization upon physiologically relevant behaviors for information processing (e.g., learning) is still understudied. Encouragingly, recent reports have begun to tackle this deficit and suggest that experience induces plasticity in a dendritic compartment-specific fashion that could reflect information processing that takes place during learning, development, and sensory processing. Exposure to an enriched environment leads to compartmentalized changes in the distribution of dendritic spike propagation within particular dendritic branches of CA1 principal neurons (Makara et al. 2009), indicating that the electrical properties of individual dendritic branches can be modified by in vivo experience. Similarly, spontaneous activity that occurs during development is shown to functionally cluster CA3 synapses (based on glutamate receptor activity and insertion, and intracellular Ca^{2+} distribution) on developing dendrites (Kleindienst et al. 2011). Lastly, sensory experience was shown to produce synaptic potentiation of nearby (clustered) dendritic synapses (Makino and Malinow 2011). Interestingly, this clustered synaptic potentiation was eliminated when animals were deprived of sensory experience (Makino and Malinow 2011).

These outstanding studies strongly demonstrate that experience drives compartmentalized synaptic changes in neuronal dendritic domains; suggesting compartmentalization of plastic events may prove fundamental for the development and function of neuronal circuits.

2.9 Multiple Levels of Integration of Information

Multiple compartments with different functional sizes are thought to dramatically enhance the associative properties of a neuron that receives multiple streams of information in its different synapses. According to the functional compartmentalization model, the size of a compartment depends on an activity function/wave; there is no restriction in the size of a compartment, and therefore, in the extent of

plasticity-mediated associativity within it. A neuron's functional compartmentalization could be confined to a small cluster of synapses within a dendritic branch, or to larger portions of the dendrite (including primary and secondary branches). In theory, compartmentalization could extend throughout the entire dendritic tree and even across the whole neuron. What would be the distinction of processing information within compartments, across compartments and at the cell-wide levels? And, what are the functional and behavioral consequences of each form of information processing?

Compartment-specific associativity can be overridden (Alarcon et al. 2006). As described above, compartment-specific synaptic capture of LTP is observed within basilar or apical CA1 dendrites, but not from one dendrite to another. However, when a stronger synaptic stimulation (different from the one normally used for synaptic capture of LTP experiments) was used to activate (tag) the capture of plasticity products at synapses in the opposite dendrite, LTP associativity across these formerly independent compartments was observed (Alarcon et al. 2006; Sajikumar et al. 2007a). Compartment-specific and across-compartments plastic associativity may depend on the cumulative recruitment of synaptically driven molecular mechanisms; therefore, they are a function of the strength of synaptic activation (Alarcon et al. 2006; Fonseca et al. 2004; Sajikumar et al. 2007a; Young and Nguyen 2005). Different activity thresholds that regulate the magnitude of compartmentalization could, in theory, allow for the expression of separate, independent, functional compartments within a single neuron or facilitate the functional overlapping between two or more compartments (Schacher et al. 1997; White et al. 1990).

Associative processes that encompass the whole cell would require stronger synaptic activation to initiate a larger recruitment of plasticity mechanisms– not at the branch or the dendrite level but at the cellular level. Somatic depolarization that strongly increases neuron's excitability is known to promote associative phenomena at the cell-wide level in CA1 pyramidal neurons. After strong somatic depolarization, synaptic capture of LTP is observed at any set of synapses in either basal or apical dendrites (Alarcon et al. 2006; Dudek and Fields 2002). Conceivably, somatic depolarization that generates spike back-propagation into both basal and apical dendrites (Kloosterman et al. 2001) could mitigate the functional boundaries of previously established compartments. This biophysical process could trigger cellular mechanisms that may facilitate the unbiased sorting and distribution of gene products within the cell. Likewise, enhanced CREB-mediated gene expression initiated in the absence of synaptic activity increases overall cell excitability, and that too promotes cell-wide synaptic capture of LTP (Alarcon et al. 2006; Barco et al. 2002; Casadio et al. 1999; Dong et al. 2006; Lopez de Armentia et al. 2007; Marie et al. 2005). Increased cell excitability and gene expression resulting from substantial neuromodulatory activity, as in aroused, highly attentional or alarmed behavioral states, could expedite cell-wide associative processes (Berke and Hyman 2000; Reymann and Frey 2007). Moreover, environmental enrichment is shown to increase intrinsic excitability (Irvine et al. 2006), which could facilitate cell-wide facilitation of associative processes (Adams and Dudek 2005; Lee et al. 2005).

Vis-à-vis plasticity-mediated associative processes, the computing capability of a neuron could involve compartment-specific, inter-compartmental, and cell-wide integration. These levels of integration could represent separate, independent mechanisms for encoding information in different neurons. That is to say, some neurons (upon particular synaptic activation) could undergo one type of integration, while others (upon different regimes of synaptic activation) could undergo a different type of integration. Alternatively, these different levels of integration could be dynamic transitional stages; adding more richness to the neuron's temporal and spatial integrative capability. For instance, compartment-specific changes in synaptic function elicited by sensory stimulation progress into a cell-wide change in synaptic function after sensory deprivation (Makino and Malinow 2011). Moreover, whereas cognitive behaviors may induce selective compartment-specific changes of synaptic function, stressful experiences would cause extreme neuromodulatory function to break down compartmentalization and promote cell-wide changes (Sajikumar et al. 2007b). Cell-wide and compartmental processes may be part of overlapping learning mechanisms which can be essential for behavior (Hulme et al. 2013). Transitions between compartment-specific, inter-compartmental, and cell-wide integration stages may also serve for the temporally correct processing of information. Acquisition of information may require the use of one integrative mechanism, whereas consolidation of information may require another. Hence, the cellular mechanisms that subserve the processing of information at each level as well as the transit from one level to another become most relevant for the proper association and segregation of information. Failure of these mechanisms may underlie the manifestation of disorders that have improper information processing as their core feature.

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Chapter 3
Synaptic Cooperation and Competition: Two Sides of the Same Coin?

Rosalina Fonseca

Abstract Activity-dependent plasticity of synaptic connections is a hallmark of the mammalian brain and represents a key mechanism for rewiring neural circuits during development, experience-dependent plasticity, and brain disorders. Understanding the rules that determine how different neuronal inputs interact with each other, allow us to gain insight on the cellular and molecular mechanisms involved in memory establishment and maintenance. One of the most intriguing aspects of memory formation is the observation that past and ongoing activity can influence how information is processed and maintained in the brain. At the cellular level, the synaptic tagging and capture (STC) theory states that the maintenance of activity-dependent synaptic changes is based on the interaction between synaptic-specific tags and the capture of plasticity-related proteins. The STC has provided a solid framework to account for the input specificity of synaptic plasticity but also provides a working model to understand the heterosynaptic interaction between different groups of synapses. In this chapter, I will discuss the evidence regarding the cooperative and competitive interactions between different groups of synapses. In particular, I will address the properties of synaptic cooperation and competition that contribute to the refinement of neuronal connections during development. Later, I will address the evidence that similar rules operate during the induction and maintenance of synaptic plasticity. Due to the intricate relationship between synaptic plasticity and memory formation, understanding the cellular rules of cooperative and competitive interactions between synapses, will allow us to further dissect the rules underlying associative learning.

Keywords Synaptic plasticity • Synaptic cooperation • Synaptic competition • Neuronal connectivity • Synaptic capture

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3.1 Introduction

The most striking property of the nervous system is its ongoing ability to learn and adapt to the stimulus of the environment. However, this constant ability to adapt raises a fundamental problem: how to be able to change without losing identity. Indeed, the nervous system has evolved to be a highly plastic system but maintaining the identity of the individual and preserving the responses necessary for its survival. It is now well accepted that developmental and learning changes in the nervous system are implemented through modifications in synaptic strength and ultimately in neuronal connectivity (Malenka and Nicoll 1997, 1999). In this respect, Donald Hebb postulated "When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place, in one or both cells so that the efficacy of cell A in firing B is increased" (Hebb 1949). This learning rule, commonly referred as "neurons that fire together, wire together," implies that correlated activity between two connected neurons leads to a strengthening of their connectivity (Miller 1996). The observation that high-frequency electrical stimulation of hippocampal afferents results in a long-term potentiation (LTP) of synaptic strength was the first demonstration that this learning rule could be implemented in biological systems (Bliss et al. 2003; Bliss and Collingridge 1993). After this, it was also demonstrated that synaptic transmission can be decreased by the induction of long-term depression (LTD) (Becker et al. 2008; Malenka and Bear 2004; Malenka and Nicoll 1998). Since then, a substantial amount of work has been devoted to understand the rules underlying the induction and the maintenance of LTP and LTD (Kauer et al. 1990; Lisman et al. 1997).

It is also clear that learning is an ongoing process, in which past and present neuronal activity can influence how information is processed in the brain and ultimately how memories are formed and maintained (Redondo and Morris 2011). Similarly, at the cellular level, it is now well established that previous neuronal activity can modulate the induction and maintenance of LTP and LTD (Ehlers 2003; Fonseca et al. 2006a, b; Fonseca 2012; Sajikumar et al. 2005, 2007; Sajikumar and Frey 2004a, b). This continuous processing of information allows different groups of activated synapses to interact, modulating the ability to induce and maintain LTP and LTD (Alarcon et al. 2006; Fonseca et al. 2004; Govindarajan et al. 2011). In this chapter, I will provide a brief outlook of these dynamic interactions between activated synapses, particularly discussing the evidence that synapses can engage in synaptic cooperation or synaptic competition. Although the cellular mechanisms involved in LTP and LTD are in general similar, I will focus on the cooperative and competitive synaptic interactions involved in the induction and maintenance of LTP.

Classically, LTP is divided into three stages or phases, an induction phase, an early-LTP phase, not dependent on protein synthesis and a late-LTP phase, dependent on de novo protein synthesis (Bramham 2008; Bramham et al. 2010; Frey et al. 1988; Huang et al. 1996; Kelleher et al. 2004; Reymann and Frey 2007; Wikstrom et al. 2003). This distinction, based on pharmacological or genetic manipulations of the neuronal protein synthesis machinery, is clearly an artificial division, as protein

synthesis is activated at the time of LTP induction and activity-dependent mechanisms can modulate the length of these phases and their dependence on protein synthesis (Djakovic et al. 2009; Fonseca et al. 2006a, b). Nevertheless, I will maintain this classic distinction for the purpose of clarity.

At this point, it is also useful to define what one considers being synaptic cooperation and synaptic competition. Synaptic cooperation is any cellular mechanism that allows two distinct groups of synapses to synergically trigger the induction or the maintenance of LTP. Conversely, synaptic competition is any cellular mechanism in which distinct groups of synapses interact by a defined rule such that one of the participants emerge as a winner (Van Essen et al. 1990). This does not necessarily mean that the winner has to be potentiated nor does it consider the mechanism by which the winner is achieved. Indeed, there are two possible forms of competition. In an independent competition, there are no interactions between the different participants. In this case, each participant does not influence each other, but rather the winner is selected based on its own performance (Colman and Lichtman 1992). In an interdependent competition, the participants interact with each other that is the performance of each participant is influenced by other participants (van Ooyen 2001). In this form of competition participants can interact in a consumptive way, competing for a limited resource, or by interference, in which one input has a direct negative interaction with a second input (van Ooyen 2001). Since LTP can be divided, at least, in three phases, synapses can interact cooperatively and competitively during any of these phases, during the induction, the early-phase or the late-phase of LTP. This idea that synapses or neuronal inputs can cooperate or compete is not new. It was first described, more than 60 years ago, in the developing nervous system, when studying the formation of a cell receptive field (Hubel et al. 1977; Stent 1973). However, the fundamental question regarding the cooperative and competitive interactions between synapses remains to be unanswered: what are the rules underlying these interactions? Or in other words, which patterns of neuronal activity leads to synaptic cooperation or to synaptic competition? In this chapter, I will address this question by first making a brief overview of the rules of synaptic cooperation and competition in the developing nervous system and further discuss what is known in the adult learning brain.

3.2 Synaptic Cooperation and Competition in a Developing Nervous System

The first indication that synapses can engage in synaptic cooperation and competition to establish new connective partners, came from studies of the developing nervous system. Since Cajal's observations of the nervous system, it is clear that the development of the nervous system is based on pruning of synaptic connections. Moreover, it is now clear that long-lasting changes in neuronal connectivity in the developing and the mature brain share many common principles. For example, the Hebbian rule described above, in the context of synaptic plasticity, also applies to

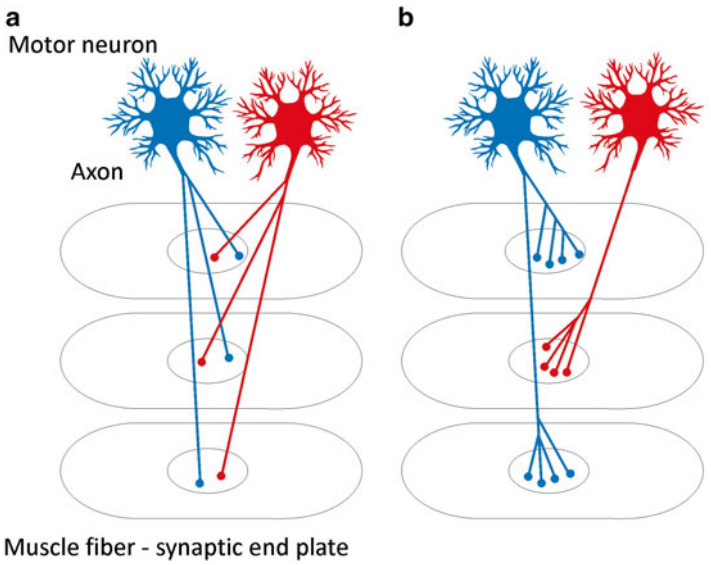

Fig. 3.1 Development of the neuromuscular junction. (**a**) Initially, each muscle fiber is innervated with axonal inputs originating from multiple motor neurons. (**b**) During development, synaptic cooperation and competition leads to neuronal refinement and single innervation of the neuromuscular junction

the developing nervous system, in which coincident spike activity leads to the strengthening of neuronal connections whereas non-coincident activity leads to the weakening of connections (Lo and Poo 1991; Stent 1973).

Although there are numerous examples described in the literature, the development of a mature neuromuscular junction is by far the most studied and clear example how synaptic cooperation and competition can shape the nervous system. In a mature system, in mammals, each muscle fiber is innervated by a single motor neuron. During development, however, this connective pattern is initially much less refined with each muscle fiber being innervated by several inputs originating from several motor neurons (Fig. 3.1). How does this system mature? For a muscle to function there are certain pre-requisites that need to be preserved: first, there must be a sufficient number of inputs terminating in a muscle fiber. This allows the neuromuscular junction to be sufficiently activated and overcome the contractility threshold so that the muscle can contract in an effective manner. Second, the correct target must be found so that groups of muscles are activated in a coordinated fashion. For example, during a simple moving such as walking, flexors and extensors muscles need to be contracting and relaxing in a coordinative manner so that their action does not oppose. During development, several mechanisms operate to achieve this level of coordination. Genetic mechanisms are clearly involved in the targeting of muscle cells by specific neuronal inputs and hence in their initial localization, but the connectivity pattern is highly unspecific, with each motor neuron innervating several targets simultaneously (Fig. 3.1a).

The initial unspecific innervation of muscle fiber is gradually being replaced by a single motor-neuron innervation (Fig. 3.1b). While the detailed cellular mechanisms involved in the refinement of the neuromuscular junction are still not entirely clear, there is substantial evidence that local synaptic interactions leads to the alteration of the functional connective pattern. This process of axonal refinement is gradual and asynchronous, linked to changes in synaptic efficacy, with inputs gradually retracting while others occupy their post-synaptic sites, once they become available (Colman et al. 1997; Walsh and Lichtman 2003). It is now clear that this activity-dependent remodelling of connections involves molecular cues that determine the best match between axonal input and muscle fiber, but synaptic cooperation and competition between axonal terminals of the same motor neuron and between different motor neurons (intra-neuronal and interneuronal) plays a fundamental role (Laskowski et al. 1998; Laskowski and Sanes 1987; Walsh and Lichtman 2003).

How can synaptic cooperation and competition ensure the refinement of the connective pattern between motor neurons and muscle fibers? In the mature neuromuscular junction, spike activity of motor neurons of the pool which innervates a given muscle is asynchronous (Buffelli et al. 2002, 2004). This ensures that muscle contraction is smooth. This asynchronous activity creates a local instability that may constitute the substrate for synaptic competition. Consistently, induction of synchronous activity by electrical stimulation or NMDA glutamate receptors inhibition blocks synaptic competition leading to a poly-innervated neuromuscular junction (Buffelli et al. 2004; Personius et al. 2008). Recent evidence suggests that individual axon branch removal occurs randomly, leaving a post-synaptic site unoccupied. This creates a triggering signal for neighboring axons to sprout. The re-occupation favors axons that better drive the post-synaptic target or in other words favors the motor neuron with the highest number of neighboring axons (Turney and Lichtman 2012). Eventually, this process leads to single innervation. Interestingly, there is also evidence that the same principle applies to synaptic rearrangements occurring in other areas of the nervous system. For example, climbing fibers on Purkinje cells elaborate new connections as other axons are eliminated. This process is highly complementary with losses being compensated with growth (Hashimoto et al. 2009). As in the neuromuscular junction, in the Purkinje cell—climbing fiber system, there is evidence that the limited resource is space. In both systems, the number of synaptic sites is mainly determined by the target cell, and under normal conditions input fibers can establish more connections than the ones available. This, of course, generates a competitive pressure for occupancy of the functional synapses.

Interestingly, there is also evidence that the synaptic instability described above can lead to synaptic cooperation. In a model of retinotopic refinement, in the Goldfish, if the number of retinotectal projections is low, a cooperative interaction between input projections is the dominant mechanism involved in the refinement of the connections (Olson and Meyer 1994). Because there is no competitive pressure in this situation, the authors suggest that the synaptic instability by itself would lead to the de-innervation of the target cells and only the inputs that are active in correlation with the target cell, following the Hebbian rule, would be reinforced, possibly

by a positive feedback signal (Olson and Meyer 1994). This positive feedback signal can actively promote an adjustable convergence of coactive fibers without the necessity of competition.

In an attempt to conciliate all these observations, Turney and Lichtman (2012) proposed a model in which the initial event leading to the refinement of the neuromuscular junction is the loss of motor-neuron synaptic contacts. This can occur following a Hebbian-based loss of connectivity in which non-correlated motor neurons are depressed, progressively becoming less and less efficient at stimulating their post-synaptic partners. There is evidence of a direct negative interaction by diffuse released proteins, such as proteases that are released by neuronal activation and precede synaptic elimination (Liu et al. 1994a, b). Once a post-synaptic site is vacant, neighboring neurons receive a potent signal to grow. One possible trigger for this growth is the release of diffusible neurotrophic factors from Schwann cells upon loss of contact with neuronal terminals (Henderson et al. 1994; Yan et al. 1995). Indeed, exogenous application of glial growth factors to postnatal muscles or overexpression of those factors in the developing system leads to polyneuronal innervation, which suggests that activity-dependent release of neurotrophic factors can function as the positive feedback signal stabilizing neuronal connections. Synaptic competition for non-occupied sites favors motor neurons that have the biggest number of axonal terminals, leading to single innervation (Turney and Lichtman 2012). This increase in the elaboration by a single motor neuron might also be the key for this stabilization since it increases the release of the positive feedback signals by the post-synaptic partner. During development, this system progresses from a dynamic competitive state to a long-lasting stable system. Although the detailed molecular orchestration involved in the neuromuscular junction development is still being revealed, the rules underlying the developing and the learning brain are quite similar and provided us with a strong conceptual framework to test the mechanisms of synaptic cooperation and competition in the context of learning and memory.

3.3 Synaptic Cooperation and Competition During LTP

As stated above, LTP can be divided in several stages or phases (Reymann and Frey 2007). This division opens the possibility for synapses to interact cooperatively and competitively in all these time periods. Interestingly, the induction of LTP is by itself a cooperative process (Froemke et al. 2010). LTP induction requires that multiple inputs have to be activated simultaneously so that the post-synaptic neuron is depolarized enough to induce a large calcium influx and downstream activation of signalling cascades (Sanhueza et al. 2011; Sanhueza and Lisman 2013). This form of synaptic cooperation allows "weaker" stimulus to summate electrically, leading to a sufficient membrane depolarization and induction of LTP (Mehta 2004). In this cooperative effect of synaptic plasticity, timing is everything: the level of temporal correlation is translated in the post-synaptic intracellular concentration of calcium. When activity is correlated, intracellular [Ca^{2+}] transiently increases leading to the induction of synaptic potentiation; non-correlated activity leads to a small but

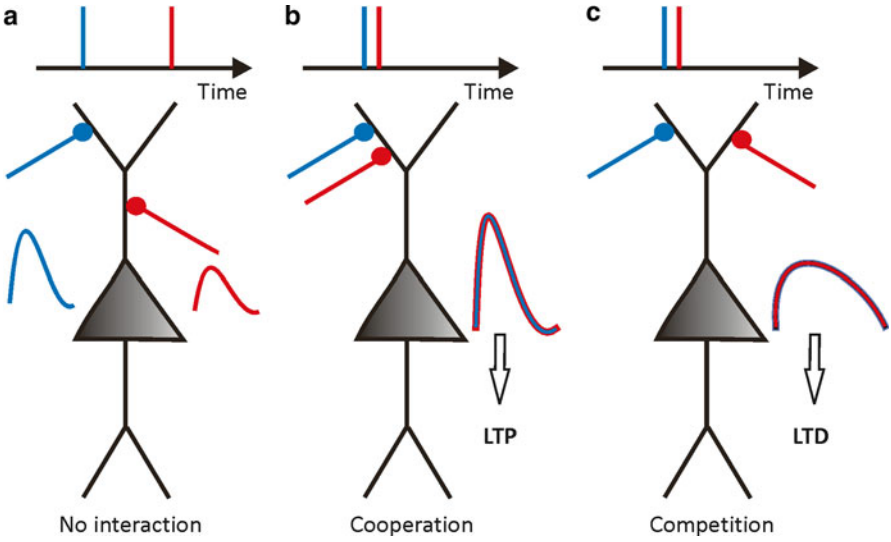

Fig. 3.2 Synaptic cooperation and competition at LTP induction. (**a**) Synaptic potential evoked by activation of two distinct inputs have no impact on each other, due to distinct timing of activation. (**b**, **c**) In the case where the two inputs are activated within a temporal significant window, they can either interact cooperatively or competitively depending on the localization within the dendritic arbor or the timing of activation relative to each other. In (**b**) the two inputs are localized close together leading to the summation of synaptic potentials and the induction of LTP. In (**c**) due to the localization of the two inputs the timing of arrival of the synaptic signals relative to the spike initiation zone leads to a broader and small signal leading to LTD induction

prolonged intracellular [Ca^{2+}] rise leading to a depression of synaptic strength. This synaptic plasticity rule, later on denominated as Spike-time dependent plasticity (STDP) (Bar et al. 2011; Froemke et al. 2010), relates the timing between synaptic-evoked potential and back-propagating action potentials or dendritic calcium spikes and can explain how two inputs can interact cooperatively or competitively depending on the timing of activation and relative position in the dendritic arbor (Fig. 3.2). Detailed analysis of this form of synaptic cooperation revealed several intriguing properties and constraints. Since it is based on the summation of local electrical signals, it is spatially limited for several reasons: first, most EPSPs in vivo have relative small amplitude so several EPSPs would need to cooperate to generate a signal over the threshold for LTP induction. Due to the cable properties of dendrites, the spatial spreading of those signals is very limited. This implies that cooperation is spatially limited. Second, active inhibition temporally and spatially significantly reduces the probability of two inputs to cooperate (Bar et al. 2011; Froemke et al. 2010). Together, these two properties create a temporal and anatomical constrain that restricts synaptic cooperation to temporally contiguous events. This also implies that the dendritic organization of synapses contains information about the temporal relationship of events. Such a mapping has several advantages, such as fast associative recall of entire sequences with a limited number of inputs (Mehta 2004).

On the other hand, it favors particular associations to be formed and reduces the plasticity of the system (Fig. 3.2b). It is interesting to note that, in this case, the limiting factor is space, similarly to what has been described in the developing neuromuscular junction.

Following the reasoning of the STDP, synaptic competition can also occur during LTP induction. Inputs that consistently are the best predictors of post-synaptic activation become the strongest inputs of the neuron. This can lead to the weakening of other inputs since the stronger input can more efficiently trigger spiking of the post-synaptic neuron, altering the correlation timing to other inputs (Fig. 3.2c). Also, in this form of synaptic competition, the dendritic localization of the inputs in relation to the spike initiation zone is critical (Bar et al. 2011; Song and Abbott 2001). Again, space seems to be the critical factor.

3.4 Synaptic Cooperation and Competition During LTP Maintenance

One of the critical features of memory formation is that not all learning events are maintained in the brain. Similarly, once synaptic plasticity is induced, it goes through a process of consolidation before it is stabilized as a functional and morphological change in neuronal connectivity. Synthesis of proteins, generally described as plasticity related proteins (PRPs), is necessary for the maintenance of synaptic plasticity (Barco et al. 2002; Bramham 2008). However, how to conciliate the input specificity of synaptic plasticity with the requirement of PRPs for plasticity maintenance? The working model that arose from the initial work of Frey and Morris, proposed that activated synapses are "tagged" so that newly synthesized PRPS could be specifically localized to these activated synapses allowing input-specific maintenance of plasticity (Frey and Morris 1997). This working model, later evolved into the synaptic tagging and capture model (STC), was the first demonstration that synapses could cooperate by sharing PRPs (Fig. 3.3). The authors showed that the induction of a long-lasting form of LTP in one set of synapses can stabilize a transient form of LTP induced in a second independent set of synapses (Frey and Morris 1997, 1998a). The stabilization of the transient form of LTP, induced by weak synaptic stimulation, is blocked if protein synthesis inhibitors are applied during the induction of the long-lasting form of LTP, suggesting that this form of synaptic cooperation is achieved by an interaction between the activity-dependent input-specific "synaptic tags," set by the weak synaptic activation, and the capture of (PRPs) induced by the strong synaptic activation. It is now clear that the setting of the "synaptic tag" and the long-lasting maintenance of LTP are independent processes and can occur separately in time (Fonseca 2012; Frey and Frey 2008; Frey and Morris 1998b; Redondo et al. 2010; Sajikumar et al. 2005, 2007).

Further analysis of this form of synaptic cooperation has revealed that the time in which the synaptic tag is able to capture the PRPs is limited, ranging from 1 to

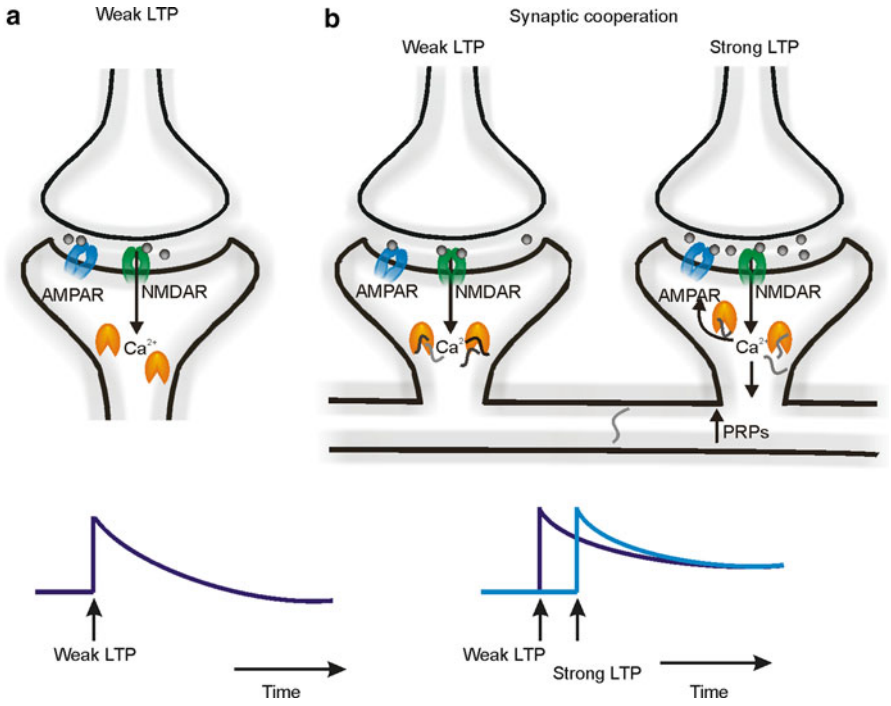

Fig. 3.3 Synaptic cooperation during LTP maintenance. (**a**) LTP induced by weak LTP induction leads to a transient form of LTP that generates tags (*yellow triangles*) at potentiated synapses but not the synthesis of PRPs and therefore decays with time. (**b**) If the weak synaptic stimulation is followed by a strong stimulation of a second set of synapses, the induction of long-lasting form of LTP leads to the synthesis of PRPs that are shared between the two activated inputs. This allows a cooperative maintenance of LTP in both activated groups of synapses

2 h (Fonseca 2012; Frey and Morris 1998b; Govindarajan et al. 2011). This transient activity of the synaptic tag limits the time interval in which synaptic cooperation can be induced, but it still allows different learning events to be associated in relatively larger time interval than the one described for LTP induction.

A second interesting property of this form of synaptic cooperation is the observation that synapses do not cooperate in a cell wide manner but that this interaction is space restricted. Using extracellular recording that lack the fine-space analysis, there was already an indication that different dendritic branches in pyramidal cells do not cooperate (Alarcon et al. 2006; Fonseca et al. 2004). Recently, using 2-photon uncaging of glutamate to spatially restrict synaptic activation, it was shown that the ability to induce synaptic competition was inversely correlated with distance, and had a bias towards the same branch (Govindarajan et al. 2011). This space constrain is extremely intriguing, since during the development of the neuronal connective pattern there is already a bias for correlated neurons to establish connections in proximity (Turney and Lichtman 2012). It is, therefore, plausible that the rules of

synaptic cooperation and competition operating during the developing of the nervous system determine the cooperative and competitive interaction that one observes in the mature brain. It is also interesting to note that the synaptic cooperativity that occurs during LTP induction is also dependent on the localization of the interacting inputs (Mehta 2004). Inputs that terminate in the same dendritic branches have a higher probability to summate and to be able to induce LTP and the formation of synaptic tags. This supports the hypothesis that there is a bias during the development of the nervous system to establish clustered connections between correlated neurons, which are maintained in the mature brain. This hypothesis of clustered plasticity (Govindarajan et al. 2006), is quite attractive since it would allow in a highly efficient way to associate neutral or less relevant information into a single memory engram (Frey and Morris 1998a) and it would allow a faster and easier reactivation of the engram (Govindarajan et al. 2006).

Interestingly, this clustering of plasticity also increases the probability of activated inputs to engage in synaptic competition. If PRPs are limited, activation of multiple inputs can generate a competitive pressure since PRPs would be distributed among all activated synapses (Fig. 3.4a, b). In such case, the strength of the tags, the distance at which the activated synapses is from the translational initiation site as well as the time elapsed between the two events, would determine which activated synapses are stabilized (Fig. 3.4c). Although this competitive maintenance was initially demonstrated using protein synthesis inhibitors (Fonseca et al. 2004; Govindarajan et al. 2011), limitation of the initial available pool of PRPs, using a more naturalistic patterns of stimulation, can induce synaptic competition without blocking protein synthesis (Fonseca et al. 2004). Moreover, the degree of synaptic competition is directly proportional to the degree of synaptic potentiation induced at the winner input (Fonseca et al. 2004). This suggests that the activity of the synaptic tag is proportional to the degree of synaptic activation and that an increase in the tag activity leads to an increase in the capture of PRPs.

What is the relevance of these forms of synaptic cooperation and competition to memory formation and maintenance? Recently, a couple of studies have shown that novelty, presumably through activation of dopamine receptors, induces the synthesis of PRPs converting a short-lasting memory into a long-lasting memory (Moncada et al. 2011; Moncada and Viola 2007; Wang et al. 2010). However, these studies do not address the possibility that activation of different groups of synapses can interact either in a cooperative or competitive fashion to modulate memory formation.

3.5 Synaptic Cooperation in the Lateral Nucleus of the Amygdala: Link to Behavior?

As stated above, one question that remains unanswered is the relevance of synaptic cooperation and competition during learning. To tackle this question, I have recently studied the cooperative interaction between the cortical and thalamic afferents to projection neurons of the lateral amygdala, a circuitry necessary for the formation

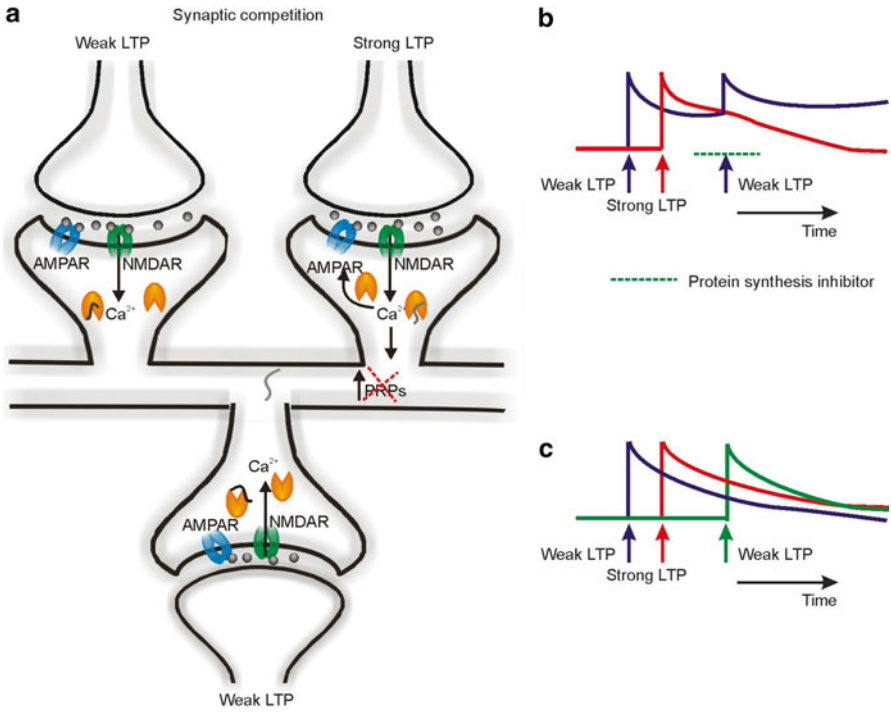

Fig. 3.4 Synaptic competition during LTP maintenance. (**a**) LTP induced by weak synaptic stimulation leads to a transient form of LTP that generates tags (*yellow triangles*) at potentiated synapses but not the synthesis of PRPs. The strong stimulation of a second set of synapses, up-regulates the synthesis of PRPs that are shared between the two activated inputs. (**b**) If protein synthesis is limited, by application of a protein synthesis inhibitor the reactivation of one of the previous activated synapses increases the number of tags creating a competitive pressure in the non-reactivated synapses. (**c**) If protein synthesis is not blocked but a third group of synapses is activated with a stimulus that generates synaptic tags but not the synthesis of PRPs, a similar scenario is created, with multiple groups of tagged synapses competing for a limited pool of proteins

of fear-conditioning memories (Fonseca 2013). I found that cortical and thalamic inputs to the lateral nucleus of amygdala can cooperate during LTP maintenance, similarly to what have been described in hippocampal synapses. Interestingly, the cooperation between cortical and thalamic inputs is bi-directional but asymmetrical (Fig. 3.5). I found that the ability to capture PRPs by the thalamic tag decays much faster than the ability of the cortical tag to capture PRPs. This argues for a restriction mechanism in thalamic cooperation. Consistent with this, inhibition of synaptic activation, inhibition of the metabotropic glutamate receptors (mGluR) or inhibition of the endocannabinoid receptor CB1, can extend the time window of thalamic cooperation. This is the first observation that synaptic cooperation can be asymmetrical, supporting the view that the synaptic tag is not a single molecule but a cellular process that allows the expression of LTP in an input-specific manner.

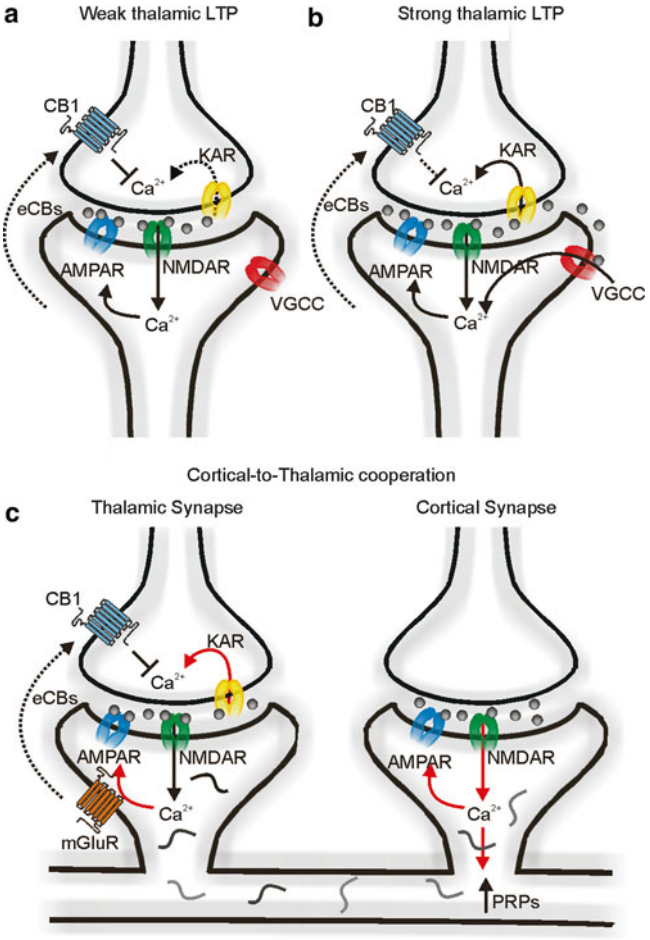

Fig. 3.5 Synaptic cooperation between thalamic and cortical inputs to the lateral nucleus of the amygdala. (**a**) LTP induced by weak stimulation of the thalamic input leads to a transient form of LTP that decays with time. (**b**) Strong stimulation of the thalamic input leads to the induction of a long-lasting form of LTP that is dependent on the activation of kainate glutamate receptors (KAR). (**c**) If the weak thalamic stimulation is followed by a strong stimulation of the cortical inputs the thalamic tag can capture the PRPs synthesized upon strong cortical stimulation. This occurs only if the time interval between thalamic and cortical stimulation is within a short time interval, to avoid the inhibitory effect of CB1 receptor activation

What might be the significance of this asymmetrical thalamic and cortical synaptic cooperation? It is possible that the association between cortical and thalamic projection is necessary for a discriminative form of fear-learning. While the activation of either the cortical or thalamic inputs is sufficient for fear-conditioning learning (Campeau and Davis 1995; Kwon and Choi 2009), in auditory discriminative fear-learning, co-activation of both inputs might be necessary for discrimination

(Antunes and Moita 2010). It is therefore conceivable, that synaptic cooperation between cortical and thalamic inputs underlies the establishment of a discriminative fear memory.

What could be the functional consequence of the time asymmetry? One possibility is that restricting the time window of thalamic cooperation, protects from generalizing fear responses. Increasing the expression of CREB in the direct thalamic-LA input enhances fear-learning and leads to generalization in discriminative fear-learning task (Han et al. 2008). It is, therefore, conceivable that restricting the time window for cortical-to-thalamic cooperation decreases the induction of incorrect associations and hence generalization. Although this is highly speculative, this is a powerful system to test whether synaptic competition and cooperation has a fundamental role in learning.

3.6 Conclusion Remarks

Synaptic cooperation and competition are powerful cellular mechanisms that in one hand contribute to maintain the overall activity of the neuron constant, but also determine the pattern of connectivity between neurons and ultimately the information that is stored in the brain. There are however, several open questions that remain. Due to the properties of signal processing in neurons it is clear that the anatomical organization of inputs determines the probability of synaptic cooperative and competitive interactions to occur. Since the pattern of connectivity is already determined following the same principles of neuronal cooperation and competition then in the mature brain the possible cooperative and competitive synaptic interactions are quite limited. This argues in favor of the clustered plasticity theory, suggesting that events with similar properties may be mapped in similar groups of neurons and on close by locations in the dendritic arbor. As stated above, this is a highly efficient manner to optimize associations but also to keep a constant update of the relative strengths of the various components of the engram. Further analysis of the relevance of synaptic cooperative and competitive synaptic interactions in associative learning will allow us to construct better models of memory formation and maintenance.

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Chapter 4
Neuropsin-Dependent and -Independent Synaptic Tagging and Modulation of Long-Term Potentiation: A Quest for the Associated Signaling Pathway(s)

Yasuyuki Ishikawa and Sadao Shiosaka

Abstract Synaptic tagging is plausible hypothesis that can potentially explain relational memory. However, it has not yet been cleared why and how the tagged synapses can be distinguished from the other non-activated synapses. Early-phase long-term potentiation (E-LTP)-related signaling molecules and intracellular molecular trafficking for capturing these toward tagged synapses have been considered as essential for synaptic tagging apparatus. In this chapter, we will describe a new mechanism of synaptic tagging which shares the common set of E-LTP induction mechanisms as above; that is, the E-LTP-specific proteolysis by neuropsin, an extracellular serine protease, is involved in neuropsin-dependent form of synaptic tagging.

Keywords E-LTP • Gate keepers • Extracellular • Neuropsin • NRG1 • ErbB4 • CaMKII • β1 integrin • LVDCC

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4.1 Introduction

Relational memory refers to the postulated role of the hippocampus in forming a collective representation of the various aspects of an experience. Thus, relational memory allows an individual who has experienced a traumatic event (e.g., a traffic accident) to remember even the most trivial details from the scene, as well as their spatial, sequential, and causative interconnections. This process permits the consolidation of signals from a number of weakly stimulated synapses into a single, sometimes unforgettable memory. However, little is known about the neural mechanism(s) underlying relational memory.

Synaptic tagging is a plausible hypothesis that can potentially explain relational memory. In 1997, Frey and Morris presented the hypothesis that active synapses are marked with a tag(s), and that newly synthesized, plasticity-related proteins (PRPs) must be targeted into the tagged synapses for prolongation of the potentiation state (Frey and Morris 1997). Because a weak synaptic stimulation is thought to produce a tag only at a single specific synapse and not to generate PRPs, a coordinated strong stimulation of a remote synapse arising from many weakly stimulated synapses is required to induce the production of new PRPs. These PRPs must then be transported into additional, specifically tagged synapses for the persistence of signal transmission between neurons. Therefore, the capturing and subsequent stabilization of new PRPs (such as the subunits of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor) at the tagged postsynapses are both essential for prolonged synaptic potentiation.

General intracellular signaling molecules have recently been considered as candidate molecular components of synaptic tagging apparatus (including those involved in the capture of PRPs) (Navakkode et al. 2005; Huang et al. 2006a; Sajikumar et al. 2007; Lu et al. 2011). However, extracellular molecules have not heretofore been taken into account as participants in synaptic tagging mechanisms. We now speculate that weak synaptic stimulation affords some initial change in extracellular matrix molecules and/or the extracellular domain of transmembrane signaling molecules found on the surface of the postsynaptic neuron, and that the resultant outside-in signaling permits PRP capture into the tagged dendritic spines. To explore this hypothesis, we focused on an established type of weak electrical stimulation, the single-pulse train (100 Hz, 1 s). The single-pulse train evokes only the early phase of long-term potentiation (E-LTP), lasting 1–3 h, and therefore provides a model of weak synaptic transmission. On the other hand, a four-pulse (repeated) train provokes the late phase of LTP (L-LTP, the protein-synthesis-dependent phase of LTP), lasting more than 4 h, and thus provides a model of strong synaptic stimulation (Huang and Kandel 1994).

Numerous E-LTP-related molecules (e.g., extracellular and transmembrane proteins, as well as their intracellular effectors) are found in the CA1 region of the hippocampus, where they might contribute to synaptic tagging-related signal transduction cascades. Data from mutant animals, especially knockout (KO) mice, indicate that these molecules participate in spatial learning and memory (Table 4.1).

Table 4.1 Mutant mice, E-LTP, spatial learning, and memory

Mutant[a]	E-LTP in CA1	Spatial learning/memory	Reference
CaMKII	Impaired	Impaired spatial learning	Hinds et al. (1998), Giese et al. (1998), Silva et al. (1992a, b)
Protein kinase C (PKC)-γ	Impaired	Impaired spatial learning	Abeliovich et al. (1993a, b)
Fyn	Impaired	Impaired spatial learning	Grant et al. (1992), Kojima et al. (1997)
Synaptic Ras GTPase-activating protein (SynGAP) (+/−)	Reduced	Impaired spatial learning	Komiyama et al. (2002)
Protein-tyrosine phosphatase (PTP)-δ	Enhanced	Impaired spatial learning	Uetani et al. (2000)
Neurogranin	Impaired	Impaired spatial learning	Huang et al. (2004)
LIM kinase 1	Enhanced	Impaired spatial learning	Meng et al. (2002)
Integrin α3	Impaired	Impaired spatial learning	Chan et al. (2007)
Integrin α8	Impaired	Spatial learning unaffected	Chan et al. (2010)
Integrin β1	Impaired	Impaired spatial learning and working memory	Chan et al. (2006), Huang et al. (2006b)
EphB2	Impaired	Impaired spatial learning	Henderson et al. (2001), Grunwald et al. (2001)
TrkB	Impaired	Impaired spatial learning	Minichiello et al. (1999)
Dopamine D1	Reduced	Impaired spatial learning	Granado et al. (2008)
Neural cell adhesion molecule (NCAM)	Impaired	Impaired fear memory	Muller et al. (2000), Senkov et al. (2006)
Tenascin-R	Impaired	Spatial learning unaffected	Saghatelyan et al. (2001)
Neuropsin	Impaired	Impaired spatial learning and working memory	Tamura et al. (2006)
BDNF	Reduced		Korte et al. (1995)

[a]Data are derived from KO animals unless otherwise noted

Moreover, the E-LTP-specific proteolysis of extracellular components by neuropsin, a neuronal serine protease, is a potential cellular/molecular mechanism involved in synaptic tagging (i.e., neuropsin-dependent synaptic tagging). This concept will be discussed in detail below.

4.2 E-LTP-Related Signaling Molecules That Are Modulated by Weak Stimulation

Signaling pathways responsible for the initial process of E-LTP and "gating" (the process whereby neuronal networks control input by inhibiting or promoting specific synaptic activity) from E-LTP into L-LTP still remain to be elucidated.

However, the induction of E-LTP after a single-tetanus stimulation in the CA1 region of the hippocampus is known to require Ca^{2+} influx through the *N*-methyl-D-aspartate (NMDA) receptor (Lisman 2003). Intracellular signaling begins with a slow (maximal 1–2-min) Ca^{2+}-dependent after depolarization, followed by activation of the NMDA receptor and elevation of postsynaptic cyclic AMP (cAMP) levels (Blitzer et al. 1995). Coincidentally, several cAMP-related molecules, such as cAMP-dependent protein kinase (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), participate in E-LTP induction.

In addition to their role in E-LTP induction, cAMP-activated PKA and CaMKII also block a protein phosphatase 1 (PP1)-operated inhibitory synaptic gating pathway. The gate is thereby maintained in the open state to execute synaptic responses for persistent plasticity (Blitzer et al. 1995; Otmakhova et al. 2000). The slow gating pathway is then closed by calcineurin to terminate LTP (Winder et al. 1998). CaMKII and PKA therefore control the gating mechanism from E-LTP into L-LTP by promoting synaptic modifications when Ca^{2+} levels increase (Okamoto et al. 2009). CaMKII is probably also involved in the setting of LTP-specific tags (Sajikumar et al. 2007; Redondo et al. 2010). Thus, E-LTP induction and synaptic tagging might share common and overlapping signal transduction mechanisms.

The role of PKA and CaMKII as "gate keepers" that alleviate the inhibitory constraint of PP1 and allow the transition from E-LTP into persistent plasticity/L-LTP was convincingly demonstrated by a number of studies employing hippocampal slices, electrophysiology techniques, and specific kinase inhibitors (Huang and Kandel 1994; Blitzer et al. 1995; Winder et al. 1998; Lisman 2003). The role of PKA in E-LTP/L-LTP was also explored in subsequent studies by using genetically engineered mice. For example, transgenic animals expressing a dominant negative form of the regulatory subunit of PKA showed a 50 % reduction in basal PKA activity, but continued to exhibit normal Schaffer-collateral E-LTP as induced by one- or two-pulse 100 Hz tetanic stimulation. However, L-LTP was clearly impaired (Abel et al. 1997). Thus, PKA is more likely to be necessary for L-LTP than for E-LTP itself. Nevertheless, conventional genetic approaches utilizing PKA regulatory subunit-KO animals failed to establish any decrease in PKA activity, or changes in Schaffer-collateral E-LTP and/or L-LTP (Brandon et al. 1995; Qi et al. 1996).

The G-protein-coupled dopamine D1/D5 and D4 receptors regulate postsynaptic cAMP and reportedly participate in the induction and regulation of E-LTP and L-LTP, respectively. The mesolimbic dopaminergic system projects from the ventral tegmental area to the limbic system via the nucleus accumbens, the amygdala, and the hippocampus, and is responsible for the relationship between memory acquisition and learning reinforcement (Lemon and Manahan-Vaughan 2006). The effect of the D1/D5 receptor on LTP has long been controversial, because the experimental results fluctuate according to the agonist employed and the experimental conditions (Mockett et al. 2004). Nevertheless, pharmacological studies using dopamine agonists have generally indicated that dopamine positively regulates E-LTP as well as L-LTP via the D1/D5 receptor (Frey et al. 1993; Huang and Kandel 1995; Otmakhova and Lisman 1996). In addition, in the recent gene-engineering study, E-LTP and

L-LTP were both markedly impaired in the hippocampus of D1 receptor gene-KO mice (Granado et al. 2008). Because further impairment was not imparted by supplementation of a D1/D5 antagonist to D1 receptor-deleted hippocampal slices, the investigators argued that the D1 receptor rather than the D5 receptor is critical for both types of LTP.

On the other hand, the D4 receptor triggers downregulation of intracellular cAMP levels by inhibiting the adenylyl cyclase-mediated G-protein α subunit, Gi. Current studies suggest that the dampening effect of D4 on E-LTP might be essential to the cognitive process (Herwerth et al. 2011). Furthermore, D4 modulation apparently occurs through NMDA receptors containing NR2B subunits, because such modulation is ablated in the hippocampus of mice lacking NR2B, but remains unaltered in the hippocampus of mice lacking NR2A (Herwerth et al. 2011). Notably, D4 receptor agonists increase γ oscillations, a risk factor for schizophrenia, in a manner similar to that afforded by neuregulin 1 (NRG1) (Fisahn et al. 2009; Andersson et al. 2012).

NRG1 and dopamine signaling pathways potentially crosstalk in gamma-aminobutyric acid (GABA)ergic interneurons to regulate the frequency of γ oscillations. Recently, Tamura et al. (2012) showed that neuropsin cleaves mature NRG1 to remove its heparin-binding domain, releasing the active form of NRG1 from the mature glycoprotein (Tamura et al. 2012). ErbB4 signaling induced by neuropsin-dependent proteolytic processing and subsequent release of NRG1 then modulates E-LTP via regulation of GABAergic transmission in the hippocampus (Fig. 4.1). Collectively, the convergence of dopamine- and neuropsin/NRG1-mediated signaling regulates intracellular cAMP levels in GABAergic neurons to control E-LTP.

In addition, certain neuromodulatory receptor signaling systems contribute to the regulation of E-LTP. Although only limited studies are available to date, some of these are briefly discussed below. For instance, the integrins comprise an important family of transmembrane cell adhesion receptors that function as heterodimers of α- and β-subunits. Integrins mediate diverse signaling processes in numerous cell populations, including neurons. Currently, 19 different α-subunits and eight different β-subunits are known in vertebrates, and over 20 different α/β heterodimers have been described. Mice with reduced expression of α3, α5, and α8 integrin subunits are defective in E-LTP (Chan et al. 2003, 2007), substantiating the involvement of the integrins in learning and memory. Furthermore, the integrin peptide antagonist, GRGDSP, as well as an infusion of function-blocking antibodies against the β1 integrin subunit, suppressed E-LTP and stabilize LTP in hippocampal slices (Stäubli et al. 1998; Kramár et al. 2006). Although the contribution of α5-containing integrins to LTP modulation has yet to be confirmed by a conditional genetic technology approach, conditional deletion of the β1 integrin subunit at a later postnatal stage compromised L-LTP induced by a two-tetanus protocol (Huang et al. 2006b). Further studies to reveal possible interactions between integrin signaling and LTP are necessary to clarify the precise role of these receptors.

Ephrin type-B receptor 2 (EphB2) interacts with and controls NMDA receptor activity, and as a result, EphB2 can modulate synaptic plasticity. EphB2 interacts

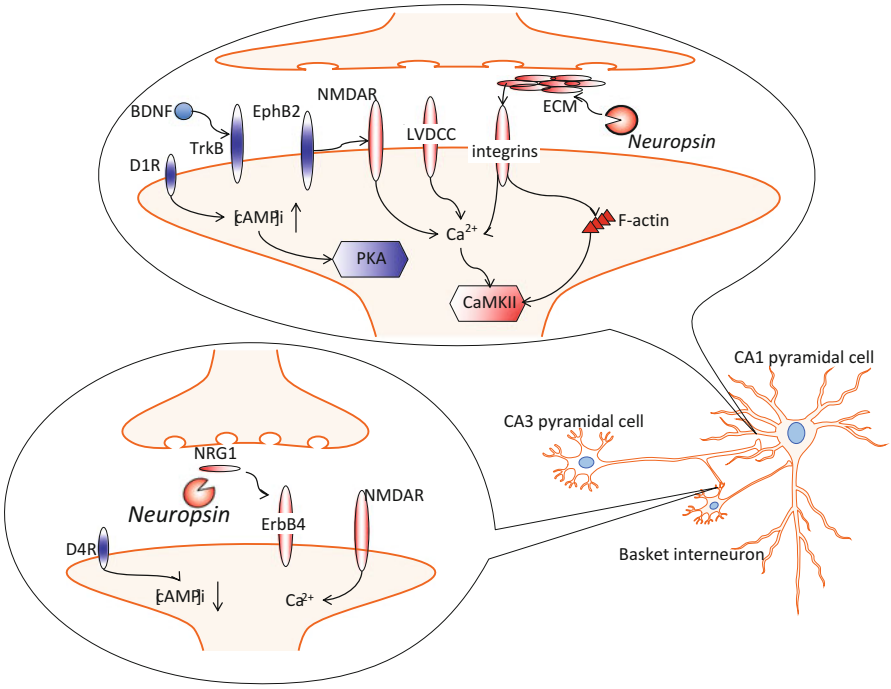

Fig. 4.1 E-LTP-induced signaling cascades are activated postsynaptically. Neuropsin modulates E-LTP and synaptic tagging via proteolysis-dependent postsynaptic signaling pathways. Neuropsin-dependent pathways are shown (*red*), one that involves integrin/CaMKII signaling, and another that involves NRG1/ErbB4 signaling

via its extracellular domain with the NR1, NR2A, and NR2B subunits of the NMDA receptor (Dalva et al. 2000). This interaction does not appear to be a simple two molecular interaction, but rather, a more complex heterogeneous interaction. In fact, activation of EphB2 results in clustering of NMDA receptors with other synaptic proteins, including αCaMKII (Dalva et al. 2000). EphB2 activation also enhances Ca^{2+} influx through the NMDA receptor, and is itself dependent on the phosphorylation of specific tyrosines in the NR2B subunit (Takasu et al. 2002). In addition, EphB2 deletion leads to deficits in synaptic plasticity (Grunwald et al. 2001; Henderson et al. 2001).

Investigations involving brain regions other than the hippocampus suggest that the molecular interaction between EphB2 and the NMDA receptor is, like the activation of NRG1, regulated by neuropsin-dependent proteolytic processing. In the amygdaloid complex, neuropsin cleaves EphB2 in response to stress. EphB2 regulates stress-induced plasticity and anxiety-like behavior, and its cleavage stimulates a dynamic interaction between the EphB2 and NMDA receptors, leading to an increase in the expression of an anxiety-related gene, FKBP5 (Attwood et al. 2011). The neuropsin/EphB2/NMDA receptor interaction is fast and specific for E-LTP, as evidenced by its disruption in the lateral-basal pathway of neuropsin-KO mice in

response to a weak stimulation protocol. The dynamic neuropsin/EphB2/NMDA receptor interaction ultimately results in increased NMDA receptor activity and manifestation of the behavioral signatures of anxiety.

Brain-derived neurotrophic factor (BDNF) causes synaptic plasticity in the fully developed brain, as well as in the immature brain. BDNF is produced by post-translational cleavage of a precursor protein termed proBDNF (Seidah et al. 1996; Pang et al. 2004; Matsumoto et al. 2008). BDNF is apparently involved in the regulation of E-LTP, because deletion of *Bdnf* in mice disrupted the induction of E-LTP in the CA1 region of hippocampal slices (Korte et al. 1995). The defect was rescued by reintroducing BDNF via viral transduction or by supplying exogenous BDNF (Korte et al. 1996; Patterson et al. 1996; Pozzo-Miller et al. 1999). Therefore, BDNF might be associated with the initiation of E-LTP.

Furthermore, pharmacological studies have demonstrated that the maintenance of L-LTP was also significantly impaired in hippocampal slices pretreated with tropomyosin receptor kinase (Trk) B antiserum, indicating an involvement of BDNF/TrkB signaling in L-LTP (Kang et al. 1997; Korte et al. 1998). Conditional deletion of *Trkb* from forebrain principal neurons also provided evidence for the involvement of TrkB in both E- and L-LTP, as assessed in hippocampal slices (Minichiello et al. 1999, 2002; Xu et al. 2000). The BDNF/TrkB system is controlled by Ca^{2+} influx through NMDA receptors and Ca^{2+} channels, enhancing TrkB receptor tyrosine kinase activity and facilitating ligand-induced internalization of TrkB (Du et al. 2003). In addition, cAMP expedites sorting of TrkB into the post-synaptic density (Ji et al. 2005). Because the BDNF/TrkB system participates in postsynaptic labeling by virtue of the molecular localization of TrkB, it may act as a tag-associated signaling system (Lu et al. 2011).

4.3 The Extracellular Protease Neuropsin Contributes to E-LTP

Neuropsin belongs to the family of secreted-type serine proteases, which are thought to be essential for many aspects of neuronal activity and function (Chen et al. 1995; Komai et al. 2000; Davies et al. 2001; Tamura et al. 2006; Ishikawa et al. 2008, 2011; Attwood et al. 2011). As described above, neuropsin stimulates GABAergic neurons via NRG1/ErbB4 signaling (Fig. 4.1). Recombinant neuropsin (produced by insect cells) modulates Schaffer-collateral E-LTP in a dose-dependent manner in hippocampal slices, and neuropsin enzyme activity (measured with synthetic neuropsin substrates) is transiently activated in the hippocampus during in vivo E-LTP in an NMDA receptor-dependent manner (Komai et al. 2000; Tamura et al. 2006; Ishikawa et al. 2008). Furthermore, electrophysiology investigations employing an E-LTP-preferential protocol (i.e., weak stimulation) showed that E-LTP is almost completely eradicated in hippocampal slices derived from neuropsin-KO mice. Consistent with this result, bath-application of a neuropsin-specific inhibitor to hippocampal slices derived from wild-type mice confirmed the E-LTP-specific

involvement of neuropsin in the CA1 region of the hippocampus (Hirata et al. 2001; Tamura et al. 2006; Ishikawa et al. 2008).

Interestingly, enzymatic activation of neuropsin is rather slow (requiring at least a few minutes) after a single tetanus-triggering protocol. The slow response of neuropsin may represent a sequential up-regulation of the enzyme after Ca^{2+}-dependent afterdepolarization (Blitzer et al. 1995; Tamura et al. 2006). Notably, neuropsin activation was abolished by a pharmacological NMDA receptor inhibitor. Behavioral studies showed that neuropsin protease deficiency caused a significant impairment of working memory-like behavior in the Y maze, as well as during the early stage of training in the Morris water maze (Tamura et al. 2006). Thus, the regulatory activity of neuropsin in the hippocampus (and likely in the amygdaloid complex as well; see above) was temporally restricted in E-LTP, rather than in L-LTP.

4.4 Neuropsin-Dependent and Independent Synaptic Tagging

Due to the distinctive feature of neuropsin's function in E-LTP alone, we postulated that the protease might contribute to an initial, protein synthesis-independent step in synaptic tag production. According to the original synaptic tagging hypothesis set forth by Frey and Morris (1997), two independent [weak (S1) vs. strong (S0)] synaptic inputs to the same neuronal population can be monitored by using a single recording electrode in the CA1 stratum radiatum. When the S0-mediated pathway is initiated by a strong stimulus, subsequent synaptic persistency is evoked in the S1-mediated pathway by a weak (single-tetanus) stimulus, which normally produces only E-LTP. This process represents the association of the strong and weak synaptic pathways.

However, our work showed that the S0/S1 association completely disappeared in hippocampal slices derived from neuropsin-KO mice, but recovered to normal levels by bath-application of an enzymatically active form of recombinant neuropsin. This observation suggests that neuropsin does indeed participate in an early step in synaptic tagging, as well as in the acquisition of persistency at the S1 site, where the single tetanus was delivered. We hypothesize that this neuropsin-dependent step encompasses the capture of PRP, as opposed to their synthesis. Additionally, a second form of synaptic association was evoked by a stronger (two-tetanus) stimulus at the S1 synapse, which perseveres in the neuropsin-KO mouse. Taken together, our findings support the existence of at least two types of synaptic association: neuropsin-dependent and neuropsin-independent synaptic association (Ishikawa et al. 2008).

Ample experimental evidence indicates that the neuropsin-dependent form of synaptic association is concomitantly driven by integrin/actin signaling and an L-type voltage-dependent Ca^{2+} channel (LVDCC)-mediated pathway (Ishikawa et al. 2008). For example, blockade of integrin function by the GRGDSP peptide, by an antibody against the β1 integrin subunit, by the actin polymerization inhibitor

cytochalasin D or by the LVDCC inhibitor nitrendipine all impaired neuropsin-dependent synaptic association (Fig. 4.1). The integrin-, the actin polymerization- and LVDCC-mediated signaling pathways probably converge into one or more common Ca^{2+}-dependent signaling pathways downstream of neuropsin, such as the CaMKII-dependent and/or the cAMP-dependent pathway. In support of this idea, KN93, a CaMKII inhibitor, was bath-applied to a recombinant neuropsin-supplemented (rescued) neuropsin-KO hippocampal slice. KN93, together with weak stimulation at S1, completely blocked the late associativity elicited at S1 in the stratum radiatum by a strong stimulation at S0 (Ishikawa et al. 2011). However, no study to date has directly examined whether neuropsin alters any molecular component(s) of the CaMKII-dependent or the cAMP-dependent signaling pathway to influence synaptic tagging. As such, further investigation is required to elucidate the detailed biochemical mechanism underlying neuropsin-dependent synaptic association.

Complicating matters further, the neuropsin-independent form of synaptic association may also involve LVDCC. A two- or more-tetanus stimulus induces NMDA-independent and LVDCC-dependent L-LTP and the formation of longer-lasting memories, particularly those based on stress-driven memory tasks (Grover and Teyler 1990; Cavuş and Teyler 1996) (e.g., food exploration in the radial maze under conditions of severe starvation (Borroni et al. 2000), and fear conditioning (Moosmang et al. 2005; McKinney and Murphy 2006). Thus, neuropsin (integrin/actin signaling)-dependent and neuropsin-independent late associativity apparently come together into the same LVDCC-mediated intracellular signaling pathway (Ishikawa et al. 2008).

4.5 Conclusions

Recent studies have revealed several novel potential mechanisms of synaptic tagging in which integrin, neuropsin, dopamine receptors, PKA, protein kinase Mzeta (PKMz), TrkB, and CaMKII all participate in local and synapse-specific regulation of E-LTP signaling and E-LTP transition into L-LTP (Sajikumar et al. 2007; Ishikawa et al. 2008, 2011; Redondo et al. 2010; Attwood et al. 2011; Lu et al. 2011; Tamura et al. 2012). These signaling molecules are probably shared among common and overlapping E-LTP and synaptic tagging pathways.

The mechanisms underlying synaptic tagging are triggered by Ca^{2+} influx through synaptic NMDA receptors and other Ca^{2+} channels, followed by an enhancement in cAMP- and CaMKII-dependent signaling at local synapses. A series of studies from our group revealed the existence of neuropsin-dependent and -independent forms of synaptic tagging in the hippocampus. Other investigations demonstrated that CaMKII may function as a component of a "gating" mechanism for the acquisition of persistency from E-LTP into L-LTP by promoting cAMP-dependent protein modifications (Okamoto et al. 2009) and by situating LTP-specific tags at appropriate sites (Sajikumar et al. 2007; Redondo et al. 2010). Thus, the outside-in signaling

associated with synaptic gating may utilize several independent intracellular pathways that converge into a single CaMKII-mediated regulatory mechanism for setting the tag at a specific synapse. As described above, LVDCC-mediated signaling possibly also supports the acquisition of synaptic persistency.

Neural activity-dependent proteolytic processing of neuropsin substrates (e.g., NRG1 and various extracellular matrix molecules) results in the exertion of multiple signals toward the acquisition of synaptic plasticity, thus contributing to changes in synaptic configurations. Neuropsin-dependent synaptic tagging via outside-in signaling, as mediated through NRG1/ErbB4 and integrins/CaMKII (Fig. 4.1), might place some as yet unidentified mark on Schaffer-collaterals and interneuronal synapses related to the procurement of late associativity (Ishikawa et al. 2008, 2011; Tamura et al. 2012). Although neuropsin is apparently crucial for this process, direct mechanisms for the attainment of synaptic persistency are still unknown. One possibility is that a CaMKII-dependent modulation of F-actin induces delivery to and capture of PRPs within a specific tagged dendritic spine (Okamoto et al. 2009).

In addition, theoretical work suggests that theta rhythms might act as a type of "tag" for short-term memory processing in the hippocampus (Vertes 2005). Theta rhythms selectively appear in the rodent during periods of active exploratory movement. If the exploratory information is temporally coupled to theta rhythms, the theta rhythm-induced storage mechanism of novel information in the hippocampus may be similar to that of synaptic tagging-induced initiation of E-LTP.

Although molecular and cellular cognition studies have provided compelling evidence that synaptic plasticity and synaptic associativity are required for learning and memory, it is still unclear where and how they act in the brain. The field is full of major questions, including the nature of the molecular and cellular mechanisms of plasticity and memory that encode, edit, and use stored information. Certainly, a more complete understanding of the fundamental signaling pathways responsible for LTP and synaptic tagging will continue to further our understanding of the identity and functioning of the neuronal networks behind learning and memory.

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Chapter 5
PKA Anchoring and Synaptic Tagging and Capture

Alan Jung Park and Ted Abel

Abstract Synaptic tagging and capture (STC) hypothesis has been receiving increasing attention because it reflects heterosynaptic association of information processing during memory formation in the brain. Indeed, electrophysiological and behavioral studies suggest that STC is a better cellular model for memory formation than the conventional homosynaptic experiment. In STC, a short-lasting potentiation in one pathway becomes persistent when it is paired with a long-lasting potentiation in the other independent pathway. It has been proposed that the setting of synapse-specific tag and capture of non-synapse-specific diffusible gene products by the tag determines the fate of each pathway. However, the mechanism of STC is still elusive and three major questions should be answered: (1) What is the tag and how does it modulate synapse-specific plasticity? (2) How does the tag capture gene products? (3) What are the gene products and how are they produced? Although several molecules and processes have been suggested to answer to these questions, they only provide partial explanations about the phenomenon. Here, this article will discuss how PKA modulates synapse-specific neuronal processing by coordinating signaling molecules and processes through PKA anchoring proteins, and how anchored PKA is involved in the generation and capture of plasticity-related gene products. Having PKA as a key molecule, the goal of this article is to provide a unified model of STC that addresses the key questions.

Keywords cAMP • PKA • AKAP • PKA anchoring • Synaptic tagging and capture

5.1 Introduction

Synaptic plasticity, the activity-dependent change in synaptic strength, has been extensively studied as a cellular/physiological correlate of memory storage (Mayford et al. 2012). Memory is stored in the hippocampus, and physiological and behavioral studies have been conducted to unravel the mechanism of memory processes

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in this brain area. Hippocampal long-term potentiation (LTP) has been studied as the primary model for memory storage because of its long duration, input specificity, and associativity (Bliss and Gardner-Medwin 1973; Bliss and Lomo 1973). The hippocampal Schaffer collateral—CA1 region is an output area of the hippocampus to the cortex, and damage to CA1 impairs memory formation (Zola-Morgan et al. 1986; Auer et al. 1989). Various forms of synaptic plasticity in CA1 have distinct molecular requirements. Early-LTP (E-LTP), the neural correlate of short-term memory (STM), lasts 1–2 h and requires NMDA receptor and Ca^{2+}/calmodulin-dependent protein kinases II (CaMKII) activation, but does not require PKA activation, transcription, and protein synthesis (Huang and Kandel 1994; Tsien et al. 1996). In contrast, late-LTP (L-LTP), the neural correlate of long-term memory (LTM), lasts several hours and requires PKA activation, transcription, and protein synthesis in addition to NMDA receptor and CaMKII activation (Frey et al. 1996, 1988; Huang and Kandel 1994; Nguyen et al. 1994; Tsien et al. 1996; Nguyen and Kandel 1997; Abel et al. 1997; Matsushita et al. 2001; Otmakhov et al. 2004). As with L-LTP, studies of hippocampus-dependent behavioral tasks also demonstrate that PKA activation, transcription, and protein synthesis are critical determinants discriminating STM from LTM formation (Barondes and Jarvik 1964; Abel et al. 1997; Pittenger and Kandel 1998; Meiri and Rosenblum 1998).

These findings provide critical groundwork to understand the mechanisms underlying L-LTP and LTM. However, studies on homosynaptic LTP recordings and LTM from a single behavioral experience using naïve animals have substantial limitations because they do not reflect the complex nature of memory processing that requires integration of multiple synaptic inputs from several interacting experiences. Memory formation is continuously influenced by past, present, and future experiences. Memories linked to events that require more attention or involve emotional arousal are more persistent than ordinary memories (Richter-Levin and Akirav 2003). To account for this complex memory processing, a synaptic model addressing integration of multiple inputs is needed. Synaptic tagging and capture (STC), first described in rodent hippocampal CA1 and *Aplysia* neurons in 1997 (Frey and Morris 1997; Martin et al. 1997), demonstrates the association and integration of synaptic activities of two independent sets of synapses. Frey and Morris placed two stimulating electrodes on either side of a recording electrode in the hippocampal area CA1 in order to stimulate two independent sets of synapses that were converging onto the same population of CA1 neurons (Fig. 5.1a). In this experiment, they showed that weak stimulation-induced E-LTP in one pathway became persistent when the weak pathway was paired with strong stimulation-induced L-LTP in the other pathway. According to the STC hypothesis, strong stimulation (S1) not only *tags* the activated synapses, but also *induces* production of gene products (Plasticity Related Products, PRPs). These tagged synapses then *capture* the gene products that migrate within the neurons. The capture of PRPs by the tag allows L-LTP expression in the S1 pathway (Fig. 5.1b). On the contrary, weak stimulation (S2) only generates tags, and therefore L-LTP is not induced because PRPs are not available for the tag to capture (Fig. 5.1c). However, once this S2 pathway is paired with the S1 pathway, the tag in the S2 pathway can capture PRPs produced from the S1 pathway and thereby transform E-LTP to L-LTP in the S2 pathway (Fig. 5.1d). Input specificity described by

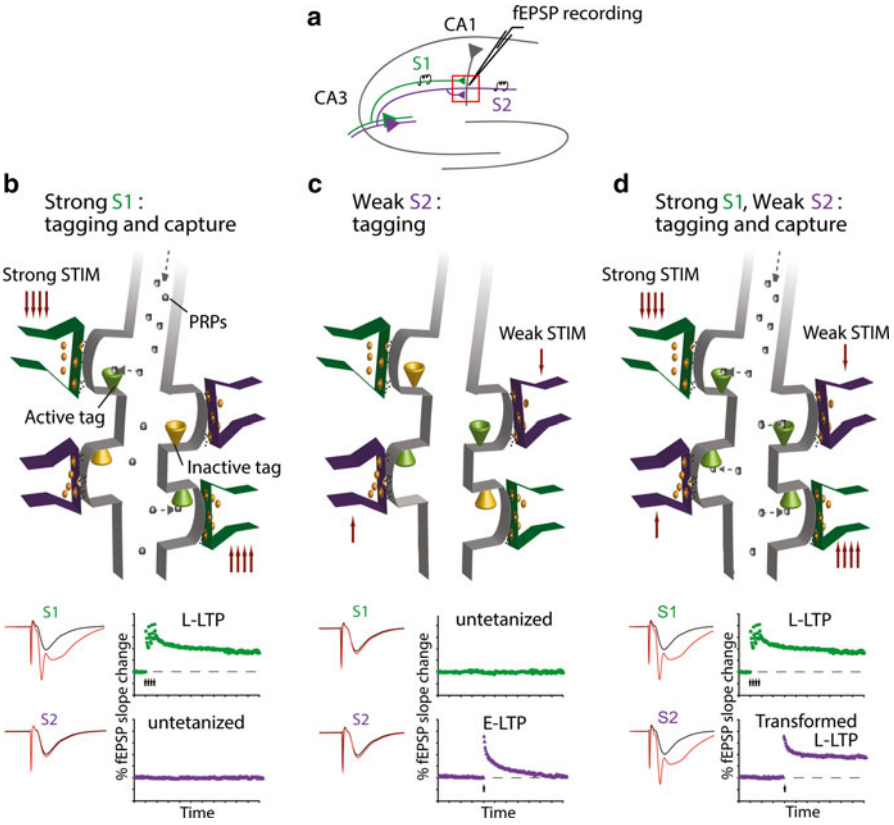

Fig. 5.1 Synaptic tagging and capture. (**a**) Schematic diagram of the two-pathway experiment. Two stimulating electrodes are positioned to stimulate two independent pathways (S1 and S2) converging onto the same CA1 neurons. (**b**) A strong stimulation activates local tags and induces PRP production. The capture of PRPs by the activated tags allows L-LTP expression in the S1 pathway. (**c**) A weak stimulation only activates local tags and induces E-LTP in the S2 pathway. (**d**) Pairing of S1 and S2 pathways results in capture of PRPs from the S1 pathway by activated tags in the S2 pathway which transforms E-LTP to L-LTP in the S2 pathway. Left lower insets are representative traces (*black*: baseline, *red*: hours after stimulation), and right lower insets are representative slope recordings of field excitatory postsynaptic potentials (fEPSP) over hours. (Modified with permission from Ted Huang, Ph.D.)

STC is efficient in that the tagged synapses can use the same pool of PRPs produced by the strong stimulation. The time window or the duration of the tag, which allows for successful L-LTP capture, is about 1–2 h (Frey and Morris 1998). Behaviorally, weak task-induced STM can also become long-lasting by a strong independent task that produces protein synthesis-dependent LTM (Moncada and Viola 2007; Ballarini et al. 2009; Wang et al. 2010b). Hence, heterosynaptic capture and the longer period of associativity described by STC provide a better representation of the complex integrative nature of memory processing.

Although STC has been studied by many researchers over the last decade, the identity of the tag remains elusive. The tagging process is the most critical component of STC because PRP production alone is not sufficient for L-LTP expression (Barco et al. 2002). Lines of evidence suggest multiple requirements for being a tag. A tag should be (1) spatially restricted to activated synapses, (2) transient and reversible, (3) interacting with PRPs, and (4) independent of protein synthesis (Martin and Kosik 2002; Kelleher et al. 2004b). As many molecules and processes (e.g. NMDA receptor, PKA, CaMKII, TrkB, actin polymerization, etc.) have been independently proposed as tags (Martin and Kosik 2002; Redondo and Morris 2011), tagging likely involves the coordination of multiple molecules or processes rather than a single molecule or a single process. Kinase-mediated processes are the strongest candidate tagging mechanisms. By reversible phosphorylation of their targets, kinases provide a history of activated synapses. The most convincing evidence supporting this idea is that low frequency stimulation (LFS) resets tags and this tag-reset is mediated by increased phosphatase activities. Also, LFS-induced detagging does not affect already established STC and gene expression (Barco et al. 2002; Sajikumar and Frey 2004; Young and Nguyen 2005; Young et al. 2006). More importantly, increased phosphatase activities by LFS interfere with PKA activity (Young et al. 2006). If PKA is the central molecule coordinating tagging processes, the next question that arises is how such a diffusible molecule can modulate spatially restricted STC processes. In fact, PKA signaling is highly localized by a family of scaffold proteins known as A-kinase-anchoring proteins (AKAPs) (Michel and Scott 2002). In this article, we will discuss PKA as a key tagging molecule that modulates proposed tagging mechanisms and how AKAPs compartmentalize PKA and its targets to ensure synapse-specific tagging processes.

5.2 The Role of PKA in Synaptic Plasticity and Memory Formation

In the brain, the heterosynaptic neuromodulatory system is critical for information processing, and likely a deterministic factor making memory long-lasting. Major neuromodulators in the brain are the dopaminergic, adrenergic, and serotonergic systems. These neuromodulators project to various brain regions, including the hippocampus. At the synaptic level, they may modulate L-LTP by lowering the threshold for tag setting or PRP production (Richter-Levin and Akirav 2003). Dopaminergic and noradrenergic signaling not only modulate electrically induced L-LTP but also facilitate L-LTP and STC expression (Frey et al. 1991; Gelinas and Nguyen 2005; Gelinas et al. 2008; Connor et al. 2011; Havekes et al. 2012). Interestingly, these modulatory innervations are linked to G-protein coupled receptors (GPCRs). Upon ligand binding, GPCRs activate adenylate cyclases (ACs) to produce the ubiquitous secondary messenger, cAMP. Targets of cAMP are PKA, exchange proteins directly activated by cAMP (Epac), and the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Although Epac and HCN channels are involved in regulation

of the neuronal function, their roles in synaptic plasticity and memory are less well understood (Laurent et al. 2012; Benarroch 2013). As a major target of cAMP, the role of PKA in L-LTP and LTM has been extensively studied. Mice expressing a dominant negative PKA regulatory subunit have significantly reduced L-LTP in the area CA1 and exhibit deficits in hippocampus-dependent memory (Abel et al. 1997). The PKA inhibitors Rp-cAMP and KT5720 block L-LTP (Matthies and Reymann 1993; Frey et al. 1993), and PKA activation mediates a form of L-LTP facilitated by β-adrenergic receptor activation in the area CA1 (Gelinas et al. 2008). Additionally, PKA activation by dopaminergic innervations is involved in LTM formation, and pharmacological activation of PKA enhances LTM formation as well as L-LTP expression (Bernabeu et al. 1997; Barad et al. 1998). In the next section, we will expand our knowledge on the contribution of PKA to synaptic plasticity by providing evidence that supports the role of PKA in STC.

5.3 The Requirement for PKA in Synaptic Tagging and Capture

The most compelling evidence for PKA as a critical molecule for L-LTP is that direct activation of PKA by pharmacological agents such as Sp-cAMP (a cAMP analog), forskolin (FSK, adenylate cyclase activator that enhances cAMP production), and inhibitors of phosphodiesterases (PDE, cAMP degrading enzyme) successfully induces transcription- and protein synthesis-dependent long-lasting potentiation which occludes electrically induced L-LTP (Frey et al. 1993; Huang and Kandel 1994; Slack and Walsh 1995; Abel et al. 1997; Selbach et al. 1998; Woo et al. 2002). The fact that PKA activation itself is sufficient for L-LTP expression strongly suggests that PKA mediates tagging and capture processes. Indeed, PKA is required for STC. PKA inhibitor KT5720 treatment and expression of a dominant negative PKA regulatory subunit impair STC (Young et al. 2006). PKA activation by a PDE inhibitor, caffeine facilitates STC (Sajikumar et al. 2009). Also, PDE4 inhibitor, rolipram augments protein synthesis-dependent L-LTP and STC (Navakkode et al. 2004). Moreover, LFS activates phosphatases that resets tagging by dephosphorylation of PKA targets (Sajikumar and Frey 2004; Young et al. 2006). In line with this, inhibition of protein phosphatase 1 (PP1) by PKA is critical for L-LTP expression, and PP1 inhibitors rescues L-LTP deficits in mice expressing a dominant negative PKA regulatory subunit (Blitzer et al. 1998; Woo et al. 2002). Importantly, PKA inhibitors are effective only when they are treated during the induction phase of STC (Barco et al. 2002; Young et al. 2006). This transient involvement of PKA in STC complies with the requirement for being a tag molecule (see Sect. 5.1). However, PKA is not the only tag molecule that has been proposed, and we will discuss how PKA interacts with other candidate tagging mechanisms in the next section.

5.4 Other Potential Tagging Mechanisms Possibly Mediated by PKA

NMDA Receptors (NMDARs) are required for L-LTP and LTM (Morris et al. 1986; Nguyen and Woo 2003). The NMDAR has been suggested as a tagging molecule because the NMDAR inhibitor AP5 blocks STC, although PRPs are still available for being captured (Barco et al. 2002). It is worth noting that Ca^{2+}-influx through NMDARs activates Ca^{2+}-dependent ACs, which results in the activation of PKA. Also, increased Ca^{2+} permeability of the NMDAR by PKA phosphorylation facilitates LTP (Skeberdis et al. 2006). Therefore PKA, at least in part, mediates tagging processes involving NMDARs.

CaMKII is activated by Ca^{2+}-influx through NMDARs after LTP induction, and is another strong candidate tag molecule. Pharmacological inhibition of CaMKII blocks STC, providing evidence that STC requires CaMKII. Also, inhibition of CaMKII does not affect PRP production and subsequent capture of PRPs (Redondo et al. 2010). Following NMDAR stimulation, an active form of CaMKII moves to the postsynaptic density (PSD) of activated dendritic spines, then phosphorylates its targets (Shen and Meyer 1999). Because PKA modulates NMDAR-mediated Ca^{2+} signaling, it is likely that PKA and CaMKII act in concert in the process of tagging. Indeed, it has been reported that inhibition of PP1 by PKA gates CaMKII signaling by preventing dephosphorylation of CaMKII during L-LTP expression (Blitzer et al. 1998). However, the role of CaMKII as a tag is not clear. Unlike PKA phosphorylation of AMPA receptor (AMPAR) subunit GluR1 at S845, CaMKII-mediated phosphorylation of GluR1 at S831 does not increase open probability of the receptor (Banke et al. 2000). In addition, a GFP reporter flanked by the 5′- and 3′-UTR of CaMKII shows local dendritic synthesis after stimulation, and the protein level of CaMKII increases in dendrites within 5 min after tetanization (Ouyang et al. 1999; Aakalu et al. 2001). Dendritic local synthesis of CaMKII does not comply with the criteria for being a tag, since the tagging process is independent of protein synthesis. Therefore, CaMKII is possibly a component of PRPs.

TrkB is a tyrosine kinase that has been suggested as a potential tag. Its ligand is brain-derived neurotrophic factor (BDNF), which will be discussed later as a strong candidate PRP (see Sect. 5.5). Bath application of BDNF induces protein synthesis-dependent long-lasting potentiation, suggesting that BDNF-TrkB signaling is sufficient to elicit tagging and capture processes as PKA activation does (Kang et al. 1996; Messaoudi et al. 2002). Inhibition of TrkB blocks STC and a behavioral version of tagging, and TrkB activation after stimulation lasts about 1–2 h. Also, TrkB activation does not require protein synthesis (Lu et al. 2011). Therefore, TrkB satisfies the requirements for a tag. In fact, PKA activation gates BDNF-induced TrkB phosphorylation (Ji et al. 2005). In addition, TrkB phosphorylation is increased after PKA activation by forskolin (Patterson et al. 2001). This suggests that PKA is upstream of BDNF-TrkB signaling and that the described roles of TrkB as a tag are likely modulated by PKA.

Actin Dynamics are important for the structural modification of synapses and memory formation (Krucker et al. 2000; Lisman 2003; Chen et al. 2007; Hou et al. 2009). NMDAR-dependent actin polymerization in dendritic spines is critical for L-LTP (Lin et al. 2005), and BDNF signaling modulates this process (Rex et al. 2007). LFS reverses LTP by depolymerizing actin (Kramár et al. 2006). Moreover, inhibition of actin polymerization impairs tagging process but does not affect PRP production (Ramachandran and Frey 2009). Actin remodeling is controlled by cofilin, an actin depolymerizing factor. Cofilin-mediated actin dynamics regulate spine morphology and AMPAR trafficking during synaptic plasticity (Chen et al. 2007; Gu et al. 2010). Phosphorylation of cofilin by LIM kinase inhibits its activity, which allows actin polymerization. In fact, the activity of LIM kinase is modulated by PKA (Lamprecht and LeDoux 2004; Nadella et al. 2009).

5.5 Plasticity-Related Products

As a tag, PKA interacts with plasticity-related products (PRPs) by regulating the synthesis and function of these gene products. PRPs are produced from gene transcription and protein synthesis after neuronal activity. While it was initially believed that only proteins produced in the soma serve as PRPs, it is now widely accepted that mRNAs also serve as PRPs by its dendritic targeting and subsequent local dendritic translation. mRNAs packaged in RNA granules are transported close to synapses in a translationally silent state (Mayford et al. 1996; Krichevsky and Kosik 2001; Wang et al. 2010a). Upon LTP induction, polyribosomes and local translation machinery at spine necks are activated to translate these locally targeted mRNAs (Steward and Schuman 2001; Ostroff et al. 2002; Kelleher et al. 2004b).

PKA activates the transcription factor cAMP response element (CRE)-binding protein (CREB) to promote CRE-driven gene expression critical for both L-LTP and LTM (Impey et al. 1996, 1998b). In addition, CREB-dependent gene expression facilitates synaptic plasticity including STC in both *Aplysia* and mice (Martin et al. 1997; Casadio et al. 1999; Barco et al. 2002). Thus, CREB-mediated CRE-driven gene expression provides a pool of PRPs critical for both L-LTP and LTM. Infusion of PKA inhibitor into the nucleus blocks CREB phosphorylation and impairs L-LTP, but not E-LTP (Matsushita et al. 2001). PKA facilitates nuclear translocation of extracellular-signal-regulated protein kinase (ERK), which leads to CREB phosphorylation (Impey et al. 1998a; Roberson et al. 1999; Patterson et al. 2001). Therefore, PKA directly and indirectly activates CREB. In addition to nuclear gene transcription, translation of mRNAs contributes to PRP production. Dendritic protein synthesis is mainly controlled by mitogen-activated protein kinase (MAPK), BDNF-TrkB signaling pathways, and actin dynamics (Kelleher et al. 2004a; Kuczewski et al. 2010; Santos et al. 2010). PKA cross-talks with the MAPK pathway at multiple levels (Gerits et al. 2008) and modulates BDNF-TrkB signaling and actin dynamics as described earlier (see Sect. 5.4). Collectively, PKA attributes to PRP production both at the level of transcription and protein synthesis.

As a CREB target gene, BDNF has been proposed as the strongest candidate PRP (Tao et al. 1998; Barco et al. 2005). Activity-dependent dendritic targeting and expression of BDNF and TrkB support this idea (Tongiorgi et al. 1997). BDNF promotes synaptic remodeling through actin dynamics, PSD reconstitution, and local translation during L-LTP (Kang and Schuman 1996; Liao et al. 2007; Rex et al. 2007; Yoshii and Constantine-Paton 2007). Additionally, BDNF deletion in the hippocampus impairs STC (Barco et al. 2005). These observations suggest multiple roles of BDNF such that it is not only produced as a PRP but also induces production of other PRPs and aids tag setting, all of which are modulated by PKA (also see Sect. 5.4 TrkB).

Another well-known candidate PRP is the AMPAR. AMPAR trafficking and incorporation into synapses that are regulated by both actin and PKA are critical for L-LTP expression (Malinow et al. 2000; Sheng and Lee 2001; Esteban et al. 2003). PKA phosphorylation at S845 of GluR1 promotes an increase in open probability, frequency, and duration of the receptor that leads to stable LTP expression (Greengard et al. 1991; Banke et al. 2000; Esteban et al. 2003). Although AMPARs can be considered as tags, since their activity and incorporation make synapses more excitable, they are more likely PRPs because their requirement for synaptic plasticity is not transient and their synthesis is required for their rapid turnover. Indeed, the maintenance phase of LTP requires a PKA-mediated increase in AMPAR synthesis 3 h after LTP induction (Nayak et al. 1998; Yao et al. 2008). Taken together, PKA regulates the property, trafficking, as well as synthesis of AMPARs to ensure L-LTP expression.

5.6 The Role of PKA Anchoring in Synaptic Plasticity and Memory Formation

In the previous sections, we discussed PKA as a key molecule mediating the processes of STC, in which synaptic inputs from the two independent pathways (S1 and S2) are integrated. This pathway-specificity is a unique property of neuronal communication that can only be achieved by highly compartmentalized and spatially restricted cellular signaling. This is surprising because secondary messengers including cAMP, kinases such as PKA, mRNAs, and many proteins are diffusible throughout the cell. This suggests that there is a way to localize signaling molecules together to ensure spatially restricted signaling. Spatially compartmentalized PKA signaling is achieved by PKA anchoring proteins, or AKAPs. There are more than 50 AKAPs that are localized to specific intracellular regions. By binding PKA regulatory subunits as well as other signaling molecules, AKAPs provide a compartmentalized pool of PKA signaling (Colledge and Scott 1999; Michel and Scott 2002). The importance of the compartmentalized PKA signaling in synaptic plasticity and memory formation is confirmed by pharmacological and genetic disruption of PKA anchoring by the PKA anchoring disrupting peptide Ht31. This peptide is derived from the human thyroid anchoring protein that binds PKA and has been used to block anchoring of PKA without affecting PKA activity (Colledge and Scott 1999).

Conditional expression of Ht31 in neurons within the hippocampus impairs hippocampal L-LTP and hippocampus-dependent spatial memory, and reduces GluR1 S845 phosphorylation (Nie et al. 2007; Kim et al. 2011). Similar to PKA, the application of Ht31 peptide at different time point reveals that PKA anchoring is transiently required for L-LTP, not E-LTP, and STC during the induction phase (Huang et al. 2006; Havekes et al. 2012). In addition, PKA anchoring disruption by Ht31 reduces synaptic AMPARs and AMPAR currents and occludes long-term depression (LTD) (Rosenmund et al. 1994; Snyder et al. 2005). It should be noted that the effect of Ht31 directly matches the effect of PKA inhibition in synaptic plasticity and memory formation (see Sects. 5.2 and 5.3). This suggests that PKA exerts its activity through anchored signaling complexes controlled by AKAPs.

5.7 Examples of AKAPs Modulating Neuronal Function

In the brain, several AKAPs have been identified as scaffold proteins that tie PKA signaling to Ca^{2+} signaling, MAPK signaling, cytoskeletal dynamics, and gene expression mechanisms. Therefore, AKAPs contribute to the formation of highly coordinated signalosomes that are critical for synaptic plasticity and neuronal information processing.

AKAP5/79/150 is targeted to the plasma membrane and associated with PSD-95. It recruits NMDARs, AMPARs, $GABA_A$ receptors, L-type Ca^{2+} channels, K^+ channels, synapse-associated protein (SAP)-97, PKC, protein phosphatase 2B (PP2B or calcineurin), β-adrenergic receptors (β-ARs), as well as PKA (Bregman et al. 1989; Carr et al. 1992; Coghlan et al. 1995; Gao et al. 1997; Fraser et al. 2000; Colledge et al. 2000; Gomez et al. 2002; Brandon et al. 2003; Hoshi et al. 2003). Deletion of AKAP5/79/150 leads to impaired synaptic plasticity, altered AMPAR currents, and disrupted hippocampus-dependent spatial memory. AKAP5/79/150 also mediates nuclear PKA signaling, and perturbation of PKA anchoring reduces nuclear CREB phosphorylation (Feliciello et al. 1996, 1997).

Gravin (*AKAP12/250*), also known as Src-suppressed C kinase substrate (SSecks) in mice, binds not only PKA but also other signaling molecules including PKC, calmodulin, PP2B, β-ARs, actin, and PDE4D (Lin et al. 1996; Nauert et al. 1997; Shih et al. 1999; Lin and Gelman 2002; Willoughby et al. 2006). By bringing PDEs close to ACs, Gravin provides cAMP gradients to shape compartmentalized PKA signaling. Gravin is also localized to the actin cytoskeleton and regulates actin remodeling (Lin et al. 1996; Gelman et al. 1998). Its localization to the plasma membrane, the endoplasmic reticulum, and the perinuclear region has also been reported (Streb et al. 2004). Along with AKAP 5/79/150, it mediates PKA phosphorylation of β-ARs that leads to desensitization of the receptor and activation of the MAPK pathway (Daaka et al. 1997; Baillie et al. 2003). Mice lacking the α-isoform of Gravin show impaired PKA-dependent L-LTP, β-AR-mediated metaplasticity, and hippocampus-dependent contextual fear memory, possibly due to reduced phosphorylation of β-ARs and MAPK (Havekes et al. 2012). Interestingly, FSK-mediated long-lasting potentiation is not affected in these mice, suggesting

that the cellwide activation of PKA overcomes compartmentalization barriers or that the presence of other AKAPs is sufficient to support this form of potentiation (Havekes et al. 2012).

Microtubule-Associated Protein 2 (MAP2) is the first identified AKAP. As a predominantly expressed AKAP in the brain, MAP2 binds a third of neuronal PKA, and regulates microtubule stabilization and long-distance transport along dendrites and axons (Theurkauf and Vallee 1982; Sánchez et al. 2000). As a dominant AKAP, MAP2 establishes a pool of PKA along dendritic shafts so that, upon cAMP elevation, catalytic subunits of PKA can rapidly translocate to dendritic spines for synaptic plasticity (Zhong et al. 2009). In addition, loss of MAP2 results in reduction of the total amount of PKA and CREB phosphorylation (Harada et al. 2002). The latter suggests MAP2-mediated synapse-to-nucleus signaling of PKA. Deletion of the PKA binding site of MAP2 results in abnormal CA1 architecture and disruption of contextual fear memory (Khuchua et al. 2003). Also, MAP2 mRNA is the first mRNA found to be targeted to dendrites for subsequent local synthesis of the protein (Garner et al. 1988; Steward and Halpain 1999).

5.8 PKA-Centric Unified Model of Synaptic Tagging and Capture

Since first being described in 1997, a large number of studies have proposed various molecules and processes as the mechanisms of synaptic tagging and capture. Although the identity of tagging and capture processes is still elusive, it is likely the collective interaction of molecules, rather than a single molecule, that accounts for these processes. Considering the crucial role of PKA in synaptic plasticity and memory formation, a AKAP-mediated compartmentalized pool of signaling complexes could contribute to the heterosynaptic nature of information processing in the brain, here represented as STC.

When a set of synapses receives supra-threshold stimulation, Ca^{2+}-influx through NMDARs and activation of neuromodulatory GPCRs trigger a large increase of cAMP production by ACs. Following the cAMP wave, a large amount of PKA is activated from both the reserve pool in the dendritic shafts maintained by MAP2 and the local pool maintained by AKAPs in the spine. Activated dendritic PKA then enters the activated spine and interacts with NMDARs, AMPARs, TrkB, and Ca^{2+} signaling cascades in concert with locally activated PKA in the spine (tagging). Having a reserve pool in dendritic shafts is an efficient way to supply PKA to activated synapses on demand. PKA from this reserve pool promotes gene transcription by activating CREB in the nucleus. Also, the cross-talk between PKA and MAPK signaling initiates protein synthesis to produce PRPs such as BDNF. PKA gates subsequent BDNF-TrkB signaling to augment protein synthesis and synaptic remodeling. In addition, PKA regulates AMPAR trafficking via actin dynamics (capture). AKAPs tightly regulate all of these processes by clustering signaling components so that stable L-LTP in this set of synapses is ensured (Fig. 5.2a).

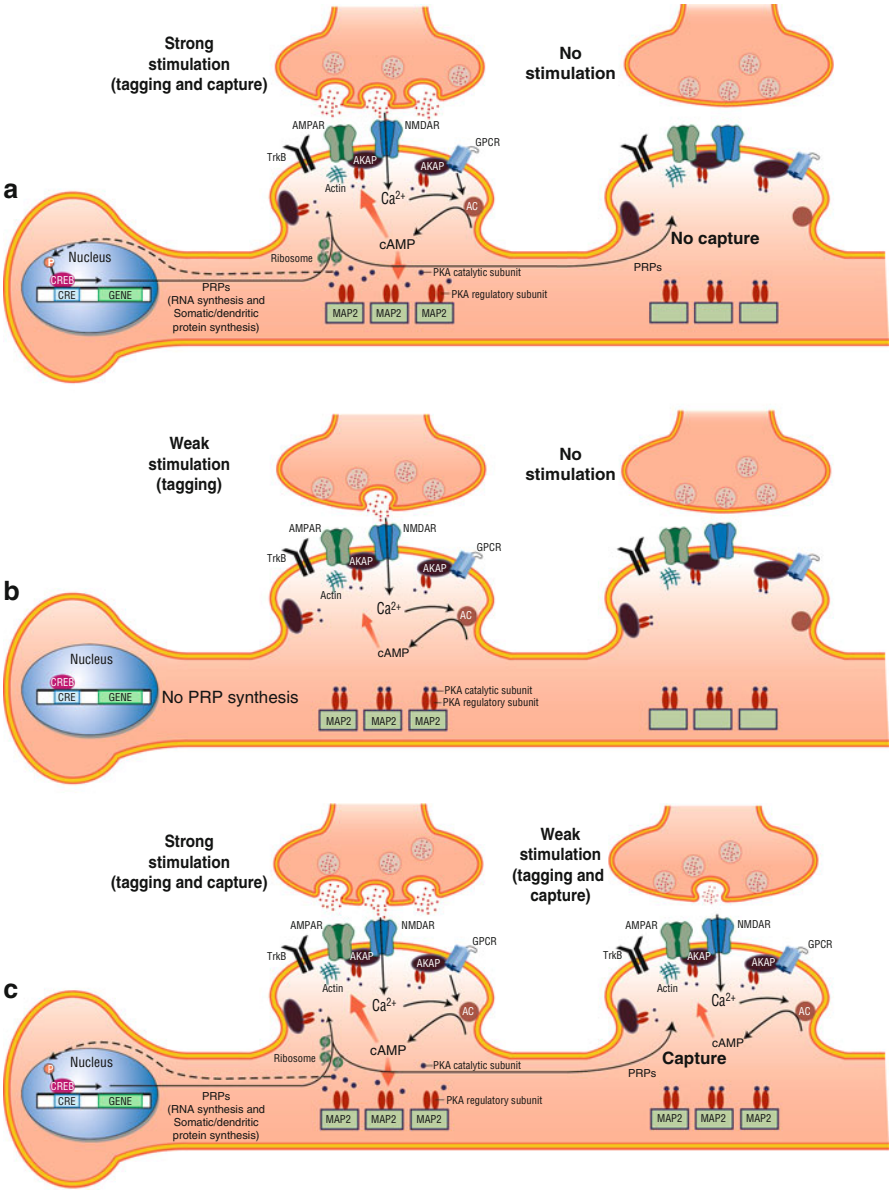

Fig. 5.2 A PKA-centric model of synaptic tagging and capture. (**a**) In the strong pathway, a large amount of cAMP is produced by ACs through NMDAR- and GPCR-mediated processes, which in turn activates PKA from both the local AKAP pool in the spine and the dendritic MAP2 pool. Once activated, PKA catalytic subunits are liberated from PKA regulatory subunits to induce CREB-mediated gene expression and somatic/dendritic protein synthesis to produce PRPs, as well as tagging the activated synapses by priming molecules in the spine (e.g. NMDARs, AMPARs, and TrkB). Upon arrival of PRPs into the spine, only the tagged synapses interact with/capture PRPs (possibly through AMPAR trafficking or BDNF-TrkB signaling) to strengthen the pathway. (**b**) The weak pathway has a sub-threshold level of cAMP produced by NMDAR-mediated Ca^{2+}-dependent AC activation resulting in local activation of PKA only in the spine, which establishes tags in the synapses but does not induce PRP production. (**c**) The weak pathway is strengthened if PRPs from the strong pathway are captured by the tags when the two pathways are paired

When a set of synapses receives sub-threshold stimulation, the amount of activated PKA is not sufficient to induce PRP production, and E-LTP is induced. PKA bound to only local AKAPs tags the set of synapses by priming synaptic proteins such as NMDARs, AMPARs, and TrkB (Fig. 5.2b). If this E-LTP pathway is paired with the L-LTP pathway, PRPs produced by the L-LTP pathway can be captured by the E-LTP pathway, so that E-LTP is transformed to L-LTP. For example, BDNF produced by the L-LTP pathway can strengthen synaptic connection of the weak pathway by interacting with the *primed* signaling molecules such as TrkB (Fig. 5.2c).

In summary, PKA activation through NMDAR activity alone can only set tags at a subset of synapses by priming local targets such as NMDARs, AMPARs, and TrkB. PKA phosphorylation of these targets fades over time, which creates a limited time window of tagging. However, PKA activation through both NMDARs and neuromodulatory GPCRs triggers PRP production as well as local tag setting. PKA-mediated capture processes involve the interaction of PRPs with their signaling partners that have already been primed by PKA (e.g. BDNF-TrkB signaling gated by PKA). Finally, AKAP supervises the heterosynaptic coordination of complex signaling by tethering signaling participants together at the synapse.

5.9 Future Directions

Most of the literature included in this chapter focuses on the postsynaptic mechanisms of STC. Due to technical challenges, it is hard to assess potential presynaptic components of STC. However, there is evidence supporting the presynaptic role of PKA activity and PKA anchoring in L-LTP and memory formation. Long-lasting potentiations induced by Sp-cAMP or FSK relies on increased presynaptic transmitter release (Chavez-Noriega and Stevens 1994; Bolshakov et al. 1997). Additionally, studies using transgenic mice expressing Ht31 suggest that presynaptic CA3-PKA anchoring is required for theta-burst L-LTP and spatial memory (Nie et al. 2007). Moreover, the induction of theta-burst L-LTP increases the release of BDNF as well as synaptic vesicles from presynaptic terminals (Zakharenko et al. 2003). Also, BDNF deletion in both CA3 and CA1 results in complete impairment of STC, while BDNF deletion in only postsynaptic CA1 has a delayed impairment suggesting that BDNF in presynaptic CA3 has a role in the early phase of STC (Barco et al. 2005). To investigate the presynaptic role of PKA and PKA anchoring in STC, genetic approaches specifically blocking PKA anchoring or PKA activity in presynaptic CA3 will be necessary.

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Chapter 6
Activity-Dependent Protein Transport as a Synaptic Tag

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Abstract The "synaptic tagging and capture" hypothesis proposed that a hypothetical, cell biological mark is activated in the synapses undergoing early-phase plasticity. Newly synthesized plasticity-related proteins (PRPs) are assumed to establish late plasticity only in the marked synapses after unspecific transport along dendrites from soma. Demonstration of the "synaptic tagging and capture" hypothesis will be achieved by showing that a specific cell biological activity regulates behaviors of an exemplifying PRP in accordance with several unique characteristics assumed by the original hypothesis. We hypothesized that synaptic activity affects synaptic localization of PRPs on transport, namely, active spines receive PRPs, while no transport to inactive spines. We observed transport of Vesl-1S (also called Homer-1a) protein, one of PRPs, by measuring fluorescence of fused protein with EGFP (VE) in spines, and found that somatic Vesl-1S protein prevailed in most dendritic branches, and was translocated into spines where NMDA receptors were activated. The NMDA receptor-dependent translocation of VE protein from dendrite to spine fulfilled many of the hypothesized conditions of synaptic tagging, demonstrating the synaptic tagging hypothesis with Vesl-1S as an exemplifying PRP.

In addition to summarizing our findings, we would like to discuss the relevance of synaptic tagging as an input-specificity mechanism of late plasticity. An input-specificity mechanism restricts synapses where the expression mechanism of plasticity is activated. An essential feature of late plasticity is that it depends on synaptic functions of multiple PRPs, which is newly synthesized in various loci and lags. Late expression mechanism may require integrated functions of multiple PRPs, each of which likely has distinct localization, regulation, and function in the synapse. Synaptic tagging is a mechanism that allows synapse-specific function of PRPs,

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thereby assumed as a late input-specificity mechanism. Considering diversity in cell biological and biochemical properties of PRPs, it is suggested that multiple cell biological activities work as synaptic tagging, each of which is specific to a subset of PRPs and differently regulates synaptic localization and function of the PRPs at distinct timing. Activity-dependent spine translocation of Vesl-1S/Homer-1a may be an example of the diverse spectrum of synaptic tagging mechanisms.

Keywords Synaptic tagging • Late plasticity • Spine • PRP • Transport • Input-specificity • Expression mechanism

6.1 Relevance of Synaptic Tagging in Late Plasticity

The synaptic tagging hypothesis was proposed in 1997 by Frey and Morris as a mechanism underlying late associative plasticity which depends on new protein synthesis (Frey and Morris 1997). Although activation of synaptic tagging can be detected by associative late-phase long-term potentiation (L-LTP) in two-pathway experiments (for details see other articles in this book such as one by Redondo and Morris), it was difficult to define synaptic tagging in a cell biological sense. Martin and Kosik (2002) listed three conditions for a synaptic tag: it should be (1) spatially restricted, (2) time-limited and reversible, and (3) able to interact with cell-wide molecular events that occur after strong stimulation to produce long-term synapse-specific strengthening. Now that associative late-phase long-term depression was reported to involve a synaptic tagging mechanism (Sajikumar and Frey 2004), and taking into account of the fact that the essential difference of late plasticity from early phase is dependent on protein synthesis, the third condition can be substituted with the following; a synaptic tag should be able to interact with cell-wide molecular events that occur after particular stimulation to produce a protein synthesis-dependent, synapse-specific, and persistent modification of synapse strength.

Nevertheless, wide variety of cell biological activities can be nominated as candidates of synaptic tag. Thus, to figure out the molecular entity and cell biological mechanism of synaptic tagging, we had to start from considering its neurobiological relevance. In this short review, we would first like to discuss the relevance of synaptic tagging as an input-specificity mechanism of late plasticity and relationship to the expression mechanism of late plasticity.

6.1.1 Synaptic Tagging as an Input-Specificity Mechanism of Late Plasticity

Studies on the early phase of long-term potentiation (E-LTP) have revealed major molecular mechanisms for the input-specificity (Collingridge 2003) and the expression (Nicoll 2003). Of the two, the input-specificity mechanism is an upstream component that launches the expression mechanism in restricted synapses.

E-LTP can be evoked by various methods such as high frequency stimulation (Bliss and Lømo 1973), theta burst stimulation (Larson et al. 1986), pairing of presynaptic activation, and postsynaptic depolarization (Magee and Johnston 1997), and synchronized firing of both pre- and postsynaptic cells (spike timing-dependent plasticity) (Markram et al. 1997). In these protocols, both pre- and postsynaptic cells are coincidently excited, as Hebb (1949) assumed in his famous monograph. Depolarization input-specifically releases the NMDA receptor channel from blockade by magnesium, and another immediately following glutamate activates the same receptor to elicit calcium influx through it (Novak et al. 1984). In the hippocampal CA1 pyramidal-Schaffer-collateral synapses, or the dentate gyrus granule cell-perforant path synapses, calcium influx through NMDA receptors is known to be necessary and sufficient to elicit E-LTP (Malenka et al. 1988) and believed to be the coincidence detector for the input-specificity mechanism (Collingridge 2003).

On the other hand, given the dependency of late plasticity on induction of a new set of gene expression (Krug et al. 1984; Frey et al. 1988), the late expression mechanism must involve synaptic functions of new PRPs. Competition among associative L-LTP of multiple synapses was observed when protein synthesis was limited (Fonseca et al. 2004), supporting the idea that late expression depends on synaptic functions of PRPs. The late input-specificity mechanism should trigger synaptic functions of PRPs. Synaptic tagging was proposed as a late input-specificity mechanism that enables PRP functions in the restricted synapses.

The synaptic tagging hypothesis assumes that a synaptic tag is activated by synaptic inputs that evoke early-phase plasticity. Furthermore, an important feature of PRP was revealed by the experiments comparing with a counter-hypothesis, the mail hypothesis, in which each PRP is born to be delivered specifically to the synapse that had received the plasticity-evoking inputs. Two-pathway experiments excluded the mail hypothesis (Frey and Morris 1997), and supported the view of synaptic tagging hypothesis in which PRPs are transported in an unspecific manner along dendrites thereby available in all synapses as suggested in Fonseca et al. (2004). Thus, synaptic tagging hypothesis proposes not only an input-specificity mechanism of late plasticity, but also a thorough story for PRPs, in which PRPs synthesized in soma prevail in all dendrites and function only in the "tagged" synapse to express late plasticity.

6.1.2 Two Possibilities of Synaptic Tagging Action

The idea that the input-specificity mechanism triggers the expression mechanism suggests that two processes involved in late expression can be regulated by synaptic tagging, namely, synaptic localization of PRP and synaptic adaptation. A simpler possibility of the cell biological activity for synaptic tagging is input-specific regulation of PRP localization in synaptic area, because PRP transported unspecifically along dendrites should be captured by the tagged synapse before functioning there. Our research showed an example of this mechanism (Okada et al. 2009).

On the other hand, synaptic adaptation involves input-specific modulation of the synapse. The modulation is induced by expression mechanisms of early plasticity and results in PRP acceptance in the synapses. Increase in surface expression of synaptic GluA receptors (electrophysiological plasticity) and enlargement of spine head (morphological plasticity) are considered as major expression mechanisms of the early plasticity, whereas up to present, consensus on the expression mechanism of late plasticity has not been reached. It is likely that late expression mechanisms modulate the functional structure of the synapse built by the early expression and make it persistent. For example, synaptic F-actin increased in an L-LTP-dependent manner (Fukazawa et al. 2003). This increase may be preceded by a transient decrease in synaptic F-actin which was NMDA receptor-dependent (Ouyang et al. 2005), suggesting remodeling of F-actin in spines was promoted by early plasticity. Thus, early plasticity likely alters molecular composition, size, and activity of the postsynaptic protein complex. If the altered state of the complex is a prerequisite for integration of new PRPs, only synapses expressing early plasticity can express late plasticity, and we refer to this alteration of molecular states in the synapse as synaptic adaptation. Although a special case of synaptic adaptation was experimentally excluded (Frey and Morris 1998a), we guess that the general idea of synaptic adaptation mechanisms may be consistent with synaptic functions of some PRPs. Furthermore, synaptic adaptation and synaptic localization are not mutually exclusive as principles of synaptic tagging.

6.2 Strategy to Reach the Cell Biological Activity of Synaptic Tagging

In this chapter, we would like to describe our strategy to define cell biological activity for synaptic tagging.

6.2.1 Advantage and Limitations of Two-Pathway Protocol

The two-pathway experiment, which measures associative L-LTP, has been the exclusive method to detect activation of synaptic tagging (Frey and Morris 1997). By the use of inhibitors, two-pathway experiments revealed many molecules involved in associative L-LTP and L-LTD, such as PDE4 (Navakkode et al. 2004), PKMζ (Sajikumar et al. 2005), PKA, ERK1/2, CaMKII and PKMζ (Sajikumar et al. 2007), AKAP (Huang et al. 2006), PKA (Young et al. 2006), neuropsin (Ishikawa et al. 2008), and F-actin (Ramachandran and Frey 2009). However, involvement of these molecules in synaptic tagging is difficult to be concluded from these experiments.

Accumulating knowledge on mechanisms underlying late plasticity suggests that concerted actions of multiple internal processes are required for late expression. Internal processes include preceding early plasticity, induction of new PRP gene expression, PRP production and transport, synaptic tagging and capture, and PRP functioning in the targeted synapses (Reymann and Frey 2007). Furthermore, one molecule can be involved in many of the multiple internal processes. For example, NMDA receptors are known to contribute to multiple processes involved in late plasticity such as early plasticity (Collingridge 2003), induction of gene expression (Cole et al. 1989), and PRP transport into spine (Okada et al. 2009). Under such a complicated situation, an inhibitor specific to a molecule that is involved in only synaptic tagging process should be used to conclude involvement of a molecule in synaptic tagging.

To circumvent such an agnostic difficulty, it is obvious that synaptic tagging activity itself should be directly measured. But, what activity should be measured? We decided to first hypothesize a possible activity that conforms to synaptic tagging hypothesis, and then accumulate experimental evidence supporting it.

6.2.2 Controlled Transport Across Dendrite–Spine Boundary

As discussed in previous section, the late expression is achieved by function and localization of PRPs in synapses and synaptic tagging enables PRPs to do so. We paid much attention to PRP transport, which is a prerequisite process for synaptic functions of PRPs. Since PRP transport from soma to dendrites is unspecific (Frey and Morris 1997), we focused PRP transport from dendrite to synapse. This transport may have two steps, namely, entry from dendrite to synaptic area and integration to preexisting synaptic protein complex. Both of them can be activity-dependent (as discussed in Sect. 6.1.2), thereby promising candidate for synaptic tagging.

Proteins and vesicles are transported in dendrite along cytoskeletons, a major part of which is microtubule–kinesin system (Brady 1995), while dendritic transmembrane proteins are carried by myosin motors (Lewis et al. 2009). Microtubules are not found in spines where F-actin–myosin system is predominant (Lebeux and Willemot 1975). Cargoes should switch from microtubule to F-actin to enter spines, and actually such switching was observed in neurons (Shakiryanova et al. 2006; Correia et al. 2008) and non-neuronal cells (Kuroda and Fukuda 2004), while direct interaction between kinesin and myosin was also observed (Huang et al. 1999). Protein trafficking in and out of a spine is limited, and sometimes activity-dependent (Bloodgood and Sabatini 2005). Spine localization of several proteins such as CaMKII (Shen and Meyer 1999), AMPA receptors (Ehlers et al. 2007; Matsuo et al. 2008), profilin (Ackermann and Matus 2003), Arc (Moga et al. 2004), and neurabin/Lfc (Ryan et al. 2005) was reported to be regulated by synaptic activity.

These observations suggest that transport of some synaptic proteins from dendrite to spine depends on synaptic activity, which suggests an example of synaptic

tagging in accordance with the synaptic localization theory discussed in Sect. 6.1.2). This idea can be directly tested by tracking the movement of a PRP from soma to spines. We decided to ask whether the PRP prevails in all dendrites before it enters spines activity dependently.

6.2.3 Critical Assumptions of Our Hypothesis

We hypothesized that a synaptic tagging mechanism activity dependently captures PRP in dendrite to translocate it into spines. To test our hypothesis, we referred to known characteristics of synaptic tagging (Frey and Morris 1997, 1998a, b), which can be summarized as follows: (1) PRPs prevail in all dendrites, namely, they are transported unspecifically in dendrites. (2) E-LTP evoking stimulus, such as NMDA receptor activation, activates synaptic tagging input-specifically. (3) Activation of synaptic tagging is independent of protein synthesis. (4) Once activated, synaptic tag is active for a few hours. These are essentially identical to the synaptic tagging conditions described in Kelleher et al. (2004).

6.2.4 Use of Vesl-1S for the Tracer PRP

Next, we selected a PRP suitable for the tracer of input-specific transport to spines. The tracer protein should be newly synthesized in soma after intense neuronal activity evoking late plasticity, localized in the synapse, and needed for late plasticity. We selected Vesl-1S (Homer-1a) protein as the tracer among our list of genes induced during late plasticity (Matsuo et al. 2000). Vesl-1S protein is one of the proteins that fulfill all of these criteria for the tracer PRP. Furthermore, its role in the late plasticity was known as following. Vesl proteins are one of the postsynaptic scaffolding proteins which bind to mGlu1/5 receptors (Brakeman et al. 1997; Kato et al. 1998), IP3 receptors (Tu et al. 1998), Shank (Tu et al. 1999), TrpC channel (Yuan et al. 2003) and so on. Long forms of Vesl/Homer proteins are tetrameric (Hayashi et al. 2006), make a postsynaptic network with Shank (Hayashi et al. 2009), and regulate constitutive activity of mGluRs (Ango et al. 2001). On the other hand, short form of Vesl/Homer protein such as Vesl-1S/Homer-1a is an immediate-early gene product inducible in L-LTP in the dentate gyrus (Kato et al. 1997; Brakeman et al. 1997). Knock in mice lacking Vesl-1S shows abnormal long-term memory (Inoue et al. 2009). Its mRNA was detected in soma, but so far not in dendrites (Kato et al. 1997; Matsumoto et al. 2007). Vesl-1S/Homer-1a works as a dominant negative form of long form Vesl/Homer proteins (Xiao et al. 1998). Glutamate stimulation caused biphasic changes in long-form Homer-1c clusters in neurons; first reduced then later increased (Inoue et al. 2007). This transient decrease was coincided with Homer-1a accumulation and biphasic changes were totally dependent on Homer-1a expression. These observations strongly suggested that

Vesl-1S/Homer-1a dissociated the preexisting Homer clusters, which was required for later enhancement of the cluster. Thus, Vesl-1S/Homer-1a is not directly involved in persistency of late plasticity, but it plays a key role as an initiator of PSD rearrangement prerequisite for late plasticity.

6.3 Results

6.3.1 Activity-Dependent Regulation of Spine Translocation of Vesl-1S/Homer-1a Protein as a Synaptic Tagging

We tested our hypothesis by measuring fluorescence of EGFP-fused Vesl-1S/Homer-1a (VE) proteins in dendritic spines of primarily cultured neurons of rat hippocampus (Okada et al. 2009). We tested whether synaptic activity regulates Vesl-1S localization in the activated spines, and whether this activity-dependent spine translocation fulfills above-mentioned four known characteristics of synaptic tagging.

Cells were seeded on a coverslip at a high density (10^5 cells/1.8 cm^2) and transfected with the Vesl-promoter-driven VE plasmid on DIV9-12. Fluorescence was observed after growth of spines on DIV18-25. The Vesl promoter has moderate induction power restricted in neurons, thus, a limited portion of neurons expressed moderate levels of VE protein. VE fluorescence was observed homogeneously in entire neurons including soma, axon, and dendrites. Mushroom-shaped spines of pyramidal cell-like neurons and granule cell-like neurons were tested, and difference among them was not observed. Spine head size was not usually changed by the applied stimuli, and spines with significant change in size and shape were excluded from analysis.

During growing-up incubation, elaborate synaptic connection was autonomously built among neurons. These neurons fire spontaneously, evoking glutamatergic transmission in many spines. Since this transmission is spontaneous, synaptic NMDA receptors can be selectively activated by changing the extracellular medium into magnesium-free artificial cerebrospinal fluid (Mg-free ACSF). Brief application of Mg-free ACSF increased spine fluorescence gradually and persistently, which was not affected by inhibitors of proteasome and protein synthesis, suggesting the increase is resulted by translocation of VE proteins from dendrites to spines (#3 of the synaptic tagging characteristics described in Sect. 6.2.3).

Translocation of soma-derived Vesl-1S/Homer-1a into spines was detected by the use of photoactivatable GFP (PAGFP) instead of EGFP. PAGFP-Vesl1S (PAV) was photoconverted only in soma by scanning illumination of small laser spots. The fluorescing PAV prevailed in most dendritic branches, but do not enter into spines (#1 of the synaptic tagging characteristics described in Sect. 6.2.3). PAV entered spines which received local activation of NMDA receptor by means of microperfusion (#2, see Fig. 6.1a). Furthermore, spine translocation activity lasted for 3 h (#4, see the next section for details). Similar movement was not found when EGFP

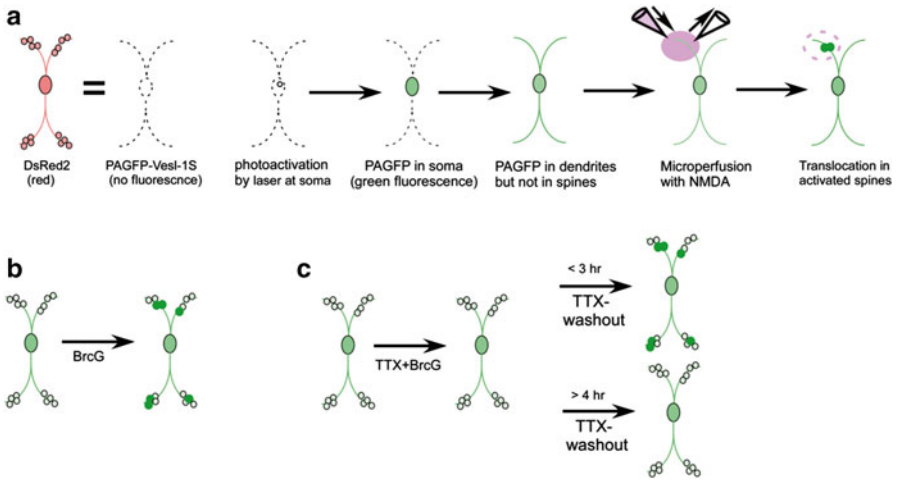

Fig. 6.1 The cartoon illustrates neurons with spines (*small circles*). Fluorescence protein translocation is shown by darker colors in the spines. (**a**) Experiments with VPA, indicating input-specific spine translocation of soma-derived VPA. (**b**) 8-Bromo cyclic GMP (BrcGMP) application for 20-min activated VE translocation. (**c**) BrcGMP failed VE translocation in the presence of TTX. VE translocation activity regained by TTX washout 0.5–3 h after BrcGMP, but not 4 h, suggesting VE translocation was reversibly and persistently activated

was used instead of VE or PAV, indicating that Vesl-1S/Homer-1a, a PRP, is the target of the transport. We concluded that NMDA receptor-dependent control of VE transport to spine conforms to the synaptic tagging hypothesis (Fig. 6.2). Thus, synaptic tagging hypothesis was demonstrated with an exemplifying PRP, Vesl-1S/Homer-1a.

6.3.2 Molecular Mechanisms Underlying VE Protein Transport to Spine

Further studies revealed that spine translocation of VE protein involved two distinct reactions, protein kinase G (PKG) and a TTX-sensitive component. We searched downstream signals of NMDA receptor activation and found involvement of extracellular calcium, indicating that calcium influx through NMDA receptors activates both early phase expression and the synaptic tagging. Intracellular increases in calcium ions usually trigger multiple reactions and we found that calcium-dependent nitric oxide (NO) production was involved in spine translocation of VE proteins. NO can spread across membranes, however, a water-soluble NO scavenger, *N*-(dithiocarboxy)-sarcosine complexed with iron, in the extracellular medium (a condition under which extracellular NO is scavenged) did not affect NMDA receptor-dependent spine translocation of VE proteins, suggesting that the generator

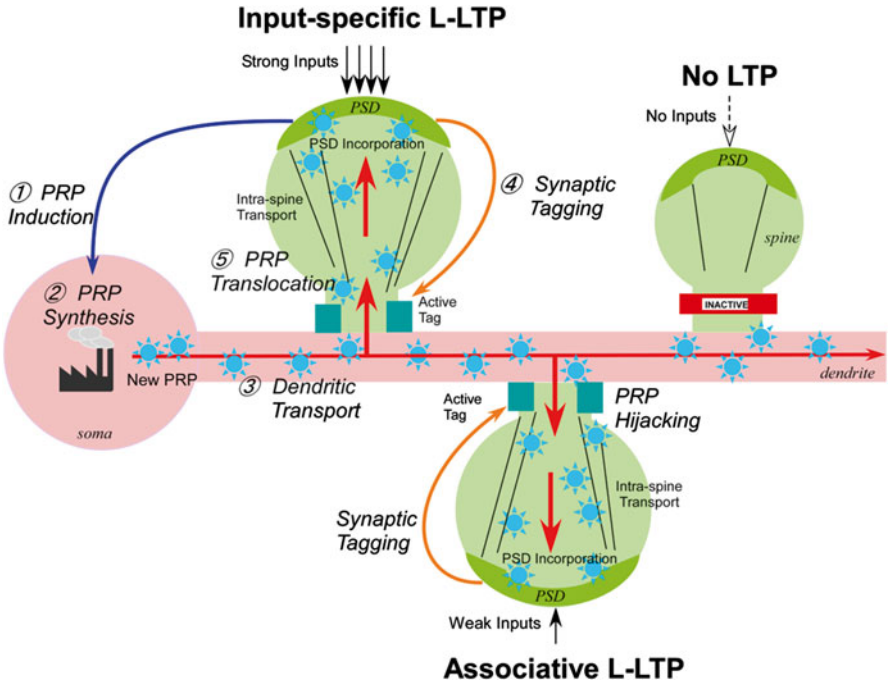

Fig. 6.2 "Synaptic tagging and capture" by activity-dependent PRP translocation into spine. Strong inputs to one synapse evoke input-specific late-LTP through the following processes. First, synaptic inputs (*1*) activate somatic PRP synthesis (*2*). New PRPs are transported along dendrites unspecifically (*3*). On the other hand, some downstream signals of the strong inputs activate synaptic tagging (*4*). Activated synaptic tag facilitates PRP translocation from dendrite to spine. We speculate that PRPs are translocated from the dendritic transport system to the intraspine transport. PRPs are thus to be incorporated into PSD or other synaptic machinery and contribute to expression of input-specific late-LTP. PRP translocation takes place also in the synapses received weak inputs because weak inputs that can evoke early LTP are assumed to activate synaptic tagging. Synapses without receiving inputs are not tagged, therefore, PRP translocation does not occur there, resulting in failure in LTP

and target molecules of NO were in the same intracellular space. NMDA receptor activation was mimicked by a membrane permeable cyclic GMP analogue, 8-bromo cyclic GMP (BrcGMP), suggesting that the major contribution of NO in the VE transport was PKG activation through cyclic GMP production. Involvement of NO, a diffusible activator of soluble guanylyl cyclase, indicates an involvement of a moderately remote interaction between the postsynaptic site near the NMDA receptor and the spine–dendrite border, which is consistent with the idea that synaptic activity regulates interaction with material transport in the dendrite. The nature of this interaction is the subject of the future study.

Frey and Morris (1998a) reported that the synaptic tagging has a lifetime of 1–2 h from observations of time-dependent decay of the associativity between weak and strong stimuli for L-LTP. We observed that BrcGMP could not activate VE

translocation in the presence of tetrodotoxin (TTX), while washout of TTX within 3 h after BrcGMP restored VE translocation (Fig. 6.1b, c). These results suggested that spine translocation of VE proteins was promoted by some unknown factor released in an activity-dependent manner. These results also suggested that PKG or its downstream was persistently active for 3 h, which is longer than the time window observed in slice preparation (Frey and Morris 1998a). A shorter time window (30 min) for the association between activities of the perforant path and the basolateral amygdala in behaving animals was reported (Frey et al. 2001). Behavioral tagging experiments showed ~1–2 h of the time window (Ballarini et al. 2009). The life time of associativity and VE translocation may be different, and depends on the experimental configuration, such as temperature and complexity of the processes. Higher stringency (shorter lifetime) is expected for more complex systems.

We also tested involvement of mGluR1, mGluR5, PKA, PKCα/β2, and trkB, in VE translocation using specific inhibitors such as CPCCOEt, MPEP, PKAI, GF109203X, and K252a, respectively (Okada et al. 2009). All of these drugs had no effect on VE transport into spines, while some of them were implicated in associative late plasticity, for example, PKA (Sajikumar et al. 2007) and trkB (Lu et al. 2011). They may be involved in the internal processes of late plasticity other than PRP translocation. In addition, AKAP (Huang et al. 2006), CaMKII (Okuno et al. 2012), CaMKIV, MEK1/2 (Sajikumar et al. 2007), PKMζ (Ling et al. 2002; Sajikumar et al. 2005), and PDE4B4 (Navakkode et al. 2004) are reported to be involved in associative late plasticity, but we did not confirm their involvement in VE translocation.

6.4 Perspectives

6.4.1 Multiple Mechanisms for Synaptic Tagging

It is noted that late plasticity requires induction of hundreds of new PRPs (Nedivi et al. 1993; Matsuo et al. 2000). These PRPs occur in synapses after varied lags, depending on their promoters or transport systems. It is therefore likely that the synaptic tag should be persistently active to cover lags of all PRPs required for late plasticity. It is not clear whether an exclusive tag is activated during the entire lifetime of synaptic tagging, or multiple tags for synaptic functions of individual PRPs are activated during portions of the lifetime. Since synaptic tagging is closely linked to the expression mechanism, we speculate each PRP may be introduced in the late expression by distinct synaptic tagging mechanisms suitable for each PRP. The idea of the multiple tagging emphasizes that the late plasticity is established as the final consequence of concerted actions of PRPs having distinct localization and roles (Fig. 6.3). PRP may be classified into several groups in the light of tagging mechanism. Proteins interacting each other may be transported in the same vesicles, such as Homer/Vesl and class I metabotropic glutamate receptors (Ango et al. 2000). Cell biological activities other than spine translocalization can be synaptic tagging, such as synaptic adaptation.

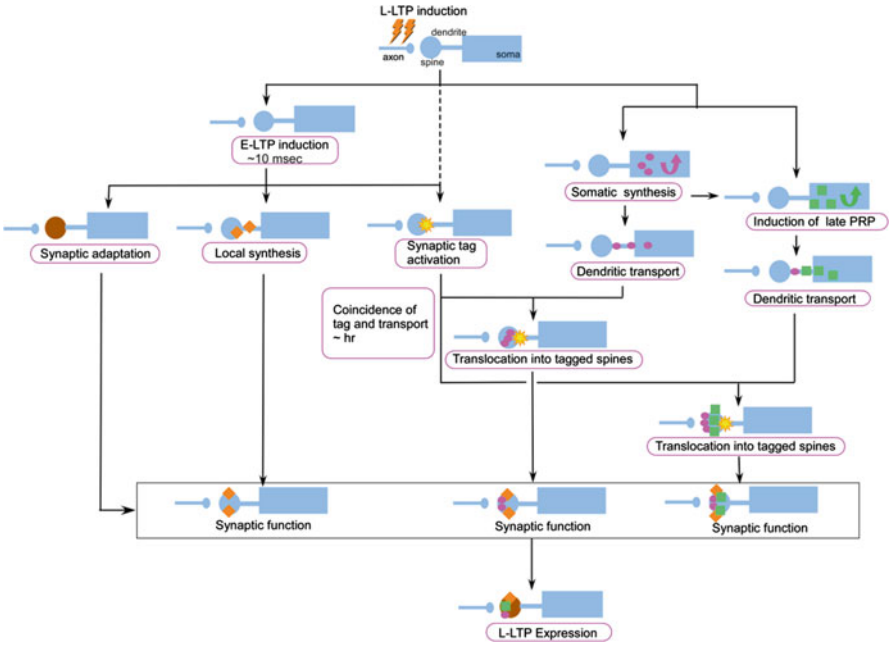

Fig. 6.3 A hypothesis of multiple PRP-specific tagging for late expression. This hypothesis assumes that each PRP contributes to late expression in distinct manners, because they are produced at discrete timing and location, and require dissimilar localization and conditions for individual synaptic functions. L-LTP inducing inputs activate somatic synthesis of PRPs and the early phase of LTP (E-LTP). E-LTP in turn activates three processes, synaptic adaptation (expressed by *brown spine*), local synthesis, and synaptic tag activation (indicated by *yellow spiny symbols*). Local synthesis supplies new PRP at a shortest lag behind synaptic activation, and these PRPs (depicted by *orange diamonds*) may contribute to synaptic strengthening as well as adaptation. Synaptic tagging depicted here is activity-dependent PRP translocation and cooperates with dendritic transport of PRPs. Since hundreds of PRPs are newly synthesized with ranged lags (depicted as to two kinds of PRPs indicated by *magenta circles* and *green squares*, respectively), coincidence of the tag activation and PRP synthesis-transport should be persistent for hours, while the coincidence time window of E-LTP is as narrow as 10 ms. Cell biological activities other than the translocation regulation can also be synaptic tag but not included in this figure. All PRPs are gathered in the tag-activated synapses and contribute in late expression in concert (indicated by the square integrating synaptic functions of classes of PRPs)

6.4.2 Molecular Events Involved in Late Expression

In this section, we discuss molecules implicated as major contributors in the late expression mechanism. These proteins may also characterize distinct groups in synaptic tagging diversity.

AMPA Receptor Insertion

Increase in surface expression of GluA receptors is considered as a major expression mechanism for E-LTP (Bliss and Collingridge 2013). GluA receptor surface expression is dynamically regulated by membrane-vesicle fusion in a manner depending on the subunit composition (Shi et al. 2001). Extrasynaptic receptors are also highly mobile and GluA receptors conjugated with Stargazin proteins are fixed in the synaptic region by binding to PSD-95 proteins (Opazo et al. 2012). This slot hypothesis for receptor surface expression is a good model for stabilization of membrane-sorted receptors and one of the possible underlying mechanisms of the early expression. Scaffolding proteins such as Homer and Shank also contribute to the receptor stabilization by forming sub-membrane networks that regulate receptor localization, recycling, and postsynaptic reconstruction (Xiao et al. 1998; Lu et al. 2007; Hayashi et al. 2009). While the early expression is reversible and lasts for a few hours in vitro, the late expression lasts far longer. One of the possible late expression mechanisms may be increments in the slot number using new component proteins supplied as new PRPs. This situation is associated with an increased number of total molecules in a spine, leading to PSD increment (Desmond and Levy 1986) and spine head enlargement as observed in matured spines (Matsuzaki et al. 2004). Thus, GluA receptor trafficking is supported and regulated by structural components of postsynaptic density. Activity-dependent regulation of these proteins is one of the possible major expression mechanisms, thereby the targets of synaptic tagging.

F-actin Network

F-actin content in spines is essential for morphological plasticity. Spine head size is enlarged transiently in E-LTP (Matsuzaki et al. 2004) and persistently in L-LTP in a BDNF- and protein-synthesis-dependent manner in the hippocampus (Tanaka et al. 2008), but not in the cerebellum (Sdrulla and Linden 2007). Accordingly, E-LTP is associated with a dynamic increase in spine F-actin (Okamoto et al. 2004), while F-actin is accumulated in synapse layer of dentate granule cells in L-LTP (Fukazawa et al. 2003).

F-actin is the major cytoskeleton and the transporting rail in the spines; therefore, its increase is necessary in the era of spine enlargement to transport the increasing numbers of molecules for the support of the increased functions. This consideration suggests that F-actin network should be stabilized in the late plasticity. Synaptopodin is an actin-binding protein, expressed a few hours after L-LTP induction as a late PRP (Yamazaki et al. 2001) and enhanced surface expression of GluA receptors through Ca^{2+} release from the spine apparatus (Vlachos et al. 2009). Overexpression of Synaptopodin stabilized spine head enlargement after NMDA receptor stimulation of cultured hippocampal neurons (Okubo-Suzuki et al. 2008). Synaptopodin-deficient mice lacked spine apparatus, and did not show E- and L-LTP (Deller et al. 2003). Thus, Synaptopodin can be involved in the late expression mechanism through F-actin stabilization.

Degradation of Preexisting PSD

Postsynaptic protein complexes including receptor scaffolding and F-actin networks are reconstructed during L-LTP by posttranslational modification of preexisting PSD proteins. Phosphorylation regulates protein–protein interaction, while polyubiquitination triggers protein degradation and was involved in LTP maintenance (Fonseca et al. 2006) and memory reconsolidation (Lee et al. 2008). Molecules that destroy or destabilize the integration of the postsynaptic protein network should be immediately degraded after disintegration of preexisting structure. For example, Vesl-1S/Homer-1a possesses a PEST sequence for proteasomal degradation and rapidly degraded in spines (Ageta et al. 2001). Other mechanisms of protein degradation such as Caspase3 (Li et al. 2010), and autophagy (Shehata et al. 2012) were reported to have influence on plasticity and memory.

Extracellular Component

Extracellular molecules such as Cadherins (Tang et al. 1998) and β-catenin (Murase et al. 2002) were implicated in LTP. These molecules are known to be involved in intracellular signaling as well as a cell adhesion, and affect synaptic size, transmission efficacy, and transcription regulation.

EphB receptor is a cell adhesion molecule interacting with the Ephrin ligand, and involved in spine formation and synaptic plasticity by facilitating glutamate receptor clustering (Henkemeyer et al. 2003). Although regulatory mechanisms of these extracellular molecules are not well known, they may fall in the important category of synaptic tagging or expression mechanism of late plasticity. For example, Neuropsin is an extracellular protease implicated in late associative LTP (Ishikawa et al. 2008) and reported to cleave EphB2 in the amygdala which triggers modulation of anxiety (Attwood et al. 2011).

Brain-Derived Neurotrophic Factor (BDNF)

BDNF and its receptor trkB are also implicated in synaptic and behavioral tagging. BDNF is essential for protein-synthesis-dependent persistent structural plasticity of dendritic spines (Tanaka et al. 2008). TrkB is transiently activated by E-LTP evoking theta burst in confined synapses in a manner independent of protein synthesis, which was necessary for associative L-LTP and behavioral tagging (Lu et al. 2011). Although TrkB activation seems to fulfill conditions of synaptic tagging, input-specific TrkB phosphorylation implies spatially restricted action of BDNF. The release mechanism of BDNF is not elucidated (Bramham and Messaoudi 2005).

Distributed Plasticity

Although input-specific expression was considered as an essential feature of E-LTP, distributed expression has been consistently observed, which appeared within the same dendritic branch (Engert and Bonhoeffer 1997). Input-specific E-LTP persistently activated Rho-GTPase within surrounding area of 5 μm distance, suggesting distribution of plastic changes among synapses in vicinity (Murakoshi et al. 2011). Computational and electrophysiological studies suggested that NMDA spike propagation within a dendritic segment is the key for distributed LTP (Polsky et al. 2004). These observations suggest that molecules or activities spreading or propagating within dendrites are involved in tagging or expression mechanisms underlying distributed plasticity. In accordance with these observations, cluster plasticity hypothesis was suggested (Govindarajan et al. 2006) with experimental demonstrations using hippocampal slices (Govindarajan et al. 2011).

6.4.3 Local Synthesis

PRP supply does not necessarily require the synaptic tagging, when the PRP is translated by local synthesis, another input-specificity mechanism for late plasticity. Subcellular fractionation and electron microscopic studies demonstrated that dendrites and postsynaptic area contained mRNAs for some PRPs which are induced and functioning in late plasticity (Eberwine et al., 2001). Dendritic transport of mRNA involves RNA granules which contain various mRNAs (Anderson and Kedersha 2006), and stability of mRNA is regulated in part by miRNAs (Shouten et al. 2013). Local synthesis was excluded from associative late plasticity in the original report of synaptic tagging (Frey and Morris 1997), while it is involved in persistent plasticity in other conditions. For example, mRNA for GluA1 receptor was found in dendrites, and its translation was activated by synaptic activity, enabling input-specific supply of new receptors even in spines of dendrites without physical connection to the soma (Ju et al. 2004). However, local synthesis and somatic synthesis are not mutually exclusive; rather they may work in concert. For instance, PRP such as α-calcium-calmodulin-dependent protein kinase II may be supplied by both mechanisms (Miller et al. 2002). It is noted that local synthesis achieves PRP delivery to the activated synapses more rapidly than somatic synthesis does. PRPs supplied by these mechanisms may have distinct roles in early and late expression mechanisms. Availability of mRNA for particular PRPs in relation with the translational machinery may function as the synaptic tagging as reported in Aplysia neurons (Wang et al. 2009).

It is noted that protein synthesis dependence of late plasticity has been often shown by the use of protein synthesis inhibitors such as anisomycin, a blocker of peptidyltransferase reaction in eukaryotic mRNA–ribosomal complex. Anisomycin is also known to activate MAP kinases (Takenaka et al. 1998), and enhance noradrenaline release in the amygdala (Sadowski et al. 2011), suggesting its effects on cellular function other than protein synthesis.

6.4.4 *Synaptic Tagging as a Cellular Mechanism of Memory Association*

According to Hebb's theory, the neuronal entity of a brain function is a cell assembly built up through plastic changes in synaptic connection (Hebb 1949). According to sparse coding theory, only a few synapses among 10^3–10^5 undergo plastic changes in an activity-dependent manner (Olshausen and Field 2004). Consistently, small portions of neurons were activated during learning episodes, and recall of the late memory reactivated fewer neurons (Reijmers et al. 2007). Optogenetical manipulation showed that reactivation of a set of neurons that had been activated during learning caused recall of the experience, supporting Hebb's theory (Liu et al. 2012).

We usually do not remember every component of an experience. Especially in the late memory, fewer components can be recalled later. This well-recognized feature of our memory, selection of recallable subjects in an experience, may be closely related to the function of synaptic tagging in the late memory. Original synaptic tagging is proposed for cellular level observation and considered as an input-specificity mechanism for late plasticity, which selects synapses undergoing plastic changes. Under the above-mentioned assumption by Hebb, this cellular role of synaptic tagging can be translated into systems level. Because individual axons transfer separate sets of information composing an experience, selection of emphasized synapses is equivalent to that of memory subjects.

Another feature of our memory is association. Above consideration on the role of synaptic tagging in the subject selection suggests that two distinct memories can be associated when the cell assembly representing each memory contains synapses that are activated commonly by the two memories. This idea for neuronal basis of memory association was also suggested by the behavioral tagging revealed by the "behavioral two-pathway" protocol (Ballarini et al. 2009), in which weak conditioning of a task (causing short memory) was reinforced (becoming persistent memory) by strong conditioning of another task.

Episode memory first represented in the hippocampal network is thought to be sent to the cerebral cortices representing individual modalities of the episode, to form remote memory. During memory transfer from hippocampus to multiple cortices, there must be some rule to recall many of the memory components and reproduce the episode memory as a whole. This rule is not known, but a possibility was suggested as memory tag (Lesburgueres et al. 2011). In this way, synaptic, behavioral and memory tagging contribute to the consistency of restored and recalled memory, by generating proper sets of synaptic connections and neuron networks. Deficits of synaptic tagging may thus disrupt such life-spanning association among experiences. For example, post-traumatic stress disorder may occur through association of a traumatic component and any details of the event.

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Chapter 7
mTOR and the Regulation of Translational Capacity in Late Forms of Synaptic Plasticity

Panayiotis Tsokas and Robert D. Blitzer

Abstract Stimulus-induced changes in gene expression, mediated by translation and transcription, are required for persistent forms of synaptic plasticity (late-LTP and late-LTD) and for memory consolidation. In recent years, the search for the translational control mechanisms that operate during synaptic plasticity has centered on signaling within the dendritic compartment, guided by the accumulating evidence that proteins synthesized within dendrites, and not in the cell body, are required for late-LTP and late-LTD. Among the first such control mechanisms to be discovered was the activation of mechanistic target of rapamycin (mTOR; formerly called mammalian TOR), a ubiquitous translational regulator that is required for the induction of late-LTP and late-LTD. The consistent requirement of mTOR in multiple forms of late plasticity suggests that it is involved in the synthesis of both LTP- and LTD-related plasticity-related proteins (PRPs).

This chapter focuses on the importance of mTOR-regulated translational capacity in neurons and other cells that must rapidly synthesize proteins in response to appropriate stimuli. The role of mTOR in synaptic plasticity, and the potential mechanisms by which synaptic activity activates mTOR, are addressed. We review the behavioral studies that established the relevance of mTOR to long-term memory (LTM), including the consolidation and reconsolidation of hippocampus-dependent LTM, and the acquisition and expression of drug-seeking behavior. Finally, we outline a model of synaptic capture in which activation of mTOR raises the

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translational capacity of the dendrite from a low resting state, facilitating the input-specific synthesis of PRPs from dendritic mRNAs that are locally de-repressed by synaptic activity.

Keywords Hippocampus • mTOR • Translational capacity • Long-term potentiation • Long-term depression • Long-term memory • Addiction • Synaptic tagging • Synaptic capture

Abbreviations

4EBP	eIF4E-binding protein
AMPK	5′ Adenosine monophosphate-activated kinase
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CP-AMPAR	Ca^{2+}-permeable AMPA receptor
eEF	Eukaryotic elongation factor
eEF2K	eEF2 kinase
eIF	Eukaryotic initiation factor
FMRP	Fragile X mental retardation protein
GSK3	Glycogen synthetase kinase 3
HFS	High-frequency stimulation
IRS-1	Insulin receptor substrate 1
LFS	Low-frequency stimulation
mTOR	Mechanistic target of rapamycin
PABP	Poly(A)-binding protein
PDK1	Phosphoinositide-dependent kinase 1
PI3K	Phosphoinositide 3-kinase
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate (PIP3)
PRP	Plasticity-related protein
PSD	Postsynaptic density
S6K	rpS6 kinase
TOP	Terminal oligopyrimidine tract
TSC	Tuberous sclerosis complex
VGCC	Voltage-gated Ca^{2+} channel
ZBP1	Zipcode-binding protein 1

7.1 The Concept of Translational Capacity and Its Regulation

The process of mRNA translation in eukaryotes is intricate and highly orchestrated, involving approximately 100 different proteins that are considered core components of the translational machinery: eukaryotic initiation factors (eIFs), eukaryotic elongation factors (eEFs), and ribosomal proteins (for a review, see Jackson et al. 2010).

In addition, a large number of other molecules—not only proteins, but also various classes of RNAs (transfer, ribosomal, and messenger, as well as microRNAs and other non-coding RNAs)—participate in the translational response to signals arising from extracellular transmitters or from within the cell. A key determinant of the *translational capacity* of the cell is the availability of the necessary core components.

Depending on the rate of protein synthesis that is required for normal cell function, maintaining sufficient translational capacity can consume a large fraction of the cell's metabolic resources (Warner 1999). However, translational demand is not invariant: many types of cell are intermittently faced with an acute need to synthesize proteins at an accelerated rate in response to growth factors, cytokines, and other excitatory events. To accommodate periods of high translational demand while remaining metabolically efficient, cells increase translational capacity in response to appropriate cues, and allow capacity to decline during the intervening periods of relative quiescence. This form of regulation is mediated by mTOR. Importantly, such an on-demand increase in translational capacity is prominent in the dendrites of hippocampal neurons during the induction of late forms of synaptic plasticity (Cammalleri et al. 2003; Takei et al. 2004; Tsokas et al. 2005).

7.2 mTOR Signaling

7.2.1 The Two mTOR Complexes: mTORC1 and mTORC2

mTOR is a serine/threonine kinase that is found in two molecular complexes that differ in their regulatory inputs and substrates: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Laplante and Sabatini 2012). Of the two, mTORC1 has been much more extensively studied, and its role in regulating translation is relatively well understood.

The substrate selectivity of mTORC1 is conferred by raptor (regulatory-associated protein of mTOR), which brings together mTOR and target proteins that contain the TOS (target of rapamycin substrate) sequence. mTORC1 activity rapidly up-regulates protein synthesis in two general ways: (1) by facilitating the synthesis of translational machinery, and (2) by activating certain pre-formed translation factors. Both of these mechanisms have been implicated in the induction of late forms of synaptic plasticity. At longer time scales, activation of mTORC1 leads to ribosome biogenesis (in keeping with its role in setting translational capacity) and the induction of autophagy. While we owe much of our current understanding of the mTORC1 pathway to experiments on proliferating cells, it has become apparent that mTORC1 also is highly regulated in many terminally differentiated cells, including neurons of the adult brain.

Much less is known about the inputs and functions of mTORC2. With respect to the composition of the complex, the most important difference from mTORC1 is the absence of raptor, and the presence of rictor (rapamycin-insensitive companion of mTOR), which is obligatory for the phosphorylation of at least some mTORC2

substrates. In neurons, mTORC2 is involved in cytoskeletal remodeling, and recently was implicated in morphological changes associated with LTP induction (Angliker and Ruegg 2013; Huang et al. 2013). In addition, mTORC2 is necessary for full activation of the mTORC1 pathway, as discussed in Sect. 7.2.2.

7.2.2 The Regulation of mTORC1

Most events that activate mTORC1 do so through the PI3K–Akt–TSC2 pathway (Fig. 7.1). As an example, the activation of mTORC1 by brain-derived neurotrophic factor (BDNF) will serve to introduce the components of this canonical pathway (for a recent overview of mTORC1 regulation, see Shimobayashi and Hall 2014). Upon binding BDNF, TrkB receptor subunits dimerize and cross-phosphorylate cytoplasmic tyrosine residues, creating a binding site for the adapter protein insulin receptor substrate 1 (IRS-1). Activated IRS-1, in turn, stimulates phosphoinositide 3-kinase (PI3K), a membrane lipid kinase that causes phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to accumulate in the cell membrane. PIP3 recruits certain cytosolic proteins that contain a pleckstrin homology (PH) domain, including phosphoinositide-dependent kinase 1 (PDK1) and its substrate, the protein kinase Akt. Co-localization of these proteins at the membrane allows PDK1 to stimulate Akt by phosphorylating Thr-308 in the activation loop. Akt then phosphorylates and inhibits the tumor suppressor TSC2 (tuberous sclerosis 2; also called tuberin). TSC2, functioning as a complex with TSC1 (also called hamartin), is the GTPase-activating protein for the small G-protein Rheb; thus, stimulation of Akt leads to an increase in active, GTP-bound Rheb. Finally, Rheb•GTP, through an as-yet-unknown mechanism, activates mTORC1.

The phosphorylation of Akt by PDK1 is a key step in the activation of mTORC1, but this is not sufficient to confer full Akt activity, which requires an additional phosphorylation at Ser-473 in the hydrophobic region (Alessi et al. 1996). For a decade, the kinase responsible for this second phosphorylation was unknown and referred to by the placeholder name "PDK2," until it was identified as mTORC2 (Sarbassov et al. 2005). Thus, mTORC2 acts upstream to enhance mTORC1 signaling.

There are other signaling pathways through which stimuli can activate mTORC1. In the context of synaptic plasticity, one of the most important of these is the ERK/MAPK pathway. Both ERK and its substrate RSK can phosphorylate and inhibit TSC2 at Akt-independent sites (Ma et al. 2005; Roux et al. 2004). In addition, RSK stimulates PDK1, suggesting that increases in ERK/MAPK signaling may compensate for modest co-localization of PDK1 and Akt at the membrane (Frödin et al. 2000; Tsokas et al. 2007a). Indeed, even when PI3K activity is at its resting level, ERK signaling leads to Akt phosphorylation in the hippocampus, although not necessarily to mTORC1 activation (Gelinas et al. 2007; Ma et al. 2011).

Under conditions of energy depletion (e.g., when glucose levels are low), mTORC1 fails to respond to stimuli that call for increased protein synthesis, ensuring

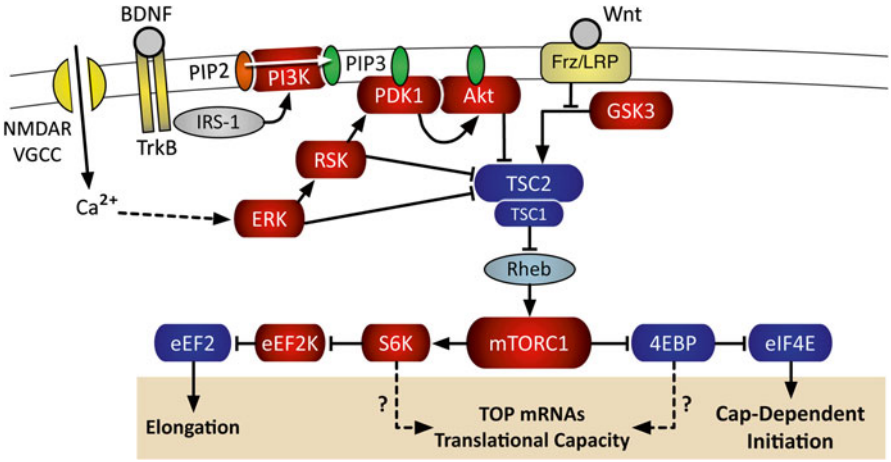

Fig. 7.1 Overview of the mTORC1 signaling pathway. The network depicted includes a subset of the known inputs to mTORC1, emphasizing mechanisms that are likely to be engaged by synaptic stimulation. BDNF binds to and activates TrkB receptors, which recruit the adapter protein IRS-1 and consequently activate the lipid kinase PI3K, leading to accumulation of PIP3 in the inner leaflet of the cell membrane. By binding to PIP3, the protein kinases PDK1 and Akt co-localize at the membrane, resulting in the activation of Akt. Among the many known targets of Akt is TSC2, which has GAP activity towards the small G-protein Rheb. Since Rheb in its GTP-bound form stimulates mTORC1 (through an unknown mechanism), TSC2 is a suppressor of mTORC1. TSC2 is inhibited by Akt-mediated phosphorylation, resulting in mTORC1 stimulation. This canonical cascade is subject to input from the Ras-ERK pathway at multiple points: ERK inhibits TSC2, and the ERK effector RSK not only inhibits TSC2 at different sites, but also acts upstream to stimulate PDK1. Ca^{2+} entry through VGCCs or NMDA receptors is likely to recruit Ras-ERK upon synaptic stimulation. Another synaptically activated input to mTORC1 is the Wnt signaling pathway, which disrupts the ability of basal GSK3 activity to phosphorylate and activate TSC2 that resides within the destruction complex. Downstream of mTORC1, two substrates are responsible for translational up-regulation through several mechanisms. The protein kinase S6K phosphorylates eEF2 kinase, resulting in disinhibition of the elongation factor eEF2. The other relevant mTORC1 substrate is 4EBP, which binds to the initiation factor eIF4E and thus interferes with cap-dependent initiation; phosphorylation of 4EBP by mTORC1 relieves this interference. Finally, mTORC1 facilitates the translation of TOP mRNAs and the consequent increase in translational capacity. The mechanism is uncertain; S6K and 4EBP both have been proposed as mediators. Not shown is the S6K-mediated phosphorylation of eIF4B, which facilitates ribosome scanning through secondary structure in the 5′ UTR (see text)

that available ATP equivalents are conserved for use in essential maintenance reactions. This brake on mTORC1 is mediated by 5′ AMP-activated protein kinase (AMPK), an enzyme that is active when the ratio of 5′ AMP to ATP is high. AMPK phosphorylates and *activates* TSC2, thus decreasing Rheb•GTP levels and inhibiting mTORC1 (Inoki et al. 2003). Another limitation on mTORC1 activation is the availability of amino acids, especially leucine. However, amino acids do not signal to mTORC1 through the PI3K–Akt–TSC2 pathway, but rather through the Rag family of small GTPases, which are required to recruit mTORC1 to the lysosome membrane where it can be activated by Rheb (Kim et al. 2008; Sancak et al. 2008).

7.2.3 Downstream of mTORC1

The acute effects of mTORC1 on protein synthesis are mediated through two effectors: S6 kinase (S6K) and eIF4E-binding protein (4EBP). Here, we discuss the mechanistic consequences of mTORC1 activation on translational proteins, and the roles of S6K and 4EBP in these effects.

Translation of TOP mRNAs: Building a Platform for Synthesis

mTORC1 preferentially induces the synthesis of a group of proteins encoded by the TOP mRNAs. In all members of this small group of abundant transcripts, there is an uninterrupted stretch of 4–14 pyrimidines—the terminal oligopyrimidine tract (TOP)—within the 5′ UTR immediately after the 5′ cap, beginning with an invariant cytosine (Meyuhas 2000). TOP mRNAs encode components of the protein synthesis machinery, including all of the 40S and 60S ribosomal proteins and all translation elongation factors. Additional proteins that are encoded by TOP mRNAs include subunits of translation initiation factor 3 (eIF3), and poly(A)-binding protein (PABP), which facilitates the translation of many mRNAs and has been implicated in plasticity-related protein synthesis (Udagawa et al. 2012; Khoutorsky et al. 2013). In response to a stimulus that activates mTORC1, the TOP mRNAs are recruited, in a nearly all-or-none manner, from a repressed state to the highly translated polysomal pool. Based on the rapid and coordinate increase in the translation of TOP mRNAs upon stimulation, it is probable that mTORC1 relieves a trans-acting repressor that recognizes the TOP motif; however, the mechanism of de-repression is unknown. For some time, activation of S6K was thought to be crucial for regulating TOP mRNA translation; however, this explanation was challenged by the observation that S6K is dispensable for the rapamycin-sensitive recruitment of TOP mRNAs under some conditions (Pende et al. 2004; Stolovich et al. 2002). As discussed in the next section, recent work points to a role for mTORC1-mediated phosphorylation of 4EBP in the regulation of TOP mRNA translation.

By increasing the translational capacity of the cell—or possibly a region of the cell, in the case of highly differentiated large cells such as pyramidal neurons—mTORC1 essentially builds a platform on which a second wave of synthesis can take place. Thus, it is not surprising that mTORC1 can affect the levels of many proteins that are not encoded by TOP mRNAs. In fact, inhibition of mTORC1 inhibition was found to reduce the expression of nearly the entire proteome of mouse embryonic fibroblasts (Thoreen et al. 2012). However, such a widespread effect of mTORC1 on protein expression might be more characteristic of proliferating cells than adult neurons, in which many mRNAs are conditionally repressed (see Sect. 7.5.5) and therefore unavailable for translation in the absence of an appropriate stimulus (Batish et al. 2012; Kindler et al. 2005). Thus, the set of proteins whose expression is acutely increased by mTORC1 activity in adult neurons would be limited to those whose mRNAs are in a de-repressed state, and therefore available for translation.

mTORC1 Effects on Cap-Dependent Translation

All eukaryotic mRNAs begin with a 5′ cap, which is a methylated guanine that is linked to the next nucleotide by a unique 5′–5′ triphosphate bond. In most cases, the initiation of translation requires that the cap be recruited to the 40S ribosome subunit, and this event constitutes a key translation control point. The recruitment depends on an association between initiation factors eIF4E (which binds the 5′ cap) and eIF4G (a scaffold that associates with the 40S subunit via eIF3). In the absence of mTORC1 activity, the interaction between eIF4E and eIF4G is impeded by a family of eIF4E-binding proteins (4EBPs; the predominant isoform in the mammalian brain is 4EBP2). mTORC1 phosphorylates 4EBP, thereby reducing its affinity for eIF4E and allowing the mRNA to bind to eIF4G.

Until recently, the ability of mTORC1 to inhibit of 4EBP was thought to be unrelated to the mTORC1-dependent up-regulation of TOP mRNA-encoded proteins. Surprisingly, deletion of 4EBP1 and 4EBP2 in mouse embryonic fibroblasts led to increased translation of TOP mRNAs, and this enhancement was largely independent of mTORC1 activity (Thoreen et al. 2012). However, the importance of 4EBP in regulating TOP mRNA translation in other cell types, including non-proliferating cells, remains to be established.

Effects of S6K on Initiation and Elongation

Although the role of S6K in TOP mRNA regulation is in doubt, this enzyme is known to enhance translation in other ways. First, S6K phosphorylates two stimulatory sites in the initiation factor eIF4B, greatly increasing the helicase activity of its partner eIF4A (Raught et al. 2004). This activity relaxes secondary structure that is present in the 5′ UTRs of many mRNAs, increasing the efficiency of ribosome scanning through to the start codon, where translation commences. In this manner, S6K may preferentially enhance the rate of initiation for mRNAs that have extensive structure in their 5′ UTRs.

S6K directly suppresses the activity of eukaryotic elongation factor 2 kinase (eEF2K), an enzyme that slows the rate of elongation by phosphorylating and inhibiting elongation factor 2 (eEF2) (Wang et al. 2001). Since eEF2 and the other elongation factors are encoded by TOP mRNAs, mTORC1 influences the rate of elongation in two distinct ways. This coherent feedforward effect of mTORC1 on elongation is noteworthy because translation usually is regulated mainly at the initiation step, when the decision is made to commit substantial translational machinery, including ribosomes and many initiation factors, for at least one round of translation. However, there are conditions where regulation of elongation can become relatively important (Browne and Proud 2002). One such situation is likely to occur in neuronal dendrites, where RNA granules contain mRNAs whose translation already has been initiated (Anderson and Kedersha 2006) (described in more

detail in Sect. 7.5.5). Such mRNAs are poised for rapid translation upon de-repression, and the rate of protein synthesis may be determined largely by the rate of elongation. Interestingly, eEF2K is directly stimulated by AMPK, which thus opposes mTORC1 activity both upstream by stimulating TSC2, and downstream at eEF2K (Browne et al. 2004).

Finally, by phosphorylating and inhibiting IRS-1, which mediates stimulation of the PI3K–Akt–TSC2 pathway by several growth factors, S6K negatively feeds back on mTORC1 signaling (Gual et al. 2005).

7.2.4 Rapamycin and Other Inhibitors of mTOR

Efforts to characterize the consequences of mTORC1 activity have relied heavily on rapamycin, a macrolide drug for which mTOR was named. Rapamycin (also called sirolimus) is the founding member of a class of mTORC1 inhibitors called rapalogs, which are used clinically to inhibit cell proliferation associated with transplant rejection and certain cancers. The rapamycin receptor is not mTOR itself, but rather the immunophilin FKBP12, and it is the FKBP12-rapamycin complex that inhibits mTORC1 (Sabatini et al. 1994). However, results from adult hippocampus indicate that FKBP12 might have some intrinsic inhibitory effect on translation even in the absence of rapamycin (Hoeffer et al. 2008). The FKBP12-rapamycin complex binds the FRB domain of mTOR and hinders substrate access to the active site; thus, rapamycin is not strictly a kinase inhibitor, but instead acts as a gate (Yang et al. 2013). However, the FKBP12-rapamycin gate is not equally effective against all mTORC1 substrates, which may explain why a relatively low concentration of rapamycin that effectively inhibits the phosphorylation of S6K is less effective against the phosphorylation of 4EBP (Patursky-Polischuk et al. 2009; Choo et al. 2008).

With respect to the selectivity of rapamycin for mTORC1 over mTORC2, a distinction must be made based on the duration of treatment: while rapamycin application for less than an hour largely spares mTORC2, more prolonged treatment can interfere with mTORC2 assembly, and strongly inhibits mTORC2 in some cell lines (Sarbassov et al. 2006). For this reason, studies in which rapamycin is administered for extended periods must be interpreted cautiously, since effects on plasticity or behavior may involve reduced mTORC2 function.

A more recently developed class of ATP-competitive inhibitors, including Torin 1 and PP242, act at the catalytic site of mTOR. These inhibitors block mTORC1-mediated phosphorylation of 4EBP and TOP mRNA up-regulation equally effectively (Thoreen et al. 2009, 2012; Benjamin et al. 2011). Unlike rapamycin and the other rapalogs, the ATP-competitive drugs inhibit intact mTORC2, and thus block all mTOR-mediated processes.

7.3 Evidence for a Role of mTORC1 in Late Forms of Synaptic Plasticity and Long-Term Memory

7.3.1 Long-Term Facilitation in Aplysia

Direct evidence for a role of mTORC1 in synaptic plasticity was first obtained in *Aplysia* sensorimotor cultures, where strong stimulation of individual synapses with serotonin induces a persistent, presynaptic, and synapse-specific long-term facilitation (LTF) that requires protein synthesis (Casadio et al. 1999). Application of rapamycin near the stimulated synapse prevented the induction of LTF, revealing a requirement for localized mTORC1 activity in LTF. Interestingly, the induction of LTF enabled synaptic capture by a weak stimulus at a second sensorimotor input, and this capture lasted depended on local protein synthesis as well as mTORC1 activity near the capturing synapse. Subsequently, serotonin was reported to activate mTORC1 in *Aplysia* synaptosomes, providing further evidence that mTORC1 is present and can be regulated near stimulated synapses (Khan et al. 2001).

7.3.2 Late-LTP in the Hippocampus

Following the initial report in *Aplysia*, a role for mTORC1 was demonstrated for mammalian synaptic plasticity. At the CA3-CA1 synapse, rapamycin blocks the expression of late-LTP, whether induced by synaptic stimulation or treatment with BDNF (Cammalleri et al. 2003; Tsokas et al. 2005; Raymond et al. 2002; Tang et al. 2002) (Fig. 7.2a$_1$). Importantly, mTORC1 activity is required specifically during induction of late-LTP by synaptic stimulation, and not during maintenance (Cammalleri et al. 2003). mTOR and its substrates S6K and 4EBP2 are expressed within the dendrites of CA1 pyramidal neurons, consistent with a role for mTORC1 in plasticity-related local protein synthesis. Moreover, a strong HFS protocol that induces late-LTP, but not a weak HFS protocol, results in the phosphorylation of dendritic S6K (Cammalleri et al. 2003; Tsokas et al. 2005; Tang et al. 2002) (Fig. 7.2a). This effect, which depends on PI3K and mTOR activity and NMDA receptors (NMDARs), suggests that dendritic translational capacity is responsive to synaptic stimulation and increases acutely during the induction of late-LTP (Cammalleri et al. 2003).

The concept of regulated dendritic translational capacity was strengthened by the discovery that strong HFS results in a rapid increase in the dendritic expression of multiple TOP mRNA-encoded proteins, including elongation factors 1A and 2 (eEF1A, eEF2), PABP, and the ribosomal protein S6 (Tsokas et al. 2007a) (Fig. 7.2b, c). Several lines of evidence pointed to the local elevation of capacity within dendrites, independent of any contribution from the cell body. For example, the mRNA encoding eEF1A was detected in the dendrites of CA1 pyramidal cells (Job and Eberwine 2001), and the expression of several TOP mRNA-encoded proteins increased

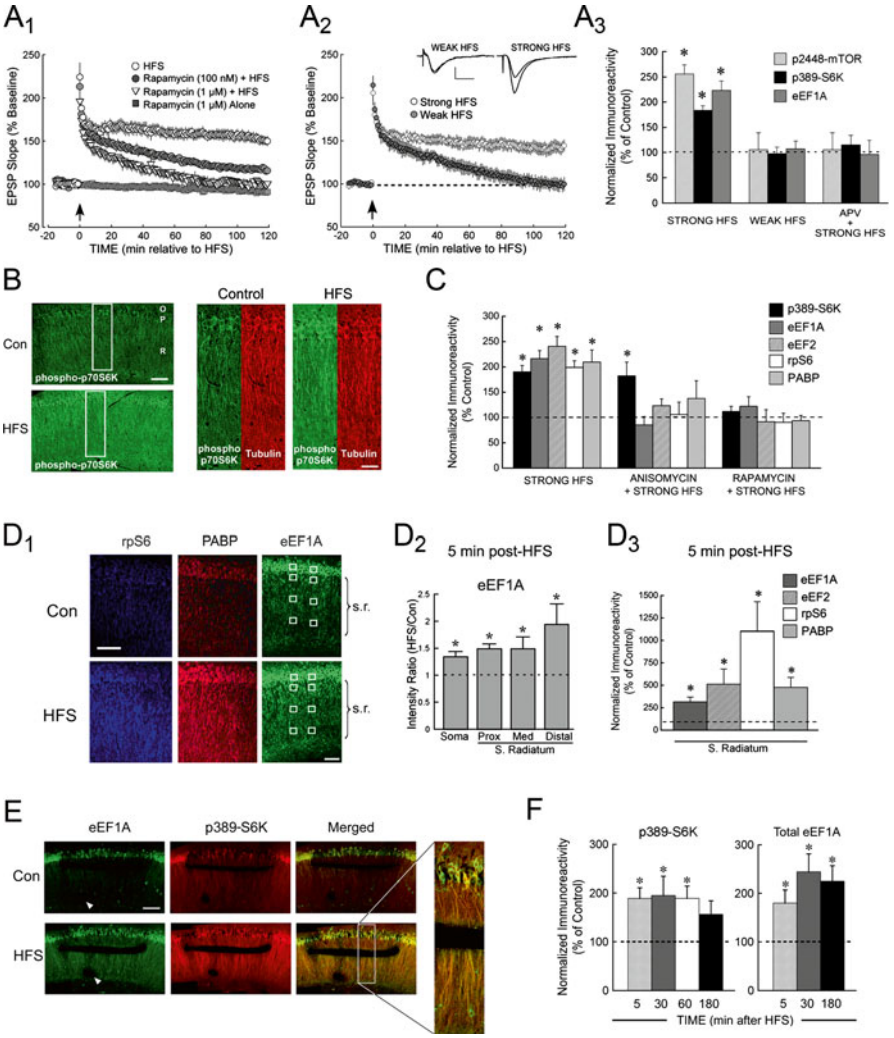

Fig. 7.2 Strong HFS activates the mTORC1 pathway in area CA1 and causes a general and long-lasting increase in the dendritic expression of TOP mRNA-encoded proteins, via de novo mTORC1-dependent local protein synthesis. (**a**) Strong HFS of the Schaffer collaterals induces rapamycin-sensitive late-LTP ($\mathbf{a}_1$) and activates the mTORC1 pathway in area CA1 in an NMDA receptor-mediated manner, as indicated by phosphorylation of S6K and increased expression of the TOP mRNA-encoded elongation factor 1A (eEF1A) ($\mathbf{a}_3$). Note that rapamycin produces a dose-dependent block of late-LTP, but has no effect on basal synaptic transmission, indicating that the mTORC1 requirement is specific to potentiated synapses. In contrast, weak HFS that produces only early-LTP ($\mathbf{a}_2$) fails to activate mTORC1 ($\mathbf{a}_3$). (**b**) Strong HFS of the Schaffer collaterals produced a widespread activation of mTORC1 signaling in the apical dendrites and cell bodies, as indicated by phosphorylation of S6K at the mTORC1-dependent site T389. The slices were fixed 30 min after control stimulation or HFS. Scale bar: 100 μm (**c**) Strong HFS induces a coordinate increase in the expression of TOP mRNA-encoded proteins (eEF1A, eEF2, rpS6, and PABP) in area CA1. All of these increases are mediated by de novo protein synthesis via the mTORC1 pathway,

throughout the apical dendritic compartment within 5 min after stimulation (Fig. 7.2d). In fact, at this early time point the increase in eEF1A protein expression was most pronounced in the most distal apical dendrites and weakest in the cell body (Tsokas et al. 2005). The most direct evidence for local regulation of dendritic translational capacity has come from studies on CA1 pyramidal cell dendrites that are physically severed from their cell bodies (Tsokas et al. 2005; Cracco et al. 2005; Vickers et al. 2005). HFS delivered to these isolated dendrites causes an increase in eEF1A expression that strongly co-localizes with activity in the mTORC1 pathway (Fig. 7.2e). Moreover, the induction of late-LTP in isolated dendrites depends on local mTORC1 activity, since it is blocked by rapamycin (Cracco et al. 2005; Vickers et al. 2005).

mTORC1 activation by strong HFS produces relatively persistent effects on downstream effectors. For example, S6K in the apical dendritic layer of CA1 remains hyperphosphorylated for at least 60 min after stimulation, and eEF1A expression is increased for at least 3 h (Tsokas et al. 2005) (Fig. 7.2f). Note that this duration of effect covers the interval over which hippocampal synaptic capture occurs in strong-before-weak experiments, and thus it is likely that translational capacity remains elevated at the time of capture in such studies (Frey and Morris 1997; Sajikumar and Frey 2004).

Several genetic approaches in mice have been used to probe the role of mTORC1 in late-LTP at the CA3-CA1 synapse. In general, manipulations that enhance mTORC1 signaling facilitate the induction of late-LTP. Thus, deletion of 4EBP2, mimicking the effect of mTORC1 activity on cap-dependent initiation (and possibly increasing translational capacity by up-regulating TOP mRNA translation; see Sect. 7.2.3), enabled weak HFS to induce late-LTP (Banko et al. 2005). Surprisingly, in these mice the normal induction of late-LTP by strong synaptic protocols was deficient. In another strategy to increase mTORC1 activity, mice with a heterozygous loss-of-function mutation of TSC2 also showed enhanced LTP after weak HFS (Ehninger et al. 2008). mTORC1 activity also was elevated in FKBP12 knockout mice, providing the first evidence that FKBP12 suppresses mTORC1

Fig. 7.2 (continued) as shown by their sensitivity to anisomycin (10 μM) and rapamycin (1 μM). (**d**) The dendritic expression of multiple TOP mRNA-encoded proteins increases as early as 5 min after strong HFS. $\mathbf{d}_1$: Immunoreactivity for rpS6, PABP, and eEF1A in CA1 *stratum radiatum* (s.r.) in slices fixed 5 min after control stimulation or HFS. Scale bar: 100 μm. $\mathbf{d}_2$: The HFS-induced increase in eEF1A expression is most pronounced in distal dendrites 5 min after HFS. The somatic, proximal, medial, and distal values were determined by densitometry for the regions indicated by the small squares in $\mathbf{d}_1$. $\mathbf{d}_3$: Strong HFS-induced de novo translation of TOP-RNA-encoded proteins results in their enriched expression in CA1 stratum radiatum 5 min post-HFS (compare with increases in entire CA1 region in panel **c**). (**e**) In isolated apical dendrites of CA1, local protein synthesis is sufficient for the HFS-induced increase in eEF1A expression, which occurs throughout apical dendritic arbor. Slices were fixed 1 h after stimulation. The *arrowhead* indicates the placement of the recording electrode. Scale bar: 150 μm (**f**). The HFS-mediated recruitment of mTORC1 effectors (phosphorylation of S6K and increased expression of eEF1A) in area CA1 is rapid and sustained. All panels are modified from Tsokas et al. (2005), except for panels **c**, $\mathbf{d}_1$, and $\mathbf{d}_3$, which are modified from Tsokas et al. (2007a)

even in the absence of rapamycin (Hoeffer et al. 2008). In agreement with the other transgenic mice in which mTORC1 was deregulated, these mice expressed enhanced late-LTP in response to an HFS protocol.

These different transgenic strategies all suggest that an increase in background mTORC1 activity essentially converts a weak stimulation protocol to a strong one, defined as one that can induce late-LTP. One possible explanation for this effect is that a persistent up-regulation of translational capacity allows LTP-related PRPs to accumulate, and these are available for capture by weak stimulation that generates the appropriate synaptic tag. An alternative hypothesis is when translational capacity is high, weak HFS becomes capable of producing PRPs from dendritic mRNAs. This second hypothesis is discussed further in Sect. 7.5.

Fewer studies have addressed the role of mTORC2 in synaptic plasticity, mainly because no selective small-molecule inhibitors are available. Recently, conditional deletion of rictor was used to selectively inhibit mTORC2 signaling (Huang et al. 2013). These mice showed reduced actin polymerization (consistent with the role of mTORC2 in cytoskeletal modeling) and deficient late-LTP, which could be rescued by pharmacologically enhancing actin polymerization. These results suggest that the two mTOR complexes might play complementary roles in late-LTP: mTORC1 contributes to the synthesis of new proteins that must be incorporated into dendritic spines or synapses, and mTORC2 accommodates this process through cytoskeletal remodeling. By the same reasoning, the cytoskeletal changes mediated by mTORC2 might serve a synaptic tagging function, enabling weakly stimulated synapses to accept PRPs that have been synthesized in response to strong stimulation of other synapses. A better understanding of the regulation of mTORC2 by synaptic events, and the degree to which this regulation is localized to stimulated synapses, will shed light on these possibilities.

7.3.3 Late-LTD

The activation of group I mGluRs in hippocampus, either pharmacologically or by low-frequency stimulation of the Schaffer collaterals during NMDAR blockade, induces rapamycin-sensitive late-LTD (Hou and Klann 2004; Huber et al. 2000). Moreover, transgenic mice that lack 4EBP2 (thus mimicking mTORC1-mediated inhibition of 4EBP) thus show enhanced DHPG-induced LTD (Banko et al. 2006). Direct activation with the mGluR agonist DHPG increases mTORC1 signaling within the stimulated dendrites of CA1 pyramidal cells, as indicated by increased phosphorylation of Akt (at the PDK1-dependent site) and mTOR within the stimulated dendrites. This localization is consistent with a known physical link between mGluRs and PI3K: long-isoform Homer proteins serve as scaffolds, binding the C-terminal tail of mGluRs as well as PIKE, a family of PI3K-stimulating proteins that contain pleckstrin homology and GTPases domains (Ronesi and Huber 2008).

It is interesting that BDNF and DHPG both activate mTORC1 but induce opposing forms of late plasticity (late-LTP and late-LTD, respectively). One proposed explanation for this dichotomy is that the relevant TrkB and mGluR receptors couple to different, highly localized pools of mTORC1 (Hoeffer and Klann 2010). An alternative explanation for the contrasting effects of BDNF and DHPG is that the two drugs both produce a distributed increase in translational capacity, but they de-repress different plasticity-related mRNAs whose synthesis then leads to either LTP or LTD.

7.3.4 mTOR in Hippocampus-Based Long-Term Memory

LTM for hippocampus-dependent tasks depends on a period of mTORC1 activity during acquisition. This requirement was first shown in spatial learning, using the Morris water maze (Dash et al. 2006). A single intrahippocampal administration of rapamycin, injected immediately after the rat had reached criterion for response acquisition, dramatically reduced performance when measured 48 h later. This study also implicated mTORC1 in the ability of glucose, acting through the inhibition of AMPK, to improve LTM. Most subsequent studies of the role of mTOR in hippocampus-based learning have relied on one-trial training procedures: inhibitory avoidance (IA), contextual fear conditioning (CFC), or novel object recognition (Bekinschtein et al. 2007; Gafford et al. 2011; Jobim et al. 2012a, b; Slipczuk et al. 2009). These procedures provide the opportunity to more precisely define the time-course for mTORC1 activation, as well as the window during which mTORC1 activity is needed for LTM. Among these studies, there is general agreement that the mTORC1 pathway is activated immediately and persistently following training. For example, phosphorylation of S6K was elevated 1 h after CFC, and 3 h after IA training (Gafford et al. 2011; Slipczuk et al. 2009). Interestingly, a detailed examination of mTOR phosphorylation at S2448 (a feedback event catalyzed by S6K) following IA training revealed two waves of activation, one occurring immediately after training, and the second 3 h later (Slipczuk et al. 2009; Chiang and Abraham 2005). The mechanism for this biphasic activation of mTORC1 might reflect an immediate release of BDNF, followed by the delayed release of newly synthesized BDNF. This biphasic activation of mTORC1 during learning is conserved across different brain regions, since a similar pattern was observed in the gustatory cortex after conditioned taste aversion training: mTORC1 signaling increased 15 min and 3 h after training, but not at intervening times (Belelovsky et al. 2009).

Intrahippocampal injection of rapamycin 15 min before training consistently interferes with LTM (Bekinschtein et al. 2007; Jobim et al. 2012a, b; Slipczuk et al. 2009). In some experiments, but not all, delaying the injection of rapamycin until immediately after training left LTM intact (Bekinschtein et al. 2007; Gafford et al. 2011; Jobim et al. 2012a). Taking into account the time required for rapamycin to accumulate within the relevant neurons after injection and inhibit mTORC1, it seems that the initial window during which mTORC1 activity is required for LTM

probably closes a few minutes after training. Remarkably, under conditions where mTORC1 was elevated biphasically, rapamycin injection at the corresponding times, but not at others, interfered with the consolidation of LTM. Since the window during which rapamycin interferes with LTM is brief, the finding of Dash et al. (2006) that rapamycin injected at the end of water maze training was effective might seem surprising: substantial learning occurred before the criterion was reached, so mTORC1 presumably was activated well before rapamycin was injected. A possible explanation comes from experiments on reconsolidation, which showed that rapamycin injected at the time of memory reactivation disrupted subsequent LTM (Jobim et al. 2012a, b), consistent with the requirement for protein synthesis in the reconsolidation of hippocampus-dependent memories (Milekic and Alberini 2002; Debiec et al. 2002).

Transgenic manipulations of the mTORC1 pathway, discussed in Sect. 7.3.2 in the context of late-LTP, also affect hippocampus-dependent LTM. However, while these manipulations (deletion of TSC2, FKBP12, or 4EBP2) consistently enhanced the ability of a weak synaptic protocol to induce late-LTP, their effects on LTM were variable. For example, hippocampal LTM as assessed by Morris water maze and contextual fear conditioning was deficient in the TSC2 and 4EBP2 knockout mice, while deletion of FKBP12 improved LTM for contextual fear (Banko et al. 2005; Ehninger et al. 2008; Hoeffer et al. 2008).

7.3.5 mTOR Signaling in Models of Drug Addiction

The synaptic changes that are associated with drug addiction can be regarded as an aberrantly persistent form of learning. Based on the evidence from the hippocampus for a specific role of mTORC1 in late forms of synaptic plasticity and in LTM, it is not surprising that mTORC1 contributes to the synaptic and behavioral effects observed in animal models of drug addiction. Here we describe several of the relevant studies, and we refer the reader to recent reviews for a more complete perspective on this very active field of research (Neasta et al. 2014; Dayas et al. 2012).

With respect to synaptic effects, most work on the role of mTOR in addiction has examined brain regions within the reward circuit, especially nucleus accumbens and the ventral tegmental area. One striking conclusion from these studies is that mTORC1 signaling is acutely involved in behavioral or synaptic phenomena that are observed long after exposure to a drug of abuse. For example, in mice trained in a cocaine-based conditioned place preference (CPP) task, daily systemic injections of rapamycin did not interfere with the acquisition of CPP (Bailey et al. 2012). However, when these mice were tested 2 days after training, an injection of rapamycin 1 h before the test interfered with CPP performance. Moreover, locomotor sensitization to cocaine measured 3 weeks after training was abolished when rapamycin was injected 1 h before the test. Similar findings were reported in mice exposed to alcohol: both CPP and locomotor sensitization were disrupted by acute systemic treatment with rapamycin (Neasta et al. 2010). A role for mTORC1 also has been

shown for reinstatement of drug seeking, which is a widely used model for drug craving. In rats trained to self-administer cocaine followed by extinction of drug-seeking behavior, subsequent cue-induced reinstatement was abolished by acute injection of rapamycin into the nucleus accumbens core region (Wang et al. 2010).

Electrophysiological data also support an ongoing requirement for mTORC1 signaling in the delayed sequelae of drug exposure. More than 40 days after rats were trained to self-administer cocaine, synaptic currents recorded from medium spiny neurons in striatal slices revealed an increase in the fraction that was carried by Ca^{2+}-permeable AMPA receptors (CP-AMPARs, which lack GluA2 subunits), and this effect was abolished by pretreatment with rapamycin for 60 min (Scheyer et al. 2014). The translation inhibitor cycloheximide was equally effective in reversing the cocaine-induced increase in CP-AMPARs, suggesting that a sustained high rate of synthesis for GluA1 subunits or some other protein maintains an altered balance of GluA subunits in the synapse. It seems unlikely that persistently elevated mTORC1 activity mediates such a remote phenomenon; a more plausible explanation for the normalizing effect of rapamycin is that an acute reduction in translational capacity preferentially depletes a protein that is being synthesized at a high rate (and presumably has a short half-life), restoring the normal balance of GluA subtypes at these synapses. Based on these results and the behavioral evidence that rapamycin disrupts remote addiction-related behaviors, it is tempting to speculate that mTORC1 presents a possible target for treating substance abuse. However, another study suggests that rapamycin might actually *protect* cocaine-induced synaptic effects (Mameli et al. 2007). Here, one day after a single injection of cocaine in mice, synaptic potentials recorded from dopaminergic neurons in slices of ventral tegmental area showed increased CP-AMPARs. This effect could be reversed by treating the slices with DHPG, which induced the dendritic synthesis of GluA2 subunits and the replacement of the synaptic CP-AMPARs with GluA2-containing AMPARs. However, this reversal was *prevented* by rapamycin, presumably because it interfered with the DHPG-induced synthesis of GluA2.

7.4 How Does Synaptic Stimulation Activate mTORC1?

A central question in the field of synaptic plasticity is: What key process is engaged specifically by strong synaptic protocols, defined as those that induce late-LTP or late-LTD, as opposed to weak stimulation that is sufficient to create a synaptic tag but fails to induce late forms of plasticity? We propose that it is the ability to recruit mTORC1 that distinguishes strong stimulation from weak.

Numerous signaling pathways that are engaged by strong synaptic activity could, in principle, stimulate the PI3K–Akt–TSC2 cascade at various points. For example, ERK is a well-established activator of the mTORC1 pathway, and one of the first studies to investigate the mechanism of mTORC1 activation by HFS implicated ERK (Kelleher et al. 2004). However, a decade later the relative importance of ERK

compared to other signaling pathways that can stimulate mTORC1 remains unclear. Here, we will consider potential mechanisms for the synaptic activation of mTORC1, focusing on signaling pathways that have been demonstrated to participate in persistent forms of hippocampal synaptic plasticity or LTM.

7.4.1 Activation of TrkB Receptors by Secreted BDNF

Several lines of evidence point to BDNF, acting at TrkB receptors, as a prime candidate for mediating the activation of mTORC1 and the induction of late-LTP by strong synaptic protocols. BDNF, like other growth factors, can activate mTORC1 through the canonical PI3K–Akt–TSC2 pathway (Fig. 7.1). In addition, BDNF stimulates the Ras-ERK pathway through the adapter protein Grb2 and SOS, which is a guanine nucleotide exchange factor for Ras. Increased Ras-ERK signaling, in turn, activates the mTORC1 pathway at several points: ERK, as well as its substrate RSK, can phosphorylate and inhibit TSC2 and thus disinhibit mTORC1, and RSK acts further upstream to activate PDK1, thereby stimulating Akt (Ma et al. 2005; Roux et al. 2004; Frödin et al. 2000).

The earliest evidence that BDNF might be involved in synaptically induced hippocampal LTP was the finding that HFS, delivered to the perforant path in vivo, increased mRNA levels for BDNF and TrkB receptors in dentate gyrus granule cells in an NMDA receptor-dependent manner (Bramham et al. 1996; Dragunow et al. 1993). The role of BDNF in LTP was extended to the CA3-CA1 synapse in acute slices, where HFS increased the expression of BDNF mRNA in CA1 pyramidal cells (Patterson et al. 1992), and exogenous BDNF produced a persistent LTP that depended on local protein synthesis (Kang and Schuman 1995, 1996). However, to date no studies have directly implicated BDNF in the activation of mTORC1 by synaptic stimuli. Moreover, there are important differences between BDNF- and HFS-induced LTP that cast doubt on BDNF as an essential mediator of synaptically induced LTP. Unlike HFS-induced LTP (Manabe et al. 1993), BDNF causes a substantial decrease in paired-pulse facilitation, suggesting a presynaptic component. In addition, the potentiation that is produced by BDNF only partially occludes subsequent LTP induced by a saturating HFS protocol (Kang and Schuman 1995). Finally, blocking TrkB receptors prevented LTP induction by some but not all synaptic protocols; in particular, multiple closely spaced trains of HFS, which produce persistent LTP that other studies have shown to be translation- and mTORC1-dependent, was insensitive to TrkB blockade (Tsokas et al. 2005; Kang et al. 1997; Osten et al. 1996). Notably, late-LTP following theta-burst stimulation was not blocked by the translation inhibitor anisomycin when BDNF was co-applied, raising the possibility that BDNF might be able to stabilize early-LTP without requiring de novo protein synthesis. In this regard, it is interesting that some synaptic protocols can produce very persistent LTP that is translation independent, but does depend on PI3K activity (Villers et al. 2012). Since PI3K

promotes the synaptic insertion of GluA2 receptors (Man et al. 2003), it is possible that BDNF-mediated stimulation of PI3K can prolong early-LTP by altering the balance between AMPAR exocytosis and endocytosis without requiring newly synthesized proteins or mTORC1 activity.

7.4.2 Increased Intracellular Ca^{2+}

The rise in the intracellular concentration of Ca^{2+} that accompanies strong synaptic stimulation offers a second potential mechanism for activating TORC1, particularly by entry through NMDA receptors (NMDARs) since synaptic stimulation in the presence of an NMDA receptor antagonist fails to activate mTORC1 (Cammalleri et al. 2003; Tsokas et al. 2005; Ma et al. 2011). In addition, voltage-gated Ca^{2+} channels (VGCCs) may be important for mTORC1 activation by certain types of stimuli (Ma et al. 2011); the potential importance of VGCCs in determining the spatial extent of mTORC1 activation in dendrites is discussed below (Sect. 7.5.4).

There are several known pathways by which an increase in intracellular Ca^{2+} can signal to mTORC1. First, Ca^{2+}/calmodulin directly activates RasGRF1 and RasGRF2, which act as guanine nucleotide exchange factors (GEFs) for Ras and thereby couple Ca^{2+} to the Ras–ERK pathway (Agell et al. 2002; Grewal et al. 1999), which in turn can activate mTORC1 signaling as described in the previous section. RasGRF1 also can be stimulated by Ca^{2+}-dependent phosphorylation (Schmitt et al. 2005). Both RasGRFs respond to Ca^{2+} influx through synaptic NMDA receptors; notably, RasGRF1 directly associates with the NR2B subunit, situating it optimally for stimulation during trains of HFS (Krapivinsky et al. 2003).

Ca^{2+} also can signal to the Ras-ERK pathway through a less direct route, by activating the Ca^{2+}/calmodulin-dependent isoforms of adenylyl cyclase, AC1 and AC8, which are necessary for normal hippocampal LTP and contextual LTM (Wong et al. 1999). The resulting increase in cyclic AMP concentration is sensed by Epac, an Ras-GEF that activates the Ras–ERK pathway and enhances late-LTP (Gelinas et al. 2008).

It is important to note that ERK also affects protein synthesis through effects that do not depend on mTORC1, and in fact the two kinases regulate some important translational proteins in a cooperative manner. The two major mTORC1 effectors, 4EBP and S6K, are phosphorylated and regulated by both mTORC1 and ERK (see Tsokas et al. 2007a and references therein). In addition, ERK regulates some components of the translational machinery that are not under the control of mTORC1, including the cap-binding protein eIF4E, which is phosphorylated in an ERK-dependent manner following induction of late-LTP by BDNF, or late-LTD by stimulation of mGluRs (Banko et al. 2006; Kanhema et al. 2006).

Finally, there is some evidence that Ca^{2+}/calmodulin can directly stimulate PI3K, and that this effect can be produced in neurons by activation of glutamate receptors (Joyal et al. 1997; Perkinton et al. 1999).

7.4.3 Activation of Group I mGluRs

The role of group I metabotropic glutamate receptors (mGluRs) receptors in synaptic plasticity has been studied mainly in the context of LTD, and their possible contribution to synaptically induced LTP is controversial (Bortolotto et al. 1994; Manzoni et al. 1994; Breakwell et al. 1996). However, there is little doubt that mGluRs are stimulated by HFS protocols that induce late-LTP, and synaptic activation of group I mGluRs receptors does increase mTORC1 signaling. Thus, it is likely that, depending on the particular stimulation protocol, mGluRs can participate in mTORC1 activation during the synaptic induction of late-LTP.

7.4.4 Involvement of FMRP in mTORC1 Activation

Studies on the effect of deleting *Fmr1*, the gene that encodes fragile X mental retardation protein (FMRP), have been influential in assigning a general role of mTOR to late forms of synaptic plasticity. Loss-of-function mutations in *Fmr1* are associated with Fragile X syndrome, an autistic spectrum disorder that is the most common genetic cause of intellectual disability (Hagerman et al. 2009). *Fmr1* knockout mice show elevated mTORC1 signaling, as well as facilitated induction of mGluR-dependent LTD that is not sensitive to rapamycin or to inhibitors of protein synthesis (Huber et al. 2002; Hou et al. 2006; Sharma et al. 2010). This facilitation is consistent with the established role of mTORC1 in late-LTD, and the ability to dispense with mTORC1 activity and de novo protein synthesis during induction suggests that PRPs accumulate in *Fmr1* knockout mice as a result of persistently high mTORC1 activity. According to this hypothesis, weak low-frequency stimulation (LFS) would set LTD-specific synaptic tags at the stimulated synapses, allowing the capture of their cognate LTD-related PRPs. An argument against this hypothesis is that it does not explain why elevated mTORC1 activity would not also increase the levels of LTP-related proteins, yet *Fmr1* knockout mice show either normal or deficient synaptically induced LTP at the CA3-CA1 synapse (Huber et al. 2002). A possible explanation for the divergent effects of *Fmr1* deletion on LTP and LTD is the ability of FMRP to bind and regulate the translation of a subset of mRNAs; consequently, *Fmr1* deletion might preferentially up-regulate LTD-related PRPs to specifically enable LTD capture by weak stimuli, and not LTP capture (Darnell et al. 2011; Miyashiro et al. 2003).

7.4.5 GSK3 and Wnt Signaling

mTORC1 activity is under the control of TSC2, which in turn integrates signals from multiple kinases. Some of these kinases (Akt, Erk, and RSK) inhibit TSC2, while AMPK stimulates TSC2 and thereby inhibits mTORC1. Another kinase that

stimulates TSC2 is glycogen synthetase kinase 3 (GSK3), which is notable for its autonomous activity: in the absence of any regulatory input, GSK3 acts as a tonic brake on mTORC1. Thus, GSK3 inhibitors activate mTORC1, and this effect can be observed in the adult hippocampus (Ma et al. 2011; Inoki et al. 2006).

While GSK3 is a widely distributed enzyme with many substrates, the fraction that is relevant to mTOR regulation and synaptic plasticity is present within the "destruction complex." Here, the scaffold protein Axin brings together GSK3 and at least one of two substrates: the transcriptional regulator β-catenin, and TSC2. The destruction complex is under the control of Wnt proteins, a family of secreted molecules that act as autocrine or paracrine factors. Through a mechanism that is not yet understood, the binding of specific Wnts to their surface co-receptors (Frizzled and LRP5/6) disrupts the function of the destruction complex, preventing GSK3 from activating TSC2 (the consequence of Wnt signaling for β-catenin is to protect it from proteasomal degradation, allowing it to enter the nucleus and affect transcription). Thus, Wnt signaling can acutely stimulate mTORC1, as first demonstrated in non-neuronal cell lines (Inoki et al. 2006).

Chen et al. (2006) showed that Wnt3a, which is one of the Wnt isoforms that regulates the destruction complex, is present in the dendrites of CA1 pyramidal neurons and is depleted (presumably due to secretion) in response to trains of HFS (Chen et al. 2006). Moreover, the same study showed that late-LTP depends on Wnt signaling, and that exogenous Wnt3a enhances the induction of late-LTP without affecting basal synaptic efficiency. However, synaptically induced activation of Wnt signaling by itself does not appear to activate the mTOR in the hippocampus. Rather, Wnt signaling plays a permissive role, allowing Akt to stimulate the mTOR pathway (Ma et al. 2011). One explanation for this finding is that TSC2 within the destruction complex might be protected from Akt, and that Wnt removes this protection by disrupting the destruction complex.

7.5 A Proposed Role for mTORC1-Induced Translational Capacity in Synaptic Capture

7.5.1 Background and Rationale for an Alternative Model of Capture

The phenomenon of synaptic capture usually has been demonstrated by strongly stimulating one set of synapses, and delivering either weak stimulation, or strong stimulation in the presence of a translation inhibitor, to an independent set of synapses on the same population of neurons. Here, we will use the term *conditioning* to refer to the molecular events that are set into motion only by strong stimulation, enabling even weak stimulation at other synapses to express late forms of plasticity. Conditioning is distributed over some dendritic distance, and can interact with both LTP tags and LTD tags, as established in "cross-capture" experiments

(Sajikumar and Frey 2004). Thus, conditioning is a nondirectional process, and the synaptic tag specifies the direction of plasticity. Furthermore, the molecular mechanism that is responsible for conditioning must persist for more than 1 h to participate in the extended associativity that characterizes synaptic capture. We will use the term *capture* to describe the molecular events that result directly from the interaction between the conditioning process and the synaptic tag, leading to the expression of late-LTP or late-LTD.

In the prevailing synaptic tagging and capture (STC) model (Frey and Morris 1997; Sajikumar and Frey 2004; Barco et al. 2008; Martin and Kosik 2002), conditioning is equated with the synthesis and distribution of plasticity-related proteins (PRPs) that interact specifically with LTP tags or LTD tags. A strong synaptic protocol, regardless of stimulation frequency, produces both types of PRPs. The most convincing evidence in favor of the STC model is that capture can occur even when protein synthesis is blocked, indicating that synaptic tags are generated by post-translational modifications and interact with PRPs that are synthesized before or after synthesis was blocked. However, there have been several reports in which protein synthesis was required *at the time of synaptic capture*. In some of these studies, capture of late-LTP at a second input was substantially reduced, although not eliminated, when it was attempted in the presence of the translation inhibitor anisomycin (Alarcon et al. 2006; Barco et al. 2002). It is noteworthy that in these cases, the inhibitor was washed out about an hour after the capturing stimulation, as has been customary when demonstrating capture in the absence of protein synthesis. Even more striking are those experiments where translation was inhibited for a longer time during the capture phase: *under these conditions*, *synaptic capture was abolished*, *while late-LTP that had been induced at other synapses before translation was blocked remained intact* (Ris et al. 2009; Tsokas et al. 2007b). These findings argue for a role of input-specific PRP synthesis in synaptic capture, and suggest that conditioning by strong stimulation enables such synthesis to take place at tagged synapses in response to weak stimulation. In those experiments where the translation inhibitor was washed out and capture was successful, it is possible that the conditioning process as well as the local synaptic tag persisted until translation was allowed to resume, allowing PRPs to be synthesized at the capturing synapses.

In this section, we consider an alternative model of synaptic capture that incorporates the evidence for input-specific PRP synthesis. The distributed translational capacity (DTC) model explains synaptic capture as an interaction between two molecular events: an increase in translational capacity that is mediated by mTORC1 activation in the dendrites (the conditioning process), and the input-specific de-repression of plasticity-related mRNAs (the synaptic tag). This model is schematized in Fig. 7.3, and the concepts of conditioning and synaptic tagging in the context of the model are discussed below. As with other hypotheses of synaptic capture, the DTC model applies equally to homosynaptic capture that occurs directly at strongly stimulated synapses, and heterosynaptic capture as explicitly studied in two-pathway experiments.

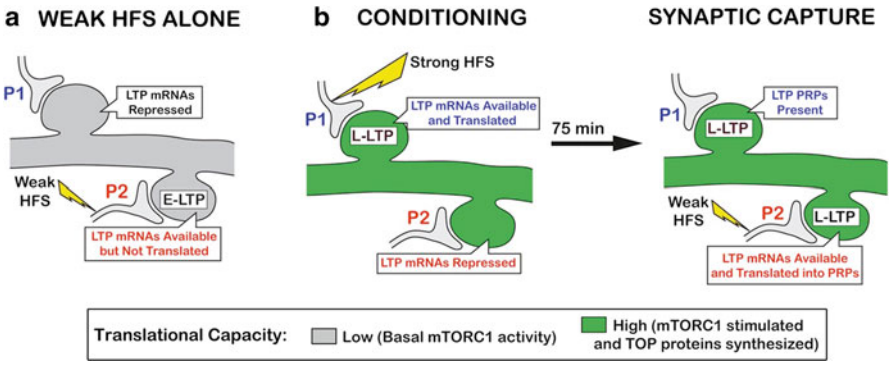

Fig. 7.3 The distributed translational capacity (DTC) model of synaptic capture. A region of dendritic branch with two spines is depicted, representing discrete *populations* of synapses that are activated by different input pathways (P1 and P2). (**a**) Under basal conditions, the translational capacity of the dendrite is low, and mRNAs that encode plasticity-related proteins (PRPs) are repressed. Weak HFS at P2 locally de-represses mRNAs encoding LTP-specific PRPs, but only early-LTP (E-LTP) is induced because the low resting translational capacity is insufficient for PRP synthesis at the rate needed to express late-LTP. (**b**) In the context of synaptic capture, conditioning occurs when strong HFS at P1 (*left*) activates mTORC1, leading to a distributed synthesis of TOP mRNA-encoded proteins and increased translational capacity throughout the dendritic branch (*green*). At the P1 synapse, mRNAs for LTP-related proteins are de-repressed; accordingly, LTP-specific proteins are synthesized locally at this synapse, resulting in homosynaptic late-LTP (L-LTP). Subsequent delivery of weak HFS to P2 (*right*) makes LTP-specific mRNAs available at that synapse, and the distributed increase in translational capacity enables the local synthesis of LTP plasticity proteins, resulting in capture of late-LTP. Had the weak stimulation to P2 been LFS instead of HFS, mRNAs for LTD-specific PRPs would have been de-repressed, and late-LTD would have been captured

7.5.2 Elevated Translational Capacity as a Conditioning Mechanism

The DTC model assumes that the translational capacity of a pyramidal cell dendrite ranges from a low (resting) state to a high (stimulated) state. In the resting state, translational capacity is sufficient for the synthesis of proteins that manage routine homeostatic activities, including the maintenance of synaptic efficiency as determined by earlier events. However, the induction of late-LTP or late-LTD demands a high rate of synthesis in order to accumulate PRPs, and this need is accommodated by an mTORC1-mediated increase in translational capacity as described in Sect. 7.2.3. For this reason, late plasticity can only be induced by synaptic or pharmacological stimuli that activate mTORC1, such as BDNF, DHPG, strong HFS, or weak HFS in the presence of isoproterenol.

Findings in support of the general role of mTORC1 in the conditioning effect include the following:

(1) Transient activation of group I mGluRs enables weak HFS to induce late-LTP at Schaffer collateral-CA1 synapses, whether mGluR activation preceded or followed the weak HFS, and this conditioning effect is blocked when mGluRs are activated in the presence of rapamycin (Cammalleri et al. 2003; Raymond et al. 2000).
(2) Antidromic stimulation of CA1 pyramidal cells, which evokes back-propagating action potentials and activates mTORC1 throughout the pyramidal cells without inducing synaptic plasticity, enables subsequent weak HFS or weak LFS to capture late-LTP or late-LTD, respectively; as with mGluR-induced conditioning, the stabilizing effect of antidromic stimulation was blocked by rapamycin (Tsokas et al. 2007b; Dudek and Fields 2002).
(3) Inhibitors of GSK3 also stimulate mTORC1 (mediated by the relief of suppression by TSC2; see Sect. 7.4.5), and produce a rapamycin-sensitive conditioning effect that allows weak HFS to induce late-LTP (Ma et al. 2011).
(4) The conditioning effect of strong HFS is lost when it is delivered in the presence of rapamycin (Tsokas et al. 2007b).
(5) A strong LFS protocol that induces late-LTD at CA3-CA1 synapses (Sajikumar and Frey 2004) reliably activated mTORC1 in stratum radiatum and increased the expression of TOP mRNA-encoded proteins, effects that were not produced by weak LFS (Tsokas et al. 2007b). Moreover, the conditioning effect of strong LFS was abolished when such stimulation was delivered in the presence of rapamycin.

7.5.3 Why Does Synaptic Capture Occur When Translation Has Been Blocked?

If input-specific synthesis of PRPs is required for late forms of plasticity, why do some studies show that capture succeeds at a second pathway even when translation is inhibited at the time of the capturing stimulation, and then allowed to recover after about 1 h? The explanation offered by the DTC model is that at the time of recovery from translation inhibition, dendritic translational capacity remains elevated (Tsokas et al. 2005) and PRP-encoding mRNAs remain de-repressed at the capturing synapses, in accordance with the observed lifetime of the synaptic tag (Frey and Morris 1998). Depending on the strength of the conditioning and capturing stimuli, this procedure will allow PRPs to be synthesized at the capturing synapses, although under some experimental conditions capture is only partially successful, presumably occurring only at a subset of the stimulated synapses (Alarcon et al. 2006; Barco et al. 2002). However, if inhibition of translation is extended throughout the capture phase, synaptic capture necessarily fails.

7.5.4 What Is the Spatial Extent of Increased Translational Capacity?

To date, all studies that have examined the activation of mTORC1 in response to plasticity-inducing protocols have employed diffuse forms of stimulation: pharmacological/chemical (e.g., BDNF, DHPG, forskolin, or high K^+) or synaptic (field stimulation). Such stimuli generally produce a widely distributed activation of mTORC1 in dendrites, with little evidence for compartmentalization or heterogeneity between dendritic branches (Cammalleri et al. 2003; Tsokas et al. 2005; Ma et al. 2011; Gobert et al. 2008). However, discrete synaptic stimulation that more accurately approximates the natural distribution of synaptic activity during learning, such as glutamate uncaging at a subset of spines within a dendritic branch, might produce a much more spatially limited activation of mTORC1. According to the DTC model, such a compartmentalized increase in translational capacity will favor synaptic capture within that branch ("clustered" plasticity), as observed by Govindarajan et al. (2011). Even with global stimulation there is a suggestion that mTORC1 can be activated locally: primary hippocampal neurons that are exposed to high K^+ show a punctate activation of mTORC1 in the dendrites (Cammalleri et al. 2003). A possible explanation for this compartmentalization is that Ca^{2+} spikes generated in specific dendritic branches during high K^+ might serve as the trigger for mTORC1 activation; as discussed earlier, there are several signaling pathways that can couple a rise in intracellular Ca^{2+} to mTORC1. In fact, dendritic spikes may play a major role in the activation of mTORC1 by synaptic stimulation. For example, back-propagated dendritic spikes, which are characteristic of CA1 pyramidal neurons during synaptic stimulation, are carried in part by L-type VGCCs (Hoogland and Saggau 2004; Stuart et al. 1997), and mTORC1 activation by HFS in the presence of the β-adrenergic agonist isoproterenol correlates with the generation of action potentials in the apical dendritic layer of CA1 (Ma et al. 2011). Moreover, action potentials that are triggered locally in the dendrites can be restricted to individual dendritic branches; the resulting Ca^{2+} entry may activate mTOR in a spatially restricted manner, helping to explain the observation that synaptic capture occurs preferentially within a single branch (Govindarajan et al. 2011; Losonczy et al. 2008; Branco and Hausser 2010). Such a mechanism could allow activation of mTORC1 to be confined to an individual dendritic branch, or even to a region of the branch near stimulated synapses due to additional localized Ca^{2+} entry through NMDA receptors, creating transient pockets of high translational capacity within which synaptic capture will be favored.

7.5.5 Repression and Conditional De-repression of Dendritic mRNAs

The activation of mTORC1 is not sufficient to induce late plasticity. For example, the pharmacological inhibition of GSK3 increases mTORC1 activity in area CA1 pyramidal neurons but has no intrinsic effect on CA3-CA1 synaptic efficiency

(Ma et al. 2011; Peineau et al. 2007). Thus, translational capacity must interact with a tag that is produced by synaptic stimulation. In the DTC model, the tag consists of dendritic mRNAs that have been locally de-repressed by synaptic stimulation, even by stimuli that are too weak to activate mTORC1 (Fig. 7.3). Among the mRNAs thus de-repressed, some are specific for LTP (preferentially de-repressed by HFS), and others for LTD (preferentially de-repressed by LFS). It is the pattern of synaptic stimulation that determines whether LTP- or LTD-related mRNAs are de-repressed.

Several studies have shown that a heterogeneous population of translationally silent mRNAs is present in neuronal dendrites complexed with RNA-binding proteins in various types of macromolecular assemblies (for review, see Sossin and DesGroseillers 2006). RNA granules contain ribosomes and transcripts that are repressed at the stage of protein translation elongation (Anderson and Kedersha 2006). P-bodies lack ribosomes and are sites of RNA storage, degradation, and transport that reversibly repress translation of specific transcripts, presumably at the stage of initiation (Parker and Sheth 2007). Both particles are heterogeneous in composition and lack certain factors necessary for translation (Krichevsky and Kosik 2001), which may be added upon stimulation of protein synthesis in response to specific local synaptic signals, resulting in "differential translation" (Vanderklish and Edelman 2005). It is estimated that over 500 RNA-binding proteins exist (Eberwine et al. 2001), and that 68 % of all transcripts present in distal neuronal dendrites encode for such proteins (Poon et al. 2006). Based on their ubiquitous presence in dendrites and their heterogeneity, it has been postulated that different combinations of RNA-binding proteins may characterize distinct types of granules/P-bodies that regulate the transport and translation of specific subsets of functionally connected mRNAs, thus serving as "post-transcriptional operons" (Keene and Tenenbaum 2002). In the case of synaptic plasticity, the direction of long lasting synaptic change would thus be conferred by the coordinated de-repression of functionally connected subsets of mRNAs contained within specific types of ribonucleoprotein complexes.

How are RNA granules and P-bodies localized vis-à-vis synaptic spines? Several studies have shown that RNPs or RNA-binding proteins involved in mRNA transport and present in dendritic granules (FMRP, Staufen, Smaug, ZBP1, and Pum2) may be encountered at the base of dendritic spines (Zeitelhofer et al. 2008; Cougot et al. 2008; Ferrari et al. 2007; di Penta et al. 2009; Vessey et al. 2006), or even within spines and at postsynaptic densities (Ferrari et al. 2007; Eom et al. 2003) and may undergo profound modifications upon depolarization or stimulation with BDNF, NMDA, glutamate, or DHPG. Such modifications include motorized transport along the dendrites (Zeitelhofer et al. 2008; Cougot et al. 2008), movement into spines and association with the PSD, as in the case of FMRP in response to DHPG stimulation (Ferrari et al. 2007; di Penta et al. 2009), disassembly (Krichevsky and Kosik 2001), or loss of individual RNA-binding proteins (Zeitelhofer et al. 2008; Cougot et al. 2008). Importantly, specific subpopulations of P-bodies (defined by the presence or absence of various RNA-binding proteins) have been shown to respond differentially to distinct patterns of synaptic stimulation (Cougot et al. 2008). This suggests that the local regulation of diverse classes of P-bodies is under the control of different signaling pathways, and raises the possibility that by

activating specific signaling pathways, different patterns of synaptic stimulation could recruit distinct populations of granules/P-bodies.

How mRNAs that are repressed within granules and P-bodies are reactivated for translation is not presently clear. It appears that most of synaptic translational modulators are repressors (Kindler et al. 2005; Tiedge 2005); thus, the activity-dependent loss of specific RNA-binding proteins (Zeitelhofer et al. 2008; Cougot et al. 2008) or the partial disassembly of the RNP complex in response to stimulation (Krichevsky and Kosik 2001) could account for relief of repression and activation of translation. In the best-understood example of stimulation-induced de-repression in neurons, the zipcode-binding protein 1 (ZBP1) in its unphosphorylated state binds to and prevents the translation of mRNA for β-actin. Upon Src phosphorylation of a key tyrosine residue on ZBP1 at the end point of mRNA transport, ZBP1 dissociates from β-actin mRNA (Huttelmaier et al. 2005). This is a highly localized case of de-repression that essentially "tags" the region of the cell where Src is active. Since this phenomenon was observed in the growth cone of developing neurons and fibroblasts, it is likely that translational capacity was quite high in the vicinity where β-actin mRNA was de-repressed, allowing β-actin to accumulate and locally modify the cytoskeleton. Recently, in studies where the dynamics of single copies of β-actin mRNA were visualized in neuronal dendrites stimulated by a chemical LTP protocol, mRNA was unmasked due to release from RNA granules (Buxbaum et al. 2014; Park et al. 2014).

In contrast to ZBP1, the neuron-specific ELAV proteins (HuB, HuC, and HuD) are examples of RNA-binding proteins that are translational activators. In somatic cells ELAV protein HuR has been shown to relieve RNA from microRNA repression, and to promote its exit from P-bodies (Bhattacharyya et al. 2006). In hippocampal slices HuD immunoreactivity co-localizes with granules in the dendrites (Bolognani et al. 2004), and in hippocampal neurons KCl depolarization causes the localization of HuD beneath spine-like protrusions in distal dendrites, and enhances its association with several neuronal mRNAs, including α-CaMKII (Tiruchinapalli et al. 2008).

Synaptic activity-dependent de-repression of specific transcripts via changes in the structure of the RNA-induced silencing complex (RISC) has also been described. Specifically, activity-dependent degradation of MOV10, a component of the RISC complex, by the ubiquitin–proteasome system, has been linked to a coordinated increase in the local mRNA translation of several transcripts, including α-CaMKII (Banerjee et al. 2009). Importantly, the increased translation of these RISC-dependent transcripts occurred within dendritic puncta. Many instances of protein degradation at the synapse have been noted, including the association of ubiquitin ligases with synapses (Hegde et al. 1997), the activity-dependent movement of the proteasome into the spine (Bingol and Schuman 2006), and the control of positive and negative regulators by the proteasome during long-term synaptic plasticity (Dong et al. 2014). Thus, the degradation of MOV10 and possibly other components of the RISC may underlie the observations that both degradation and synthesis are required for synaptic plasticity (Ashraf et al. 2006; Fonseca et al. 2006).

Selective polyadenylation of specific transcripts could also serve as a mechanism promoting differential local translation. Phosphorylation of cytoplasmic polyadenylation element-binding protein (CPEB) by α-CaMKII, which facilitates polyadenylation, has been shown to occur in response to both weak and strong LTP-inducing

stimulation, while LTD-promoting protocols cause its dephosphorylation (Atkins et al. 2005). Further evidence suggests that the poly(A) polymerase Gld2, deadenylase PARN, and translation inhibitory factor neuroguidin are key players in dendritic CPEB-mediated polyadenylation, in response to synaptic stimulation (Udagawa et al. 2012). Thus Ngd knockdown enhances LTP and Gld2 depletion inhibits it, while the expression of specific dendritic transcripts is bidirectionally regulated by Gld2 and Ngd. Stimulation-induced rapid proteolysis by calpains of PAIP2A, a translational repressor that inhibits PABP, has also been implicated in synaptic plasticity in a recent study (Khoutorsky et al. 2013). Thus, hippocampal slices from PAIP2A knockout mice exhibit a lowered threshold for the induction of long-lasting LTP (but not LTD), and a lowered threshold for the translation of α-CaMKII, which is an LTP-related PRP. Moreover, synaptic stimulation that induces LTP causes the calpain-mediated degradation of PAIP2A. Taken together, these data strongly support the involvement of selective polyadenylation as a mechanism that determines the input-specificity and directionality of long-lasting plasticity.

7.6 Summary

The concept of the dendrite as a translational compartment is well established by functional studies of stimulation-induced dendritic protein synthesis, and proteomic and transcriptomic analyses have revealed a vast number of mRNAs within dendrites together with a wide variety of RNA-binding proteins. Clearly there is the potential for the localized de-repression of mRNAs by synaptic stimulation, and for different patterns of stimulation to de-repress distinct groups of mRNAs. In addition, dendrites show a conspicuous ability to increase the efficiency of protein synthesis, through elevated translational capacity and other mechanisms, in response to strong synaptic stimulation. Indeed, while synaptic communication is the overarching function of dendrites, their investment in protein synthesis and its regulation defines a second major function.

Our current understanding of the repertoire of translational controls that dendrites employ in the course of synaptic plasticity and learning is very limited, and many important questions remain unanswered. However, it must be recognized that many of the most interesting questions will be difficult or impossible to address with diffuse forms of stimulation that activate a great number and uncontrolled distribution of synapses, and will require more spatially constrained approaches such as glutamate uncaging and optogenetic stimulation. Such questions include: Is synaptic capture possible only in dendritic regions where translational capacity has been increased? Can weak stimulation de-repress mRNAs? How localized is protein synthesis following synaptic stimulation that induces late plasticity? Do different patterns of synaptic stimulation locally de-repress different mRNAs, and if so, what coupling mechanisms are involved? Gaining insights into this last problem—identifying mRNAs that are de-repressed by protocols known to induce or capture late-LTP or late-LTD—could allow us to identify new putative PRPs that interact with known components of the synapse. RDB is supported by NIH grants GM54508 and NS072359. PT was the recipient of an Alexander S. Onassis Public Benefit Foundation Award.

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Chapter 8
Dopaminergic Neuromodulation in Synaptic Tagging and Capture

Sheeja Navakkode

Abstract Dopamine (3,4-dihydroxyphenethylamine) is a hormone and neurotransmitter of the catecholamine and phenethylamine families which plays a major role in reward-motivated behaviour. Dopaminergic neuromodulation is critical for many forms of memory. Dopaminergic D1/D5-receptor function is required for the induction of the protein synthesis-dependent maintenance of hippocampal late-LTP (L-LTP) through activation of the cAMP/PKA-pathway. This chapter will brief about dopaminergic neuromodulation required for the establishment of L-LTP and its late-associative processes such as synaptic tagging and capture (STC) in CA1 pyramidal neurons and how it varies in the different lamina of the same neurons. In addition we discuss how neuromodulation by dopamine leads to the synthesis of the major plasticity-related protein (PRP), protein kinase Mzeta (PKMζ), and its role in maintaining STC at potentiated synapses.

Keywords Dopamine • Neuromodulation • Synaptic tagging and capture • Long-term memory • Hippocampus • LTP

8.1 Dopamine and Memory

Dopamine is a multi-faced neurotransmitter and is a catecholamine which functions both in the periphery and in the central nervous system (Best et al. 2009). Dopamine is critical for many brain functions. It affects the sleep-wake cycle (Dzirasa et al. 2006), reward learning, and the fine control of movement via the basal ganglia (DeLong 1990). Dopamine plays an important role in synaptic plasticity in brain regions such as in the hippocampus, the striatum and the prefrontal cortex (Jay 2003; Yao et al. 2004). It is also important for homeostatic mechanisms, which are critical for the balance between synthesis, storage, release, metabolism and reuptake. Dysfunction in dopaminergic systems is known to be associated with many disorders, including schizophrenia, Parkinson's disease, and Tourette's syndrome

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(Gainetdinov et al. 1998; Kienast and Heinz 2006). Considering its role in attention, motivation, novelty and for improving the long-term memory dopamine is considered as the 'feel-good hormone' of our body.

Dopamine is synthesized in the nerve terminals from tyrosine which is transported across the blood–brain barrier. Tyrosine is converted into L-3,4-dihydroxyphenylalanine (L-Dopa) by tyrosine hydroxylase (TH) and L-Dopa is converted into cytosolic dopamine by aromatic amino acid decarboxylase (AADC). Cytosolic dopamine is transported into the vesicular compartment by the monoamine transporter and vesicular dopamine is released from the vesicular compartment into the extracellular space at a rate proportional to the firing rate of the neuron. In the extracellular space, extracellular dopamine affects the autoreceptors and is taken up into the terminal by the dopamine transporters (DATs) and is removed from the system by uptake into glial cells and the blood. Dopamine is also catabolized both in the nerve terminals and in the extracellular space (Best et al. 2009).

The major dopaminergic pathways and its functions are depicted below:

Cortico-striatal projections are not dopaminergic pathways per se, but have a modulating influence on the prefrontal cortex. These projections from the premotor/motor cortex to the putamen are involved in basic volitional motor planning and motor action. They regulate the automatic and involuntary aspects of movement that originate in the putamen and related structures.

The meso-limbic pathway projects from dopamine-producing cells in the ventral tegmental area (VTA) of the midbrain to limbic structures such as the nucleus acumbens (NA), hippocampus and the amygdala. The meso-limbic pathway functions in learning and memory, social emotional behaviour, motivation, pleasure and reward.

The meso-cortical pathway projects from the VTA of the midbrain to the prefrontal cortex, especially the dorsolateral prefrontal cortex. Meso-cortical dopamine projections help to regulate and modulate functions such as attention, motivation, planning, decision making, working memory and other higher cognitive functions.

The nigro-striatal pathway projects from cell bodies within the substantia nigra to the caudate and putamen (corpus striatum). These structures are components of the basal ganglia and are part of the extrapyramidal motor system within the brain. The nigro-striatal pathway plays an important role in the coordination and maintenance of movement.

The hypothalamic–pituitary (tubero-infundibular) pathway originates in the periventricular area of the hypothalamus and projects to the anterior pituitary. The release of dopamine in the hypothalamic–pituitary pathway leads to the inhibition of release of the hormone prolactin from the pituitary. Prolactin is an important reproductive hormone that stimulates breast tissue and promotes lactation.

8.1.1 Dopamine Receptors and Hippocampus

Long-term potentiation (LTP) and long-term depression (LTD) are widely accepted cellular models of learning and memory (Malenka and Bear 2004). Large amount of work has been already reported which highlights the need and necessity of the activation of heterosynaptic inputs such as dopamine (DA) for learning and LTP in in vitro and in vivo preparations (O'Carroll and Morris 2004; Moncada and Viola 2007; Wang et al. 2010). In addition to hippocampus, one of the highly explored brain regions for synaptic plasticity, DA modulation of LTP has been performed in several other areas such as neocortex and the striatum. Since this chapter is more devoted to hippocampal plasticity modulation by dopamine, a detailed description of plasticity regulation by dopaminergic modulation in hippocampus is described. A wider perspective of how dopamine gate information in neural networks can be obtained from well-received review such as in Lisman and Grace (2005) or Hansen and Manahan-Vaughan (2014).

The hippocampal formation, especially the dorsal hippocampus, receives DA input from different midbrain groups, mainly from the meso-limbic pathway (Swanson 1982). Five DA receptor subtypes, D1–D5, have been cloned and characterized so far (Jay 2003). DA receptor subtypes in the hippocampus has a prominent labelling for D1 receptors dorsally in granular cells of the hippocampal dentate gyrus (DG) and in the subicular complex (Fremeau et al. 1991). The expression of D1 receptors in the stratum oriens and radiatum of CA1–CA3 fields are less; however, it has been reported later that D5 receptor is the predominant D1-like receptor in these non-classical recipients of DA innervation (Levey et al. 1993; Ciliax et al. 2000) and are localized in the hilus and granular cells of the DG, CA1, CA3 and in subiculum (Ciliax et al. 2000; Khan et al. 2000). The D2 receptors are more localized in septal portions of the lacunosum moleculare of CA1 and stratum moleculare of the subiculum whereas no binding to D2 receptors were noticed in the temporal hippocampus (Goldsmith and Joyce 1994). The density of D3 receptor was also detected at a low level in the hippocampus, while a high level of D4 receptor was found in DG, CA1, CA2 and CA3 regions (Defagot et al. 1997). Pharmacological and physiological studies could not differentiate the individual roles of some of the DA-receptor subtypes because of the absence of specific agonists and antagonists (Jay 2003).

8.1.2 Dopamine Receptors and Its Role in LTP

In a series of very intriguing set of experiments Frey et al. in 1990s (Frey et al. 1990, 1991) provided the first experimental evidence for the role of D1 receptor in the late maintenance of LTP. Application of the specific D1 antagonist, SCH 23390 during induction of late-LTP (L-LTP) prevented the long-term maintenance of LTP; however, application immediately after tetanization had no influence on established LTP (Frey et al. 1990, 1991). In 1995, Eric Kandel's group demonstrated that agonists of

the D1/D5 receptors (SKF38393) produced a persistent increase of the excitatory post-synaptic potentials (EPSPs), with a very slow onset synaptic potentiation starting from 50 to 90 min after drug application and stabilized at 3–4 h (Huang and Kandel 1995). Another strong experimental evidence in support of the above notion demonstrated by Matthies and colleagues that L-LTP was impaired in mice lacking D1 receptors (Matthies et al. 1997). Above all, more recent study by Sajikumar and Frey reported that dopamine itself can induce slow onset plasticity and the fate of synapses that express potentiation or depression is based on the amount of dopamine available in the synapses during baseline activity (Sajikumar and Frey 2004). In short, all these experimental evidences established the importance of heterosynaptic dopaminergic modulation during the late phase of LTP.

Dopaminergic modulation is in synergy with glutamatergic inputs such as NMDA receptors, similar to the induction of L-LTP in which it requires the activation of the cAMP/PKA-cascade in synergistic interaction with NMDA-receptor function (Frey et al. 1993). In an intriguing sets of experiments we have provided the first evidence for a synergistic role of D1/D5- as well as NMDA-receptor function for the maintenance of protein synthesis-dependent dopamine induced slow onset potentiation (Navakkode et al. 2007). We employed the two-pathway hippocampal slice experimental design (Fig. 8.1a) in which two stimulating electrodes S1 and S2 were used to stimulate two independent synaptic inputs to the single neuronal population in CA1 pyramidal neurons. After recording a stable baseline of 1 h in both inputs S1 and S2 dopamine agonists such as SKF38393 (SKF; 50 μM) and 6-bromo-APB (APB; 50 μM) were applied. Consistent with earlier reports (Huang and Kandel 1995), we observed a slow onset potentiation that stabilized within 3–6 h (Fig. 8.1b). A normal control stimulation is necessary to initiate synergistic processes required for the D1/D5-LTP. To show that, baseline recordings during the application of D1/D5-receptor agonists were suspended for a total of 3 h. In the absence of synaptic control stimulation, D1/D5-LTP was not observed, suggesting a synergistic requirement of glutamatergic NMDA-receptor function (Fig. 8.1c). When input S1 was given control-stimulation during and after D1/D5-receptor activation and input S2 had no control stimulation for 3 h (Fig. 8.1d). The input which received glutamatergic activation developed a D1/D5-LTP whereas the unstimulated input remained at baseline levels.

8.1.3 Dopamine and STC

Synaptic tagging and capture (STC) is one of the most attractive models to explain how input specificity is achieved in a protein synthesis-dependent stage and provides a conceptual basis for how short-term memory is transformed to long-term memory in a time-dependent manner (Frey and Morris 1997). Here a ‘tag’ set in a protein synthesis-independent manner will benefit from the proteins synthesized from an independent but convergent input thus converting a transient form of plasticity to long lasting form (Redondo and Morris 2011). STC has been intensively

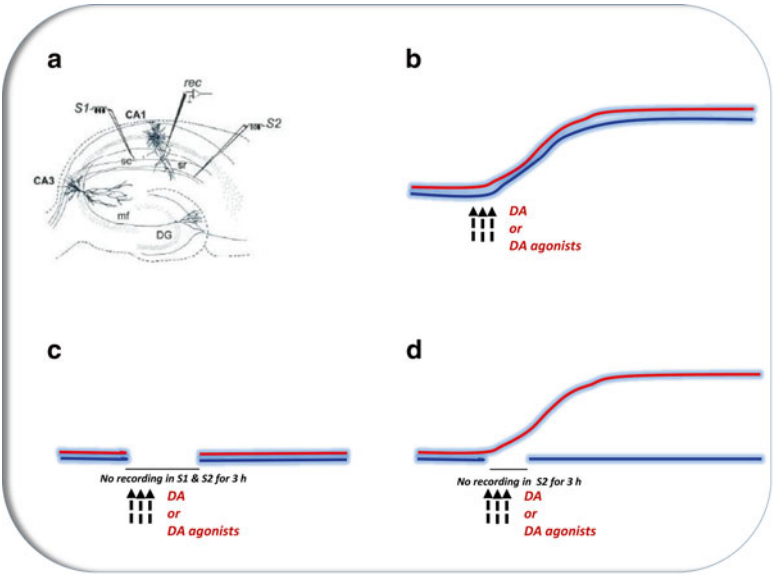

Fig. 8.1 Dopamine (DA) or its agonists induces slow onset potentiation. (**a**) Schematic representation of a transverse hippocampal slice showing the positioning of the electrodes. The two independent synaptic inputs to the same neuronal population, S1 and S2, and the recording sites (Rec) for the field excitatory post-synaptic potentials (fEPSP). (**b**) Repeated application of the D1/D5-receptor agonist or DA for 5 min (represented by *broker arrows*) with an interval of 10 min (a similar situation as compared to the 3 × tetanization protocol used for the induction of conventional late-LTP) induces a slow onset potentiation in synaptic input S1 (*red*) and S2 (*blue*). (**c**) Three times application of a D1/D5-receptor agonist or DA for 5 min with an interval of 10 min did not induce the late-onset potentiation in both synaptic input S1 (*red*) and S2 (*blue*) if the slice was not subjected to control stimulation starting from the time of drug application (point zero) until 3 h. (**d**) Three times application of the D1/D5-receptor agonist or DA for 5 min with an interval of 10 min induces a late-onset potentiation in synaptic input S1 (*red*). The second synaptic input S2 (*blue*) was not subjected to control stimulation from the time point of drug application until 3 h. Here the potentials in the second synaptic input remained stable at baseline values for the remaining period showing that D1/D5-LTP requires glutamatergic input

studied from cell culture system to living rats (Frey and Morris 1997; Martin et al. 1997; Shires et al. 2012). The role of dopaminergic activation during LTP/LTD and tagging has also been reported (Sajikumar and Frey 2004). Dopamine-mediated slow onset potentiation (DA-LTP) also displays same characteristic features of electrically induced LTP in terms of protein synthesis and NMDA-receptor dependency. Indeed, DA-LTP occludes electrically induced LTP depicting a similar mechanism for both. If electrically induced L-LTP can take part in associativity such as STC, could DA-LTP also show the same? We have addressed this question recently published work (Navakkode et al. 2010). We showed that an atypical PKC isotype PKM zeta (PKMζ) can act as a DA-induced plasticity-related protein (PRP), which exerted its action at activated synaptic inputs by processes of synaptic tagging. For these sets of experiments, again the two input models referred in Fig. 8.1a were

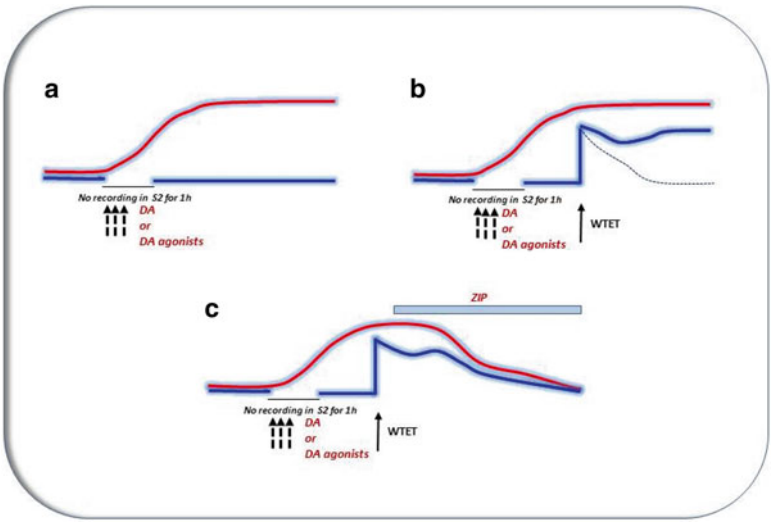

Fig. 8.2 Dopamine and STC. (**a**) Three times application of DA for 5 min with an interval of 10 min induces a late-onset potentiation in synaptic input S1 (*red*). The second synaptic input S2 (*blue*) was not subjected to control stimulation from the time point of drug application until 1 h. Here the potentials in the second synaptic input remained stable at baseline. (**b**) DA was applied to S1 and S2, and recording was suspended in S2 (*blue*) for 1 h after DA application, but not suspended for S1 (*red*). Thirty minutes after restarting recording in S2, early-LTP was induced by a weak tetanization (WTET, 100 Hz 21 pulses). Here the transient early-LTP in S2 was transformed to late-LTP by capturing the PRPs from S1. (**c**) Similar to B but PKMzeta inhibitor (ZIP) was applied 120 min after DA application. PKMzeta inhibition prevented tagging and capture in both S1 and S2. *Broken arrows* indicate the time point of DA application

employed. Baseline recording was suspended in S1 for 1 h after DA application, but not in S2 (Fig. 8.2a). Mechanistically S1 displayed LTP as expected because D1/D5-receptor is positively coupled to adenylyl cyclase which induces subsequent cAMP-dependent processes including activation of protein kinase A and the synthesis of plasticity-related proteins but S2 is expected not to have potentiation because it lacks the NMDA-receptor activity (Navakkode et al. 2007). Thirty minutes after resuming the baseline recording in S1, a protein synthesis-independent form of early-LTP was induced. Here the transient form of early-LTP was transformed to a long lasting form of LTP displaying tagging and capture (Fig. 8.2b) which was sensitive to PKMζ inhibition (Fig. 8.2c). More details about D1/D5-receptor-mediated STC at behavioural level can be referred from (Moncada and Viola 2007; Wang et al. 2010).

What are the mechanisms by which DA-induced LTP and synaptic tagging occur? PKMζ is considered as a molecule that maintains the persistence of long-term memory (Sacktor 2011), but also see (Lee et al. 2013; Volk et al. 2013). The latter findings are mainly due to the compensatory activity of other atypical PKCs such as PKMι/λ. (Personnel communication from Todd Sacktor.) PKMζ can be synthesized and regulated via a synergistic, associative interaction and activation of

dopaminergic and NMDA receptors, as it is reported that it can act as an LTP-specific PRP (Sajikumar et al. 2005, 2007) (Sajikumar et al. 2005b 2007a). Similar to PKMζ, PKA is also required for L-LTP induction (Frey et al. 1993; Abel et al. 1997). PKA also regulates the synthesis of PKMζ (Kelly et al. 2007), thus giving a clear picture that the D1/D5-NMDA-receptor-induced pathway can result in the persistent PKMζ phosphorylation necessary for maintaining LTP (Ling et al. 2002; Serrano et al. 2005). The synthesis of PKMζ in DA-LTP is more local than global (Navakkode et al. 2010). Thus, in apical CA1 dendrites the associative activation of dopaminergic and glutamatergic inputs can result in the local synthesis of PKMζ, which enhances synaptic transmission by doubling the number of post-synaptic AMPA receptors (AMPAR) through GluR2 subunit-mediated trafficking of the receptors to the synapse (Sacktor 2011), thus expressing and maintaining LTP and tagging.

Computational models predict that the temporal and spatial characteristics of input activity to a neuron determine which information is stored in the neuronal networks over a period of time for memory engrams (Govindarajan et al. 2006). Behaviourally relevant stimuli should activate storage processes proportional to both the strength of the synaptic tag and the local concentration of essential protein which where synthesized in accordance with the activation of heterosynaptic inputs such as dopamine. In contrast, behaviourally irrelevant information would not be stored because it is subthreshold for STC. To elegantly accommodate this aspect, the neural computation with regard to heterosynaptic neuromodulation is tightly controlled within a neuron. For instance, we have recently reported that DA-LTP in basal dendrites is dependent on the activation of L-type voltage-gated calcium channels (VDCC) while in apical dendrites it is independent (Navakkode et al. 2012). Activation via NMDA-receptor activity is critical for the induction of DA-LTP in both apical and basal dendrites, but there is specificity for the PRPs, because here brain-derived neurotrophic factor (BDNF) is required only for the induction and maintenance of DA-LTP in apical dendrites. Thus, dopaminergic modulation of LTP is lamina specific at the Schaffer collateral/commissural synapses in the CA1 region (Navakkode et al. 2012).

8.1.4 Dopamine and Memory in Wide Angle

Recent findings from the German Center for Neurodegenerative Diseases and the University of Magdeburg reported that dopamine improves long-term memory, specifically episodic memory (Chowdhury et al. 2012). Chowdhury and colleagues examined elderly population ranging in age from 65 to 75 years to recognize photos which they had been shown previously. Half of the test participants had first taken a placebo and the remainder had taken L-DOPA, a precursor of dopamine. L-DOPA, which is able to reach the brain from the bloodstream, and there it is converted into dopamine. In this way the researchers could exercise a targeted influence over dopamine levels in the brains of the test subjects. Neurons, which produce dopamine decline with age and their episodic memory declines.

The participants were first shown black and white photos of indoor scenes and landscapes. They were to differentiate these images from others, which they had not seen before. When they first viewed the pictures, brain activity of the participants was monitored using a special form of magnetic resonance imaging (fMRI). The photos which triggered hardly any activity in the memory centre were of particular interest to the neuroscientists because this area represents an area of little or no dopamine release. In this case the memory of these pictures should gradually fade since they have been encoded or tagged 'weakly'.

Two and six hours after the participants had memorized the photos, they were requested to recognize and distinguish them from new images. In the test after 2 h there was no significant difference between participants who had taken L-DOPA and those who had consumed a placebo. However, after 6 h memory performance changed. Test subjects with Levodopa recognized up to 20 % more photos than the members of the comparison group. These experiments in human beings bridge results of tagging and capture conducted in the animal models revealing a nice correlation as to how important is dopaminergic neuromodulation and tag-PRP interactions in making persistent memory even in old age.

8.1.5 Future Perspectives

The release of dopamine can be enhanced by strong hippocampal activation (Lisman and Grace 2005). It is not clear how much dopamine is required for the initiation of associative memory in neural networks. A good amount of studies regarding dopamine action is conducted using higher concentration at which dopamine (50 μM) or its agonists, thus it is critical to explore at what particular concentration dopamine can initiate synaptic co-operation or competition leading to memory persistence or erasure. Future works from our laboratory will highlight these aspects of dopamine-mediated associative memory at cellular level.

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Chapter 9
From Where? Synaptic Tagging Allows the Nucleus Not to Care

Shannon Farris and Serena M. Dudek

Abstract Most of us now accept that the formation of long-lasting memories requires de novo transcription of plasticity-related proteins. It is also thought that localized translation of these transcripts, at or near recently activated synaptic sites, structurally stabilizes synaptic connections, thereby consolidating the memory. However, the molecular mechanisms underlying where, when, and how these newly synthesized transcripts participate in memory storage has remained a formidable question in neuroscience. Here we discuss the hypothesis that the nucleus acts as a calculator of incoming signals from activated synapses, either in the form of an electrical signal, through calcium, or as part of a transported signal. As long as a synaptic tag is created, the form of how a signal reaches the nucleus is freed from the requirement of leaving a "trail of breadcrumbs." The nucleus can instead detect information on how the neuron fits into the network (counting number of modified or active synapses, or whether inhibitory neurons have a say, for example). We propose that it is the output of the nucleus, or nucleus-to-synapse signaling, along with the type of synaptic tag formed, that determines whether the right transcript will be translated at the right synapse at the right time. We further discuss the idea of inverse tagging and how local protein synthesis might play a role in distinguishing inactive versus active synapses.

Keywords Synaptic tagging • Action potentials • Dendritic mRNA • Arc/Arg3.1 • Local protein synthesis

The requirement of de novo transcription for long-term synaptic plasticity thought to underlie memory consolidation has been known for over 30 years (reviewed in Davis and Squire 1984 and Goelet et al. 1986). Yet, the coordinated role and function of these newly synthesized transcripts still remains largely unclear. With more recent advancements in high throughput methodologies to assess activity-dependent gene expression, such as microarray and next generation sequencing (NGS), the

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number of distinct RNA transcripts induced by activity is growing, and consists of transcription factors, neurotransmitters, receptors, scaffolding and structural proteins, as well as many others. In addition to the transcripts that are retained and translated in the cell soma, there is a subset of RNAs that are trafficked into dendrites, often hundreds of microns away from the cell body. These dendritic RNAs are thought to play a role in synapse maintenance and modification in response to internal or external stimuli, such as growth factor or neurotransmitter receptor activation. Dendritic RNAs are thought to be in a translationally repressed state, which poises their translation to be locally regulated by compartment-specific cues such as those found at individual synapses. A recent study in rats suggested that there are upwards of 2,500 RNAs present in CA1 hippocampal projections (Cajigas et al. 2012). This number is tenfold greater than the number of dendritic RNAs previously found through microarray studies (Zhong et al. 2006; Poon et al. 2006) and underscores the important role(s) these RNAs might have in terms of spatially and temporally controlling synapse-specific plasticity. However, the question remains as to how a transcript packaged and shipped from the nucleus is able to find the synapse that initially signaled for its transcription.

One attractive model is synaptic tagging and capture (Frey and Morris 1997), where a synapse is "tagged" to indicate that it had recently undergone some form of plasticity in order to be able to "capture" the correct gene products required for long-term stabilization. This model maintains input specificity if new RNA or protein synthesis is required for stabilization. Synaptic tagging has been shown to occur for both long-term potentiation (LTP, Frey and Morris 1997; Barco et al. 2002; Dudek and Fields 2002; Fonseca et al. 2004; Young and Nguyen 2005) and long-term depression (LTD, Kauderer and Kandel 2000; Sajikumar and Frey 2004). However, the search for the identity of such a tag is still an area of heavy investigation, where no tag quite fits all conceptual requirements. Here we discuss whether the signal sent from the synapse to the nucleus plays a critical role in the synaptic tag and capture hypothesis, or whether the nucleus to synapse signal is sufficient to stabilize synapses that have been tagged. We discuss potential types of tags and how local protein synthesis might play a role in distinguishing inactive versus active synapses.

9.1 Synapse-to-Nucleus Signaling: What *Counts* to the Nucleus?

We have previously proposed that synaptic activity-induced signaling from the synapse to the nucleus does not happen on a fast enough time scale to account for some of the transcriptional events that happen within minutes, namely immediate early gene (IEG) transcription (Adams and Dudek 2005). For example, the well-characterized IEG *Arc*, also known as *Arg3.1*, can be detected in hippocampal pyramidal cells within 2 min after neuronal activity (Guzowski et al. 1999). Instead, we proposed the idea that calcium influx evoked from synaptic Excitatory Postsynaptic

Potentials (EPSPs), or more likely EPSPs together with action potentials, could account for the early transcriptional events occurring in the nucleus, as these can induce calcium-dependent signals instantaneously and within close proximity to the nucleus (Saha and Dudek 2008; Dudek and Fields 2001; Zhao et al. 2005). That stated, since the time we first considered these time, distance, and volume constraints (Adams and Dudek 2005), several examples of synapse-to-nucleus signals (SNSs) that display activity-dependent synaptonuclear shuttling have been described, many of which translocate to the nucleus specifically in response to LTP-inducing stimulation. These include TORC1 (CRTC1), CREB2, JACOB, and ERK1/2 (Zhou et al. 2006; Ch'ng et al. 2012; Lai et al. 2008; Behnisch et al. 2011; Karpova et al. 2013; Davis et al. 2000; Patterson et al. 2001). In the case of ERK1/2, Yasuda and colleagues recently reported that stimulating as few as 3–7 spines on the dendrites of neurons in culture with uncaged glutamate is sufficient to lead to nuclear ERK activation as measured by a nuclear fluorescent reporter (Zhai et al. 2013). Nuclear ERK activation was inhibited by the NMDAR antagonist APV but not by blockade of Voltage Gated Calcium Channels with $CdCl_2$ (and experiments were performed in tetrodotoxin to block action potentials). Therefore the authors reasoned that uncaging-induced nuclear ERK activation was not caused by direct membrane depolarization in close proximity to the nucleus. In the same study, the experiments above were performed within 200 μm from the cell soma and when synapses activated at greater distances, the authors noted that nuclear ERK activation took ~40 min to reach the same level of activation, for example (Zhai et al. 2013). Thus, even in this study, evidence that SNS nuclear import occurs on a sufficiently short time scale required for many IEG transcription (<2 min) is lacking. Furthermore, evidence that comes from studies investigating the mechanism of ERK dendritic trafficking supports the idea that rather than being transported actively (such as with a molecular motor), ERK1/2 is propagated by passive diffusion and is imported to the nucleus via facilitated diffusion, which would make it an unsuitable synapse-to-nucleus signal unless activated in very close proximity to the cell soma (Wiegert et al. 2007).

How would genes be transcribed rapidly (with 2 min) in response to neuronal activity? We found that promoter regions of the fastest of the IEGs such as Arc (rapid IEGs) come "pre-charged" with RNA polymerase II (Pol II), in effect poising the genes for a rapid response (Saha et al. 2011). In these cases the Pol II is proposed to have already initiated transcription, but is paused, apparently awaiting a signal, a process mediated by the Negative Elongation Factor (NELF) complex (Adelman and Lis 2012). There is little question that signaling from the synapse to the nucleus is likely to have profound effects on the later transcriptional output of the nucleus, such as for the slower "delayed IEGs" (Saha et al. 2011), or the so-called second wave of transcription. Also possible is that some of the activity-dependent synaptonuclear proteins are acting as sites of integration for the nucleus, as has been proposed for αCaMKII, whose active form has been shown in vitro to increase as a function of number of inputs and frequency, whereas activity of calcineurin increases with number of inputs (Fujii et al. 2013). Despite the compelling evidence for activity-dependent SNSs and the information they may carry on the

type and amount of stimulus, it still begs the question as to how the nucleus integrates incoming signals to produce a coordinated change in gene expression that can selectively modify tagged synapses to impact synaptic function.

We propose, that as long as the synaptic tag is created, the signal that makes it to the nucleus need not contain locale-specific information. In our model, the nucleus acts as a calculator of incoming signals from activated synapses, either in the form of an electrical signal, through calcium, or as part of a transported signal. To our knowledge, evidence in support of the idea that incoming signals can specify information regarding the location of the tag, or a "follow the trail of breadcrumbs back to the synapse" model has yet to be reported. Our proposed model is not only independent of what synaptic signals come in but it also allows for the nucleus to integrate multiple electrical signals imposed upon the neuron from the network. For instance, the nucleus can integrate signals from excitatory *and* inhibitory neurons, on both the soma and distal dendrites, and if the threshold for repeatedly firing action potentials is reached, the nucleus can respond accordingly (Saha and Dudek 2013). Who gets the product? That is up to the tag!

9.2 Activity-Dependent Transport of mRNAs to the Synapse: What's in a Tag?

9.2.1 Dendritic mRNA Transport and Its Role in Tagging

Despite considerable investigation, surprisingly very little is known about how dendritically targeted mRNAs get docked at synaptic sites. Previous studies have shown that dendritic mRNAs display bidirectional transport in dendrites with rates consistent with microtubule-based transport (Köhrmann et al. 1999; Dynes and Steward 2007; Dictenberg et al. 2008; Tübing et al. 2010). Bidirectional dendritic transport suggests that mRNAs, present as ribonucleoprotein particles (RNPs) consisting of one or a few mRNA transcripts and their RNA binding proteins, might shuttle from synapse to synapse or within dendritic compartments. Another critical finding was that many of the RNA binding proteins associated with dendritically targeted RNPs are translational repressors, suggesting that mRNAs are translationally silent as they are being transported (Krichevsky and Kosik 2001; Napoli et al. 2008; Fritzsche et al. 2013). This led to the appealing notion that synaptic activity could locally remodel dendritic RNPs to allow for translation to occur in a synapse-specific manner. In the example of *Arc* mRNA, there is evidence for *Arc* RNPs being translationally repressed when associated with the RNA-binding protein fragile X mental retardation protein (FMRP) and cytoplasmic FMRP-interacting protein 1 (CYFIP1). However after brain-derived neurotrophic factor (BDNF) treatment, *Arc* RNPs are remodeled by Rac1-dependent phosphorylation of CYFIP1 that recruits proteins critical for cytoskeletal arrangement, such as the WASp-family verprolin homologous protein (WAVE) regulatory complex (De Rubeis et al. 2013). These data

suggest that dendritic RNPs are dynamic and highly regulated both spatially and temporally depending on the extracellular cues.

Because it is likely that the synaptic tag(s) differs depending on the cell type and form of plasticity, we reason that seeding the materialization of variable tags is a common underlying process. For example, local actin remodeling and/or local protein synthesis are both processes that have been shown to be required for many forms of synaptic plasticity and might be a mechanism by which tags can be built upon within a common framework depending on the microenvironment it encounters (Martin and Kosik 2002). Similarly, the sushi belt model (Doyle and Kiebler 2011) proposes that RNPs do not statically anchor to synaptic sites but rather patrol a dendritic compartment until recruited into a synapse that recently underwent activity-dependent tagging. This model complements ours in that how the signal reaches the nucleus is relevant for neither tag generation nor the synapse's ability to recruit plasticity-related proteins (PRPs). Rather, the output of the nucleus, whether transported RNPs or PRPs, gets recruited to the tag independently of the signal to the nucleus in order to stabilize plasticity of synaptic transmission.

9.2.2 The Functional Role of ARC in Inverse Synaptic Tagging

Until recently, the synaptic function of the IEG ARC has been at odds with studies implicating ARC in both the strengthening and weakening of synaptic contacts. *Arc* transcription is known to be strongly induced with neuronal activity that produces both LTP and long-term memory and *Arc* mRNA is transported to and localized near activated synapses, presumably to be locally translated (Lyford et al. 1995; Link et al. 1995; Guzowski et al. 2000; Steward et al. 1998). Furthermore, *Arc* mRNA is degraded in an activity- and translation-dependent manner in dendrites, consistent with a tight temporal and spatial burst of translation near activated synapses (Farris et al. 2014). ARC protein may play a role in stabilizing F-actin during consolidation, as depletion of ARC via antisense oligonucleotides 2 h after LTP resulted in a rapid decay of LTP and loss of F-actin at synaptic sites (Messaoudi et al. 2007). ARC protein has also been shown to interact with members of the endocytic machinery, dynamin, and endophillin, leading to the internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and therefore a weakening of synapses during both LTD and homeostatic forms of synaptic plasticity (Chowdhury et al. 2006; Shepherd et al. 2006; Rial Verde et al. 2006). How one molecule could participate in so many forms of plasticity remains unclear. Recently, however, Okuno and colleagues have discovered that ARC protein, induced from strong synaptic activation, formed a tight interaction with the inactive (calmodulin-unbound) β isoform of CaMKII at unpotentiated or weak synapses (Okuno et al. 2012). Synaptic levels of ARC were correlated with the removal of surface AMPARs, an effect that was abolished with a lack of CaMKIIβ (Okuno et al. 2012). Some evidence that such a widespread weakening of synapses,

developing 1 h after action potential firing has been presented (Bukalo et al. 2013), even though the same stimulus "rescues" early-LTP at tagged synapses from decay (Dudek and Fields 2002). These data together suggest that *Arc* induced by activity may contribute to late-phase long-term plasticity by preventing the enhancement, or even by inducing depression of unstimulated synapses, to maintain balance of synaptic weights.

What is fascinating about this idea of inverse tagging, though, is that it unites plasticity-induced gene expression, dendritic mRNA localization, and local translation with tagging and synapse-specific remodeling. CaMKII is an enzyme consisting of two subunits (α and β) in the brain. The regulatory subunit (CaMKIIβ) is thought to be translated in the soma and then transported to synapses, whereas CaMKIIα is locally translated only at active synapses. This situation is well suited for CaMKIIα to act as an initial tag at activated synapses to capture CaMKIIβ to form the active CaMKII enzyme which can then initiate downstream pathways such as actin remodeling, which can then serve as a tag for further PRPs. However, for inactive synapses without CaMKIIα, CaMKIIβ presence serves as the tag for locally translated ARC to be captured and prevent the undesired enhancement of weak synapses (Nonaka et al. 2014). This example of inverse synaptic tagging illuminates novel and interesting avenues for future tagging research as well as providing a mechanism for how ARC can participate in multiple forms of synaptic plasticity. Remaining unclear, though, is the function of ARC protein enriched in the dendritic shaft, which in the presence of calcium and calmodulin, prefers to interact with CaMKIIα.

Interestingly, inverse tagging may be consistent with the concept of "cross-tagging," a positive associative interaction of LTP and LTD in which late-LTP (or late-LTD) at one synaptic input is able to promote stabilization of the opposite form of plasticity (LTD or LTP, respectively) at another independent synaptic input where only the early phase was induced (Sajikumar and Frey 2004; reviewed in Frey and Frey 2008). The functional crosstalk between various, and seemingly opposing, forms of plasticity at separate synapses imposes additional requirements for the tagging and capture of PRPs. Namely, the tags must be specific for each type of plasticity and the genes required for both LTP and LTD must be induced either by the late-inducing stimulus or at least available from prior transcriptional history. Consistent with cross-tagging, inverse tagging also requires that PRPs are "captured" by both active and inactive synapses that were "tagged" by different mechanisms (see Fig. 9.1). Both mechanisms expand the synaptic tagging hypothesis to include a cell-wide capture process that integrates plasticity at different afferents and considerably strengthens the argument that PRPs or signals from the nucleus do not rely on locale-specific signals from their inputs. The inverse tagging model does not, however, specifically address whether or how ARC, or other IEGs might play a role in stabilizing synaptic plasticity at modified synapses or whether any input specificity to LTD can be maintained. We look forward to future studies on this front.

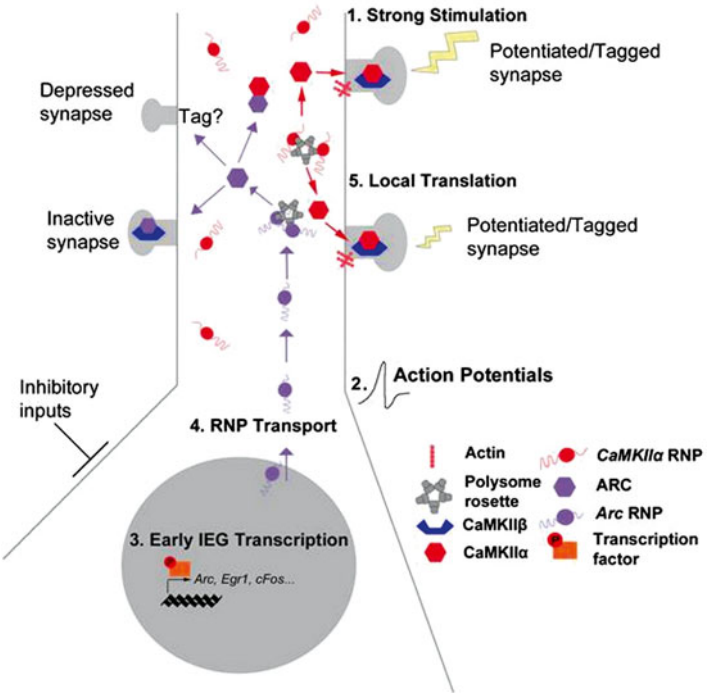

Fig. 9.1 ARC: the proverbial "tag along" in synaptic and inverse tagging. Having ARC be at the right place at the right time is critical for multiple forms of synaptic plasticity. Therefore it is not surprising that ARC is heavily regulated at every level from birth (transcription) to death (protein degradation). Here we depict the steps necessary for ARC protein to make it to a potentiated or tagged synapse. (1) A strong afferent stimulus is needed to generate (2) action potentials, which are sufficient to (3) induce *Arc*. *Arc* mRNA is then packaged into a ribonucleoprotein (RNP) particle and (4) transported out of the nucleus and trafficked via microtubule-based transport into dendrites. *Arc*'s translation is suppressed until its RNP is remodeled by extracellular cues, such as signals from a potentiated synapse, whereby it gets (5) rapidly translated by polyribosomes present in dendrites. How ARC protein finds a potentiated or tagged synapse is not known, although it may be directly due to the docking of its mRNA near active synapses. ARC co-immunoprecipitates with F-actin and CaMKII**α** and has been suggested to play a role in stabilizing F-actin during consolidation. *CaMKIIα* mRNA is present constitutively in dendrites, but has also been shown in vivo to localize near stimulated synapses. It is likely, that the local translation of *CaMKIIα* occurs soon after stimulation since it does not need to be transported from the nucleus in response to activity. Alternatively, (illustrated here), ARC protein has been shown in vitro to behave like an "inverse tag" by way of its association with the inactive form of CaMKII**β** at unpotentiated synapses, possibly ensuring the synapse remains weak by removal of AMPARs. Similarly, ARC has been shown to be required for synaptic depression by removing AMPARs from the synapse, although unlike with inactive synapses, it is not known what recruits ARC to depressed synapses and whether this occurs after a strong stimulus

9.3 Conclusions

Recent data describing mechanisms underlying nuclear to synapse signaling can shed light on our understanding of synaptic tagging and capture and how mRNA localization and local protein synthesis might fit into the picture. The extensive bidirectional dialogue between the nucleus and the synapse underscore the complexity and amount of regulation concerning these processes as they relate to plasticity and learning. However, what is even less clear is how these processes fit into the specific networks in the context of complex behaviors. New models that can integrate how mRNA targeting and local protein synthesis contribute to synaptic tagging and how multiple inputs can be calculated to form a coordinated output of gene expression to modify synaptic transmission will be critical for understanding the mechanisms of learning and memory.

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Part II
Behavioral and Metaplastic Aspects of Synaptic Tagging and Capture

Chapter 10
BDNF and TrkB-Mediated Signalling Supports Processes of Metaplasticity and Long-Term Memory Formation

Martin Korte

Abstract In this chapter a special class of small secretory peptides, the neurotrophins, will be introduced as candidate molecules in order to calibrate and possibly consolidate memory contents. Numerous reports show that the neurotrophin BDNF and its TrkB receptor are mediators of positive structural and functional plasticity in the developing and adult nervous system. In the context of memory consolidation and long-lasting synaptic plasticity it is noteworthy that BDNF is discussed as an important transformer of functional into structural changes. Results presented here suggest that BDNF indeed has a functional and specific role in the consolidation of synaptic plasticity and may exert this role by stimulating the local (dendritic) production of plasticity-related proteins (PRPs). BDNF might be itself a PRP and it might be able to orchestrate the plasticity threshold for a whole cluster of synapses, and might therefore be involved in processes of metaplasticity and homeostasis as well.

Keywords BDNF • LTP • Metaplasticity • TrkB • Synaptic tagging • Synaptic capture

10.1 Neurotrophins and Their Receptors

In this chapter a special class of small secretory peptides, neurotrophins will be introduced as candidate molecules in order to calibrate and possibly consolidate memory contents. Neurotrophins were originally considered to be involved in the regulation of development, maintenance, and function of the vertebrate nervous system (for a review see Huang and Reichardt 2001). The discovery of nerve growth factor (NGF) as the first neurotrophin (Levi-Montalcini 1987) represented a hallmark in understanding molecular guidance cues and revealed the importance of

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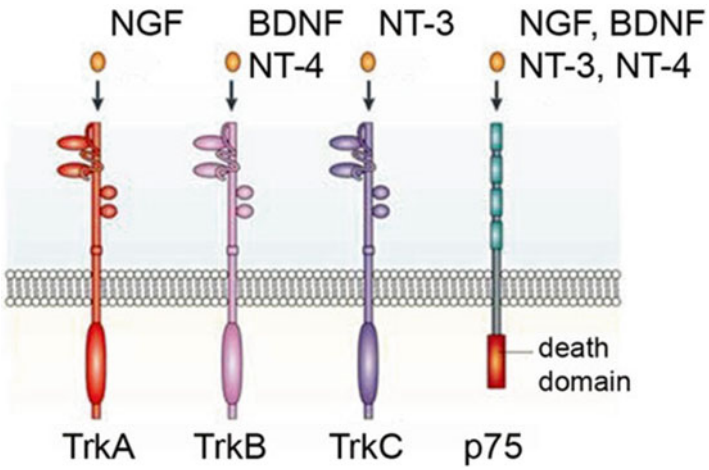

Fig. 10.1 Neurotrophins and their receptors. Neurotrophins bind selectively to one tropomyosin-related kinase receptor (Trk), whereas all bind to the pan neurotrophin receptor $p75^{NTR}$ with equimolar affinity. *NGF* nerve growth factor, *BDNF* brain-derived neurotrophic factor, *NT* neurotrophin

cellular interactions during development. Initially described as survival factors secreted by the target tissues, increasing evidence suggests that neurotrophins are as well involved in mechanisms of functional and structural plasticity (for reviews see Chao 2003; Lu et al. 2005; McAllister et al. 1999; Park and Poo 2012). In mammals, four different neurotrophins have been described (Fig. 10.1): NGF, brain-derived neurotrophic factor (BDNF), neurotrophin 3 and 4 (NT-3, NT-4). While all of them bind with equimolar affinity to the pan neurotrophin receptor $p75^{NTR}$, each neurotrophin interacts preferentially with one of the so-called Trk receptors (tropomyosin-related kinase receptors, reviewed in Minichiello 2009): NGF activates TrkA, BDNF and NT-4 are specific for TrkB, NT-3 preferentially interacts with TrkC but it is also able to activate all other neurotrophin receptors to a lesser extent. Synthesized as precursors, neurotrophins are proteolytically processed to form mature proteins. Neurotrophins have been shown to bind and dimerize Trk-receptor tyrosine kinases, resulting in the activation of the intracellular kinase through trans-phosphorylation. Endocytosis and transfer of Trk receptors to different membrane compartments control Trk-mediated signalling, especially as many of the important adaptor proteins are localized within distinct membrane compartments. Moreover, alternative splicing results in kinase-lacking isoforms of TrkB and TrkC (Klein et al. 1990). Trk receptors carry ten conserved tyrosine residues, three of which are involved in controlling the kinase activity of the receptor complex. Phosphorylation of the other residues regulates the interaction with proteins carrying phosphotyrosine-binding (PTB) or Src-homology 2 (SH2) domains (Chao 2003). Neurotrophin binding to Trk receptors activates essential intracellular pathways important for neuronal survival and differentiation (Figs. 10.1 and 10.2): Ras, PI3K (phosphatidylinositol 3-kinase), PLC-γ (phospholipase C, γ isotype) and their downstream effectors are involved in Trk-mediated signalling (Huang and Reichardt 2001). While the Trk

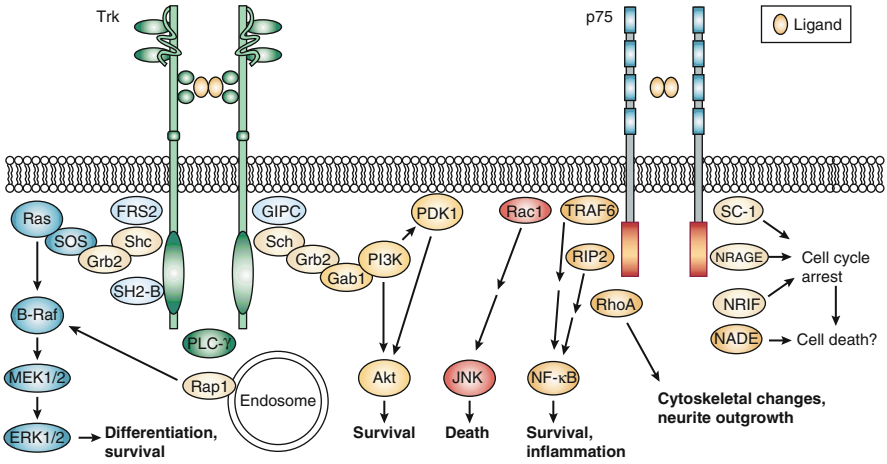

Fig. 10.2 TrkB signalling. *Akt* protein kinase B, *FRS2* fibroblast growth factor receptor substrate 2, *Gab1* Grb2-associated binder 1, *Grb2* growth factor receptor-bound protein 2, *GIPC* GAIP interacting protein, C terminus, *MEK* mitogen-activated protein kinase (MAPK)/ERK kinase, *NADE* neurotrophin-associated cell death executor, *NRIF* neurotrophin-receptor-interacting factor, *NRAGE* neurotrophin-receptor interacting MAGE homologue, *PDK1* phosphoinositide-dependent kinase 1, *RIP2*-receptor-interacting protein 2, *SC-1* Schwann cell protein 1, *SH2B* Src-homology 2-B, *SOS* son of sevenless, *Shc* Src homologous and collagen-like adaptor protein, *TRAF-6* tumor necroses factor receptor-associated factor 6 (Adapted after Chao, M. 2003)

receptors exert well-defined trophic functions, $p75^{NTR}$ is reported to mediate such diverse effects as cell survival and apoptosis. $P75^{NTR}$ binds all neurotrophins with similar affinity (Rodriguez-Tebar et al. 1990). $P75^{NTR}$ is a member of the tumor necrosis factor superfamily with an extracellular domain comprised of four cysteine-rich repeats and a cytoplasmic tail including a "death" domain comparable to those present in other members of this family. Remarkably, $p75^{NTR}$ was also reported to be a co-receptor for the Trk receptors. Co-expression of $p75^{NTR}$ can increase the affinity of Trk receptors for their neurotrophins and is able to further enhance their ligand specificity (Bibel et al. 1999). Numerous reports show BDNF and its TrkB receptor as mediators of positive structural and functional plasticity in the developing and adult nervous system (Zagrebelsky and Korte 2013). Yet, growing evidence indicates that $p75^{NTR}$ could act as the opposing player of Trk receptors involved in long-term decrease of synaptic efficacy as well as in negatively regulating dendrite structure. This idea is supported by studies in $p75^{NTR}$ knockout mice showing an impairment in the maintenance of long-term depression (Rösch et al. 2005; Woo et al. 2005). Long-term potentiation (LTP), however, was found to be unaltered in these animals. Furthermore, dendritic complexity and spine density are increased in organotypic hippocampal slice cultures of $p75^{NTR}$ knockout mice (Zagrebelsky et al. 2005). In the same study, the overexpression of $p75^{NTR}$ in pyramidal neurons led to a reduction in dendrite structure and spine number. Taken together the Trk receptors and the $p75^{NTR}$ emerge as a dual receptor system whose precisely regulated action and expression patterns may provide the neurons with the ability to tightly control both their synaptic weight and structural plasticity.

10.2 BDNF: Gatekeeper and Mediator of Synaptic Plasticity

The most prominent candidate of all neurotrophins playing a significant and instructive role in processes of synaptic plasticity is BDNF. Here, BDNF stands out among all neurotrophins in the activity-dependent regulation of its expression and secretion. It has been very early on implicated in modulating neuronal activity, and its own production and release have been shown to be activity-dependent (reviewed in Thoenen 1991, 1995). BDNF has been shown to enhance synaptic transmission in the peripheral as well as in the central nervous system (reviewed in Gottmann et al. 2009; Park and Poo 2012; Zagrebelsky and Korte 2013). In particular the role of BDNF in modulating the long-term enhancement of synaptic efficacy in hippocampal pyramidal neurons has been studied intensively. Specifically, BDNF-deficient mice show an impairment in hippocampal LTP (see e.g. Korte et al. 1995) that could be rescued by reintroduction of exogenous BDNF (Korte et al. 1996; Patterson et al. 1996). At the same time, neuronal activity increases the number of TrkB receptors at the surface of hippocampal neurons thereby promoting the action of BDNF (Du et al. 2009). Interestingly, the activation of TrkB can be seen as a link between changes in synaptic strength and structural alterations. Neurotrophins have in fact been shown to regulate cortical growth in an activity-dependent manner (McAllister et al. 1999). In addition, due to its effects on neurite outgrowth and differentiation of certain subtypes of neurons, BDNF is a strong candidate which can transform functional changes into structural changes, either during development or as a consequence of changed neuronal activity (see e.g. Korte 2008; Tanaka et al. 2008). Deletion experiments targeting TrkB in cortical pyramidal neurons reported dendrite retraction and neuronal loss, further underlining its role as a positive modulator of dendrite structure (Xu et al. 2000). Furthermore, the BDNF-TrkB signalling is reported to positively modulate axonal branching (Cohen-Cory and Fraser 1995) as well as spine density (Tyler and Pozzo-Miller 2003). In addition, upon high-frequency stimulation, BDNF is secreted in a manner, which is dependent on Ca^{2+} influx through NMDA-subtype glutamate receptors or voltage-gated Ca^{2+} channels (Balkowiec and Katz 2002). The origin of BDNF release is not entirely clear. The consistent view is that it can be released presynaptically, but the postsynaptic release is under intense discussion (Dieni et al. 2012; Edelmann et al. 2013). Once secreted into the synaptic cleft, BDNF can bind to TrkB localized at both pre- and postsynaptic sites of glutamatergic synapses (Drake et al. 1999). In the postsynaptic density (PSD), TrkB is associated with PSD95 and NMDA receptors. In addition, the expression of BDNF, particularly the transcription of the BDNF gene initiated at promoter III, is tightly controlled by neuronal activity and BDNF is sufficient to induce the transformation of early- to late-phase L-LTP in the presence of protein synthesis inhibitors, and inhibition of BDNF signalling impairs long-term memory (Pang et al. 2004). In addition to these studies it could be shown in BDNF KO mice (Korte et al. 1995; Patterson et al. 1996; Korte et al. 1998), and in conditional TrkB KO mice (Minichiello et al. 1999; Barco et al. 2005) as well as in TrkB-signalling mutant mice (Minichiello et al. 2002) that BDNF/TrkB affects two phases of LTP: the induction

phase and the maintenance, late phase of LTP. These experiments also support the notion, that BDNF is modulating LTP via the TrkB receptor, and not via the $p75^{NTR}$, which is most likely involved in mediating LTD (Rösch et al. 2005; Woo et al. 2005). Furthermore, the effect of TrkB signalling on LTP is pre- and postsynaptic (Gärtner et al. 2006) and the mature form of BDNF is released and not proBDNF (Matsumoto et al. 2008). What is not clear is how TrkB is acting at synapses in order to enhance the possibility of LTP induction and maintenance. One possibility would be that TrkB-mediated signalling influences the activity of certain kinases and one should explore further which kinase is of special importance. In this context it is important to mention that protein kinase C (PKC) consists of an amino-terminal regulatory domain, an autoinhibitory pseudosubstrate sequence, second-messenger binding sites, and a carboxy-terminal catalytic domain (Ohno and Nishizuka 2002). PKC is normally held in an inactive basal state by interactions between two domains. PKMζ is an atypical PKC isotype and is produced by a unique PKMζ mRNA, which is generated by an internal promoter within the PKCζ gene and transported to the dendrites of neurons. Both PKMζ mRNA and protein are expressed specifically in the vertebrate brain and provide an interesting link to BDNF/TrkB-receptor action.

In the context of memory consolidation and long-lasting synaptic plasticity it is noteworthy that BDNF is discussed as an important transformer of functional into structural changes. Here, a study of Tanaka et al. (2008) is of particular importance. The authors observed an enlargement of spine head size on pyramidal hippocampal neurons, which is gradual, strong, and persistent only when postsynaptic spikes were induced precisely correlated with glutamate release by uncaging of glutamate (paired protocol, fulfilling the Hebbian rule of almost simultaneous pre- and postsynaptic activity). This enlargement can only be observed in a single, activated spine, whereas neighboring spines are not affected due to the high precision of the uncaging of glutamate by using a two-photon microscope. The electrophysiological results were similar in terms of the amplitude of glutamate-induced currents. The authors also observed that while the spine head was increased in volume, spine length was reduced with the paired protocol, but at the same time, the spine neck was increased in its thickness, making it less of a barrier to the underlying dendrite. Next, the authors explored the requirement for the gradual spine enlargement. In order to stabilize changes in synaptic efficacy it has been shown earlier, that the persistence of synaptic plasticity is dependent on protein synthesis (Frey et al. 1988). And indeed, Tanaka et al. could block the observed structural changes by inhibiting protein synthesis. Most importantly in the context of this book chapter they could show that BDNF is necessary and sufficient for the induction of long-lasting structural changes at the analyzed spine, as indicated by the application of BDNF itself or by a blockade of BDNF signalling via TrkB. Tanaka et al. focus on postsynaptic changes and they conclude that BDNF is released by a mechanism that depends on postsynaptic spikes, but due to the low abundance of BDNF in the brain they could not show this directly. However, alternative views are possible, e.g. it has been shown, that BDNF can act not only post- but also presynaptically. Further it should be mentioned, that instead of increased BDNF release after postsynaptic spikes it is quite possible, that the TrkB receptor becomes more sensitive to BDNF-mediated signalling. Here it is noteworthy, that the TrkB receptor can also be

phosphorylated by a transactivation mechanism including G-protein-coupled receptors (Wiese et al. 2007). But for sure the link between BDNF and possible local protein synthesis is an important one, as this is the bottle neck for the persistence of synaptic plasticity. In the future it would be important to see if the newly manufactured proteins tag the synapse for further activity-induced changes or try to produce enough plasticity-related molecules in order to make sure that plastic processes can also happen in future events. It is of high interest in this context that BDNF also enhances the synthesis of CaMKII and Arc in synaptodendrosomes and synaptoneurosome. Lessman and colleagues (Kolarow et al. 2007) recently reported that activity-dependent BDNF secretion is correlated with activation of CaMKII and PKA signalling and this opens the possibility that also BDNF/TrkB signalling and PKMζ are linked to each other.

Other reasons why BDNF among all neurotrophins is considered to be the one mediating synaptic plasticity is evident from studies in humans indicating BDNF as an important regulator of specific memory processes: evidence comes from a common single-nucleotide polymorphism in the human BDNF gene (a methionine for a valine substitution at the codon 66; Val66Met), which leads to an impairment of BDNF secretion. This is most likely due to the misfolding and a less efficient sorting of the proBDNF protein in the Golgi apparatus. Especially the activity-mediated release of BDNF seems to be impaired. In humans with the Val-Met genotype the performance in hippocampus-dependent learning tasks is compromised in comparison to Val-Val genotypes, showing the relevance of the BDNF signalling system for processes of hippocampus-dependent learning and memory formation (Egan et al. 2003).

10.3 BDNF as Plasticity-Related Protein

As mentioned above, it has also been reported, that BDNF is not only important for the induction of LTP, but that it is also promoting specifically the protein synthesis-dependent phase of L-LTP (long-lasting LTP) (Kang and Schuman 1996; Figurov et al. 1996; Korte et al. 1998). Here, the BDNF–TrkB receptor-CaMKIV-mediated activation of CREB leads to changes in the transcription of certain genes, promoting synaptic plasticity. In addition, BDNF has been shown to induce and accelerate local protein synthesis in dendrites (Kang and Schuman 1996; Aakalu et al. 2001). Furthermore, it could be shown, that BDNF is released not only during and directly after the induction of LTP, but also during the maintenance phase of LTP (Kang et al. 1997).Additionally, it is noteworthy that in some forms of synaptic plasticity, BDNF maintains synaptic weight changes via the induction of local dendritic translation (Kang and Schuman 1996; Huang and Kandel 2005). These results suggest that BDNF indeed has a functional and specific role in the consolidation of synaptic plasticity and may exert this role by stimulating the local (dendritic) production of PRP (plasticity-related protein).

PRPs are relevant in the context of the synaptic tagging and capture (STC) hypothesis, which could explain the associative interaction between two sets of

synapses within the same neuronal network (for a review see Frey and Morris 1998; Martin and Kosik 2002). A synaptic "tag" or "mark" initiated by a transient event sequesters PRPs from a nearby strong event, thus resulting in the consolidation of synaptic potentiation in an input-specific manner. Barco et al. (2005) indeed carried out an STC type of a two-pathway experiment in hippocampal slices of BDNF +/– KO mice and stimulated two independent inputs to the same CA1 neuronal region (these mice have only a limited amount of BDNF available). They found evidence, that in BDNF heterozygous KO mice STC was significantly impaired (Barco et al. 2005). Following up on these findings, in a recent study Sajikumar and Korte (2011) studied the role of BDNF in the context of the STC hypothesis. Here, BDNF might only come into play in some forms of long-term memory, highlighting its specificity and instructive role: The form of L-LTP which Sajikumar and Korte (2011) have studied is a local form of L-LTP (Huang and Kandel 2005) and this model allows them to investigate synapse-specific L-LTP to be achieved by a modest stimulation that does not involve the nucleus and therefore processes of transcription. The local form of LTP in this study is exclusively maintained by BDNF which might stimulate local protein synthesis whose products (other PRPs) are only available to a specific dendritic compartment, thus not for sharing with other (Sajikumar and Korte 2011). This indicates, that also PRPs can be spatially restricted to a cluster of synapses e.g. on a certain dendritic branch. Surprisingly, LTP induced by a theta burst stimulation (TBS) was accessible to cross-capture, a positive associative interaction of LTP and LTD (Sajikumar and Frey 2004). This indicates that a local form of LTP can initiate cross-tagging, and that the tag set during the induction of early-LTD utilizes BDNF as a PRP for its maintenance, whereas other processes of synaptic plasticity were dependent on PKMζ, but not BDNF. Sajikumar and Korte conducted experiments addressing the question how dendritic compartments with different plasticity threshold act as a functional unit in the neuronal networks for storing long-term memory. In this context the term plasticity thresholds refers to the capacity of a synaptic unit to process incoming information (Govindarajan et al. 2006). Sajikumar and Korte (2011) could also show that hippocampal neurons have different synaptic compartments and within these compartments independent "synaptic units" or "clusters" exist. The synaptic clusters within the dendritic compartments process the information based on the strength of incoming information. It was thought earlier that each of these dendritic compartments will have similar levels of a "modification threshold", called "plasticity threshold" for coding a particular information. For example, short-term memory storage does not require a plasticity threshold, while long-term memory storage (consolidation) requires to overcome a certain level of plasticity threshold. These results revealed that the synaptic clusters have different plasticity thresholds and are able to process information independently. The threshold is modifiable based on the previous activity of a neuron by a process called "metaplasticity". And here, BDNF has a special role (Fig. 10.3): it is necessary for establishing certain particular types of memory (cross-capture, tagging between potentiated and depressed synapses), whereas PKMζ, an atypical PKC isotype, is necessary for coding other types of memory, like tagging of potentiated synapses (Sajikumar and Korte 2011).

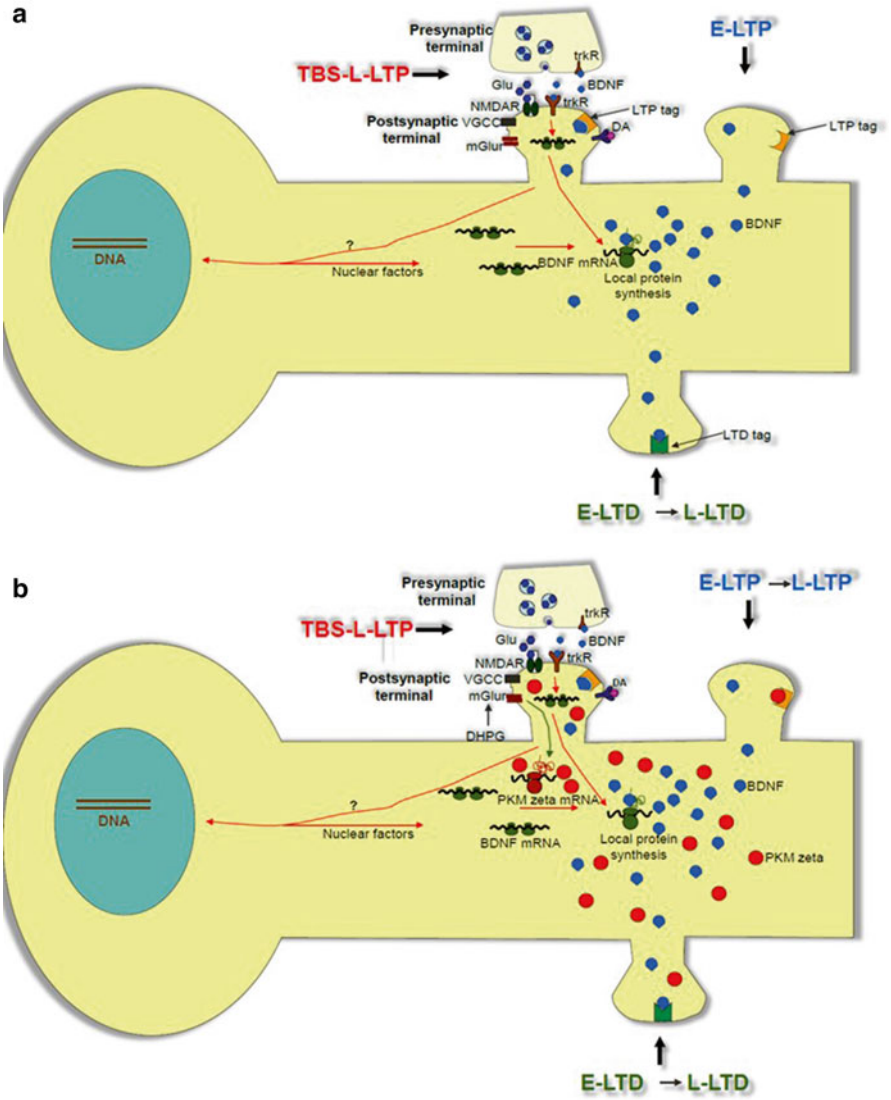

Fig. 10.3 Metaplasticity governs compartmentalization of STC party via BDNF. (**a**) Scheme representing synaptic tagging and capture (STC) and cross-tagging initiated by TBS-LTP in synaptic input S1 (*red*). Application of a TBS (5 Hz, 30 s) results in the presynaptic release of glutamate (Glu) and BDNF, which then activates presynaptic and postsynaptic tyrosine kinase-B receptors (TrkB). Glutamate along with depolarization leads to the activation of *N*-methyl-D-aspartic acid receptors (NMDAR), voltage-gated calcium channels (VGCC), activation of D1/D5 receptors by dopamine (DA), and TrkB receptors that lead to long-lasting maintenance of LTP in this synaptic pathway. Continuous activation of TrkB receptors initiates the translation processes of mRNAs located in the activated dendritic compartment and leads to the upregulation of BDNF. The secreted BDNF disperses to nearby clusters such as S2 (*blue*) and S3 (*green*). The LTP tag (*orange*) in synaptic input S2 is unable to use BDNF as a plasticity protein and fails to show STC in these clusters,

10.4 How BDNF Mediates (or Supports) Synaptic Plasticity?

To further explore the pre- and or postsynaptic function of BDNF in STC the study of Barco et al. (2005) is of special interest. The authors used mice in which the genetic deletion of BDNF was restricted either to the entire forebrain, including both the CA3 and CA1 pyramidal neurons of the hippocampus, or only to the postsynaptic CA1 neurons. And indeed the authors observed that both types of conditional BDNF KO mice had normal L-LTP in input pathway 1 (S1) but showed a defect in synaptic tagging in input pathway 2 (S2). This LTP-defect was stronger in complete hippocampal $BDNF^{-/-}$ (CA3-CA1) mice, whereas it was only significant at later time points in the more spatially restricted CA1-specific BDNF KO mice. This suggests that BDNF might play a dual role in synaptic capture and tagging. The decay of the late phase observed in the more restricted $BDNF^{-/-}$ (CA1) KO mice implies a late, postsynaptic role in the maintenance of captured L-LTP. In addition, the rapid decay of associative LTP in the S2 pathway in slices lacking pre- and postsynaptic BDNF suggests that the presynaptic release of BDNF into the synaptic cleft after high-frequency stimulation may participate in the postsynaptic tagging of the synapse. The study by Barco et al. (2005) used exogenously applied BDNF (similar to Kovalchuk et al. 2002). Their experiments suggest that, regardless of the site of BDNF release, increased levels of BDNF in the synaptic cleft lead to a facilitation of LTP in CA3–CA1 synapses, probably by acting on both pre- and postsynaptic TrkB receptors, which supports the studies reported by Gärtner et al. (2006). An enhanced release of BDNF accumulated in postsynaptic spines of CA1 neurons after high-frequency stimulation might indeed contribute to sustaining an otherwise transient potentiation by stimulating local protein synthesis (Aakalu et al. 2001; Kang and Schuman 1996) or enhancing the neurotransmitter release from presynaptic CA3 neurons (Lessmann et al. 1994; Tyler and Pozzo-Miller 2001; Zakharenko et al. 2003). In addition, the experiments on STC suggested a distinct role for pre- or postsynaptically released BDNF. This is further supported by a study in neuronal primary cultures, which has demonstrated that BDNF-induced plasticity exhibits a bimodal profile and has an early presynaptic component and a later postsynaptic component (Alder et al. 2005). The results by Barco et al. imply a similar

Fig. 10.3 (continued) whereas the LTD-specific tag (*green*) could capture BDNF. By this means, E-LTD is transformed in this cluster to an L-LTD. In short, TBS-LTP-initiated processes are capable of establishing cross-tagging in some clusters within the same compartment, although incapable of establishing STC for LTP in other clusters within the same compartment. (**b**) Effects of priming via mGluR1 before the induction of TBS-LTP inside a synaptic cluster. Activation of group I mGluRs by DHPG before the induction of TBS-LTP leads to the production of local synthesis of plasticity-related proteins (PRPs). Now the dendritic cluster in one compartment contains a pool of PRPs that contains a potentiation-specific PRP, PKMζ, and a potentiation/depression PRP, BDNF. The LTP- or LTD-specific tags capture the required PRPs for its maintenance. The LTP tag is set at the synaptic input S2 (*orange*) that specifically captures PKMζ, whereas the LTD tag specifically captures BDNF. Thus, BDNF can transform transient plasticity forms to late ones within the same compartment (Adapted from Sajikumar and Korte, 2011a, PNAS)

bimodal action. Thus, presynaptically released BDNF contributes to the formation of those forms of LTP that recruit a presynaptic component (Zakharenko et al. 2003) and might participate in tagging the synapse for subsequent capture of PRPs, necessary for L-LTP, while postsynaptically released BDNF might contribute to the maintenance of different forms of L-LTP at later time points (Korte et al. 1998; Patterson et al. 2001). And here the most likely BDNF receptor for promoting memory storage is the TrkB receptor, for the following reasons: blockade of BDNF-TrkB interaction by TrkB-receptor bodies (Figurov et al. 1996) or via the neutralization of BDNF via anti-BDNF antibodies (Chen et al. 1999) strongly reduces hippocampal LTP. Experiments employing conditional KO mice in which TrkB is deleted postnatally from the forebrain have demonstrated that BDNF is mediating the effect on synaptic plasticity via activation of TrkB receptors (Minichiello et al. 1999, 2002). Here, binding of BDNF leads to dimerization of TrkB receptors and to autophosphorylation of its tyrosine residues. This activates the Shc-pathway and on the other hand leads to the activation of PLCγ. It was previously shown that for hippocampal LTP the PLCγ pathway plays a dominant role (reviewed in Minichiell 2009), while the Shc-pathway is of lesser if any importance (Korte et al. 2000). The activated PLCγ translocates to the plasma membrane and cleaves there phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) into diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). DAG stimulates PKC, while IP3 releases Ca^{2+} from internal stores by binding to IP3 receptors. According to this model, the resulting cytosolic Ca^{2+} increase then raises the likelihood of LTP induction and maintenance. But it is not clear so far, if this is really the case, as the experiment that directly links TrkB-mediated Ca^{2+} release from internal stores to synaptic plasticity has still to be done. In addition, it is not clear, if the PLCγ signalling is promoting LTP in a pre- or postsynaptic manner. In the study by Minichiello and colleagues (2002) the TrkB-receptor was point-mutated in the whole hippocampus, and also in the earlier study by Minichiello and colleagues (1999) using conditional TrkB KO mice, the TrkB receptor was removed from all excitatory neurons in the hippocampus. Since the TrkB receptor is expressed pre- and postsynaptically, this does not resolve the issue of on which side of the synapse BDNF is acting. Indeed, there is ample evidence in the literature that BDNF have both pre- and postsynaptic effects (for a review see Gottmann et al. 2009; Park and Poo 2012). In the context of the Sajikumar and Korte study (2011), Lu et al. (2011) could show that not only BDNF is a PRP, but in addition its TrkB receptor is involved in setting a synaptic tag. Lu et al. could show that the TrkB receptor is transiently activated by a weak TBS protocol inducing only the early-phase of LTP (E-LTP). Most importantly for a tag, this TrkB receptor activation is specially restricted to stimulated synapses.

Taken together, the classic experimental paradigm for showing tagging and capture involves two convergent inputs on, e.g. on CA1 pyramidal cells as has been used by Frey and Morris (1997). Strong stimulation of input 1 (S1) generates synthesis of plasticity-related proteins (PRPs), like BDNF, as has been shown by Sajikumar and Korte (2011). These PRP can be captured by subsequent stimulation of a second input (usually called S2) receiving only weak stimulation. Weak stimulation of S2 sets a synaptic "tag" that allows the capture of a high amount of PRPs.

This capture process leads to the conversion from E-LTP to L-LTP. While the work of Sajikumar and Korte (2011) suggests that BDNF is a PRP, the study by Lu et al. (2011) in addition supports the notion that activation of the TrkB receptor sets the synaptic tag for L-LTP and therefore for memory consolidation. As mentioned above it was already known that deletion of BDNF in the CA1 region of the hippocampus abolished the ability of the weak input to undergo L-LTP in the two-pathway experiment (Barco et al. 2005). With an elegant experimental approach Lu et al. (2011) specifically studied if this defect in L-LTP was due to synaptic tagging. The authors used mice, in which the TrkB receptor was mutated in a way, that it became unresponsive, when the substance 1NMPP1 was added. By using this approach Lu et al. observed that blocking TrkB activation via application of 1NMPP1 from 40 to 60 min after a strong stimulus protocol of S1 blocks the capture of PRPs at weakly stimulated inputs (S2). Interestingly, inhibition of TrkB at the time of S1 stimulation blocks L-LTP at that input without preventing capture on S2. This suggested that L-LTP on S1 requires a TrkB-dependent synaptic tag, or possibly just TrkB activation. On S2, weak stimulation is sufficient to activate TrkB and opens the possibility to capture the previously synthesized PRPs that were induced by strong stimulation on S1 and transported to dendrites. These data support a model in which BDNF is synthesized in the postsynaptic neuron in response to a strong TBS, then released and captured at synapses by a TrkB-dependent mechanism. It is not clear whether BDNF release is synapse-specific, such that only the synapses tagged by TrkB activation are capable of releasing BDNF and sustaining LTP. Work on homeostatic plasticity in hippocampal neurons provides evidence for a presynaptic regulation of glutamatergic transmission mediated by local, postsynaptic synthesis and release of BDNF (Jakawich et al. 2010). Postsynaptic activity blockade (induced by the AMPA glutamate receptors antagonist NBQX) increases the frequency of spontaneous miniature excitatory postsynaptic currents (mEPSCs), indicating a presynaptic modification. The increase in mEPSPC frequency was blocked by local application of TrkB-Fc or treatment with the protein synthesis inhibitors, anisomycin and emetine. Taken together postsynaptically synthesized BDNF could mediate protein synthesis-independent potentiation through retrograde effects on glutamate release from presynaptic terminals.

10.5 Homeostasis and Metaplasticity

Overall the evidence listed above indicates, that BDNF might be involved as a PRP in order to orchestrate the plasticity threshold for whole clusters of synapses, and might therefore be involved in processes of metaplasticity and homeostasis. And indeed it has been reported earlier that BDNF is involved in homeostatic processes, especially in the calibration and synaptic scaling of inhibitory neurons. Rutherford et al. (1997) studied if the excitability of cortical circuits is modulated by interneurons that release the inhibitory neurotransmitter γ-aminobutyric acid (GABA). In the visual cortex of primates and rodents, activity deprivation leads to a decrease in the

expression of GABA. This suggests that activity is able to adjust the strength of cortical inhibition, and it was known that activity regulates the expression of BDNF, and BDNF has been shown to influence the phenotype of GABAergic interneurons and the modification threshold of synaptic plasticity in the visual cortex (Huber et al. 1998). Blocking spontaneous activity in these cultures reversibly decreased the number of GABA-positive neurons without affecting neuronal survival. Voltage-clamp analysis of inhibitory currents demonstrated that activity blockade also decreased GABA-mediated inhibition onto pyramidal neurons and raised pyramidal neuron firing rates. All of these effects were prevented by incubation with BDNF during activity blockade, but not by NT3 or NGF. Additionally, blockade of neurotrophin signalling mimicked the effects of activity blockade on GABA expression. These data suggest that activity regulates cortical inhibition through a BDNF-dependent mechanism and that this BDNF plays an important role in the control of cortical excitability. In a follow up study Rutherford et al. (1998) could show that the effects of activity blockade are mediated via the quantal amplitude and that this amplitude is regulated via BDNF. Exogenous BDNF prevented, and a TrkB-IgG fusion protein reproduced the effects of activity blockade on pyramidal quantal amplitude. BDNF had opposite effects on pyramidal neuron and interneuron quantal amplitudes and modified the ratio of pyramidal neuron to interneuron firing rates. These data demonstrated a role for BDNF in the homeostatic regulation of excitatory synaptic strength and in the maintenance of the balance of cortical excitation and inhibition.

Desai et al. (1999) also could show that this activity-dependent regulation of intrinsic excitability depends on BDNF, possibly as a means of homeostatically regulating firing rates during periods of intense change in synapse number or strength. In experiments on rat visual cortical cultures, they observed that exogenous BDNF prevented, and a TrkB-IgG fusion protein reproduced the change in pyramidal neuron excitability produced by activity blockade. Most of these effects were also observed in bipolar interneurons, indicating a very general role for BDNF in regulating neuronal excitability.

Taken together, these results suggest that BDNF may be a PRP controlling a coordinated regulation of synaptic and intrinsic properties aimed at allowing cortical and hippocampal networks to adapt to long-lasting changes in activity-dependent, and spatially restricted (plasticity clusters) manner. BDNF might indeed be an instructive major player in the functional strengthening of synaptic connections as a PRP and it might in addition be involved in processes of homeostasis (synaptic scaling and metaplasticity).

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Chapter 11
Prescient Synapses: Gating Future Neuronal Consciousness Through Synaptic Tagging and Metaplasticity

Steven A. Connor and Peter V. Nguyen

Abstract Restriction of synaptic plasticity to time frames dictated by fast synaptic transmission would yield neuronal networks incapable of encoding qualitatively rich memories. The ability to associate and encode temporally disparate aspects of a memory confers significant survival advantages. The temporal spread of everyday experiences necessitates broad time windows for synaptic encoding of multiple related events. By extending the time frame in which events can be associated at a synaptic level, and biasing synapses towards a plasticity-conducive state, synaptic tagging and metaplasticity provide potent mechanisms for enhancing memory quality in the brain. Tagging and metaplasticity serve as gateways for augmenting neuronal consciousness. Priming of future synaptic plasticity can enhance neuronal detection, encoding, and association of salient future events, and it can facilitate storage of detailed memories. We review key intracellular signalling mechanisms that initiate lasting changes in the ability of synapses to undergo metaplasticity, along with leading candidate synaptic tags that facilitate metaplasticity. We also speculate on how these phenomena bolster neuronal consciousness to sculpt the brain's capacity to dynamically encode and store information.

Keywords Synaptic tagging • Metaplasticity • Protein kinases and phosphatases • Memory • Cellular consciousness

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11.1 Introduction

Real-time monitoring and response to changes in synaptic activity are crucial for regulation of structural and functional changes necessary for memory encoding. Activity-dependent alterations in synaptic strength, such as long-term potentiation (LTP) and long-term depression (LTD), are canonical cellular mechanisms linked to learning and memory (Bliss and Lomo 1973; Kandel 2001; Whitlock et al. 2006; Dong et al. 2012). Disruption of LTP and LTD prevents retention of newly encoded information (see Andersen et al. 2007 for review) indicating significant overlap between synaptic plasticity and the neural processing required for memory formation. The prolific amount of polymodal sensory information flowing into the hippocampus, a brain structure necessary for memory genesis (Scoville and Milner 1957), necessitates a process for *neuronal selection* of behaviorally salient information. Additionally, the processes that enable memory formation should be synapse-specific, bidirectional (potentiating/depressing), and capable of being updated on extended time scales (minutes-hours). How have neurons ensured memory stability while maintaining synaptic modifiability needed for encoding new events related to previously encoded memories? What are the molecular events mediating these processes? How is neuronal consciousness optimized to facilitate discrimination of important information?

Nervous systems are awash with signalling molecules capable of inducing synaptic plasticity. However, their effects persist well beyond the time scales associated with presynaptic transmitter release and postsynaptic receptor activation. The downstream signalling cascades engaged by these transmitters can prime the future ability of synapses to undergo long-term changes in synaptic strength, a process known as "metaplasticity" (Abraham and Bear 1996). Additionally, synaptically localized events can enhance or diminish the ability of these synapses to undergo lasting changes. A key problem was how to maintain synaptic specificity despite the fact that the translation products required for long-term synaptic plasticity (plasticity-related proteins, PRPs) are expressed in a cell-wide manner (Frey and Morris 1997, 1998). An elegant and testable solution to this dilemma stated that a local (synaptic), activity-dependent, and reversible molecular process could "tag" synapses (Frey and Morris 1997). It is important to note that these tags can serve as both synaptic mediators of immediate plasticity and primers for future plasticity (Frey and Morris 1998; Abraham et al. 2001; Tenorio et al. 2010).

11.2 Synaptic Tagging and Capture (STC)

"Tagging" refers to activity-dependent marking of activated synapses. This could occur as a molecular or functional process that changes the ability of synapses to interact with PRPs (Frey and Morris 1998; Martin and Kosik 2002; Redondo and Morris 2011). PRPs are believed to interact with synaptic tags to convert short-term

alterations in synaptic strength to more persistent plasticity. A key feature of synaptic tagging is that the tag itself outlasts the initial tag-setting stimulus. The lifetime of the tag is determined by numerous factors, including the stimulus protocols used (Li et al. 2012), the previous activity profile of synapses (Young and Nguyen 2005), and network homeostasis (Hou et al. 2011; Vitureira et al. 2012). Generally, once set, tags can last from minutes to hours, with evidence indicating that metaplastic processes can persist for over 5 h (Li et al. 2012). Tags can interact with PRPs to further consolidate changes in synaptic strength and memory. Synaptic tagging can be expressed through several mechanisms, many of which overlap with the general mechanisms of canonical forms of plasticity. In fact, the same stimulus can set synaptic tags and increase the production of PRPs (Frey and Morris 1997). However, tags set by a stimulus temporally distinct from the PRP-inducing stimulus can nevertheless interact with PRPs, provided that the tags are set within a time frame shorter than the degradation rate of PRPs (Frey and Morris 1998; Sajikumar and Frey 2004; Connor et al. 2011b).

Evidence attempting to elucidate the mechanisms by which mRNA and proteins are appropriately targeted during synaptic plasticity was provided through dual synaptic pathway stimulation protocols. These experiments demonstrated that multiple trains of high-frequency stimulation (HFS) applied to one synaptic pathway generated LTP that was captured following a single train of HFS applied heterosynaptically (Frey and Morris 1997, 1998). Induction of LTP triggers a local synaptic event which sets an activity-dependent "tag". This tag serves as a local indicator of synaptic activity to enable "capture" of PRPs that are generated by strong HFS. Once generated, these PRPs are available for capture at other synapses converging on the same postsynaptic cell, provided those synapses have experienced activity capable of producing synaptic tags (Frey and Morris 1997; Barco et al. 2002; Redondo and Morris 2011 for review). This tag-generating activity does not need to be strong enough to produce PRPs. Importantly, the time window for heterosynaptic capture of PRPs is determined by the lifetimes of both the tags and the PRPs (Frey and Morris 1998). Additionally, synaptic capture can still take place when the tags are set prior to the generation of PRPs (Frey and Morris 1998), allowing for molecular dissection of the tag-setting and PRP-producing mechanisms.

11.3 Metaplasticity

The thresholds for eliciting synaptic plasticity can be altered by prior stimulation, a process known as *metaplasticity* (reviewed by Abraham 2008). Metaplasticity confers neuronal networks with flexibility, to prime, or limit, the processing and encoding of information. High levels of synaptic activity in parallel with neuromodulation can increase synaptic sensitivity to future activity. Synaptic activity (Young et al. 2006), neuromodulatory receptor activation (Sajikumar et al. 2009; Navakkode

et al. 2010; Tenorio et al. 2010), and prior behavioral experience (Zelcer et al. 2006) can elicit metaplasticity (Abraham and Bear 1996). Metaplasticity is often enduring, synapse-specific, and is mediated through induction of intracellular mechanisms that may not detectably alter synaptic strength. It allows neurons to implement past experiences (stored as changes in synaptic weights or altered thresholds for changing such weights) to guide future plasticity. An example from the behavioral literature demonstrated that upregulation of intrinsic excitability of CA1 pyramidal neurons associated with odor discrimination training enhanced the acquisition of a future hippocampus-dependent task (Zelcer et al. 2006). Thus, a learning-induced metaplastic state enables an apparently broadened enhancement of learning in hippocampal networks. Additionally, studies using transcranial magnetic stimulation in humans suggest that metaplasticity may underlie enduring behavioral changes associated with memory formation, and that aberrant metaplasticity may contribute to disorders of learning and memory (Hulme et al. 2013). As induction of metaplasticity may be a viable treatment for some neurological disorders (Nardone et al. 2012), understanding the cellular underpinnings of metaplasticity is crucial for providing the knowledge base for innovative treatments going forward.

There are differences between some of the mechanisms underlying STC and metaplasticity. The main distinction lies in the localization of expression of these mechanisms. STC requires molecular mechanisms occurring at, or near, synapses to fulfil the tagging criterion. Metaplasticity, however, can be expressed in a cell-wide manner. Indeed, low-frequency stimulation (LFS) applied to hippocampal slices inhibits the future induction of LTP in a cell-wide manner, through phosphatase activation (Young et al. 2006). Conversely, learning can upregulate neuronal excitability through PKA-dependent inhibition of I_h channels, thereby facilitating conversion of short-term memory (STM) to long-term memory (LTM) through metaplastic mechanisms (Parsons and Davis 2012). Neuromodulators can initiate metaplastic processes that do not require HFS to be triggered (Navakkode et al. 2010; Tenorio et al. 2010). Finally, translocation of calcium/calmodulin-dependent protein kinase II (CaMKII) can occur in hippocampal cell cultures following localized application of glutamate and glycine onto contiguous dendritic spines (Rose et al. 2009). Contrary to a localized effect, catalytically active CamKII spread throughout the somato-dendritic compartment and promoted the synaptic accumulation of GluR1-YFP (yellow fluorescent protein), consistent with that observed during LTP. This heterosynaptic potentiation expanded the known mechanisms of CaMKII function to those not requiring synaptic specificity during STC. Regardless of the mechanistic distinction, STC, metaplasticity, or both may be engaged, depending on the memory demands elicited by ongoing experience. Experiences in which the predictability of future events are low (e.g., navigating a new environment (Zelcer et al. 2006) may preferentially be encoded through metaplastic mechanisms which would boost plasticity in a cell-wide manner, allowing for incorporation of synapses into the engram in a more flexible manner. Distinct spatial aspects of the environment may be initially encoded at spatially distinct dendritic regions of a neuron. Alternatively, associating separate but related events that would activate

dendritic spines in a partially overlapping manner could take advantage of tagging to maintain synapse specificity required for neuronal cognition of event correlations. The collaboration of cell-wide mechanisms that would globally upregulate neuronal sensitivity, and STC processes that would selectively prime individual synapses, would be ideal for encoding salient and specific aspects of memory.

Despite the mechanistic divergence, several key signalling molecules have been implicated in STC and metaplasticity, including CaMKII, cAMP-dependent protein kinase (PKA), and protein kinase M-zeta (PKMζ; a brain-specific form of PKC-zeta) (Fig. 11.1). The following is a discussion of the evidence for these kinases in synaptic tagging and metaplasticity.

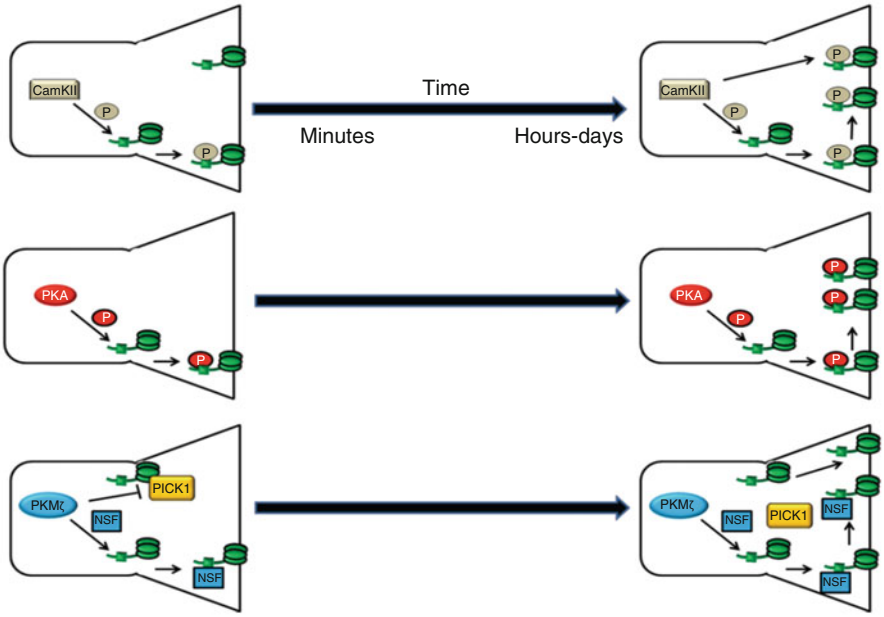

Fig. 11.1 CaMKII (*top*), PKA (*middle*), and PKMζ (*bottom*) all enhance memory formation through metaplastic and synaptic tagging and capture mechanisms. Once triggered, the enduring modifications to plasticity can extend from minutes to days. CaMKII can prime AMPAR trafficking and synaptic membrane insertion through phosphorylation. Similarly, PKA modifies the plasticity threshold through phosphorylation of AMPARs which results in their translocation. The enhanced extrasynaptic localization prepares AMPARs for activity-induced lateral diffusion within the postsynaptic membrane. Additionally, PKA can inhibit channels to regulate dendritic excitability, thereby enhancing postsynaptic depolarization in response to synaptic stimulation which could work in concert with AMPAR regulation to boost potentiation. PKMζ, working in concert with PICK1 and NSF, can liberate AMPARs from extrasynaptic pools and decrease their constitutive endocytosis. The combination of these three kinase cascades results in a boosted synaptic sensitivity to future inputs that renders the neurons harboring these synapses more conscious of their synaptic activity profiles across time and space

11.4 Canonical Synaptic Tags: CaMKII

Induction of LTP and LTD requires calcium influx into postsynaptic cells. The local elevation of calcium initiates signalling cascades coupled to several key kinases and phosphatases that are critical for bidirectional plasticity (Pi and Lisman 2008). Downstream of NMDAR activation, CaMKII qualifies as an ideal candidate tag due to its local induction, its ability to regulate plasticity processes including AMPAR function and trafficking, and the enduring nature of its activity following autophosphorylation (Sanhueza and Lisman 2013). CaMKII was originally shown to be required for the induction of LTP and LTM formation (Giese et al. 1998); however, the potential functional overlap with other CaMKs made determination of its specific roles in tagging difficult. Experiments demonstrating a role for CaMKII as a synaptic tag were provided in two-pathway recordings in stratum radiatum of the CA1 region of slices of the rat hippocampus. This protocol allows strong stimulation of one pathway, which should both set tags and generate PRPs, and weak stimulation of a second synaptic pathway to monitor tag setting per se (Frey and Morris 1997, 1998). By varying the time points between stimulation of the pathways and inhibiting different signalling components during either the weak or strong protocols, the properties and identity of the tags and PRPs can be elucidated.

Data implicating CaMKII as a synaptic tag were provided by applying a CaMKII inhibitor, KN-62, overlapping with weak stimulation of a second synaptic pathway following induction of late-LTP (L-LTP, >3 h duration, requires de novo synthesis of PRPs) at another set of synapses converging on the same postsynaptic cells (Sajikumar et al. 2007). PRPs should be available following induction of L-LTP, and unless the activity-dependent tags induced by heterosynaptic weak stimulation are "reset", the PRPs should be captured at neighboring activated synapses. KN-62 prevented the capture of L-LTP by either strong or weak stimulation, consistent with a role for CaMKII in tagging (Sajikumar et al. 2007). Activity-driven interaction of CaMKIIs with NR2B subunits of NMDARs could constitute the formation of a molecular complex that interacts with synaptic adhesion proteins and auxiliary subunits of AMPARs to regulate synaptic tagging (Sanhueza and Lisman 2013). Evidence for this model was demonstrated by preventing the association of CaMKII with NR2B using peptides that bind to the CaMKII binding site on NR2B. Preventing this interaction allowed previously saturated synapses to undergo LTP, suggesting a role for CaMKII/NMDAR complexes in maintaining synaptic strength (Sanhueza et al. 2011). This model predicts that calcium-dependent translocation of CaMKII and subsequent binding to NMDARs constitute a tag capable of capturing scaffolding proteins and AMPAR binding proteins to supply new docking sites for AMPAR stabilization (Sanhueza and Lisman 2013). Interactions of this complex with transsynaptic adhesion proteins would facilitate the structural changes necessary for subsequent expression of LTP. Further research is needed to determine if this is the key mechanism mediating CaMKII's function as a synaptic tag.

The specific functions of the CaMK isoforms in mediating different aspects of synaptic tagging and capture (STC) have been delineated (Redondo et al. 2010).

When CaMKII was specifically blocked, the setting of synaptic tags, but not the induction of PRPs, was prevented. This was demonstrated by pairing KN-93 application overlapping with strong tetanization at one pathway while inducing weak LTP at neighboring synapses. Although KN-93 prevented the capture of LTP at the strongly tetanized pathway, PRP synthesis appeared to be intact, as stimulation of a second pathway that would set tags, but not generate PRPs, nevertheless elicited L-LTP at the second pathway (Redondo et al. 2010). This result is consistent with mechanistically distinct processes driving tag setting and PRP synthesis, with the former requiring CaMKII. Conversely, when CaMKIV was inhibited, PRP synthesis was prevented whereas tagging remained intact (Redondo et al. 2010; reviewed by Redondo and Morris 2011). These results suggested a mechanistic divergence of CaMK isoforms: CaMKII could mediate tag formation, whereas CaMKIV elicits PRP synthesis.

Behavioral analogues of STC have produced results consistent with in vitro data. Brief exposure to a novel open field is a behavioral stimulus capable of inducing PRP synthesis. Weak inhibitory avoidance (IA) training, normally subthreshold for LTM formation, results in LTM when coupled with prior open-field exposure (Moncada and Viola 2007; Moncada et al. 2011). It is believed that weak IA training generates synaptic tags capable of interacting with PRPs generated during open-field exposure, thus enhancing subsequent memory duration. Mechanistic similarities between this "behavioral tagging" and STC in brain slices have been observed. Inhibition of NMDARs, and presumably downstream CaMKII activation, prevented the setting of learning tags by weak IA training. Similarly, infusion of KN-62 into dorsal hippocampus prior to, or shortly after, weak IA training prevented conversion of STM to LTM (Moncada et al. 2011).

CaMKs can also regulate synaptic tagging associated with LTD. The immediate early gene *Arc* interacts with the endocytotic proteins, endophilin and dynamin, to enhance the removal of AMPA receptors during LTD (Chowdhury et al. 2006; Park et al. 2008). Recent evidence suggests that CaMKIIβ targets activity-induced ARC from the cell soma to individual synapses (Okuno et al. 2012). This "inverse tagging" mechanism controls clearance of upregulated GluR1 at inactive or weakly potentiated synapses. This process required the interaction between ARC and CaMKIIβ, as siRNA knock-down of CaMKIIβ prevented the depression of weakly activated synapses, as did preventing the translocation of ARC to synaptic spines. Thus, ARC is maintained at weakly activated synapses through interaction with CaMKIIβ, which together might constitute a "weak activity sensor" capable of mitigating robust potentiation of weakly activated synapses (Okuno et al. 2012). This might accentuate the integration of robustly potentiated synaptic signals across spatially complex dendritic networks, much like a center-surround receptive field organization would enhance detection of boundaries between dark and bright regions in a visual field. Overall, the combination of in vitro and in vivo data provides strong evidence that CaMKII is crucial for STC and may also serve as an *inverse tag* in the hippocampus.

11.5 Priming Plasticity: CaMKII as a Metaplasticity Effector

CaMKII fulfils many of the criteria necessary to be a mediator of metaplasticity. It can bias plasticity bidirectionally, depending upon which amino acid residues of CaMKII are phosphorylated. Generation of a transgenic mouse model expressing constitutively active CaMKII (through modification of threonine-286: CaMKII-T286D) shifted plasticity thresholds in favor of LTD (Mayford et al. 1996; Elgersma et al. 2002). Conversely, blocking T305/306 inhibitory autophosphorylation while maintaining T286 phosphorylation lowered the threshold for LTP induction (Mayford et al. 1995; Pi et al. 2010a). Phosphomimetic strategies have confirmed that maintaining phosphorylation at T305/306 induces LTD, and blocking inhibitory autophosphorylation promote LTP (Pi et al. 2010b).

In an example of metaplastic and STC processes synergistically regulating plasticity thresholds, Sajikumar et al (2009) have shown that RYR agonists (ryanodine or caffeine) applied shortly before the induction of short-term potentiation (STP) can prime synapses for long-lasting potentiation. This primed LTP required CaMKII-dependent tag setting which lasted up to 1 h. Inhibitors of CaMKII, KN-62 and AIP, prevented the conversion of STP to L-LTP (Sajikumar et al. 2009). These data suggest that the temporal window for plasticity and synaptic strength at weakly potentiated synapses can both be increased through a CaMKII-mediated, enduring, priming event. Therefore, metaplasticity, synaptic tagging, and synaptic capture of PRPs can be modified through activation of CaMKII.

11.6 PKA: Tagging Along

For local molecular events to perform as synaptic tags, they should be transient and reversible to prevent network saturation and to maintain synaptic specificity (Martin and Kosik 2002). Protein kinases and phosphatases demonstrate properties consistent with a capacity to function as synaptic tags (Young and Nguyen 2005; Young et al. 2006; Sajikumar et al. 2007; Redondo et al. 2010). PKA is well suited to function as a synaptic tag; its interaction with synaptically localized AKAPs (A-kinase anchoring proteins) provides a means for precisely localizing PKA so it can interact with substrates that participate in tagging. Additionally, the time course of PKA activation coincides with time frames required for the synaptic tag. Indeed, genetic reduction of cAMP-dependent protein kinase (PKA) impaired synaptic capture (Young et al. 2006) and pharmacological inhibition of interactions between PKA and AKAPs prevented STC (Huang et al. 2006).

It has been established that once induced, L-LTP is resistant to depotentiation (DPT), an activity-dependent reversal of previously potentiated synapses (Fig. 11.2) (Woo and Nguyen 2003; Young et al. 2006). Resistance to DPT is mediated mechanistically through initiation of protein synthesis, which can homosynaptically and

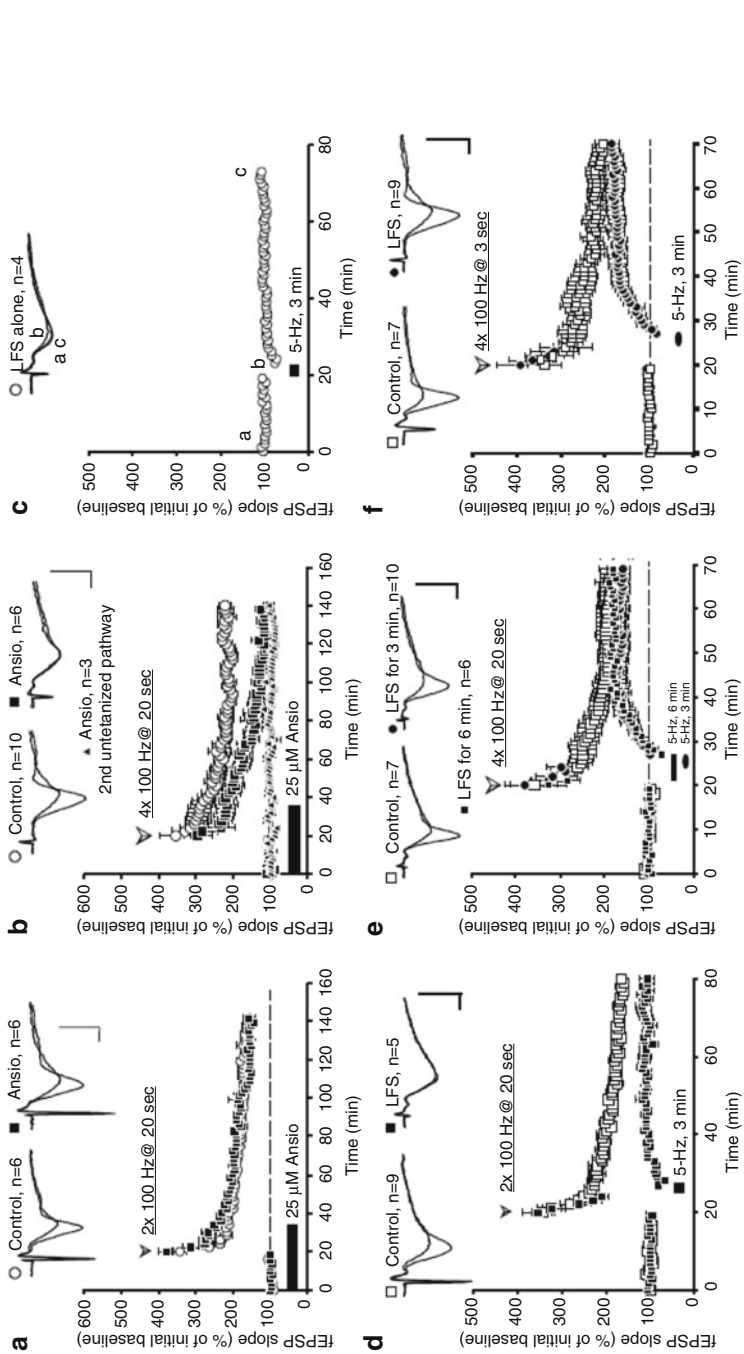

Fig. 11.2 Synaptic immunity to depotentiation (DPT) is activity-dependent. (**a**–**c**) Anisomycin did not affect two-train LTP (**a**, *squares*), but impaired maintenance of four-train LTP (**b**, *black squares*). Low-frequency stimulation (LFS, 5-Hz, 3-min) alone transiently depressed field EPSPs which recovered to pre-LFS values (**c**). (**d**–**f**) LFS given 5 min after two-train LTP induction persistently erased potentiation (**d**). In contrast, when LFS was given after four trains of 100-Hz spaced 20-s apart, persistent DPT was not observed (**e**, *black circles*). Note gradual recovery of field EPSPs to potentiated levels. Even 6 min of LFS could not persistently erase four-train LTP (**e**, *black squares*). Similarly, when the spacing between 100-Hz trains was compressed to 3-s, no persistent DPT was observed after LFS (**f**, *circles*). Sample traces from DPT experiments were taken 5 min before 100-Hz and 40 min after LFS. Calibration bars: 5 mV, 3 ms [Adapted from Woo and Nguyen (2003) with permission]

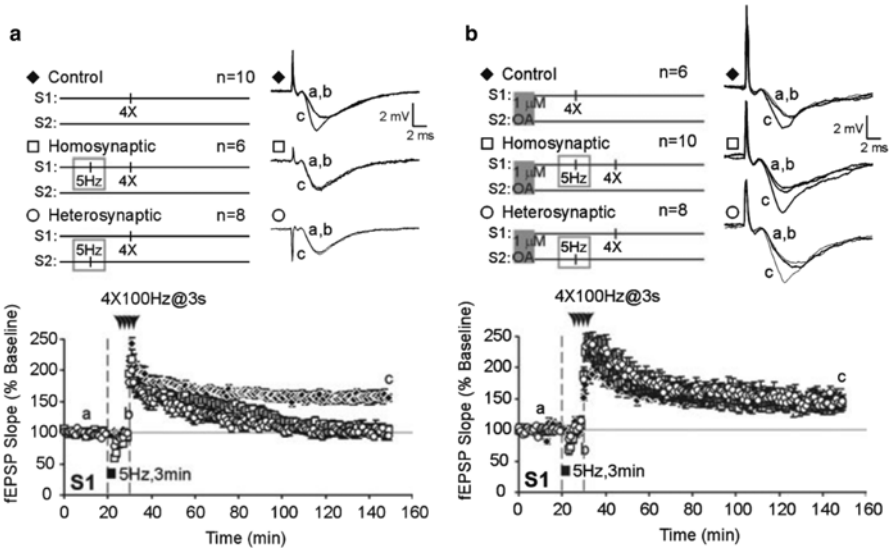

Fig. 11.3 (**a**) Prior low-frequency stimulation (LFS) impairs subsequent L-LTP at homo- and heterosynaptic inputs. Four 100-Hz trains induced stable L-LTP (*black diamonds*). LFS (5-Hz, 3 min) given prior to tetanization significantly reduced the persistence of L-LTP in homosynaptic (*squares*) and heterosynaptic (*circles*) inputs. (**b**) Protein phosphatase 1/2A activation is required for homo- and heterosynaptic inhibition of L-LTP. Pre-incubation of slices in okadaic acid (OA, 1 μM) blocked homosynaptic (*squares*) and heterosynaptic (*circles*) inhibitory effects of LFS on subsequently induced L-LTP. Note persistence of L-LTP in all groups. Control slices (*black diamonds*) received OA and 100-Hz, but no prior LFS [Adapted from Young et al. (2006) with permission]

heterosynaptically protect L-LTP from DPT (Barco et al. 2002; Woo and Nguyen 2003; Young and Nguyen 2005). Chemical or genetic inhibition of PKA renders late-phase-potentiated synapses susceptible to DPT (Young et al. 2006). Young et al. (2006) also demonstrated that LFS recruits PP1/2A and suppresses PKA activation to heterosynaptically inhibit L-LTP (Figs. 11.3 and 11.4). This inhibition was prevented by pre-incubation with okadaic acid, an inhibitor of PP1/2A. The effects of LFS were similar for LTP induced by tetanization or by chemical activation of PKA (Young et al. 2006). However, LFS only suppressed responses from protocols that induced L-LTP, as E-LTP was unaffected by prior LFS (Woo and Nguyen 2002; Young and Nguyen 2005). This suggests that PKA inhibition prevents setting of synaptic tags required for consolidation of L-LTP.

Similarly, heterosynaptic transfer of LTP following homosynaptic induction of beta-adrenergic receptor-dependent LTP is prevented by application of a membrane-permeant PKA inhibitor, PKI (Fig. 11.5) (Connor et al. 2011b). Cross-tagging

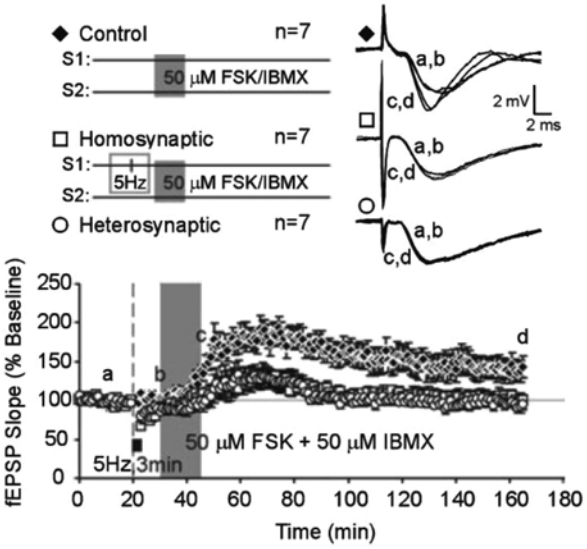

Fig. 11.4 Prior LFS impairs homosynaptic and heterosynaptic cAMP/PKA signalling. a: Chemical LTP induced by forskolin with 3-isobutyl-1-methylxanthine (FSK/IBMX) is stable over 2 h (*black diamonds*, "Control"). LFS given prior to FSK/IBMX impaired facilitation in homosynaptic (S1, *white squares*) and heterosynaptic (S2, *circles*) inputs [Adapted from Young et al. (2006), with permission]

experiments designed to determine the degree of tag compartmentalization across dendritic regions identified PKA as a tag specifically involved in mediating capture in basal dendrites of CA1 pyramidal cells (Sajikumar et al. 2007). Inhibiting PKA while inducing LTP prevented capture of heterosynaptically induced L-LTP. However, evidence linking PKA to tagging was also shown in apical dendrites of CA1 following strong tetanization, with capture of LTP being compromised by a PKA inhibitor, KT5720 (Alarcon et al. 2006).

Similar to CaMKII, injection of a PKA inhibitor in close temporal proximity to weak inhibitory avoidance training prevented conversion of short-term to LTM, suggesting interference with tagging mechanisms (Moncada et al. 2011). When PKA signalling was intact, prior novel environmental exploration was believed to have triggered the synthesis of PRPs which were captured when PKA was activated by weak IA training. Inhibiting the setting of the learning-induced tag (PKA or CaMKII) prevented memory consolidation (Moncada et al. 2011). Additional behavioral evidence for PKA as a synaptic tag was provided using weak electrical shock plus light-induced priming of LTM formation in the amygdala (Parsons and Davis 2012). Activation of PKA following pairing of light with weak shocks could serve as tag-setting stimuli, with the second stimulation triggering PRP synthesis (analogous to the "weak before strong" protocol of Frey and Morris (1997). Taken together, these data show that PKA plays a critical role in synaptic tagging; however, the putative functions of PKA in behavioral paradigms of STC remain to be elucidated.

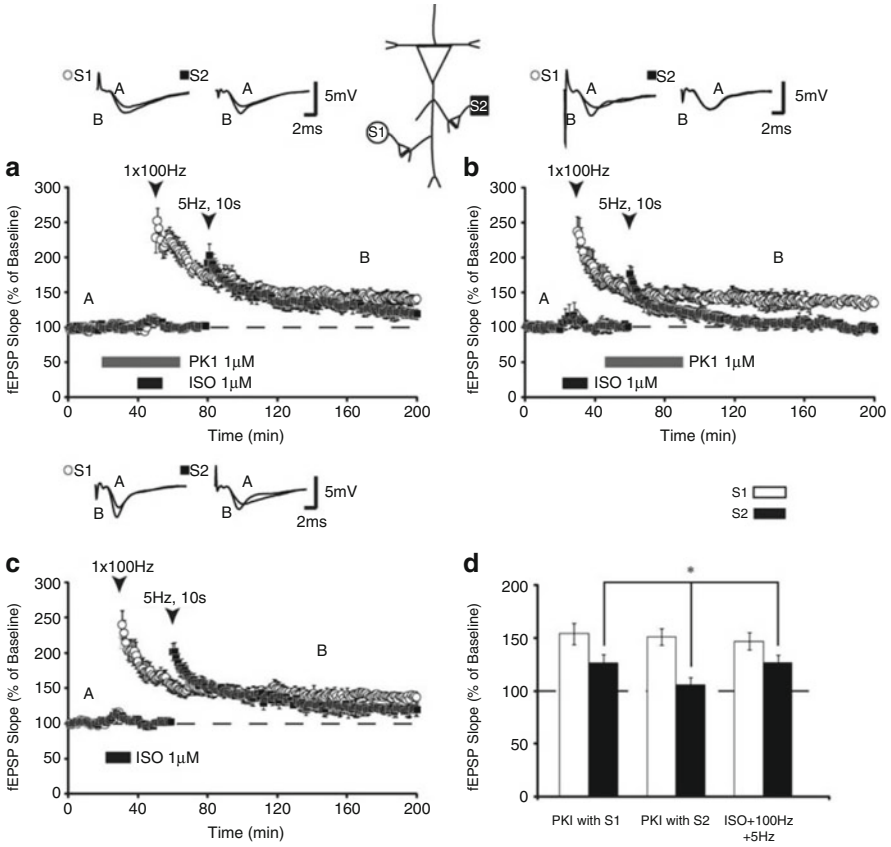

Fig. 11.5 PKA is required for heterosynaptic transfer of LTP. (**a**) Application of the membrane-permeant PKA inhibitor, PKI, overlapping with 1 × 100 Hz simulation paired with ISO (*open circles*), did not prevent the induction of homosynaptic LTP or the transfer of LTP to a second synaptic pathway (*filled squares*) (5 Hz, 10 s; $n = 8$). (**b**) Shifting PKI application to overlap with LFS prevented the heterosynaptic transfer of LTP to S2 (*filled squares*; $n = 10$). Heterosynaptic LTP was significantly reduced relative to slices treated with PKI during HFS or PKI-free controls. LTP at S1 was unaffected (*open circles*) as determined by comparisons with PKA inhibitor free controls (**c**) ($n = 6$). (**d**) Summary histogram comparing fEPSP slopes obtained 120 min after HFS (*white bars*) at S1 and 90 min after LFS at S2. Sample traces were taken 10 min after commencement of baseline recordings and 120 min after S1 stimulation. Results in (**c**) represent means ± SEM, $^*p < 0.05$ [Adapted from Connor et al. (2011b), with permission]

11.7 Setting up cAMP: PKA as a Metaplastic Molecule

The cAMP-dependent activation of PKA is only the first step in its complex signalling roles (reviewed by Abel and Nguyen 2008). Additionally, interaction with synaptically localized AKAPs ensures that PKA is spatially restricted and makes PKA

a prime candidate for mediating synaptic tagging. Beta-adrenergic receptor activation triggers signalling cascades coupling to PKA which are crucial for the induction of prominent forms of plasticity including LTF (long-term facilitation) and LTP (Chain et al. 1999; Gelinas et al. 2008; Connor et al. 2011a; see O'Dell et al. 2010 for a review). Once activated, PKA can increase the time windows for synaptic transfer of LTP between strong and weak inputs, and it can boost the induction of LTP by stimuli occurring as long as 1 h after PKA activation (Tenorio et al. 2010; Connor et al. 2011b). LFS applied 30 min after induction of beta-adrenergic receptor-dependent LTP captured LTP through a mechanism requiring PKA (Connor et al. 2011b). The ability of PKA to enhance LTP through metaplastic processes has been linked to its ability to regulate AMPAR trafficking, likely through phosphorylation of GluR1 serine-845 (Tenorio et al. 2010). GluR1 insertion is elevated immediately following the induction of LTP (Makino and Malinow 2009). During the expression of LTP, GluR1 receptors are replaced by GluR2-containing receptors which maintain potentiated synaptic responses (Makino and Malinow 2009). These data suggest that boosting the initial insertion of GluR1 could enhance LTP through PKA-dependent serine-845 phosphorylation of GluR1, resulting in an increased amount of postsynaptic AMPAR slots available for future incorporation of GluR2.

Although phosphorylation of K+ channels by PKA, which enhances cell excitability, can contribute to metaplasticity (Lin et al. 2008), the prominent role of AMPAR insertion in plasticity and memory formation suggests that the ability of PKA to mediate metaplasticity involves mechanisms that modify transmitter receptors in addition to voltage-dependent channels (Abel and Nguyen 2008). Furthermore, PKA is known to critically interact with phosphatases to effect long-lasting plasticity. LFS recruits PP1/2A and suppresses PKA to heterosynaptically inhibit L-LTP in a cell-wide manner (Young et al. 2006). This effect was prevented by pre-incubation with OA, an inhibitor of PP1/2A. Conversely, increasing cAMP activation either chemically (using an adenylyl cyclase activator, forskolin) or through stimulation of beta-adrenergic receptors (using isoproterenol) enhanced the future induction of LTP when tetanization was applied 1 h later (Young et al. 2006; Tenorio et al. 2010). Activation of beta-adrenergic receptors lowered the threshold for the future induction of L-LTP through metaplastic processes (Tenorio et al. 2010). Inhibition of PKA during beta-adrenergic receptor stimulation prevented the metaplastic lowering of the threshold for LTP induction. Phosphorylation of Ser-845 of GluR1 appeared to be a mechanism responsible for this form of metaplasticity, as Ser845 phosphorylation was significantly enhanced 1 h after β-adrenergic receptor stimulation. Phosphorylation of GluR1 is known to facilitate its activity-driven membrane insertion (Oh et al. 2006), suggesting that one mechanism for priming future plasticity is upregulation of GluR1 trafficking. PKA has also been shown to be required for transfer of heterosynaptic LTP (Alarcon et al. 2006; Huang et al. 2006; Young et al. 2006). Taken together, it is clear that PKA can enhance the modifiability of synaptic plasticity both homo- and heterosynaptically.

Consistent with in vitro data showing no detectable change in synaptic strength following stimuli that induce metaplastic mechanisms (Tenorio et al. 2010), initial

weak shock training in vivo did not generate behavioral manifestations consistent with fear responses (Parsons and Davis 2012). Thus, although underlying mechanisms that could boost synaptic modifiability had been triggered, no detectable changes in behavior were elicited. How does PKA enhance future learning? Reductions in neuronal after-hyperpolarization currents (McKay et al. 2013), which would enhance intrinsic dendritic excitability, may be linked to metaplastic boosting of learning. Altered neuronal excitability following PKA activation would satisfy the criterion of increasing the probability of encoding future events without substantially altering basal synaptic strength. Priming of future learning was induced by applying a weak electric shock anywhere from 1 h to 7 days before a second weak shock (Parsons and Davis 2012). Primed learning under this protocol required PKA activation as several substrates of PKA were phosphorylated in a time-dependent manner following weak shock training, and blocking this phosphorylation prior to the first training trial prevented the priming effect (Parsons and Davis 2012). Thus, one training trial increases excitability of neurons in the amygdala, rendering them more amenable to plasticity when a second learning event reactivates this circuit. Under these conditions, a transient, weak stimulus both lowers the threshold, and extends the duration of the reduced threshold, for subsequent encoding of long-term fear memory through PKA. Taken together, these data provide evidence that PKA activation can serve as a long-lasting mechanism for enhancing metaplasticity of behavior. PKA is capable of priming LTP and fear learning without detectably changing synaptic or behavioral responses.

11.8 PKMζ: Tagging Memory Maintenance

Strong synaptic activation triggers constitutive activation of a protein kinase C isoform, PKMζ, which is implicated in plasticity, memory and STC processes (Sacktor et al. 1993; Serrano et al. 2005; Sajikumar et al. 2005). Injection of a myristoylated Zeta inhibitory peptide (ZIP), which blocks PKMζ, strikingly prevented the maintenance of memories weeks after initial memory formation (Shema et al. 2007, 2009; see Sacktor 2011 for review). PKMζ plays a dual role as a synaptic tag and a PRP. The distinction lies in the mechanisms by which it influences synaptic function: one mechanism is PKMζ's activity-dependent synthesis and translocation to synapses (PRP function), and the other is its ability to regulate GluR2-containing AMPAR trafficking (tag function). Together, these processes allow PKMζ to boost STC and, hypothetically, neuronal consciousness.

Evidence suggests that PKMζ is a PRP that is captured at tagged synapses where it facilitates maintenance of potentiation (Sajikumar et al. 2005). Application of ZIP reversed late, but not early, LTP, consistent with a specific role in synaptic mechanisms supporting LTM. ZIP also prevented conversion of heterosynaptic early-LTP to late-LTP (Sajikumar et al. 2005; Sajikumar and Korte 2011). These data indicate

that persistent PKMζ maintains potentiation at strongly stimulated synapses and is a PRP necessary for consolidation of L-LTP. Additional "cross-tagging" experiments were designed to determine if PRPs produced by LTP or LTD could facilitate their counterpart mechanism (i.e., could L-LTP at one synaptic pathway convert E-LTD at a second synaptic pathway to L-LTD?). Surprisingly, Sajikumar and Frey (2004) found that induction of LTD, similar to induction of L-LTP, could provide PRPs necessary for transforming heterosynaptic E-LTP to L-LTP. Reversing the protocols demonstrated that L-LTP induction could foster the transition from E-LTD (induced with a weak LTD stimulus) to late-LTD. Although ZIP blocked the enhancement of E-LTP by heterosynaptic L-LTD, it failed to affect the enhancement of LTD produced by cross-tagging after heterosynaptic L-LTP. These results suggest that PKMζ acts as a potentiation-specific PRP necessary for maintaining increased synaptic strength in response to strong tetanization and for transforming E-LTP to L-LTP. Although the precise capturing mechanism required for compartmentalization of PKMζ to tagged synapses is unclear, regulation of local dendritic translation may play a role (Kelly et al. 2007). Activity-dependent local translation would provide an elegant means for maintaining synaptic specificity required for memory fidelity, although kinase interactions (cf. PKA anchoring) have not been ruled out as a mechanism for localizing PKMζ function to synapses.

PKMζ dynamically regulates AMPAR trafficking through several converging mechanisms which facilitate its function as a synaptic tag. Interestingly, phosphorylated PKMζ can prime heterosynaptic facilitation through upregulation of GluR2-dependent AMPAR trafficking (Yao et al. 2008). Once activated, PKMζ increases *N*-ethylmaleimide-sensitive fusion protein (NSF) activity, which prevents protein interacting with C-kinase 1 (PICK1)-mediated sequestration of GluR2 in extrasynaptic pools (Yao et al. 2008). Once liberated, GluR2-containing AMPARs are freely available for insertion in the postsynaptic density, thereby extending the duration of synaptic tagging. It is possible that GluR1/GluR2 subunits might themselves serve as synaptic tags, but further research is needed to test this notion.

Indeed, mimicking PKMζ function through peptide-mediated disruption of PICK1 sequestration of GluR2 extended the temporal window for heterosynaptic transfer of LTP from 30 min to 1 h (Fig. 11.6) (Connor et al. 2011b). This indicates that regulation of glutamatergic receptor trafficking may shape the lifetime of synaptic tags. This also suggests that both PKMζ-mediated GluR2 regulation and PKA-driven phosphorylation of GluR1 activation could synergistically prevent tag degradation through upregulation of synaptic incorporation of AMPARs. In vivo recordings (Barry et al. 2012) have demonstrated that spatial map stability requires PKMζ phosphorylation in order to maintain synaptic strength increase induced by map formation. Inhibiting PKMζ destabilized spatial maps without impairing future map formation. This destabilization could be mediated through a tag erasure process which would be consistent with PKMζ's function. Future research is needed to determine what processes are disrupted to decrease spatial map stability.

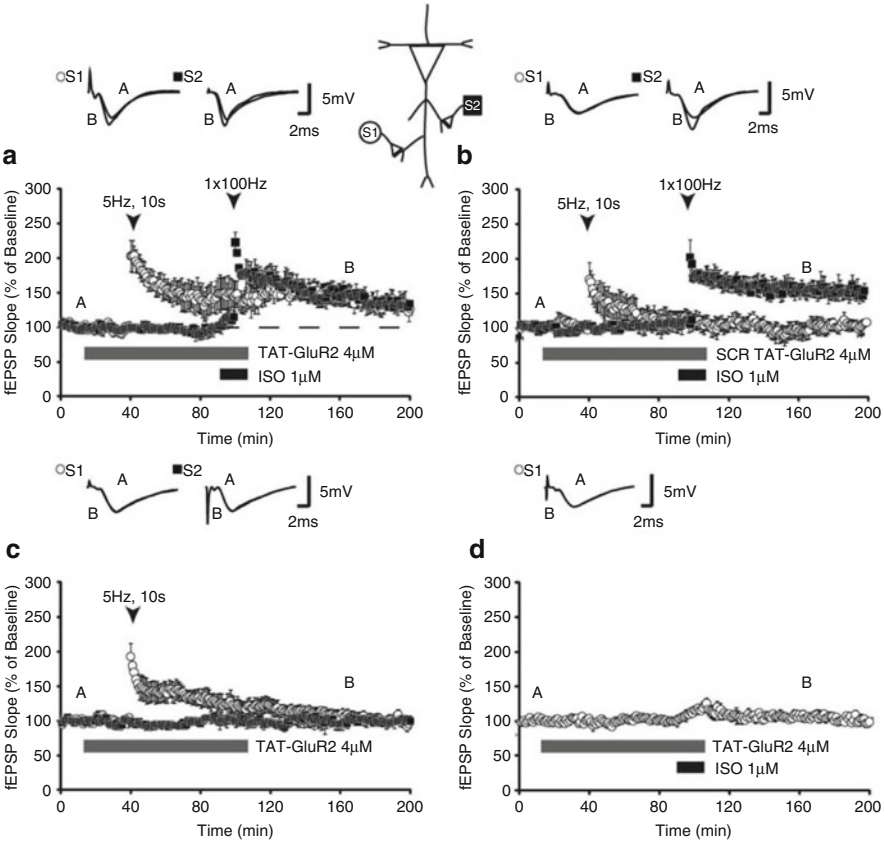

Fig. 11.6 Preventing GluR2 endocytosis extends the temporal window for transfer of heterosynaptic LTP. (**a**) When GluR2 endocytosis is prevented through application of the Tat-GluR2$_{3Y}$ peptide, LFS (5 Hz, 10 s: S1; *open circles*) still expresses LTP when HFS + ISO is applied 1 h later ($n = 4$). (**b**) Application of a scrambled, inert version of the peptide (Tat-GluR2$_{3s}$) failed to extend the time window for heterosynaptic transfer of LTP when ISO + HFS was delayed to 1 h post LFS ($n = 4$). ISO + HFS generated homosynaptic, long-lasting LTP in the presence of both peptides, indicative of intact synaptic plasticity in both groups. (**c**) Application of Tat-GluR2$_{3Y}$ peptide with 5 Hz stimulation fails to induce long-lasting LTP. When Tat-GluR2$_{3Y}$ was applied prior to and overlapping with 5 Hz, 10 s stimulation transient LTP was induced which returned to baseline in <2 h. (**d**) Pairing of ISO with Tat-GluR2$_{3Y}$ does not have any significant on baseline synaptic responses. To test for effects on basal synaptic transmission, Tat-GluR2$_{3Y}$ was paired with ISO application and basal synaptic responses were monitored. A transient, small (<20 %) increase in synaptic potentiation was observed during ISO application. fEPSPs subsequently returned to baseline levels. Sample traces were taken 10 min after commencement of baseline recordings and 120 min after stimulation [Adapted from Connor et al. (2011b), with permission]

11.9 PKMζ as a Metaplasticity Molecule

Despite the finding demonstrating that PKMζ is not required for memory formation or LTP maintenance (Volk et al. 2013; Lee et al. 2013), PKMζ continues to be a viable participant in neural representations of memory. PKMζ dually functions as a PRP and a synaptic tag; it thereby provides several avenues for metaplasticity important for memory dynamics. For example, PKMζ becomes constitutively activated, thereby providing a mechanism for maintaining upregulation of AMPAR expression despite the limited half-life and inevitable turnover of PKMζ. Interfering with extrasynaptic sequestration of GluR2-containing AMPARs would increase the readily available pool that is primed for activity-driven membrane insertion. Thus, PKMζ is capable of priming AMPAR trafficking at synapses and providing a means for influencing synaptic sensitivity subserving neuronal consciousness. This model is supported by experiments showing that mimicking the activation of PKMζ through application of Tat-GluR2$_{3Y}$ (which similarly prevents GluR2 endocytosis) significantly extended the temporal window for heterosynaptic transfer of LTP to weakly activated synapses (Connor et al. 2011b). This suggests that PKMζ primes future AMPAR insertion and prevents synaptic tag degradation to amplify future synaptic potentiation necessary for transforming transient synaptic potentiation to LTP (Sajikumar and Korte 2011).

11.10 Tag-Mediated Neuronal Consciousness

Organisms have evolved to decipher order from chaos, by learning about experiential consistencies and by recognizing inconsistencies when they occur. A brain that is capable of flagging and recording similarities (and dissimilarities) of event properties (e.g., timing and context of events) would be well-suited for preparing for future behavioral adaptations necessary for survival. Metaplasticity and synaptic tagging provide cellular substrates for addressing the difficult question of how brain circuits demarcate selected experiences as being predictive of significant future episodes requiring appropriate behavioral responses. In a broader sense, how does the brain sort out information that is relevant to an organism's survival and thus worthy of encoding and long-term storage? Part of the answer likely lies within the complex domains of neural decision-making and neuroeconomics, two burgeoning fields that are beyond the scope of this chapter (and of our own areas of expertise!). Nonetheless, we consider here a tentative premise for enhanced *neuronal consciousness.* We contend that those cells that have undergone tagging of their synapses are primed for monitoring of future synaptic activity and consolidation of the net results of this activity into molecular and network representations of memories. These attributes of priming, monitoring, and consolidation are, we submit, critical for expression of neuronal consciousness.

For example, noradrenaline acting through beta-adrenergic receptors has been linked to increased levels of attention and arousal (reviewed by Sara 2009), two closely associated processes that influence consciousness. These changes in global brain states are manifested at the cellular level through changes in cell excitability. An efficient method, in a memory formation context, for boosting neuronal consciousness would be by priming synapses located on recently activated cells for future plasticity. This would enhance the detectability of future salient stimuli (i.e., boost cellular "attention") by lowering the threshold for synaptic modification. Synaptic tags demonstrating metaplastic capabilities provide an elegant solution to the problem of how to coordinate specific synaptic modifications under conditions of global brain arousal. Depending on the circumstances, once tagged, these synapses would be more (or less) able to encode and store the attributes of future environmental events. This is the essence of "neuronal consciousness".

Metaplasticity is conducive to upregulation of neuronal consciousness by lowering the threshold for modifications of previously activated synapses (Hulme et al. 2013). Additionally, altered PRP synthesis and sequestration rates could regulate cellular consciousness by biasing synapses towards a state of increased preparedness. For example, navigating through space or exploring novel environments enhances secretion of noradrenaline, which activates beta-adrenergic receptors to translocate and activate PKA (Gelinas et al. 2008). Synaptic localization of PKA enhances AMPAR phosphorylation. Under these conditions, AMPARs are more readily incorporated into postsynaptic complexes when synaptic activity is increased subsequently at these synapses (Tenorio et al. 2010). Therefore, the cells harboring these synapses are more conscious (sensitive) to cellular events that represent (i.e., are triggered by) environmental events that should be committed to memory. As time elapses between the initial tag-inducing stimuli and subsequent activation of those synapses, neuronal consciousness will wane as a result of tag degradation. Viewed from a probabilistic stance, the less temporally contiguous two stimuli are (e.g., the longer the time interval between them), the less likely that they are associated or that their association will have predictive value. This accounts for the reversibility (Young et al. 2006) and tagging interchange (Li et al. 2012) that have been observed in studies of STC.

The finite lifetimes of synaptic tags suggest that they may serve as *strength-of-association predictors*. Hypothetically, when synaptic tags are initially activated, cell consciousness is high and synapses are primed to undergo plasticity, allowing for the association and encoding of future episodes. Over time, as the probability of events being linked decreases, synaptic tags are degraded or erased, and neuronal consciousness is reduced. Additionally, activation of other synapses on the same cells through specific patterns of activity (e.g., 5-Hz stimulation: Young and Nguyen (2005) may signal that environmental stimuli are highly divergent and unrelated. Such activity may buffer large, erratic swings of neuronal consciousness. In this scenario, suppression of heterosynaptic synaptic plasticity would prevent formation of erroneous associations between incongruous events. Interestingly, LFS erases synaptic tags in a cell-wide manner through activation of protein phosphatases (Young et al. 2006). Protein phosphatase activation would be an attractive mechanism

to suppress indiscriminate neuronal arousal, favor synaptic depression and DPT, and free up previously potentiated synapses for encoding future events.

Neuronal consciousness could also be suppressed through preferential engagement of tag degradation mechanisms or "inverse tagging" (Okuno et al. 2012). This inverse tagging, in which CaMKIIβ coalesces with ARC to reduce potentiation of weakly activated synapses, assists in honing neuronal representation at only those synapses or dendritic regions that have surpassed the activity thresholds required for triggering enduring changes of synaptic strength. Also, the compartmental restriction (Alarcon et al. 2006; Sajikumar and Korte 2011), and clustered plasticity (Govindarajan et al. 2006), models propose that PRPs should be restricted and regulated within specific dendritic compartments. This compartmentalization yields dendritic regions expressing differing degrees of perceptual bias, providing a feedback mechanism to the soma that would upregulate *somatic* consciousness by adjusting the efficacy of regulation of transcription and PRP synthesis. This synapse-to-nucleus feedback system provides a mechanism for maintaining neuronal consciousness of synaptic activity across a spatially complex dendritic area. It is not clear what mechanisms mediate these effects or their longevity. These could be part of a spectrum of mechanisms for preventing runaway synaptic potentiation/depression, resetting of synaptic weights, or preventing memory formation interference. Resetting of synaptic tags also provides a mechanism for refocusing neuronal consciousness by freeing up synapses for future priming. One can imagine that ubiquitous subthreshold sensory information triggered by trivial daily events leads to low levels of synaptic activity that favor phosphatase activation to repress tag setting and metaplasticity. Only when events elicit suprathreshold levels of synaptic activation, and/or the release of key neuromodulators, would synaptic consciousness be upregulated. Once metaplastic processes, including tag setting, are triggered, synaptic consciousness, manifested as sensitivity to future activity, would be optimized. Further research is needed to clarify the roles of tag erasure in cognition.

Data confirming a role for STC and metaplasticity in vivo are beginning to accumulate. An enduring form of metaplasticity in the amygdala has been discovered, which identified PKA as a crucial mediator of the future induction of long-term fear memory (Parsons and Davis 2013). This ability of a weak shock to prime the consolidation of LTM would be consistent with enhanced neuronal awareness of the dendritic regions initially encoding the first weak shock trial. Those synapses associated with the transient engram initiated by the first trial remained sensitive to similar stimuli for up to 7 days (Parsons and Davis 2013). The enduring phosphorylation of its substrates by PKA conferred *synaptic prescience* by maintaining these synapses in a primed state for future learning. Under this scenario, the stimulus parameters (initial learning event), the presence and activation of plasticity modulating factors such as PKA, and the enhanced neuronal awareness, all determine the future direction and magnitude of synaptic change and memory encoding.

Weaknesses in this model of STC- and metaplasticity-mediated neuronal consciousness would be the ubiquitous expression of PRPs (Frey and Morris 1998) and evidence suggesting that CaMKII can translocate beyond the dendritic region initiating its constitutive activation (Rose et al. 2009). However, the local synaptic

nature of tagging that is required for fulfilment of capture would maintain synaptic fidelity enough to bias expression of neuronal consciousness through those synapses containing *both* synaptic tags and PRPs.

11.11 Future Directions

Synaptic plasticity is a key canonical learning mechanism in the brain. Tight regulation of plasticity is crucial for ensuring that plasticity mechanisms are optimally tuned for recruitment at the proper time. Synaptic tagging and metaplasticity have evolved to synergistically aid implementation of essential plasticity constraints. A range of inter- and intracellular signalling molecules can trigger lasting changes in the ability of synapses to undergo plasticity. Determining how multiple signalling mechanisms are integrated, both spatially and temporally during synaptic plasticity, remains a critically important question in neuroscience. Evidence collected thus far has shed light on some of those processes; however, formidable questions remain regarding the key tenets of STC and metaplasticity.

Once relegated to a minor role in plasticity, LTD has gained traction as a cellular mechanism underlying specific types of memories (Dong et al. 2012). To what degree are metaplasticity and STC utilized by LTD remains to be determined. Can phosphatase activation serve as a synaptic tag? Can the temporal window for heterosynaptic transfer of LTD be enhanced through tonic suppression of AMPAR phosphorylation by phosphatases? Further research focusing on LTD-specific tagging and metaplasticity in vivo is required to address these questions.

Additionally, memories are returned to states of lability once they have been recalled, in a process known as reconsolidation (Besnard et al. 2012). Reconsolidation updates memories when new information linked to a previously consolidated memory is encountered. Similar to initial memory consolidation, reconsolidation requires protein synthesis (Duvarci et al. 2008; Da Silva et al. 2013), suggesting that it uses PRPs similar to those used in STC. Cassini et al (2013) have demonstrated that a transient spatial object recognition memory can be converted to LTM following reconsolidation of a fear memory which presumably provided the macromolecular synthesis necessary for capture of LTM. This reconsolidation-facilitated synaptic capture suggests that similar mechanisms can be recruited during initial memory formation and reconsolidation of existing memories. Further research is required to determine the degree of mechanistic divergence and convergence between these two processes and how they relate to metaplasticity.

The kinases mentioned here do not constitute an exhaustive list and many other signalling molecules, cell states (excitability, inhibition), and biosynthetic processes (translation, transcription) could play similar roles in metaplasticity and synaptic tagging. Not surprisingly PKA, CaMKII, and PKMζ engage metaplasticity and synaptic tagging through overlapping mechanisms. Several questions have yet to be addressed, including: Do different behavioral experiences engage distinct STC mechanisms involving different kinases? If so, what is the cellular mechanism for

neuronal selection of behavioral STC? Do all tags share a similar susceptibility to erasure that would limit metaplasticity? How do tagging and metaplastic mechanisms change under various developmental and pathological conditions? Data suggest that heterosynaptic processes that require metaplastic and STC-like mechanisms are altered in a mouse model of Fragile-X syndrome (Connor et al. 2011a). Further progress in studying synaptic tagging and metaplasticity will be facilitated through the integration of in vivo synapse-specific quantification of tagging combined with behavioral measures in organisms like mice. It is tempting to speculate that cases of abnormally enhanced memory (e.g., PTSD, mnemonically gifted "savants"), might be caused by excessive synaptic tagging and indiscriminate capture of PRPs. Understanding how synaptic tagging and metaplasticity are altered in animal models of these conditions could bolster our understanding of normal memory formation processes.

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Chapter 12
Metaplasticity of Synaptic Tagging and Capture: Memory Beyond the Circle

Mahima Sharma and Sreedharan Sajikumar

Abstract The Synaptic Tagging and Capture (STC) process, characterized by the tag-plasticity related protein (PRP) interactions, forms the basis of associative memories. The STC hypothesis provides a conceptual basis of how short-term forms of plasticity are transformed into long-term forms of plasticity in an associative and time-dependent manner. The capacity of a synapse to undergo plastic changes in the future is prone to modification by the previous neural activity—a phenomenon referred to as metaplasticity. The two critical components of the STC process—threshold and time-window—can be modified by a metaplastic stimulus. Metaplasticity of STC has important implications in learning and memory. It lowers the threshold for memory storage and prolongs the associativity for long-term memory. Furthermore, metaplasticity can effectively prevent synaptic competition in recently potentiated synaptic compartments. Taking these outcomes into consideration, it is clear that aberrant metaplasticity might be the basis for cognitive dysfunction. Amelioration of the cognitive dysfunction in a number of neurodegenerative diseases entails full understanding of the contributions of metaplasticity mechanisms to cognitive dysfunction. In this chapter we will review the metaplasticity of STC along with its implications in learning and memory.

Keywords Synaptic tagging and capture • Synaptic tag • Metaplasticity • Synaptic competition • Long-term memory • Associativity • LTP

12.1 Introduction

The encoding and storage of information in the brain still remains the most significant and keenly pursued challenge in neuroscience. We now know that synaptic plasticity, in the form of long-term potentiation (LTP) and long-term depression (LTD) are the fundamental mechanisms underlying the process of learning and memory (Bliss and Collingridge 1993; Siegelbaum and Kandel 1991). LTP

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strengthens the synapses in an input-specific manner, whereas, LTD selectively weakens the specific set of synapses to prevent them from reaching a ceiling level of efficacy and thereby enabling the synapses to encode new information. These activity dependent changes, which exhibit input specificity and associativity, result in the coding of memory traces in the neuronal networks (Bliss and Collingridge 1993; Martin et al. 2000; Malenka and Bear 2004). The synaptic modifications, occurring at the different synapses of a neuron, require the involvement of transcription and translation (Krug et al. 1984; Frey et al. 1988; Matthies 1989; Otani et al. 1989; Manahan-Vaughan et al. 2000). The long-lasting form of LTP termed as late-LTP (L-LTP), which is induced by strong stimulation relies on the processes of transcription and translation; however the short-lasting increase in synaptic efficacy termed as early-LTP (E-LTP) requires the modification and trafficking of existing proteins.

Input specificity of LTP refers to a long-lasting potentiation exhibited by only a set of activated synapses, not all of them. How the input specificity of long-term memory or LTP achieved in a neural network was an interesting question in the early years of LTP research. Because the transcription and translation processes are global, for many years, scientists were wondering how a neuron manages to target gene products from the nucleus to a few activated synapses out of all the inherent synapses. The picture became clear with the introduction of a new concept—Synaptic Tagging and Capture (STC) (Frey and Morris 1997). According to the STC hypothesis, strong neural activity leads to the generation of the products of gene expression, which are well distributed along the dendritic compartment. These products, specifically referred to as plasticity related proteins (PRPs) function to selectively strengthen the specific set of nearby synapses that have been "tagged" by a prior transient neural activity, that is insufficient by itself to establish L-LTP. Two sets of synapses within the same neuronal population, thus, undergo associative interactions through the processes of STC. The STC process has important implications in learning and memory. It provides us with a mechanistic explanation of the memory consolidation process, whereby E-LTP corresponding to short-term memory is consolidated to L-LTP corresponding to long-term memory. The STC processes are involved in the consolidation of newly formed associative memories (Frey and Morris 1997; Martin and Kosik 2002). Thus STC hypothesis provides a conceptual basis of how short-term forms of plasticity are transformed into long-term forms of plasticity in an associative and time-dependent manner (Frey and Morris 1997; Redondo and Morris 2011).

Let us understand the associative element of STC. Can you remember what you had for lunch yesterday? Many of us might. Do you remember what you had last Friday? Most of us won't. This could be explained by the fact that our brain processes such events as insignificant, not bothering to encode it as a long-lasting memory. Contrary to this, most of us will be able to tell what we had for lunch on our graduation. Our brain encodes the insignificant event (lunch in this case) into a long-term memory because of its associativity with a significant event—the Graduation. Here, *lunch* corresponds to a transient event that sets the "synaptic tag," and *Graduation* corresponds to the strong event that generates the "plasticity related proteins." These PRPs, when captured by the synaptic tag, result in the

encoding of the related insignificant event into a long-term memory. Thus, the STC processes form the basis of associative memories (Frey and Morris 1997; Redondo and Morris 2011).

The STC-mediated associativity is limited by a threshold and a temporal window, both of which can be modified by metaplasticity—a phenomenon by which the history of neuronal activation changes the rules of plasticity. Metaplasticity has emerged as a prominent mechanism for prolonged regulation of the network activity in neuronal populations (Abraham and Bear 1996; Abraham 1999, 2008). In this chapter, we will focus on metaplasticity and its implications in learning and memory.

12.2 Synaptic Tagging and Capture: A Historical Perspective

Frey and Morris came forward with the concept of STC in 1997. The STC process results in the consolidation of synaptic potentiation in an input-specific manner (Frey and Morris 1997; Martin and Kosik 2002). Thus two serial processes are involved in STC: (1) setting of the synaptic tag as triggered by a specific pattern of stimulation, and (2) synaptic capture, whereby the synaptic tag interacts with the newly synthesized PRPs. The synaptic tags are local molecular changes at synapses that mark synaptic plasticity as having occurred. To get a clear picture, imagine the strong event as the L-LTP input and transient event as an E-LTP input. The STC enables the E-LTP to transform into L-LTP.

12.2.1 Synaptic Tagging and Capture in Rodent

Frey and Morris made use of rodent hippocampus to exhibit the phenomenon of STC. Stimulation of two independent synaptic inputs (synaptic input 1 (S1), synaptic input 2 (S2)) to the same neuronal population in the CA1 region of the rat hippocampal slices involved repeated tetanization of S1 resulting in L-LTP, with minimal effect upon the second control input. The establishment of L-LTP depends on translation and is thus blocked by the presence of a protein synthesis inhibitor, anisomycin. Bath application of anisomycin, a reversible protein synthesis inhibitor, 35 min after the induction of LTP in S1, does not affect the L-LTP establishment in S1 as the translation process has already set in before the application of the protein synthesis inhibitor. However, the tetanization of S2 in the presence of anisomycin, with a time lag of 1 h between the two tetanizations (S1 and S2), should lead to the blockade of establishment of L-LTP. Contrary to this, L-LTP is established in S2 as well, owing to the fact that the proteins synthesized by LTP in S1 allow the induction of LTP in S2 (Frey and Morris 1997). Thus, the proteins synthesized by strong activity in the form of L-LTP at S1 are hijacked by the "synaptic tag" created by the transient neural activity at S2. The tag created is transient with a lifetime of 1–2 h and is protein synthesis

independent. Weak tetanic stimulation leading to an E-LTP, as well as strong tetanization event in the presence of protein synthesis inhibitor, initiates the creation of a synaptic tag. This synaptic tag determines the input specificity of LTP; the availability of relevant proteins along with the prior activity of the neuron determines the persistence of LTP (Frey and Morris 1997).

E-LTP, induced by the weaker stimulation of the Schaffer Collateral projections to CA1 region of the rodent hippocampus, can be stabilized to a late-form of LTP by the subsequent induction of L-LTP in the nearby set of synapses undergoing strong stimulation (weak before strong experimental paradigm). This consolidation of LTP results only if the delivery of weak stimulation occurs 1–2 h prior to or within 2.5–3 h of the strong stimulation (Frey and Morris 1998). Thus, STC ensues when a "synaptic tag" created by the weak stimulus hijacks the products of gene expression induced by the subsequent strong stimulus (see the *review* by Frey and Morris 1998) (see Fig. 12.1a, b). The molecular identity of synaptic tags and plasticity proteins are covered in some of the previous chapters of this book.

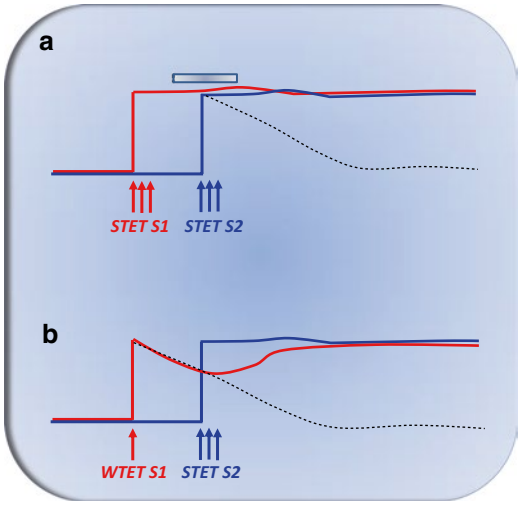

Fig. 12.1 A representation of the key experiments in synaptic tagging and capture. Frey and Morris performed these experiments in 1997 in the rodent hippocampus using two independent synaptic inputs S1 (*red*) and S2 (*blue*). (**a**) Strong tetanization of S1 (*red*) results in L-LTP (*red line*). Anisomycin, a protein synthesis inhibitor (*open rectangle*) was applied 35 min after the tetanization of S1. Strong tetanization of S2 (*blue*) 1 h after the STET in S1 (*red*) in the presence of the protein synthesis inhibitor still exhibited protein synthesis dependent L-LTP (*blue line*) (Frey and Morris 1997). (**b**) Weaker stimulation of the Schaffer Collateral projections to CA1 region (S1, *red*) of the rodent hippocampus results in E-LTP (*gray line*). However subsequent induction of L-LTP in the nearby set of synapses (S2, *blue*) within 1–2 h of weak stimulation of S1 (*red*) results in the stabilization of E-LTP to a long-lasting form of LTP in S1 (*red line*). The consolidation of LTP results from the capture of the strong stimulation induced products of the gene expression by the synaptic tag set by weaker stimulation. This is synaptic tagging/capture (Frey and Morris 1998)

12.2.2 Synaptic Tagging and Capture in Aplysia

The phenomenon of "synaptic tagging" has also been seen in the *Aplysia* system. *Aplysia* is an invertebrate sea slug from which much of scientific understanding about the different forms of synaptic plasticity stems out. Two spatially separated Aplysia motor neurons synaptically connected to a single bifurcated Aplysia sensory neuron can be studied in a culture system. Branch specific facilitation, a selective enhancement of synaptic efficacy at a certain synapse, results from the delivery of five puffs of serotonin to that contact, with no effect on the efficacy of the other contact. Application of the transcription inhibitor actinomycin D blocks the increase in synaptic potency, which is persistent for more than 24 h. Cyclic-AMP responsive element (CRE)-binding protein seems to mediate transcriptional activation, as the branch specific facilitation is blocked by the microinjection of anti-CREB antibodies. So, the change in the strength of connections of a single cell is transcription dependent and spatially restricted to a single subset of synapses (Martin et al. 1997; Martin and Kosik 2002).

The delivery of a single puff of serotonin to one contact and of five puffs to the other contact exhibits the process of synaptic tagging. A single puff of serotonin results in transient facilitation; but when five puffs are delivered to the other contact, it leads to a long-lasting facilitation at the branch receiving the single puff. It is very important to give the single pulse of serotonin within a discrete time-window (Martin et al. 1997) of either 1–4 h later or 1–2 h prior to the delivery of five pulses of serotonin to the other branch, to produce long-term facilitation. Thus, the synapse experiencing a transient activation captures the protein synthesized in a cell-wide process triggered by the long-term synaptic changes at the other synapse.

12.2.3 Synaptic Tagging and Capture in the Living Rat

For decades following the exhibition of STC processes in the in vitro studies done in Aplysia and rodent hippocampus, scientists kept on wondering if these processes are specific only to in vitro preparations. Do they have any relevance in the intact living animal? The evidence for the occurrence of STC processes in an intact animal was provided by Shires and colleagues in 2012. In a "strong before strong" paradigm study analogous to that done by Frey and Morris in 1997, strong tetanization of the first input to CA1 could rescue the decaying E-LTP, that was induced when the second input was subsequently tetanized in the presence of anisomycin. In the "weak before strong" paradigm, the later delivery of a strong tetanus to a second input could stabilize the E-LTP induced by weak tetanization. These results demonstrated that the STC is not exclusive to the in vitro preparations, but occurs in the living rat as well (Shires et al. 2012).

12.2.4 Inverse Synaptic Tagging

In recent years, the concept of inverse tagging has also come into focus (Okuno et al. 2012). Unlike the STC processes, "inverse tagging" operates at the inactive synapses. A number of neuronal activity-regulated genes exist that critically govern the molecular and cellular processes underlying memory formation and processing. One such gene, Arc has been implicated in the process of inverse tagging. Augmented neuronal activity required for cognitive processes underlies the activation of Arc (activity-regulated cytoskeleton-associated) protein. Arc mRNA is mostly localized to dendrites. Arc protein in the postsynaptic density (PSD) interacts with the components of endocytic machinery—endophilin and dynamin, thereby regulating the trafficking of the GluR1-AMPAR subunit away from the postsynaptic site. The resulting decreased surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) on the postsynaptic site leads to LTD in the respective synapse. But the ample amount of evidence for the strong induction of Arc by a LTP evoking strong stimulus (Link et al. 1995; Moga et al. 2004; Messaoudi et al. 2007) as well as the accumulation of Arc mRNA and protein in the dendritic areas receiving strong inputs (Moga et al. 2004; Steward et al. 1998) does not settle well with its role in the cell-wide weakening of glutamatergic strength. This puzzle is resolved with the demonstration of inverse synaptic tagging mechanism based on a local history of both activity and inactivity, whereby Arc-CaMKIIβ-interaction acts as a specific sensor that mediates the inactive synapse-specific control of AMPA-R clearance at weaker synapses in potentiated neurons (Okuno et al. 2012). Interaction of activity induced arc with Calmodulin (CaM) unbound CaMKIIβ leads to its anchorage at the synapses during inactivity. Dynamic interaction with CaMKIIβ mediates the inactivity dependent enrichment of Arc, clearing the upregulated GluR1 in the inactive synapses. Preferential maintenance of Arc at inactive synapses is brought about by increased affinity of Arc for the inactive CaMKIIβ at the synapses (Okuno et al. 2012). In the normal STC, PRP induced by strong activity at one synapse is captured by the synaptic tag set by transient neural activity in the nearby set of synapses. Inverse synaptic tagging mechanism involves the capture of the "negative plasticity factor" such as Arc by "inverse tag" such as inactive CaMKIIβ at the inactive synapses, leading to selective avoidance of the actively tagged synapses. Thus, this process serves to prevent the needless synaptic enhancement of the weak synapses while sparing the potentiated synapses, thereby ensuring the stability of contrast between strong and weak inputs over time (Okuno et al. 2012).

12.3 Metaplasticity of Synaptic Tagging and Capture

Metaplasticity, as the name suggests, refers to the plasticity of synaptic plasticity. The term was coined in 1996 (Abraham and Bear 1996). It is a phenomenon by which previous neural activity, without inducing a direct change in synaptic

efficacy, can alter the capacity of a synapse to undergo plastic changes in the future (Abraham 1999, 2008). Metaplasticity, in real life experience, is similar to warming up before an athletic activity which improves performance; metaplasticity enables the weakly activated synapses to undergo a long-lasting functional plasticity.

The STC process has two critical components—threshold and time-window (Frey and Morris 1997, 1998). There exists a threshold for the synaptic activity to qualify the same for the formation of a "synaptic-tag." STC initiates storage processes only when the strength of the synaptic tag and the local concentration of essential proteins are above a certain plasticity threshold. The synaptic tag-PRP interaction, which is the basis of associativity, is limited by a time-window of 60 min or so (Frey and Morris 1998; Sajikumar and Frey 2004). The previous history of synaptic activation can modify the threshold and time-window for STC process. This is the *metaplasticity of STC*.

The first correlation of metaplasticity and STC was introduced by Sajikumar et al. (2009). They revealed a novel form of metaplasticity that modifies the threshold conducive for subsequent synaptic tagging/capture. Short-term potentiation (STP) protocol was utilized, followed by L-LTP at a heterosynaptic input to study the metaplasticity of synaptic tagging/capture. We now know that a synaptic event has to reach a threshold to take part in synaptic tagging/capture. STP is unable to set the synaptic tags (Frey and Morris 1997) and thus cannot transform into L-LTP in the control set of experiments. The same synapses when primed with ryanodine receptor (RYR) agonists such as ryanodine (10 μM) or caffeine (10 mM) undergo STC, whereby an STP protocol establishes a L-LTP in response to strong tetanization of a heterosynaptic input. The delivery of low frequency depotentiating stimulation 5 or 10 min after the induction of synaptic tags in primed STP interferes with the tags, preventing the capture of newly synthesized PRPs (Sajikumar et al. 2009). It can be concluded that RYR activation, which primes weakly activated synapses for heterosynaptic interactions by lowering the threshold for subsequent synaptic tagging/capture, is one mechanism for the conversion of weak memory to strong memory. This explains that the threshold for synaptic tagging/capture is flexible and responsive to the previous history of neural activation. It becomes evident that weakly activated synapses can become enabled for long-term heterosynaptic plasticity interactions through a metaplastic priming event.

12.3.1 Metaplasticity Lowers the Threshold for Establishing STC

Synaptic threshold refers to the ability of a synaptic population to express or not to express the specific forms of plasticity. A synapse has to reach a level of threshold to exhibit different forms of plasticity. The difference in the neuromodulatory requirements for inducing long-lasting plasticity in apical and basal dendrites (Navakkode et al. 2005, 2007; Navakkode and Korte 2011) might be attributed to the difference in the threshold between these two morphologically distinct dendrites

of the CA1 pyramidal neuron (Spruston 2008). The apical dendrites of CA1 pyramidal neuron exhibit differences in the plasticity thresholds for inducing LTP and LTD in proximal and distal compartments. While the threshold for inducing LTP is higher in the distal region and decreases from the distal to the proximal part of the apical dendrites, the situation is contrary for LTD (Sajikumar and Korte 2011a, b).

It is important to regulate the over-strengthening of the synapses beyond a physiological level and this requirement is met by the existence of a homeostatic regulatory mechanism in the neurons (Young and Nguyen 2005). One of the mechanisms could be changing the threshold levels. On similar lines, the threshold for synaptic tagging/capture is adjustable and can be modified to benefit the memory storage process. It follows from the experiments which reveal that the "range of threshold" for functional plasticity is substantially increased by priming stimulation through the activation of metabotropic glutamate receptors (mGluRs) (Raymond et al. 2000; Sajikumar and Korte 2011a). The mGluR activation leads to the production of protein kinase Mζ (PKMζ) as a PRP through local protein synthesis that decreases the threshold for STC (Sajikumar and Korte 2011a). PKMζ is a constitutively active isoform of protein kinase C (PKC) that maintains the late phase of LTP as well as perpetuates the long-term memory trace (Sacktor 2011, 2012). PKMζ acts as a LTP-specific PRP and promotes synaptic strengthening by releasing AMPARs from an extrasynaptic pool, which is maintained by the protein interacting with C kinase-1 (PICK1) that binds to the GluR2-AMPAR subunit; and by enhancing *N*-ethylmaleimide-sensitive factor/glutamate receptor-2 (NSF/GluR2)-mediated trafficking towards the postsynaptic site. The resulting increased surface expression of AMPARs leads to enhanced glutamatergic transmission at the activated synapse, and thereby a persistent L-LTP is observed (Sacktor 2011). This priming activation of mGluRs enables a 5 Hz-*theta burst stimulation* (TBS-LTP) protocol to express STC, which otherwise is unable to transform E-LTP in nearby synapses to L-LTP (Huang and Kandel 2005). It is attributed to the generation of new PKMζ by DHPG (mGluR agonist) priming (Sajikumar and Korte 2011a). Different signaling cascades are activated. The activation of mGluRs leads to mammalian target of rapamycin (mTOR) signaling, facilitating the translation of terminal oligopyrimidine mRNAs. This results in the synaptodendritic synthesis of PRPs (Klen and Dever 2004). Increased PKMζ synthesis is also mediated by rapamycin sensitive pathways (Hernandez et al. 2003). The stimulation of phospholipase C and the subsequent release of Ca^{2+} from intracellular stores through RYR during priming mediate the new synthesis of PRP. Thus, the threshold for memory storage is lowered by the activation of a myriad number of signaling cascades during a metaplastic priming event (Sajikumar and Korte 2011a, b).

12.3.2 Metaplasticity Governs Compartmentalization of STC

According to the "clustered plasticity" model of long-term memory engrams (Govindarajan et al. 2006), the formation of engrams is facilitated by local translational enhancement and STC, through bidirectional synaptic weight changes among

synapses within a dendritic branch. Associativity of synaptic tagging/capture (STC) extends to the synapses in the same dendritic compartment. These compartments contain "synaptic clusters" with different plasticity thresholds. Within a dendritic compartment, a homeostatic process exists to adjust plasticity thresholds. The range in which these clusters operate can be altered by the processes of metaplasticity that operate on the cluster independently of other clusters at the same dendrite. This was revealed by a trisynaptic model of three independent inputs S1, S2, and S3 in the same compartment of apical dendrite (Sajikumar and Korte 2011a). Induction of TBS-LTP in S1, E-LTP in S2, and E-LTD in S3 results in cross-tagging but no STC (cross-tagging/cross-capture is the positive associative interaction of LTP and LTD first described by Sajikumar and Frey 2004). However, priming stimulation by an mGluR agonist DHPG significantly enhances the plasticity threshold of all synaptic units within the compartment, thus expressing STC during LTP and cross-capture (Sajikumar and Korte 2011a). Hence, the strength of incoming information and prior activity of the synapses decide the efficiency of the expression of STC. Inhibition of PKMζ and BDNF specifically prevents the transformation of E-LTP to L-LTP and E-LTD to L-LTD, respectively. Co-inhibition of PKMζ and BDNF by TrkB/Fc and ZIP prevents STC and cross-capture. Thus, even in the same compartment, the functional plasticity is both induced and maintained by different proteins. These "synaptic clusters," having been modified by the metaplastic priming event, will then prepare the synaptic network to form long-term memories (Sajikumar and Korte 2011a, b).

The metaplasticity processes before the establishment of functional plasticity substantially increase the capacity of synaptic units within a compartment for engaging in long-term functional plasticity, thereby playing an important role in long-term memory and associativity.

12.3.3 Metaplasticity Prolongs Associativity of STC

Metaplasticity not only alters the threshold for "synaptic tags," but also extends the "time-window" of the synaptic tag. In a normal scenario, the associative interaction in STC is expressed and restricted to the early phase of LTP, that is, up to a time period of 60 min (Frey and Morris 1998; Redondo and Morris 2011). The fragile nature of the synaptic tag marked during a weak synaptic potentiation event could account for the limited ability of synaptic populations to integrate information beyond this period (Qin et al. 2014). The evidence for the extension of the synaptic tag stems from the experiments demonstrating that RYR activation prior to the induction of E-LTP can increase the duration of synaptic tag from the normal 1 h to at least 5 h, making an associative interaction possible throughout this time period (Qin et al. 2014). More precise consolidation process could result by extending the time interval for the interaction of weak and strong events in a synaptic population, thereby tuning the synapses to promote or prevent long-term memory storage (Abraham 2008).

CaMKII mediates the setting of a synaptic tag in normal STC (Sajikumar et al. 2007; Redondo et al. 2010). This tag degrades probably due to dephosphorylation, and thus lasts only 60 min. This triggers a question as to how priming prolongs the duration of the synaptic tag without degradation. This query is answered by the experimental results evincing the increased "lifetime" of a synaptic tag in primed LTP. The extended lifetime of the tag is brought about by the alteration of synaptic tagging in RYR or synaptic activity primed E-LTP from a CaMKII-mediated process to a PKMζ-mediated process through the processes of metaplasticity (Qin et al. 2014) (Fig. 12.2a).

CaMKII-mediated tag setting classifies as a "short-lived tag-setting process" that limits the duration of associativity, while PKMζ-mediated tag setting is a "long-lived tag setting process" that prolongs the associativity. Thus, a stable tag-setting process is fostered by metaplasticity, thereby generating stable "synaptic tags" that prolong the encoding of memory engrams, allowing associativity in the "late" stage of LTP (Qin et al. 2014).

Let us discuss the mechanistic aspect of increase in the duration of synaptic "tag." Metaplastic priming events like mGluR activation (Sajikumar and Korte 2011a) and RYR priming (Qin et al. 2014) can generate PKMζ as a new plasticity factor. The locally synthesized PKMζ can prevent the degradation of the synaptic tag. This allows the PKMζ-mediated tag to stay intact for 4–5 h. Furthermore, PKMζ has been proposed to extend the associativity during STC by "synaptic auto-tagging" (Sacktor 2011). As discussed in the preceding sections of this chapter, PKMζ enhances the NSF-mediated trafficking of GluR2 to the postsynaptic sites by releasing the receptors from PICK1 (Sacktor 2011, 2012). The enhanced amount of GluR2 at the active synapses acts as a "tag" that captures the PKMζ-PICK1 complex. The interaction of "PKMζ-PRP" and "PKMζ-mediated synaptic tag" imparts associativity in the late phase of LTP. Thus, PKMζ mediates the synaptic tag as well as PRP, thereby ensuring an extended period of associativity (Qin et al. 2014) (Fig. 12.2b).

Fig. 12.2 (continued) When L-LTP in the neighboring synapse is induced 4 h after the E-LTP induction, the STC process does not result as the CaMKII-mediated tag degrades by that time. On the other hand, priming of the synapse undergoing E-LTP, either by ryanodine receptor (RYR) activation or DHPG (mGluR agonist) application, results in the conversion of CaMKII-mediated tag to a PKMζ mediated tag. This tag lasts for 4–5 h during which it captures the PRPs and leads to synaptic tagging/capture. (**b**) PKMζ auto-tagging. Metaplastic priming events like the mGluR activation (Sajikumar and Korte 2011a) and RYR priming (Qin et al. 2014) release the translational block on PKMζ mRNA, and thereby generate PKMζ as a new plasticity factor. PKMζ binds to PICK1 that maintains the endocytic pool of GluR2 receptors, thereby releasing the glutamate receptors from the endocytic pool. The PKMζ-PICK1 binding favors the NSF-mediated trafficking of GluR2 to the postsynaptic sites. The enhanced amount of GluR2 at the active synapses acts as a "tag" that captures the PKMζ-PRP. The PKMζ-PRP further phosphorylates its substrate, the GluR2 C-terminal or its associated proteins, which results in decreased GluR2 endocytosis, thereby potentiating synaptic transmission (Sacktor 2011). The interaction of "PKMζ-PRP" and "PKMζ-mediated synaptic tag" imparts associativity in the late phase of LTP. Thus, PKMζ mediates the synaptic tag as well as PRP

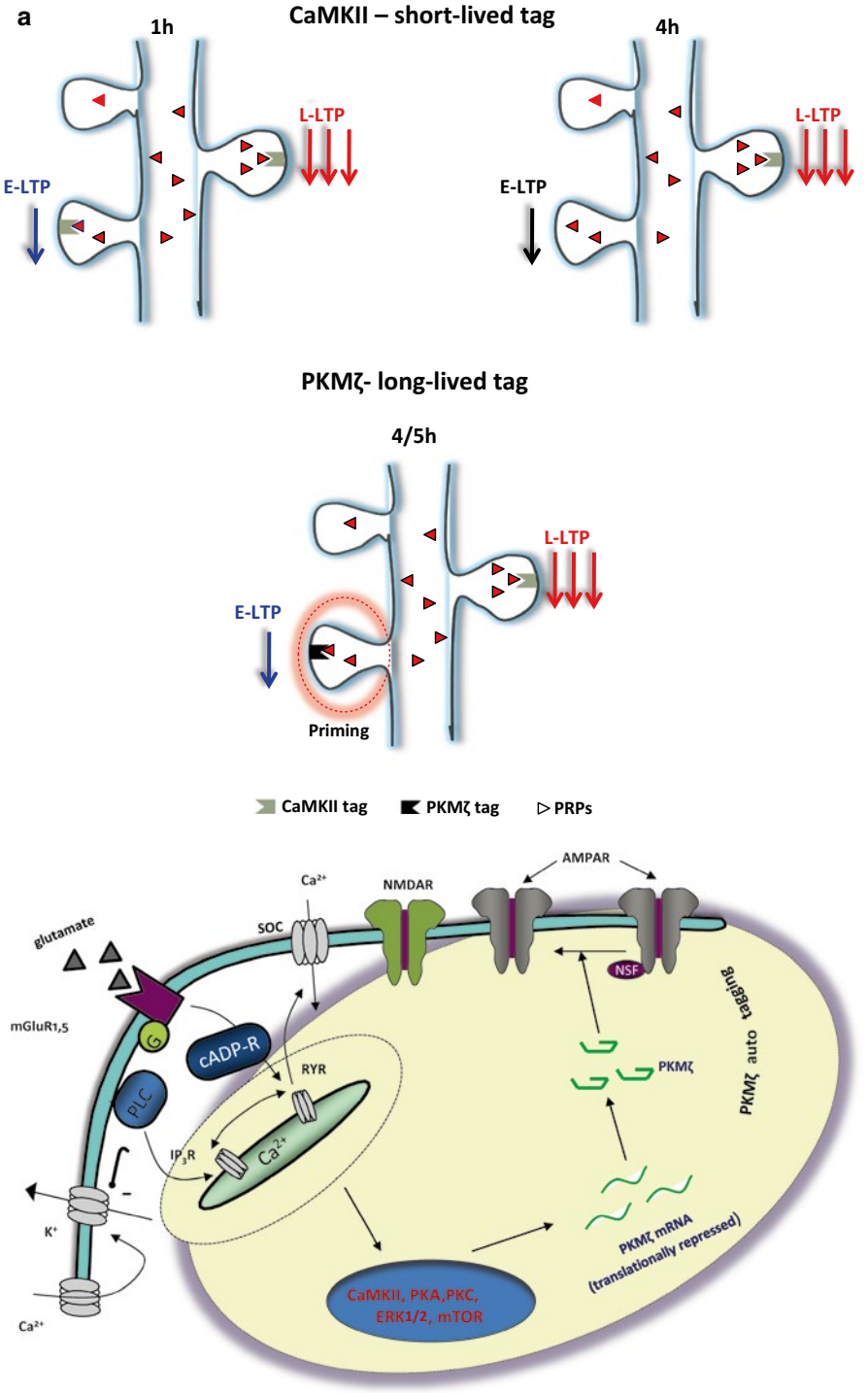

Fig. 12.2 The conversion of a synaptic tag during metaplasticity. (**a**) The CaMKII-mediated synaptic tag does not last longer than 1 h. Induction of L-LTP in the nearby synapse within 1 h of tag setting by E-LTP induction results in the capture of PRPs by the CaMKII-mediated tag.

12.4 Metaplasticity and Synaptic Competition

Synaptic competition is the theory explaining the observation that two pathways already expressing LTP will compete for scarce plasticity related products when they are further tetanized after a period of protein synthesis inhibition during the maintenance phases of LTP (Fonseca et al. 2004). For investigating synaptic competition in a physiological situation, it was important to develop a model of synaptic competition in vitro. Most of the labs study LTP and LTD only for a short period of nearly 60 min. Synaptic competition generally occurs when the availability of plasticity protein is reduced. Therefore, it was impossible to study synaptic competition within a short period of 60 min because the distribution of plasticity proteins is still not complete (Redondo and Morris 2011).

For studying synaptic competition in recently potentiated synapses, a three-input model was used (Fig. 12.3a). First we induced L-LTP in synaptic input S1 (red) followed by E-LTPs (strong before weak (Fig. 12.3b)) at 30 and 45 min in

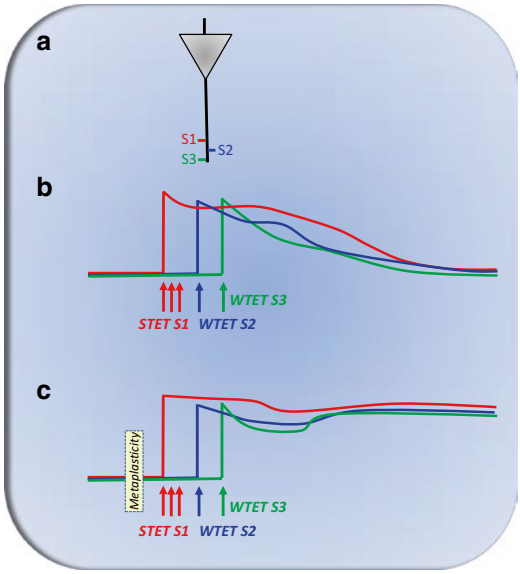

Fig. 12.3 Synaptic competition in recently potentiated synapses. (**a**) A three-input model where S1 (*red*), S2 (*blue*), and S3 (*green*) represent three different populations of synapses on to the same neuronal cell. (**b**) Synaptic competition is observed among different synaptic clusters. Induction of L-LTP in synaptic input S1 (*red*) followed by E-LTP's (Strong before weak) at 30 and 45 min in synaptic input S2 (*blue*) and S3 (*green*), respectively does not exhibit long-lasting form of LTP in S1. All these synapses compete for the scarcely available plasticity related proteins, thereby preventing all forms of plasticity. (**c**) Metaplasticity in the form of mGluR activation (represented by *yellow rectangle*) before the induction of L-LTP in S1 (*red*) effectively prevents synaptic competition. Here, the products of gene expression are captured by S2 (*blue*) and S3 (*green*) as well and result in STC. All three synaptic clusters exhibit L-LTP

synaptic input S2 (blue) and S3 (green), respectively. In a weak before strong paradigm, in which two early-LTPs (in S1 at 0 min and in S2 at 30 min) induced before the induction of L-LTP in S3 at 45 min. In both cases all forms of plasticity were prevented, providing evidence that competitive tag setting (by co-incidental induction of multiple bouts of WTETs within the temporal vicinity of STET) triggers a graded decay of long-lasting L-LTP into a shorter form of L-LTP that appears much more slowly than E-LTP decay per se. This graded and slow depreciation of L-LTP is a feature of competitive maintenance. Importantly we observed that synaptic competition was visible only if the competing partners appear within the initial 60 min (Sajikumar et al. 2014). Surprisingly, metaplastic activation of mGluR activation before the induction of L-LTP effectively prevented synaptic competition, thus providing more evidence for the tuning of synapses for long-term plasticity and memory (Fig. 12.3c).

12.5 Relevance of Metaplasticity in Behavior

The existence of a "threshold" for changes in the level of synaptic plasticity serves to prevent plasticity and learning from occurring too readily in response to nonsalient stimuli (Abraham 2008). The manipulation of the thresholds by metaplastic activity grants great flexibility to the learning process. Metaplasticity mechanisms, when activated, lower plasticity thresholds and/or increase the extent and endurance of change. These effects underlie the behavioral outcomes of metaplasticity, such as enhanced learning ability or the priming of neuronal networks to encode specific content. A neuronal network undergoing a metaplastic event gains advantage for learning the present task as well as the other tasks that engage the same neuronal network (Hulme et al. 2013). This transfer of influence between tasks has been observed in hippocampus following training in Morris water maze (MWM) task, whereby the improved acquisition of a second hippocampus-dependent task "trace eye-blink conditioning" results from the enhanced excitability of CA1 pyramidal cells (Kuo et al. 2006). Increased intrinsic excitability is reciprocated in the form of increased LTP that boosts the learning performance.

Metaplastic upregulation of protein synthesis serves as another mechanism to enhance the general willingness to learn (Hulme et al. 2013). The behavioral feature of STC involves the production of proteins by repetitive behavioral training, that remain available for a limited period of time to lower the threshold for temporally associated but weaker training stimuli at other synapses and thereby induce long-term functional plasticity changes in the form of enhanced learning at those synapses. Metaplasticity mechanisms govern the duration of the STC effect and are known to prolong the time course of associativity, thereby regulating the acquisition of associative memories. The higher efficacy of spaced trials as compared to massed trial might be based on the extended associativity period resulting from metaplasticity (DeZazzo and Tully 1995). Metaplasticity can modulate the general preparedness to learn in either way. Metaplastic stimuli, for example, an enriched environment

can enhance the learning performance, whereas stress can ablate the willingness to learn. Furthermore, metaplasticity mechanisms also serve to homeostatically regulate the balance between stability and the incessant availability of plasticity. Thus, metaplasticity constitutes a dynamic response inherently coupled to prior activity; and has the potential to provide sophisticated regulation of plasticity across space and time via diverse mechanisms (Hulme et al. 2013).

The behavioral aspect of metaplasticity can be evaluated by a paradigm where the animals are made to undergo learning tasks like the MWM task or the object recognition task. In this paradigm, the mice are exposed to a metaplastic stimulus prior to their training for the learned task. The group of animals exposed to positive stimuli, for instance, enriched environment or novel open field within a specific time frame before the real learning task will exhibit a better learning performance as compared to the control animals (unpublished observation from Sajikumar et al.). The performance of the animals exposed to negative stimuli like stress or fear, is expected to be poorer than the control animals. This paradigm might help us to evaluate and understand the outcome of exposure to a myriad number of metaplastic stimuli.

12.6 Relevance of Metaplasticity and Future Perspective

Metaplasticity phenomenon enables the neural circuits to associate events at one point in time with a much later strong stimulus/event, thereby temporally expanding a network's capacity for associating stimuli. Metaplasticity could manifest itself in a number of behavioral outcomes, such as modifications in ease of learning, or strength and duration of memory (Hulme et al. 2013). Enriched environmental stimuli and stress represent behavioral metaplastic changes that can increase and decrease the general preparedness to learn, respectively. Furthermore, metaplastic changes during a learning event help to establish the newly acquired information and keep the information from being overwritten by new learning. Thus, interfering with the establishment of metaplastic state could impede the acquisition of further information. The aberrant metaplasticity can lead to cognitive dysfunction. Inhibition of LTP through pathological engagement of metaplasticity mechanisms is thought to underlie the early memory loss in Alzheimer's disease (AD) (Zorumski and Izumi 2012). This is suggested to be mediated by NMDAR subunit-GluN2B signaling (Li et al. 2011). Models of Parkinson's disease (Nash et al. 2004) (PD) and Huntington's disease (Li et al. 2004) (HD) have also reported enhanced GluN2B signaling. Thus cognitive impairment in AD, PD, HD, and perhaps other neurodegenerative conditions results from GluN2B-mediated metaplasticity. In addition, metaplasticity effectively prevents synaptic competition in recently potentiated synaptic compartments (Sajikumar et al. 2014). Much work needs to be done to completely understand the contributions of metaplasticity mechanisms to cognitive dysfunction. Cognitive deficits arising from altered metaplasticity could be set right by appropriately restoring metaplasticity functionality. This represents a potential therapeutic strategy. Metaplasticity can reinstate or empower the desired synaptic

plasticity and thus has the potential to be exploited to improve clinical outcome. Thus it becomes very important to understand the behavioral contribution of metaplasticity so that we could harness these metaplasticity mechanisms for clinical benefit (Hulme et al. 2013).

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Chapter 13
Emotional Tagging and Long-Term Memory Formation

Gal Richter-Levin, Orli Kehat, and Rachel Anunu

Abstract Numerous studies support the notion that emotional arousal modulates the formation of long-term memories. The amygdala, a principal component of the emotional memory system, is involved in modulating memory storage in other brain areas according to the emotional content of an encountered event. According to the concept of *emotional tagging*, activation of the amygdala during emotionally arousing events "tags" the experience as important by strengthening synapses located on neurons that have just been activated in other brain regions, mainly the hippocampus.

In line with this hypothesis, research has shown that activation of the amygdala by behavioral manipulations (exposing the subject to emotional content) or by electric stimulation could transform weak memories into strong, long-lasting ones. Although many studies emphasize the enhancing effect of amygdala activation on memory consolidation, a more complex picture emerges when observing emotional arousal under different conditions. Memory consolidation may be enhanced or impaired by emotional arousal depending on such factors as the intensity of the emotional event, one's ability to cope with it and the timing of the event. Taking these complexities into consideration advances our understanding of the neural mechanisms behind *emotional tagging* and can provide insight into the neurobiology of affective disorders.

Keywords LTP • STC • Emotional tagging • Amygdale • Consolidation

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13.1 Introduction

In everyday life we are constantly exposed to a vast amount of information. An effective memory system has to learn to recognize what is important and should be retained as opposed to information that is less relevant. One of the factors that may facilitate the consolidation of a significant event is emotion arousal.

The amygdaloid complex, as part of the limbic system, is thought to represent a major hub for channeling sensory information of biological significance to storage in networks located primarily in neocortical areas (LeDoux 2000, 2003; Markowitsch and Staniloiu 2011). This function implies that the amygdala may interpret the emotional value of incoming information and attach emotional significance to different aspects of the experience (Armony and LeDoux 1997; Akirav and Richter-Levin 2006; Markowitsch and Staniloiu 2011). It is suggested that this evaluation is passed on to the hippocampus which puts a specific event into its proper context to form an accurate episodic memory (Akirav and Richter-Levin 2006). Thus, activation of the amygdala following an emotional charged event may lead to the reinforcement of consolidation of the event. The amygdala "tags" this experience as important, presumably by strengthening synapses located on neurons that have just been activated in the hippocampus. We termed this strengthening of cognitive memory by amygdala activation as *emotional tagging*. As will be discussed in the following chapter, the *emotional tagging* concept operates at a functional level, frequently mirroring the synaptic tagging concept.

In the following chapter the vast amount of data supporting the *emotional tagging* hypothesis will be discussed. Special attention will be given to the emotional modulation of long-term potentiation (LTP), a widely excepted mechanism of cellular mechanism of memory. This will allow a more straightforward comparison between the *emotional tagging* and the synaptic tagging concepts. Lastly, we will address the complexity of the *emotional tagging* concept, demonstrating that the outcome of amygdala modulation on the hippocampus is not necessarily favorable and is dependent on certain parameters of the emotional event itself.

13.2 Emotional Modulation of Memory

13.2.1 The Amygdala: Structure and Function

The research on the emotional modulation of memory identified a series of structures, neurotransmitters, and hormones involved in these processes. Concepts introduced in the first half of the twentieth century by Papez (Papez 1937), Klüver and Bucy (Kluver and Bucy 1939) and Hess (Hess and Akert 1955) linked limbic circuits and particularly the amygdala and the hippocampus to emotions and their behavioral expression. Since then numerous studies in both rats and humans indicated the importance of the amygdala in the acquisition and expression of learned fear (reviewed in LeDoux 2000).

The amygdala complex encompasses several structures with distinct connectional and functional characteristics. Among these nuclei are lateral (LA), basal (B)—sometimes grouped together as a basolateral (BLA) complex, and central (CE); each region is composed of several nuclei (LeDoux 1993; Pitkanen et al. 2000; Sah et al. 2003). Tracing studies reveal that there exists a profuse and extensive intra- and internuclear connectivity within the amygdala complex. These studies suggest that sensory information enters the amygdala through the BLA region and progresses to the CE (Maren 1996; LeDoux 2000). Afferent projections to the amygdala include all sensory modalities from thalamic and cortical origins, from other limbic structures such as the hippocampal formation, and from supramodal association cortical regions represented by the prefrontal and the entorhinal cortex (Sah et al. 2003). Efference from the amygdala projects back to the cortex and hippocampus; there is also a prominent projection to areas controlling important somatic and visceral effectors, such as the midbrain and the hypothalamus (Sah et al. 2003).

The amygdala is related mostly to negative emotions such as fear, and consequently it is considered part of the negative motivational system. In relation to memory, two different roles are attributed to the amygdala: first, it is the principal component of the emotional memory system and is involved in the processing and control of emotional behaviors and autonomic responses (LeDoux 1993, 2000) and second, it modulates memory storage in other brain areas (McGaugh 2002, 2004). With regards to the current subject of *emotional tagging*, our main focus will be on the second role of the amygdala, which is altering activity in other brain areas.

The Amygdala's Role as a Modulator of Memory Consolidation in Other Brain Areas

The modulatory role of the amygdala on consolidation of memory in other brain areas has been abundantly documented in animal (McGaugh 2002; McGaugh et al. 2002) and human experiments (Cahill and McGaugh 1995). This modulatory effect was demonstrated using memory paradigms that depend on the function of many different brain areas, including the hippocampus, the caudate nucleus or cortical regions (Packard et al. 1994; McGaugh and Cahill 1997; McGaugh 2004; Roesler et al. 2002, Roozendaal et al. 2004). It involves noradrenaline and adrenergic receptors (Cahill et al. 1994; McGaugh and Cahill 1997; Ferry and McGaugh 1999; Ferry et al. 1999a, b; Hatfield and McGaugh 1999), as well as adrenal stress hormones (Roozendaal and McGaugh 1997; Roozendaal et al. 1997, 2003; McGaugh and Roozendaal 2002), indicating that the amygdala acts as part of an extended reinforcement system also involved in attention arousal and stress.

Several lines of evidence support the claim that the amygdala has a modulatory influence on memory-related processes in the hippocampus, although the mechanism of action is not well-understood. Behavioral pharmacology research shows that activating the amygdala by post-training localized infusion of drugs modulates memory formation across tasks and in a dose- and time-dependent manner. For instance, McGaugh et al. have shown that stress hormones released during

emotionally arousing experiences activate noradrenergic mechanisms in the amygdala, specifically in the BLA, resulting in enhanced memory for those events (reviewed in McGaugh 2004). They further stressed the pivotal role of the noradrenergic system of the BLA by showing that it integrates the influences of other neuromodulatory systems on memory storage (Ferry and McGaugh 2000; McGaugh and Roozendaal 2002) Post-training intra-BLA infusions of adrenoceptors agonists, muscarinic cholinergic agonists, glucocorticoid receptor (GR) agonists, benzodiazepine receptor antagonists, opiate antagonists or gamma-aminobutyric acid (GABA) receptor antagonists were all shown to enhance retention of the inhibitory avoidance conditioning task in a dose- and time-dependent manner (Brioni et al. 1989; Introini-Collison et al. 1989, 1991, 1996; Roozendaal and McGaugh 1997; Salinas et al. 1997; Da Cunha et al. 1999; Ferry et al. 1999a, b). The enhancement effect is not specific to the inhibitory avoidance paradigm; post-training muscarinic cholinergic receptor activation of the BLA enhanced contextual fear conditioning (Vazdarjanova and McGaugh 1999), amygdala acetylcholine (ACh) release was positively correlated with performance on a hippocampus-dependent spatial working memory task (McIntyre et al. 2003) and post-training intra-BLA infusions of norepinephrine (NE) enhanced retention for the location of the hidden platform in a spatial water-maze task (Hatfield and McGaugh 1999). Post-training intra-amygdala infusions of amphetamine enhanced memory in both spatial and cued training water-maze tasks which are known to be dependent on the hippocampus and the caudate nucleus, respectively (Packard et al. 1994).

BLA lesions were also found to have an effect on hippocampal-dependent learning. For example, lesions in the amygdala block the modulatory effects of systemic and post-training intra-hippocampal injections of stress hormones on long-term memory assessed in a variety of learning tasks, including inhibitory avoidance, Y-maze discrimination, and water-maze tasks (Cahill and McGaugh 1990; Roozendaal and McGaugh 1996; Roozendaal et al. 1996, 1998).

Findings of human studies on the effects of emotional arousal of amygdala activation also support the modulatory role of the amygdala suggested by animal studies. For instance, memory for emotionally arousing material is not enhanced in human subjects with selective lesions of the amygdala (Cahill et al. 1995; Adolphs et al. 1997). Studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) showed that activation of the amygdala and hippocampal or parahippocampal regions was found to be correlated during emotional arousal (Hamann et al. 1999) and such activation was correlated with later retention (Dolcos et al. 2004; Kensinger and Schacter 2006; Ritchey et al. 2008). Kilpatrick and Cahill (2003) used PET to examine the influence of the human amygdala on other brain regions under emotional and non-emotional learning conditions. Their results indicated significantly increased amygdala influences on the ipsilateral parahippocampal gyrus and ventrolateral prefrontal cortex during the emotional relative to the neutral film viewing condition. In addition, consistent with animal studies, human studies have provided additional evidence of the importance of noradrenergic activation of the amygdala (van Stegeren 2009). β-Adrenoceptor antagonists (e.g., propranolol) block both the increase in amygdala activity and the

enhanced retention induced by emotional stimuli obtained in fMRI studies (Strange and Dolan 2004; van Stegeren et al. 2005). These findings further support the notion that the amygdala influences other brain regions particularly during emotionally arousing learning situations, thus reflecting its memory-modulation function.

Emotional Modulation of LTP

A more direct way of evaluating the *emotional tagging* concept with regard to synaptic tagging is to assess the effects of amygdala activation on synaptic plasticity in other regions, such as the hippocampus. Specific patterns of activation, such as brief high-frequency stimulation of afferent fibers to the hippocampus can result in long-lasting alterations of synaptic efficacy. The most widely studied cellular model for synaptic plasticity is long-term potentiation (LTP). LTP has gained a wide recognition as a cellular mechanism of memory (Matthies 1989; Bliss and Collingridge 1993).

Interestingly, previous studies reveal that blocking protein synthesis does not block LTP induction, but inhibits the late phase of LTP (L-LTP), the potentiation that usually occurs about 4 h after induction (Krug et al. 1984; Frey et al. 1988; Otani and Abraham 1989). This led to the recognition that LTP, like memory, has early and late phases and that protein synthesis is required for both the consolidation of memory and the late maintenance of LTP (Matthies 1989).

LTP is specific in the sense that only the activated synapses will become reinforced. Although some protein synthesis can occur at the dendritic level, the distribution of the newly synthesized protein or mRNA for local synthesis (Rodriguez et al. 2008) requires a mechanism that will guarantee specificity. The mechanism of synaptic tagging ensures specificity by stating that the activated synapses are "tagged" with a molecular marker that can capture the plasticity-related proteins (PRPs). Frey and Morris were able to prove synaptic tagging in a series of well-designed experiments (Frey and Morris 1997, 1998a, b). The synaptic tagging hypothesis claims that the stimulus that induces LTP creates a series of local transformations that ensures E-LTP and in addition it establishes a tag to ensure that the recognized PRPs are inserted only in the activated synapses. The experiments, carried out in vitro in the CA1 hippocampal subfield, also showed that E-LTP induced by a weak tetanic stimulus to one afferent set could be converted into L-LTP by applying a strong tetanus to a separate set of afferents within a given time window. In other words, although a mild stimulus was not able to activate the protein synthesis required for consolidation, it could be strong enough to set a transient tag that can "hijack" PRPs synthesized under the action of an independent stimulus, if both stimuli occurred within a definite time frame (about 1 h, in vitro). Frey and Morris (1997) argued that this could explain the flashbulb memories phenomenon, in which highly detailed (and sometimes irrelevant) information is remembered when it occurs around the time of an emotionally arousing event.

As a mechanism of memory, LTP can also be modulated by emotional and motivational events. One of the first experiments to demonstrate emotional modulation

of LTP showed that water deprived animals exhibited a longer-lasting LTP if they were allowed to drink shortly before or after (1 h) the induction of an E-LTP using mild tetanus (Seidenbecher et al. 1995, 1997). Other emotionally arousing behavioral manipulations have shown a similar effect (Straube et al. 2003a, b; Uzakov et al. 2005). The amygdala's involvement in this mechanism was confirmed by proving that temporal or permanent disruption of the BLA completely abolishes affective reinforcement (Almaguer-Melian et al. 2003; Korz and Frey 2005). An inverse approach also demonstrated such involvement: stimulating the amygdala mimics affective reinforcement of E-LTP into an L-LTP (Frey et al. 2001). The influence of the amygdala on the induction of LTP was demonstrated in a series of studies carried out in the last decade of the twentieth century (Ikegaya et al. 1994, 1995a, b, 1996; Akirav and Richter-Levin 1999a, b).

Pharmacological studies provided evidence for noradrenergic and cholinergic involvement in LTP modulation (Ikegaya et al. 1997; Seidenbecher et al. 1997; Straube et al. 2003a, b) and in LTP induced by stimulation of the amygdala (Frey et al. 2001; Akirav and Richter-Levin 2002; Vouimba et al. 2007).

Bergado et al. (2007) investigated the functional interplay among brain structures and systems which result in the conversion of a E-LTP into a L-LTP by stimulation of the BLA by using topical application of specific drugs into the dentate gyrus (DG) and other regions. Topical application at the DG showed that antagonists of both norepinephrine β_1 and acetylcholine muscarinic receptors blocked the effect of BLA stimulation on LTP-reinforcement (Bergado et al. 2007), although temporal patterns were different. It is suggested that activation of the molecular cascades that regulate protein synthesis in order to convert E-LTP to L-LTP could be one of the functions of noradrenaline and acetylcholine.

The patterns of activation described here allow an interpretation of affective reinforcement in terms of synaptic tagging mechanisms. According to this view, a mild tetanization pattern used to induce LTP is not strong enough to activate the protein synthesis regulatory cascades; therefore, only an E-LTP develops. It can however tag these synapses, allowing them to capture plasticity proteins synthesized under the influence of a temporally related affective event. The emotional event activates neuronal molecular metabolism, transcription, and translation via catecholaminergic and cholinergic projections, thus providing the tagged synapses with the proteins required to reinforce and prolong the modification in synaptic efficacy.

13.3 Factors That Influence the Emotional Tagging Process

As previously discussed, emotion-related stimuli may facilitate the transition from E-LTP to L-LTP, and allow the enhancement of hippocampal LTP and therefore enhancement of memory consolidation. However, the relationship between emotional arousal intensity and memory strength is not necessarily linear. As a first example of this complexity, it is important to remember that part of the amygdala's role in processing stimuli of an emotional nature is its involvement in stress

processing. Exposure to stress is traditionally considered a factor that impairs memory formation and most of the studies that deal with stress and LTP emphasize suppression of LTP following stressful events. This is in contrast to many studies that report that amygdala activation is generally reported to enhance LTP in the hippocampus, and may also mediate stress-related enhancement of hippocampal memory processes (Kim et al. 2001; McGaugh et al. 2002; Richter-Levin and Akirav 2003). Moreover, in contrast to the prevailing assumption that stress impairs memory, there are numerous observations suggesting that emotion and stress do not always impair memory formation, but rather they can also enhance it (Sapolsky 2003; Diamond et al. 2007). It has been suggested that through differential activation, the BLA may play a key role in both the impairing and the enhancing effects of stress on hippocampal functioning (Liang et al. 1994; Akirav and Richter-Levin 1999a, b, Kim et al. 2001; Vouimba et al. 2004; Vouimba and Richter-Levin 2005; Tsoory et al. 2008). The way emotion and stress modulate memory formation may depend on many factors such as the timing, intensity, and controllability of the stressor and the relation between stress and the information to be encoded. Some of these factors will be discussed next.

13.3.1 Nature of the Emotional/Stressful Event

Intensity-Low vs. High Stress

The intensity of the stress encountered can greatly affect the learning process and its outcome. For instance, rats trained to find a hidden platform in the Morris Water Maze (a hippocampal-dependent spatial task) under "high-stress" conditions (cold water, 19 °C) were more successful and showed better long-term memory than did rats that were trained under "low-stress" conditions (25 °C warm water, Sandi et al. 1997; Akirav et al. 2001). Moreover, in comparison to "naïve" rats, only rats trained under "high-stress" conditions exhibited significant increased extracellular signal-regulated kinase (ERK2) phosphorylation, indicative of activation of mitogen-activated protein kinase (MAPK) signaling cascades in the BLA (Akirav et al. 2001). The participation of the amygdala in learning seems to be directly dependent on the training conditions; the water temperature may have acted differently on consolidation mechanisms via the influence on the amygdala during and/or following the training, and this led to differential performance in the test.

In another study testing acquisition of spatial information in low- and high-stress conditions, a stress-related shift in the pattern of CREB activation was observed (Kogan and Richter-Levin 2008). Specifically, CREB activation was observed predominantly in the CA1 for the lower-stress learning group as opposed to significant CREB activation in the amygdala for the higher-stress learning group (Kogan and Richter-Levin 2008). This variation in activation patterns could be related to the different quality of the memory formed under lower vs. higher-stress conditions.

Numerous data suggested previously that an intermediate level of stress would provide the best memory performance, whereas a weak stimulus that induces a low level of stress and low hormonal response would result in poor memory performance (Sandi et al. 1997). In case of stronger emotion and increased hormonal response, the consolidation of memory will be facilitated, until a point of too strong stress that has been shown to be deleterious for memory. Another example comes from the two-way shuttle avoidance paradigm, in which poor performance is observed following both negligible and high doses of corticosterone (Kademian et al. 2005).

These data suggests an inverted U-shaped relation between stress and memory performance. However, rather than the generally reported curvilinear relation, Yerkes and Dodson (1908) first proposed that the level of stress might interact with the difficulty of the task to shape the degree of task achievement. More precisely, in a simple task, the relation between stress and performance is linear, such that the stronger the stress is, the better the performance will be, whereas in a more difficult task, an intermediate level of stress will provide the best response, indeed following the curvilinear relationship between stress and memory performance (Diamond et al. 2007).

Controllability

Another parameter which can modulate the effect of stress on behavior is the level of controllability that one has over the stressful situation, i.e., the possibility to cope with and/or act on it. Depending on their temporal pattern, stressors have been shown to be able to impair hippocampal plasticity (Diamond and Rose 1994; Garcia 2001), whereas controllability over a stressor has been shown to abolish this effect (Shors et al. 1989, 1990), indicating that among the components of the stressor, the psychological factor is a potent modulator of the stress influence on synaptic plasticity. Electrophysiological data revealed that controllable vs. uncontrollable stress had differential effects on LTP in hippocampal sub-regions and in the amygdala (Kavushansky et al. 2006). It was shown that an uncontrollable stressful event resulted in a greater CA1 LTP reduction than did the controllable stressful event (water maze). The pattern of CA1 LTP reactivity to stress was correlated with the effects of stress on plasma corticosterone levels: The more stressful the experience was in terms of plasma corticosterone elevation, the stronger the inhibition was of CA1 plasticity. However, in the DG, and unlike in CA1, the controllable stress groups showed a pattern of neuronal activity and plasticity similar to that of the uncontrollable stress group. In the BLA, uncontrollable stress enhanced baseline activity. Kavushansky et al. (2006) proposed that uncontrollable stress might enhance amygdala activity, which can then differentially modulate neuronal plasticity in CA1 and DG.

13.3.2 Timing of the Emotional/Stressful Event

As previously mentioned, numerous studies address the effects of emotion and stress on learning and memory abilities, mainly by observing the impairment, but occasionally also enhancement, of behavioral responses or electrophysiological responses such as LTP. It has been shown, for example, an adverse experience such as exposure to a predator before training impairs the acquisition of spatial memory (Sandi et al. 2005; Diamond et al. 2006). On the other hand, it is well known that stressful stimuli (such as foot shock) presented during aversive conditioning are easily associated with the conditioning stimuli and contribute to the buildup of strong memories. Moreover, life events associated with emotion and high arousal states are generally better remembered than neutral ones.

These inconsistencies may be explained by the timing in which the stressful event is encountered in relation to the learning task. Indeed, studies showing deficits in memory performance after stress exposure have typically involved a 30–60 min interval between the exposure to stress and the training. In fact, some studies show an improvement in the task performance when the stressful event was presented very shortly before the training (a few minutes). In this case, emotion and stress facilitated learning and memory and improved memory performance (reviewed in Diamond et al. 2006; Joels and Krugers 2007).

Electrophysiological data also show that the effect of stress on induction of LTP in the hippocampus depends on the timing between the stressful event and the electrical stimulation. Stress or amygdala activation 30 s before perforant path stimulation enhances LTP in the DG (Akirav and Richter-Levin 1999a, b, 2002). The opposite effect is obtained when stress or amygdala activation is applied 1 h before LTP induction (Akirav and Richter-Levin 1999a, b, 2002; Richter-Levin and Akirav 2003). It is proposed that a stressful event presented concomitantly with novel information or training could facilitate storage of information, whereas information presented at a later time period may not be collected with the same efficiency.

13.3.3 Differential Effects of Stress and Amygdala Activation on Different Regions of the Hippocampus

Studies have shown that CA1 and the DG of the hippocampus can display different susceptibility to stress (Gerges et al. 2001; Maroun and Richter-Levin 2003; Vouimba et al. 2004; Vouimba and Richter-Levin 2005; Tsoory et al. 2008). For instance, acute stress, resulting from exposure to elevated platform, disrupted LTP in CA1 but not in the DG (Maroun and Richter-Levin 2003; Vouimba et al. 2004). Furthermore, discriminatory avoidance learning, which evokes a substantial stress response, selectively inhibited LTP in CA1, while enhancing LTP in the DG (Izaki and Arita 1996). Such dissociations in hippocampal sub-regions may be relevant to

the understanding of the differential effect of stress on hippocampal-dependent learning and memory.

Among the mechanisms that may underlie these observations, it has was shown that BLA activation differentially affects LTP in the CA1 and in the DG (Vouimba and Richter-Levin 2005). Indeed, BLA activation enhanced cell excitability in the DG but not in CA1, and it impaired LTP in CA1 but enhanced it in the DG, when BLA stimulation was applied 30 s before or after the standard theta-like high-frequency stimulation. With a strong theta-like stimulation, amygdala stimulation had no effect in CA1 but still enhanced LTP in the DG. In CA1, depending on the intensity of stimulation used to induce LTP, the molecular mechanisms involved could be either NMDA receptor-dependent or independent. These two mechanisms seem to have different sensitivity thresholds to amygdala activation. Altogether, this suggests that under stress conditions, information processing by the DG could be favored over its processing by CA1, and the differential contribution of CA1 and DG in hippocampal network functioning could be modulated by the BLA during stress-related learning and memory. This idea is also supported by the finding that exposure to stress activated the BLA and that its lesion or its inactivation suppressed the stress effect on CA1 LTP (Akirav et al. 2001; Kim et al. 2001).

13.4 Summary

Evidence from both animals and humans support the claim that emotional arousal can enhance memory consolidation. The amygdala, and especially the BLA, is a key structure in processing and interpreting events of emotional significance and in turn modulating activity in related areas of the brain. The *emotional tagging* hypothesis claims that when an emotional event occurs, the amygdala "tags" it as important, presumably by strengthening synapses located on neurons that have just been activated in other brain regions.

Synaptic tagging and *emotional tagging* are not conflicting, but rather, complementary concepts that operate at different levels. The psychological concept of *emotional tagging* describes the process at the level of the organism; however, it is constructed upon the physiological processes of synaptic tagging, LTP, and affective reinforcement. Therefore, it is likely that both concepts rely on the same mechanisms operating at the cellular level.

The relationship between an emotionally arousing event and the strength of memory consolidation is changeable and relies on such factors as the intensity of the emotional event, one's ability to cope with it, and the timing of the event. Taking these complexities into consideration may advance our understanding of the neural mechanisms behind *emotional tagging* and provide insight into the neurobiology of affective disorders such as depression and post traumatic stress syndrome.

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Chapter 14
The Behavioral Tagging Hypothesis and Its Implications for Long-Term Memory Formation

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Abstract Memories are experience-dependent internal representations of the world that can last from short periods of time to a whole life. The formation of long-term memories relies on several biochemical changes, which inducing modifications in the synaptic efficiency change the way the neurons communicate each other. Interestingly, the formation of a lasting memory does not entirely depend on learning itself; different events occurring before or after a particular experience can affect its processing, impairing, improving, or even inducing lasting memories. The overlapping of neuronal networks involved in the processing of different types of

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learning might explain why different experiences interact at neuronal level. However, how and where this does really happen is an issue of study.

In 1997, the Synaptic Tagging and Capture (STC) hypothesis provided a strong framework to explain how synaptic specificity can be achieved when inducing long-lasting changes in electrophysiological models of functional plasticity. Ten years later, an analogous argument was used in learning and memory models to postulate the Behavioral Tagging hypothesis. This framework provided solid explanation of how weak events, only capable of inducing transient forms of memories, can result in lasting memories when occurring in the context of other behaviorally relevant experiences. The hypothesis postulates that the formation of lasting memories rely on at least two parallel processes: the setting of a learning tag that determines which memory could be stored and were; and the synthesis of plasticity-related proteins, which once captured at tagged sites will allow the consolidation of a memory for long periods of time. Therefore a weak learning, only able to induce transient forms of memories but also capable of setting a learning tag, could be benefited from the proteins synthesized by a different strong event, processed in the same areas, by using them to consolidate its own lasting memory.

In this chapter we will detail the postulates and predictions of the Behavioral Tagging hypothesis, deepen the mechanisms involved in the setting of the tag and the synthesis of proteins, and revise the universe of experiments performed from rodents to humans in order to discuss its implications on learning and memory processing.

Keywords Behavioral tagging • Synaptic tagging • Learning tag • Weak learning • Short-term memory • Long-term memory • Memory promotion • Memory competence

14.1 Introduction

Memory formation is the process that enables the retention of information about the world, acquired during learning (Lynch 2004). This cognitive function is responsible for remembering events, facts, situations, places, objects, and motor skills (Kandel et al. 2000). All this information constitutes the *acquis* of learning and memories of an individual, defining who he/she/it is and also offering a plethora of behaviors according to the circumstances that had been learned in past experiences. However, not all the information that we acquire is stored for long-term periods. The notion that multiple forms of memory exist was expressed by James (1890) and others, who distinguished between primary and secondary memory, currently called short- (STM) and long-term memory (LTM) (Nadel and Hardt 2011).

A typical feature of memory is that learning does not instantaneously induce a LTM trace; instead it takes time to be fixed. This centennial observation was reported by Müller and Pilzecker through the proposal of the perseveration-consolidation hypothesis of memory (Müller and Pilzecker 1900; Lechner et al. 1999).

They performed list-learning experiments in humans and found that memory of newly learned information was disrupted by the learning of other information shortly after the original one. These results suggested that processes underlying new memories initially rest in a fragile state and consolidate over time (McGaugh 2000; Dudai 2004). Consequently, memory is vulnerable for a certain period of time after learning, enabling endogenous processes activated by an experience, or even by another one, to modulate its strength (McGaugh 2000). In this regard, behavioral, hormonal, and neural influences acting during this fragile period can regulate memory consolidation, improving or impairing it. Thus, stress, arousal, motivation and reward can profoundly affect memory formation (McGaugh 2004; Wittmann et al. 2005; Adcock et al. 2006; Schwabe et al. 2008; Roozendaal and McGaugh 2011). This chapter will describe in detail the action of a novel experience over the formation of an independent LTM, making emphasis in the mechanism involved in this process.

Over the past decades, many molecular and cellular mechanisms underlying the formation and stabilization of LTM were well characterized (McGaugh 1966, 2000; Dudai and Eisenberg 2004). We now understand in considerable detail the molecular machinery involved in the process of "cellular or synaptic consolidation" occurring within the first hours after the encoding of information (Dudai 2004). One surprising finding, is the remarkable degree of conservation of memory mechanisms in different brain regions within a species and across species widely separated by evolution (Mayford et al. 2012). Among the shared components of this molecular machinery, the activation of synaptic neurotransmitter receptors, protein kinases, transcription factors, and gene transcription process was found to be necessary in all of them (Izquierdo et al. 2006; Romano et al. 2006; Wang et al. 2006; Won and Silva 2008; Johansen et al. 2011). In particular, the finding that protein synthesis inhibitors did not prevent the learning and the expression of STM supports the view that protein synthesis is required only for consolidation of LTM (Agranoff and Klinger 1964; Agranoff et al. 1966; Davis and Squire 1984; Montarolo et al. 1986; Quevedo et al. 2004).

This introduction led us to conceptualize that memories are experience-dependent internal representations of the world (Dudai 1989), built by biochemical changes taking place over an extended period of time after learning. However, how these representations are codified, where do they take place in our brain and, if the biochemical changes associated with LTM formation have selective functions, will be discussed below.

It is expected of memories to be encoded in spatiotemporal states of neuronal circuits. It is widely accepted that neural activity induced by learning triggers changes in the strength of synaptic connections within the brain. The Synaptic Plasticity and Memory (SPM) hypothesis states that an activity-dependent plastic change is induced at the appropriate synapses during memory formation. The plastic changes must occur in those brain areas where memory is being processed and are both necessary and sufficient for the storage of the information (Martin and Morris 2002). The most relevant aspect of a memory trace is that those changes in behavior, occurring as a consequence of a learning experience, persist in time. In this way, a model of synaptic plasticity where brief stimulations of a neural pathway induce long-lasting changes in the synapses was postulated as one plausible

clue of the mechanisms underlying the formation of lasting memories. These changes in the synaptic efficacy involve either up- or down-regulation of synaptic strength and, in the case of persisting for more than 1 h they are referred as long-term potentiation (LTP) or depression (LTD) (Bear and Malenka 1994).

Then, where does the synaptic plasticity related to LTM formation occur? Different kinds of learning are processed by different brain areas (Milner et al. 1998), resulting the substrate of the memories distributed along different and/or overlapped regions of the brain depending on their nature (Procedural, Emotional, Spatial, Declarative, etc.). Therefore, rather than being processed and stored at single neuron levels, particular memories may be thought to be distributed across multiple neurons and synapses in networks that could involve more than one particular brain area. Then, how can the synaptic specificity related to a particular memory be achieved? How can the neuronal machinery assures the delivery of proteins to those sites were plasticity should be held? Using models of synaptic plasticity, Frey and Morris (1997) postulated the hypothesis of STC and were able to explain how the system could obtain the input specificity in functional plasticity processes. The STC hypothesis declares that LTP involves the local tagging of synapses at the moment of induction. Then, those tags can capture plasticity-related proteins (PRPs) synthesized in the soma or local dendritic domains, allowing the stabilization of the potentiation for long periods of time.

The STC hypothesis opened a new approach to think about the process of LTM formation, letting us to propose that learning could signal the sites related to memory plasticity, where PRPs will be captured in order to allow its consolidation. In this context, the signaling of the site where the information will be stored seems equally as important as the synthesis of the PRPs used during consolidation which allow the formation of a lasting memory. In this frame, we display a remaining question: could these processes be dissected? Are PRPs always synthesized as a consequence of learning? Do different biochemical changes induced by learning have different function? Are some of them specifically related to establish a mark and others to trigger the synthesis of PRPs? These topics will be discussed along with this chapter.

14.2 Behavioral Tagging as a Model to Explain Long-Term Memory Formation

Thinking in tagging and capture mechanisms, it could be postulated that a particular learning leads to the activation of some particular sets of synapses in the network, which could also establish a mark ("learning tag") capable of determining the place where the PRPs should be used and for what should be used. But, how could this hypothesis be tested? In order to evidence the involvement of these two processes (tag and synthesis of PRPs) taking part in the mechanism of LTM formation, it was necessary to dissect them. To achieve this, a combination of different experiences was used, and a weak training in a task that was only able to set a learning tag was associated to a second behavioral experience that was also capable to induce the synthesis of PRPs. In this way, it has been shown that a protein synthesis independent STM

induced by a weak training can be consolidated into a LTM, if animals experience a strong event in a critical time window around the weak training. This process that depends on protein synthesis induced by the strong associated experience was originally named behavioral tagging (BT) (Moncada and Viola 2007) and it was suggested that the weak training sets a learning tag where the PRPs provided by the strong event would be captured in order to establish a persistent mnemonic trace (Fig. 14.1).

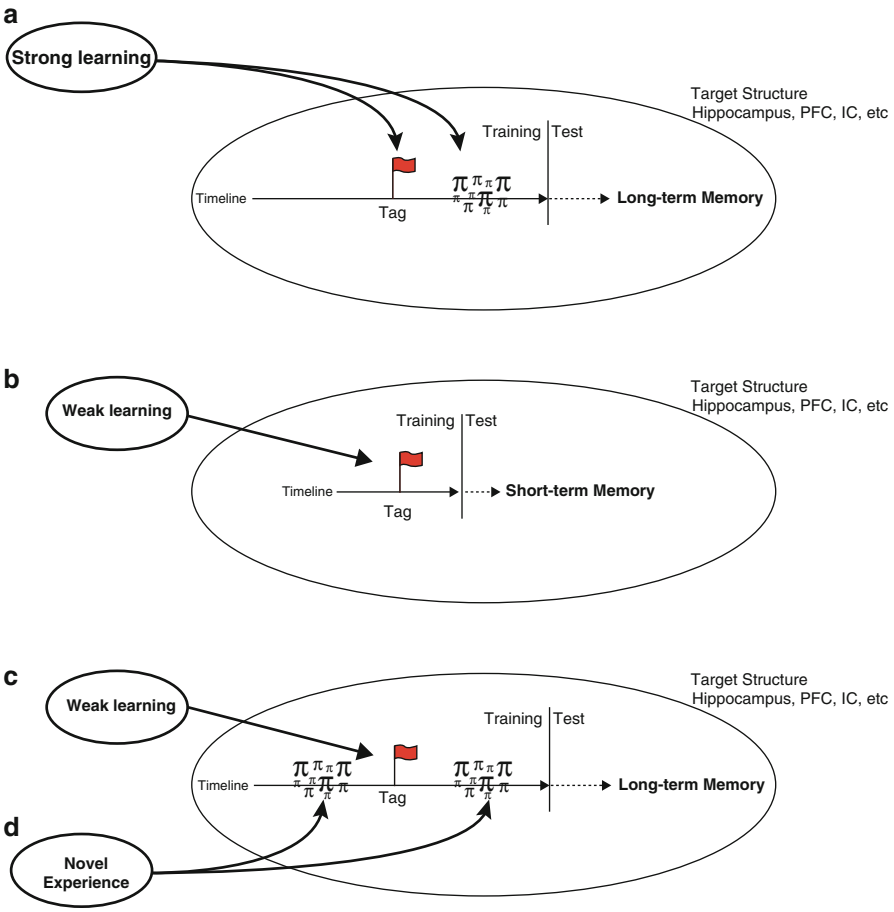

Fig. 14.1 Behavioral tagging in LTM-formation. The figure illustrates how a learning can result in LTM through a BT process and how can this process be studied by dissecting the setting of the learning tag from the synthesis of PRPs. (**a**) A strong learning experience triggers the setting of a learning tag and the synthesis of PRPs at those brain structures were learning is being processed and memory is aimed to be stored. The capture of the newly synthesized PRP by the learning tag allows the consolidation of the mnemonic trace for long periods of time. (**b**) A weak learning experience induces the setting of a learning tag but does not induces the synthesis of PRPs, triggering the formation of short forms of memories. (**c**) A weak training experience capable of setting a learning tag in association with a second strong task (such as novel experience), capable of inducing the synthesis of PRPs in same brain structures where learning tags were set, can result in the consolidation of a LTM for the weak learning experience. The process exhibits symmetry and PRPs can be captured either if they are synthesized before or after the setting of the tag

This tagging and capture hypothesis and its dynamics provide an elegant theoretical framework capable to explain why the duration of memory is not only dependent on events occurring at the moment of their encoding, but also on other events occurring previously or subsequently to learning. It also provides a wide framework to explain memory promotions, reinforcements or impairments due to interventions occurring during its consolidation phase.

The principal idea underlying BT process is that PRPs are used to originate long-lasting changes when captured by specific learning tags. Following, we list a series of requirements and processes necessary to operate BT mechanisms in LTM formation:

- The setting of tags that are able to capture PRPs in order to establish LTM. (Tags indicate the inputs activated by a given stimulus, setting local specificity, and determining the information to be stored.)
- The synthesis of proteins that are required to consolidate the mnemonic trace. These PRPs can be provided by the same learning experience that sets the learning tag (if it is strong enough) or by an independent strong event associated to it (The importance relies in the interaction of PRPs and tags rather than in the sources providing PRPs.)
- Both, tag and PRPs have a transient duration.
- In order to capture the products, tags and PRPs should be present at the same neural substrate and at the same time.
- The process exhibits symmetry and PRPs can be captured either if they are synthesized before or after the setting of the tag.

Therefore, if BT process underlies LTM formation, then a series of predictions arises:

- BT process should be evident across a diversity of learning and memory paradigms.
- BT process requires setting of tags and availability of PRPs. Thus, blocking one or both of these processes will induce LTM amnesia.
- If tags do not coincide (temporally or spatially) with the PRPs, LTM will not be formed.
- Tags set by different tasks and located in a common population of neurons, could compete for capturing available PRPs. Under limited amount of PRPs the competition will be evidenced by the expression of the prevailing LTM.
- In contrast, sufficient amount of PRPs could induce a more robust and/or persistent LTM trace.

These predictions were tested in different learning and memory tasks and activities performed in rodents and human beings. The results obtained are enumerated in the following sections. Moreover, the BT hypothesis comprised a wide theoretical framework that led us to explain many other questions about memory processing. So, other predictions derived from this hypothesis deserve investigation and some of them will be mentioned in the concluding remarks section.

14.2.1 Looking for a BT Process Across the Universe of Memory Types and Tasks

As it has been previously mentioned, to identify a BT mechanism acting in LTM formation, the processes of learning tag setting and synthesis of PRPs should be dissected in order to interfere and analyze them independently. The first research using this strategy was performed by associating two different rodent hippocampus-dependent behavioral tasks: the Inhibitory Avoidance (IA) and the exploration to a novel Open field (OF).

The exploration to a novel OF is a spatial behavioral task that even after a relatively brief training of 5 min is able to induce a LTM of habituation to the arena. This environmental novelty is also linked to the activation of the adrenergic and dopaminergic systems and to increase activated levels of the transcription factor CREB, which specifically occurs as a result of the detection of spatial novelty in this task (Viola et al. 2000; Winograd and Viola 2004). Indeed prolonged exposures to the arena leading to a familiarization process and the subsequent lack of novelty were associated to a decrease in pCREB and PKMζ levels (Moncada and Viola 2006, 2008). Moreover, the exploration to a novel arena is able to reinforce early-LTP into late forms of plasticity (Li et al. 2003; Straube et al. 2003b; Davis et al. 2004), pointing directly to the possibility of using this behavioral task as a possible PRP donor for other hippocampus-dependent behavioral tasks.

The IA is a versatile short single trial task, in which animals placed in a box with a platform on the left end of a series of metal bars that constitute the floor of the box, learn that stepping down from this platform results in a foot-shock. When the animals remember this experience, being faced again to the platform results in an increase of the time to step down (latency). This latency increase in the test session is considered as an indicator of memory formation, being a longer latency indicative of a better memory (Izquierdo et al. 2006). However, what makes this task advantageous for studying LTM formation and particularly the BT process is that the IA training triggers all the processes that can lead to memory formation after brief and defined learning session of approximately 10 s. In contrast, in multi-trial learning tasks the acquisition, retrieval, and relearning processes occur simultaneously along the successive trials performed after the first training session. A second, but a very important property of IA task relies in the fact that the strength of the training can be easily regulated simply adjusting the intensity and/or the duration of the foot-shock.

In the first approach to look for a BT process, we trained rats in the IA under weak conditions (wIA) with the intention to induce the setting of a learning tag. This training is able to induce a short transient memory of approximately 30 min, but it does not induce a protein synthesis-dependent lasting memory. As animals learned, we reasoned that IA-learning tags should have been set and therefore foreign PRPs could be captured in order to establish its own LTM. To explore this possibility we associated this wIA training with the exploration to a novel OF that should provide the required PRPs. Therefore, different group of animals were

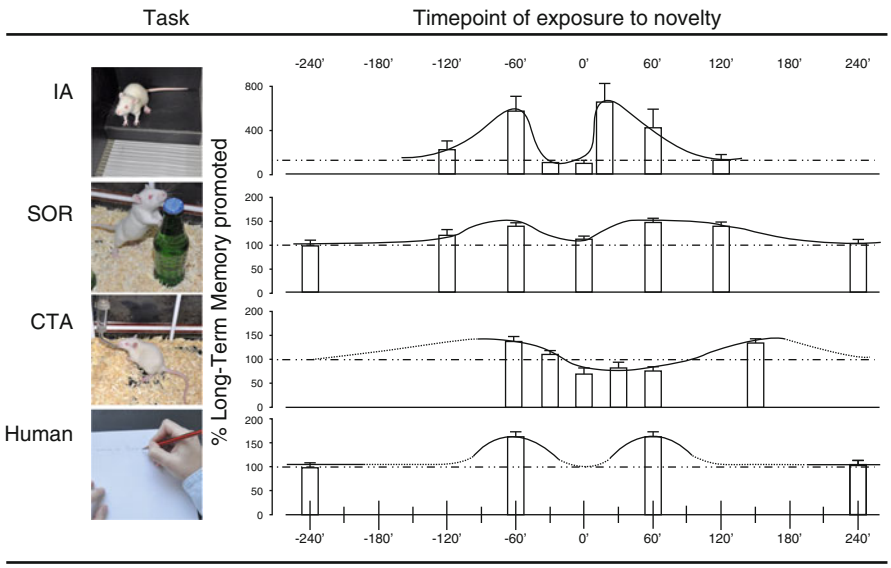

Fig. 14.2 Time course of novelty LTM promotion for different learning task. The figure shows the time point in which a novel experience is capable to promote the formation of LTM for Inhibitory Avoidance (IA), Spatial object recognition (SOR), and Conditioning taste aversion (CTA) tasks in rats and improve literary memory in school children. Long-term memory is expressed as percentage in relation to control groups that did not experience the novel event (represented by the 100 % *dashed line*). The *bars* represent experimental data and the *curves* represent the expected time course of the promoting/improving effect for the untested time points (preliminary data support the valley between promoting time point in human)

exposed to a novel OF for 5 min at several times before or after this weak training. While those animals that were only submitted to wIA were unable to form a lasting memory 24 h later, different groups that also explored the OF showed a consistent IA-LTM. This promoting effect triggered by the environmental exploration occurred in a restricted time window of approximately 1 h around the wIA training but excluding the 30 min before to immediately after (Moncada and Viola 2007) (Fig. 14.2). The symmetry, manifested by the promotion of IA-LTM when the OF was explored before or after training, put into manifest that the promoting effect of the exploratory experience on IA memory was not due to alterations in the conditions of IA-acquisition neither to sensitization or facilitation processes.

An interesting aspect of the promoting effect relies on the importance of the novel nature of the arena. We have observed that unlike the exploration to a novel arena, the exploration to a familiar OF, which had already been seen for 30 min in the previous day, is unable to promote IA-LTM (Moncada and Viola 2007). Similar results were observed studying the behavioral reinforcement of LTP, where exposure to a novel but not a familiar OF was able to reinforce early- into late-LTP (Li et al. 2003; Straube et al. 2003b).

A BT process implies that a learning tag is able to use PRPs in order to allow the consolidation of a memory. Therefore, we analyzed whether this memory promoting mechanism was dependent on the synthesis of PRPs triggered by the OF. To do this, we infused the protein synthesis inhibitor anisomycin into the CA1 region of the dorsal hippocampus, immediately after the exploration to the arena. This inhibition completely impaired the promoting effect of the novelty on IA-LTM formation, independently whether it was performed over an OF explored before or after the wIA training (Moncada and Viola 2007). In consequence, memory promotion and protein synthesis dependency are symmetric processes. To further analyze this issue it was reasoned that if the OF provides the PRPs required by a wIA training to consolidate a lasting mnemonic trace, then it should be also capable to rescue the amnesia caused by protein synthesis inhibition close to a strong (s) IA training, which typically induces a lasting IA memory. Testing this hypothesis it was observed that while anisomycin infusion (into the CA1 region of the hippocampus, 10 min before an sIA) induced amnesia 24 h later, the exploration to a novel OF 1 h before this training prevented the amnestic effect. Moreover, taking into consideration that further infusion of anisomycin after the OF session also resulted in IA-LTM amnesia, it was concluded that the novelty preventive effect is especially dependent on its capacity to provide the PRPs required to consolidate the IA memory (Moncada and Viola 2007).

Once observed that the BT process exists in the IA learning, one of the main objectives was to identify if we were facing to a general mechanism of LTM formation. Several experiments have been performed in this respect by different labs across the world using different learning tasks and studying different faces of learning. Following with the avoidance learning, Lu and collaborators (2011) showed the first evidence of a BT process in mice using a step through variation of this task. And during the next year, Dong and coworkers (2012) presented evidence that not only spatial novelty, but also the exploration of novel but not familiar objects into an arena was able to promote this avoidance memory in rats.

Moreover, BT process was also observed acting in the formation of a different aversive lasting memory using the contextual fear-conditioning (CFC) paradigm. In this hippocampus-dependent task rodents are placed into a box with metallic bars, and after a brief phase of habituation to the environment a consecutive series of foot shocks is applied during a certain period of time. Therefore, there is nothing here that the animal can do in order to avoid punishment, the shock is associated to the fact of simply being in a particular environment and leads to the formation of a usually called fear-driven memory that can be evaluated by comparing the amount of freezing during the habituation period and the test session (Kim and Fanselow 1992; Phillips and LeDoux 1992, 1994, 1995; Gould and Wehner 1999). An increase in freezing behavior is taken as an indicative of memory formation. In this task, the application of a shock that usually induces only short forms of memories, let us to observe that CFC-STM can be reinforced into a CFC-LTM by associating the learning experience with the exploration to a novel OF. Again, this promoting effect was dependent on PRP synthesis induced by the novel experience. So both, operant and classic conditioning lead to the formation of LTMs through a tagging and capture

processes (Ballarini et al. 2009). Further experiments in the CFC task, were performed to study memory extinction processes. de Carvalho Myskiw and colleagues (2013) demonstrated that exploration of a novel arena, within a critical time window around the extinction session, is able to promote the long-term extinction of the CFC memory. This extinction process is dependent on gene transcription and PRPs synthesized by the novel experience. The authors propose that extinction session is able to set a tag capable to use OF synthesized PRPs in order to induce long-term extinction. As memory extinction is indeed considered the construction of a new association and therefore a new memory that overcomes the expression of original mnemonic trace (Konorski 1967; Pearce and Hall 1980; Bouton 2004), these results show other face of the BT process acting in LTM formation.

Aversive memories, as those studied above, are important to remember things that should not be done or places that should not be visited because they might imply certain danger; however these are not the only important things to learn. Spatial memories also play a central role in our daily life, because they code an internal spatial representation of the world guiding the navigation and the pathway to find or avoid particular places or particular things. In this sense, the hippocampal region that includes the CA fields, dentate gyrus, and subicular complex is part of a system of anatomically related structures in the medial temporal lobe, which is important for mammalian memory (Broadbent et al. 2004; Buzsaki and Moser 2013). Within this lobe, the hippocampus itself is especially important for relating or combining information from multiple sources, as is required in certain spatial memory tasks (O'Keef and Nadel 1978).

The first evidence of a BT process acting in the formation of spatial LTMs came from experiments performed in the spatial version of the object recognition task (SOR). In this paradigm, which could be considered as the rodent version of a what/where memory task, the animals should recognize a change in the relative position of two objects (Dix and Aggleton 1999; Mumby et al. 2002). The task consists of letting animals to investigate an arena with two identical objects for a certain period. Then, in a further test session, one of the objects is changed from its original position and the animals are allowed to explore again. As rodents display an innate tendency to explore novel situations, an increase in the exploration time of the object placed in the novel position is considered as an indicator of memory. In this kind of learning, we observed that a weak training that only induces STM could result in a lasting memory when it was associated to the exploration of a novel arena. The OF session was able to promote SOR-LTM formation by providing newly synthesized PRPs. Similar to what was observed in the IA task, the promoting effect on SOR-LTM was dependent on the novel nature of the arena and restricted to a critical time window, which in this case extents from 1 before to 2 h after the wSOR, also excluding the exploration immediately after training (Ballarini et al. 2009) (Fig. 14.2). This extended time window puts into evidence that similar processes can have different dynamics. In accordance with these results, Cassini and colleagues (2013) have recently shown that this memory can be also promoted by a quite different source of PRPs. They observed that the protein synthesis-dependent reconsolidation process either of CFC or water-maze (WM) learning tasks can

promote SOR memory (1 but not 4 h before or after a wSOR training). This promotion was observed only when lasting reconsolidation (CFC or WM) or extinction (CFC), but not brief retrieval, sessions were associated to wSOR trainings, being the promoting effect also abolished by the infusion of anisomycin.

Further evidence of the BT process in the field of spatial memories was provided by Wang and collaborators (2010), who showed that an appetitive-driven spatial memory can be also promoted by effects of novelty. Rats trained in an event arena during several months learned to find a food reward hidden in sand-wells. After that, rats submitted to a weak-encoding session, consisting on finding one hidden pellet, remembered the proper location of the reward for 30 min but not 1 h. On the contrary, a three-pellet reward encoding session induced a 24 h lasting memory. Interestingly, animals that were subjected to a weak-encoding training could consolidate a LTM for this task if the training was associated to the exploration to a novel OF. In coincidence with our observations, this promotion was dependent on the novel nature of the arena and on the synthesis of new PRPs induced by it. These findings also support that, as well as in single trial learning experiences, encoding and storage of an everyday learning-like experience can lead to memory consolidation through a tagging and capture process.

Using a different experimental approach, Almaguer-Melian and coworkers (2012) have investigated if the WM memory could be recovered from the amnesia caused via a foot shock (FS) by submitting rats to explore a novel arena. In the WM learning task a rodent is placed into a small pool of water (with visual cues) that contains a hidden escape platform. When first released, the subject swims around the pool searching for an exit. A decrease in the time (latency) required to find the platform in the successive sessions is an indicator of memory. It was shown that four trials in the WM were sufficient for rats to learn finding the platform during training. On the other hand, the consolidation of the WM-LTM trace could be impaired by a FS session performed after training, without affecting WM-STM. In resemblance to the other results in hippocampus-dependent learning tasks, when animals were also submitted to an OF exploration 15 min before or after WM training, the memory was recovered in a protein synthesis-dependent way, overcoming the disruptive action of the FS on WM-LTM formation (Almaguer-Melian et al. 2012). In coincidence with previous observations this recovery effects are time dependent because, as the authors show, OF exposure 4 h after WM training could not prevent FS induced amnesia. It is worth to mention here that as WM could be recovered by the novelty induced PRPs, the FS did not interfere the setting neither the maintenance of the WM-learning tags. Therefore, a tempting explanation is that massive neuronal activation triggered by the strong FS depletes the system from the available PRPs, causing a long-term WM amnesia that can be reverted by providing extra proteins from an external source like novelty.

While we have shown the BT process acting in the formation of several qualitatively different LTM, all these memories have the particularity of being processed in the hippocampus. Therefore an essential question emerges: is the BT process acting in the formation of lasting memories processed in other brain structures? The answer to this question came from experiments performed in the conditioning taste

aversion (CTA), an appetitive learning task processed in the insular cortex (Berman and Dudai 2001; Merhav and Rosenblum 2008). Taste-recognition memory is also part of the essential spectrum of skills that many animals require to survive. Being able to remember whether a particular taste or flavor is associated with a malaise by intoxication or poisoning, results essential to grant survival to many animals. In that way, during the CTA task animals associate a specific flavor with a digestive disorder. During the training session animals with restricted access to water are let to consume either water or saccharine sweetened-water and, after 30 min, those animals that tasted the sweet water are intra-peritoneal injected with a LiCl solution. This substance causes an intensive digestive malaise and therefore, a decrease in the consumption of the flavored water during the test session is taken as an index of memory. Rats that receive a weak training in this task, by association of the consumption of saccharine with a low dose of LiCl, induced a negligible CTA-LTM, but expressed a strong CTA-STM 30 min after the acquisition session (Ballarini et al. 2009). In order to analyze a BT process in this memory, a PRP donor had to be found. As spatial novelty is not processed in the insular cortex, one could reason that tasting a new strong flavor (NaCl), instead of exploring a novel arena, could actually act as a novel insular dependent experience. Therefore, we combined a weak CTA training with the consumption of NaCl solution, observing that the novel taste induced a robust CTA-LTM when it was experienced 1 h before or 2.5 h after the weak CTA training, but not in between them (Fig. 14.2). In accordance to the observations performed in the IA, CFC, SOR, schemas, and WM memories, the promoting effect of novelty in CTA task also depends on both the synthesis of new PRPs induced by the consumption of NaCl and in the novel nature of this flavor, as animals familiar to this taste did not present any improvement in saccharine CTA-LTM (Ballarini et al. 2009).

All this evidence supporting that formation of lasting memories in rodents occur through BT mechanisms, leads to wonder whether human memories can be established through this process as well. A report that supports this assumption came from experiments performed with students of Argentinean elementary schools. By using a similar approach to those previously mentioned, we analyzed the memory for either literary or graphical activities when these were combined or not with novel and familiar experiences. The students' teachers performed both teaching and testing of each of the activities during a certain period of a regular lesson in their usual classroom. We observed that certain groups of students that also had the possibility to attend to a novel science lesson, presented important improvements in LTM for either of the activities, when the novelty was presented one, but not four, hour before or after the learning lesson. This effect was particularly strong on those components difficult to remember and, as well as reported previously in rodent models, it is a symmetrical effect restricted to a critical time window (Fig. 14.2) (Ballarini et al. 2013).

Similar memory improvements were also observed when the students attended a novel music lesson, but this improving effect was absent when the same lesson was familiar instead of novel. Another interesting property relies on the task's time specificity of this process. When students learnt about two different activities separated for

3 h, instead of merely one, and they attended to the novel science lesson 1 h after the second activity, they only presented memory improvements over aspects of the activity closer to the novelty (Ballarini et al. 2013). As a whole, these experiments show that a novel pedagogic experience, during regular school time schedule, can improve memory of different activities performed during class hours with the students' teacher. The fact that novelty improves memory when presented either before or after the activity, suggests that this effect is not due to an alteration of the learning performance triggered by the novel experience, stressing the idea that novelty effects might be acting through a behavioral tagging and capture mechanism.

Across this part of the chapter, we have shown how novelty and other learning activities are able to promote the formation of a plethora of memories, that otherwise would not exist, by simply providing them with those PRPs required to their consolidation. BT process has been demonstrated in aversive and non-aversive memories, classical and operant conditioning, contextual, spatial, taste-recognition, hippocampus and cortex-dependent memories, strongly suggesting that BT process is a general mechanism of LTM formation. Moreover, experiments performed in students, support the idea that BT might be acting in the formation of human memories as well, providing an interesting strategy to boost teaching activities by using novel pedagogic tasks to improve memory for those assignments of difficult learning.

14.3 Time-Related Requirements for Behavioral Tagging Processes

As it can be observed in the previous section, the effect of novelty on LTM formation is time-specific. In this section we will discuss the mechanisms involved in this phenomenon. Seminal works on synaptic plasticity led to postulate the STC hypothesis, describing that the tag is independent of protein synthesis and it has a limited duration (Frey and Morris 1997). Besides, PRPs must be close in time and space to those sites that had been or will be tagged. When these two processes are available, they can interact. Through this late associativity phenomenon, a stimulus that would only induce an early-LTP can effectively result in a late form of LTP (Reymann and Frey 2007). The tag and the PRP-dynamics, limit the time course of the STC process. Therefore, as both tag and PRPs have a transient duration, there are temporal constraints to the process. In the work by Frey and Morris (1998), the duration of the tag was shown to have a half-life of approximately 30 min, while the PRPs exhibited a half-life of 1 up to 2 h. Nevertheless, it should be noted that the duration of this coincidence window could be extended or reduced by other processes such as the regulatory mechanisms that accelerate or delay the turnover of synaptic tags and PRPs.

Likewise, for the BT process, these time-related features are fundamental for the formation of lasting memories from learning tasks that by themselves would only generate a STM. That is, there is a task of weak saliency that cannot induce protein synthesis and does not induce a long-lasting trace but is strong enough to set a

learning tag. This learning tag, considering the evidence from the experiments on BT done so far, has a half-life that goes from 30 min up to 2 h, depending on the type of task. The duration of this tag, in combination with the temporal availability of the PRPs triggered by the novel event of a strong saliency will determine the time curve of the BT phenomenon. Even though the learning tasks vary (and this could account for the time differences for the tagging process), the general outline of the time curve is similar to all of them (Fig. 14.2) (Moncada and Viola 2007; Ballarini et al. 2009, 2013).

In connection to the dynamics of the learning tag and the PRPs, one interesting characteristic of the BT experiments is that they also show a symmetrical temporal curve, similar to what has been demonstrated in STC experiments. The tag can be set before or after the PRPs' synthesis has been triggered, being these plasticity products capable of potentiating behavioral tags set in both senses (before or after the tag setting) (Moncada and Viola 2007; Ballarini et al. 2009, 2013; Cassini et al. 2013). However, if the events are separated by longer time lapses, the promoting effect is not observed. This could be explained in terms of one of them being outside the temporal coincidence window: at the time one of the requirements is available—behavioral tag or PRPs— the other has already decayed.

From Fig. 14.2 it can be concluded that the IA-learning tag seems to be rapidly established, because strong events experienced just 15 min after the wIA can induce promoting effects on IA-LTM formation. In the case of the CTA paradigm, it seems that it takes longer time for the tag to be set because the process requires the association between two stimuli which are distant in time (the ingestion of saccharin and the effect of the lithium chloride injection) and which may involve longer processing. This mechanism could determine a longer period for the establishment of the tag and also a longer period of sensitivity to its disruption. In agreement with this, there is no promotion observed in the time window between 0 and 2 h post-training.

Even though the event of a strong saliency induces protein synthesis, when it takes place at time points very close around the weak training, there is no LTM promotion for such weak training. The absence of a facilitator effect of novelty exposure in proximity to the training could be attributable to the interference or the resetting of the learning tag. Consistent with this assumption, it has been demonstrated that a short theta frequency stimulation, that resembles neural activity observed in rats exploring a novel environment, when given close to the induction of LTP, can negatively affect the setting of this tag (Sajikumar and Frey 2004; Young and Nguyen 2005; Young et al. 2006). In BT experiments, we observed that the exploration of a novel arena very close to the wIA training is not only incapable to promote IA-LTM, but it also impairs the promoting effect of a second novel OF session performed at a time where novelty usually promotes memory. These results suggest that this spatial novelty experienced too close to the IA training have negative effects on IA-learning tag and also showed that they are labile during a certain period of time after their setting (Moncada et al. 2008). On the other hand, in experiments combining the WM-spatial task with a foot-shock, it has been shown that stress from the foot-shock has a deleterious effect over LTM formation of the WM. In this case, the memory impairment can be overcome if subjects explore a

novel OF within a temporal window around the time of MWM training. Thus, these results suggest that the tag set by WM multitraining is unaffected and it can effectively capture the PRPs derived from the OF to establish a WM-LTM (Almaguer-Melian et al. 2012).

Another type of interaction that has been observed in BT experiments is that two different inputs that trigger PRPs' synthesis can be combined, as long as they share the same neural substrate and occur close in time, and a stronger memory can be induced (Martinez et al. 2012). For example, when a single wIA training is combined with two different novel OF explorations (each of them given at a time point that is effective to promote IA-LTM formation), a stronger IA-LTM is formed. Our hypothesis is that in this way the PRPs pool available to be captured by the tags is larger and allows the consolidation of a more robust memory, in comparison to the memory obtained from only one PRP-inducing stimulus. Indeed, anisomycin infusion after one of the OF sessions reverses the enhancement trigger by the double exploration, observing a memory level comparable to those of animals that only explored one OF (Moncada et al. 2008).

Experiments discussed so far are mainly focused on the memory formation phase. Recent research by de Carvalho Myskiw and colleagues (2013) has demonstrated the existence of a learning extinction tag. Briefly, authors postulate that extinction learning in a contextual fear task generates a behavioral tag that can capture the PRPs derived from a spatial learning. In contrast to other learning tags as well as synaptic tags, the setting of this extinction learning tags seems to be dependent on the activation of the translational machinery.

To summarize, for the BT process, it is fundamental that learning tags and PRPs interact within a limited temporal window determined by their rise and decay dynamics. They also need to coincide in space (the same neural substrate).

14.4 Specific Novelties Are Required to Promote Different Memory Traces

So far, conforming to the BT hypothesis, we have established that a learning experience can trigger the setting of a tag that together with the induction of PRPs synthesis in the same neural substrate will result in storage of the acquired information for long periods of time. This means that the BT process requires the integration of at least two different processes at common neural substrates within a period of few hours. This does not seem to be a problem when a particular learning induces both of them, but when weak task is associated to a strong PRPs donor experience, the process triggered by both of them should be integrated in the same or overlapped network. Based on the fact that a taste recognition induces the activation of the insular cortex, and that a spatial learning of the hippocampus, we tested if it is possible to promote the formation of CTA-LTM (a insular cortex-dependent task) using the exposure to a novel environment; and reciprocally, if a novel taste can promote the consolidation of a SOR-LTM (hippocampus-dependent task). Using the

analogue protocols described before, rodents exposed to a novel OF 1 h before or 2.5 h after a weak CTA training (times in which CTA-LTM was promoted by using a novel taste of NaCl 0.1 % for 10 min), did not present CTA-LTM. Similarly, when rats drank a novel taste in time points in which a novel OF exploration induced SOR-LTM, no promotion was observed. Therefore, neither the hippocampus-dependent task was able to promote an insular cortex-dependent memory, nor an insular dependent task was able to promote a hippocampus-dependent one, putting into evidence that spatial coexistence of tags and PRPs must occur in order to allow the consolidation of a lasting memory (Ballarini et al. 2009).

BT processes are supposed to exhibit input specificity. In other words, PRPs are supposed to be captured only by the tagged sites reinforcing only these and not all the available inputs of the network. In order to evaluate such a proposal in the formation of lasting memories, different group of rats were submitted or not to a novel arena, 1 h later weakly trained in the SOR task using two identical objects (pair 1) and after 3 h more trained again in the same condition but using a different pair of identical objects (pair 2). At the next day, half of the animals were tested for SOR-LTM changing the position of one object of the pair 1 and the other half changing the position of one object of the pair 2. Confirming that weak trainings are unable to induce a lasting memory, those animals not exposed to the novel arena did not present SOR-LTM for any pair of objects. On the other hand, of those animals exposed to the novel experience, SOR-LTM was specifically promoted for the location of the objects in pair 1, which was explored in a permissive promoting time point in comparison with the pair 2 explored beyond the usual window of efficacy. These findings indicate that BT displays input specificity, allowing LTM formation for the first learning, which set the learning tags during a permissive time in which novelty promotes spatial memories (Ballarini et al. 2009). Moreover, similar results were observed in school children, where a novel science lesson was able to improve the memory for elements of one of two different stories told by their teachers (Ballarini et al. 2013).

14.5 Mechanisms Involved in Learning Tag Setting and PRP Synthesis

The whole idea of the BT hypothesis relies in a mechanism composed of two complementary processes: the setting of a learning tag and the synthesis of those PRPs that once captured by the tag will allow the consolidation of a lasting memory. The existence of these processes requires an associated cellular mechanism capable of sustaining them. Therefore, either composed by same or different actors, a machinery of receptors, second messengers and structural proteins should be responsible of setting the learning tag and triggering the synthesis of PRPs. Taken into account that any learning leading to LTM formation should initiate both processes simultaneously, dissecting them and their machineries might represent a challenge. However the BT hypothesis provides a solution to perform such research. According to the

theory itself, the behavioral tag comprises the set of local processes and changes required to determine the information to be stored in case of obtaining the PRPs required for memory consolidation. Therefore, providing PRPs through an external event should prevent any amnesia caused by interfering with the synthesis of PRPs, in which the tag remains intact. On the other hand, as the tag is essential to determine the information to be stored as well as the substrate to do it, any intervention capable of disrupting the tag should result in an irreparable amnesia (Fig. 14.3).

Having these premises into consideration different experimental protocols can be designed in order to evaluate the role of receptors and kinases in setting the tag and/or triggering the synthesis of PRPs. For example, the combination of a weak training, capable of setting a learning tag, with a strong event capable of synthesizing PRPs, allow the identification of those processes related to the synthesis of proteins by applying drugs in the context of the strong event, as those substances that impair it shall result in amnesia. However, providing PRPs from a different source should prevent this amnesia. On the other hand, the administration of drugs in the context of the weak training will result in amnesia by interfering with the tag, as proteins provided by the strong task would still be available (Fig. 14.3). Other alternative to study these mechanism is to use a strong learning task able to induce a lasting memory and therefore to set the tags and trigger the synthesis of PRPs. In this case the infusion of drugs interfering with either one or the other process shall result in amnesia. However, if the animals are submitted to a second strong event capable of providing PRPs, such as the exploration to a novel arena, those amnesias resulted from interfering with the synthesis of PRPs should be reversed, while those others caused by interferences with tag should remain (Fig. 14.3).

But which candidates could be evaluated using these experimental designs. As it has been shown, novelty promotes memory of several learning task by providing them those PRPs required for their consolidation (Li et al. 2003; Straube et al. 2003a; Moncada and Viola 2007; Ballarini et al. 2009; Wang et al. 2010; Almaguer-Melian et al. 2012; Dong et al. 2012; de Carvalho Myskiw et al. 2013). In addition, an important body of evidence links the detection of novelty with the activation of the ventral tegmental area (VTA) and the locus coeruleus (LC), which in turn release respectively dopamine and adrenaline, to several brain structures including the hippocampus (Sara et al. 1994; Vankov et al. 1995; Kitchigina et al. 1997; Lisman and Grace 2005; Sara 2009). In turn, dopaminergic and adrenergic receptors trigger different second messenger cascade that can result in gene transcription and eventual translation process. Therefore it sounded reasonable to think that novelty could be inducing the synthesis of PRPs through the activation of these kinds of receptors. To analyze this issue, either D1/D5 dopaminergic (SCH23390) or β-adrenergic (propranolol) receptor antagonist were locally infused in the dorsal hippocampus 10 min before exposing rats to a novel OF. Sixty minutes latter rats were submitted to a wIA training and then, analyzing IA memory 24 h latter, it was observed that both SCH23390 and propranolol completely blocked the IA-LTM promotion induced by the OF exploration (Moncada et al. 2011). This dopaminergic dependency of the novelty promoting effect has also been reported before in different series of experiments that combined behavioral tasks with electrophysiological

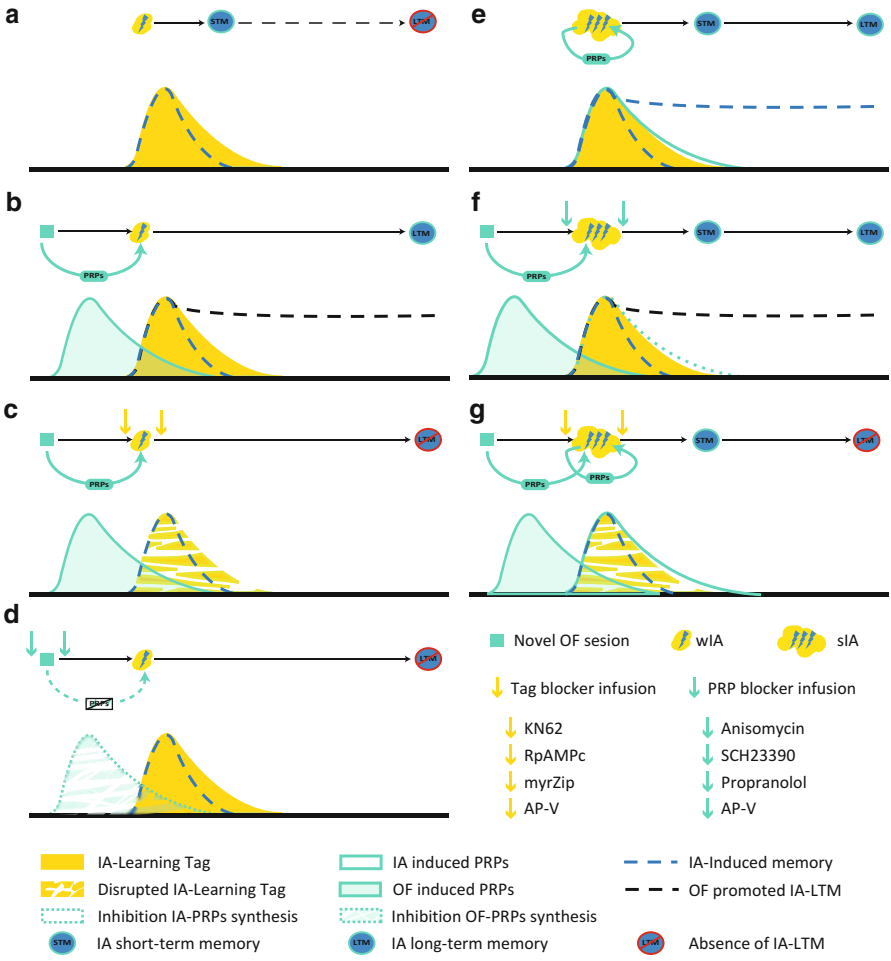

Fig. 14.3 Study of the BT process in LTM formation using the inhibitory avoidance (IA) task. Figure illustrates different strategies that can be used to study a behavioral tagging process and analyze those mechanisms associated to the setting of the learning tag or to the synthesis of PRPs. In all cases the curves represent a qualitative time course for the different processes, taking as model BT experiments performed using the IA task. (**a**–**d**): Experiments using wIA training protocol. (**a**) wIA training induces short but not long-term memory and sets an IA-learning tag. (**b**) The exploration to a novel OF induces the synthesis of PRPs that can be used by IA-learning tag to allow IA-LTM consolidation. (**c**) The infusion of different drugs (i.e. CAMKII**α** and PKA inhibitors, PKMz blocker or NMDA-receptor antagonist) in the hippocampus close to the wIA training can interfere with the proper setting and/or maintenance of the IA-learning tag impairing the promotion of IA-LTM. (**d**) The infusion of different drugs (i.e. protein synthesis inhibitors or D1/D5-dopaminergic, **β**-adrenergic and NMDA receptors antagonists) in the hippocampus close to the OF exploration can interfere with the PRPs synthesis triggered by it, resulting in an impairment to promote the IA-LTM. (**e**–**g**) Experiments using sIA training protocol. (**e**) Training sets learning tags and triggers the synthesis of PRPs resulting in the induction of an IA-LTM. (**f**) Amnesia induced by the infusion of drugs interfering with the synthesis of PRPs and upstream processes (i.e. protein synthesis inhibitors or D1/D5-dopaminergic and **β**-adrenergic receptors antagonists) can be prevented by the association of the sIA training with a strong experience (such as exploration to a novel OF) capable of providing the PRPs required for memory consolidation. (**g**) Novelty is unable to prevent amnesias caused by the infusion of drugs capable of interfering with the setting or maintenance (i.e. NMDA-receptor antagonist) of the IA-learning tags

stimulations. Both Li and colleagues (2003) and Straube and collaborators (2003a) observed that novelty induced LTP reinforcement depends on D1/D5 dopaminergic receptors functionality. Also, it has been shown the involvement of these receptors during the novel experience that allow, through a BT process, the promotion of the schemas memory and the prevention of stress-induced WM amnesia (Wang et al. 2010; Almaguer-Melian et al. 2012).

Further evidence supporting that these receptors might be responsible of triggering the synthesis of PRPs came from experiments where the novel experience was replaced by direct administration of dopaminergic (SKF 38393) and adrenergic (dobutamine) agonists. It has been shown that IA-LTM can be promoted by intraperitoneal administration of these drugs 70 min before a wIA training. These effects depend on the induction of PRPs synthesis in the hippocampus because the local infusion of anisomycin, either in CA1 or DG, prevents the IA-LTM promotion induced by dobutamine or SKF 38393, respectively. Finally, given that the injection of either of these drugs fail to promote IA memory when performed 180 min before wIA training, the promoting effects time scale of the agonists is remarkably consistent with that observed when novelty is used as memory promoter (Moncada et al. 2011).

The previous series of experiments encourage one to think that novelty indeed promotes memory through a mechanism that activating D1/D5 dopaminergic and β-adrenergic receptors of the hippocampus (and probably other brain structures) triggers the synthesis of those PRPs required for memory consolidation. But a certain memory, for example IA memory should require a particular set of proteins to be consolidated, and therefore it sounds reasonable to think that a strong IA training able to induce a lasting memory by itself could also use the mechanisms triggered by novelty to promote the IA-LTM. This issue was analyzed by studying the role of D1/D5-dopaminergic and β-adrenergic receptors during sIA induced LTM. While animals infused with vehicle solution in the dorsal hippocampus 10 min before an sIA training presented a conspicuous STM and LTM, those groups of rats infused with either SCH23390 or propranolol were completely amnestic 24 h after training but remaining intact their STM. Interestingly, this amnesia was prevented when animals were let to explore a novel OF 60 min before the sIA training (Fig. 14.3). Moreover, the infusion of anisomycin after this novel experience impaired such preventive effect (Moncada et al. 2011). The existence of this protein dependent preventive effect puts into evidence, that the activation of D1/D5-dopaminergic and β-adrenergic receptors in the hippocampus during sIA training, is specifically involved in the regulation of the synthesis of those PRPs required for the consolidation of this memory.

In contrast to the actions attributed to the studied catecholaminergic receptors, NMDA glutamate receptors have been shown to be required to establish both STM and LTM after an sIA training. Moreover, the amnesia induced by the infusion the NMDA-receptor antagonist AP-V, in the dorsal hippocampus previously to an sIA training, could not be prevented by pre-exposing the animals to a novel arena (Moncada et al. 2011). Pointing in the same direction, antagonizing the dorsal hippocampus-NMDA receptors previously to a wIA training impaired the

promotion of IA-LTM by the exposure to a novel arena. As in both cases, PRPs synthesized by action of novelty are available to be used by IA-learning tags to allow memory consolidation, thus, the failure of novelty in preventing the amnestic effect of AP-V and its impossibility to promote IA-LTM, show that NMDA receptors activity is essential for the setting of the IA-learning tags. Similar results were obtained by Cassini and colleagues (2013) who have observed that blocking NMDA receptors previously to a wSOR training impairs the promoting effect that a CFC reconsolidation event, able to provide the PRPs, has over the SOR memory. Beyond the role of NMDA receptors in the setting of the learning tag, there is an extensive body of evidence showing how they can trigger signal transduction processes leading to the translation of proteins that can be required to establish lasting memories (Cammarota et al. 2000; Izquierdo et al. 2006; Gao et al. 2009; Im et al. 2009). Also BT experiments in which the local infusion of AP-V previously to a novelty session, experienced 60 min before a wIA training, impaired the usual promotion of a lasting IA memory, certainly support a role for this receptor in PRPs synthesis process as well as in the setting of the learning tag (Moncada et al. 2011).

The current vision considers the tag as an ensemble of molecules tending to modify the morphology of the dendrite (Frey and Frey 2008; Ramachandran and Frey 2009; Redondo and Morris 2011). We believe that NMDA receptors are one of the first echelons in the tagging machinery. This raises the enigma about the other components involved in establishing the learning tag. In that sense, protein kinases result interesting targets due to their fast activation and to their speed in modifying the response of receptors and structural morphology. Among these, αCAMKII, PKA, and ERK 1/2 are suitable candidates because of their well-established involvement in the formation of LTMs (McGaugh 2000; Izquierdo et al. 2006). Their specific role in LTM formation was studied by infusing their inhibitors close to a wIA training with the expectation to block the IA-learning tags. Disrupting the tag should result in the impairment of the capacity of an associated novel experience to promote IA-LTM by providing PRPs. Our experiments showed that local administration of KN-62 (αCAMKII inhibitor) and Rp-AMPc (PKA inhibitor) impaired the novelty induced IA-LTM when infused (in CA1 region of the hippocampus) between 10 min before and 15 min after wIA training, but not 1 h latter (Moncada et al. 2011). A third kinase, PKMζ, resulted to be partially necessary in the very initial moments of the tag setting but showed to be required even 1 h after wIA training (Moncada et al. 2008). This result suggests that αCAMKII, PKA, and PKMζ play an essential role in the setting of the learning tag close around training, being only the PKMζ necessary to maintain a late phase of the tag (Fig. 14.3). Therefore, this kinase that has been shown to be required for late maintenance of memory and functional plasticity processes (Pastalkova et al. 2006) could be also required to maintain early plastic changes as well. In the same direction, recent findings from Li and colleagues (2012) show that metaplastic changes induced by previous synaptic pre-activation (either through Ryanodine or metabotropic Glutamate receptors activation) extend the usual life time of electrophysiological established synaptic tags through a mechanism dependent on PKMζ activity, allowing a prolongation of the permissive association times between tag setting stimulus and PRP providing

stimulus to allow a tagging and capture process. However, the role of PKMζ in long-term memory and plasticity processes is currently in the center of a debate due to possible unspecificities of its blocker Myr-zip (Volk et al. 2013). In contrast, neither U0126 (MEK inhibitor) nor anisomycin were able to impair the promoting effect of novelty when applied close to a wIA training, showing that the setting of the IA-learning tag uses already synthesized proteins and do not require the activity of ERKs 1/2 (Moncada et al. 2011). Further information of the tagging machinery came from experiments performed with TrkB knock-in mice. Lu and collaborators (2011) have demonstrated that inhibition of this receptor's kinase activity, during weak training in the IA, also impaired the promotion process induced by novelty. The same results were obtained in analogue in vitro experiments where TrkB inhibition during weak tetanization also blocked the reinforcing effect of a strong tetanization on LTP, leading to the postulation of this receptor as potential component of the behavioral and synaptic tag (Lu et al. 2011). Interestingly, while the setting of IA-learning tag as well as LTP- and LTD-tags are protein synthesis independent processes, recent experiments show that the tag setting during CFC extinction learning might depend on it (de Carvalho Myskiw et al. 2013).

In general, all the components of the BT tagging machinery are consistent with those identified in the electrophysiological model of synaptic tagging. Functional plasticity experiments show that the LTP-tags require the activity of αCAMKII, PKA and PKMζ and that is also a protein synthesis independent mechanism (Sajikumar et al. 2005, 2007; Redondo et al. 2010). On the contrary, ERK1/2 have been shown to be specifically required for the setting of synaptic tags associated to LTD (Navakkode et al. 2005; Sajikumar et al. 2007). Interestingly, using an active avoidance task Whitlock and collaborators (2006) showed that this learning is processed through mechanism associated to LTP induction. Consistently our results show that IA tags require αCAMKII and PKA but not ERK to be properly set. Their activity is required during a certain time period within which learning tag could be affected. Indeed, as it is depicted in Sect. 14.3, behavioral interventions can also impair the setting or can reset the learning tag. In that way, recent findings show that OF exploration promotes avoidance memory through a LTD-like process (Dong et al. 2012). This kind of processing of the exploration to the OF can explain why this novel experience interferes with the setting of the probably LTP-like IA tags, when its experienced too close to the wIA training. Supporting this view it has been recently shown that LTP induction is able to reinforce IA memory in those times close to wIA training were OF exploration interferes with the setting of the IA tags (Moncada and Frey 2011).

14.6 Memory Competence: Another Aspect of BT Process

Learning tags can capture PRPs derived from novel experiences but, what would happen if the number of tags is larger than the available PRP supply? Evidence from LTP experiments shows that under regimes of limited protein synthesis two potentiated pathways can compete for protein resources needed for the establishment of a

late-LTP (Fonseca et al. 2004). Furthermore, in a smaller scale, cellular level studies suggest that stimulated synapses would compete for limiting PRPs synthesized at the dendrite compartment for the establishment of LTP (Govindarajan et al. 2011). All these characteristics gave rise to the following question: would there be a similar mechanism at a behavioral level?

In the model of BT, one experience can promote the memory of an unrelated event, as long as the two events occur in a limited time window and they are processed in overlapping structures of the brain. Another requisite to observe this promoting effect is that one of the events triggers the synthesis of PRPs that will enable the consolidation of the trace. In this framework, the findings on BT suggest that both traces are sharing a common pool of PRPs. One possibility that could take place in such conditions is that learning tags set by different learning experiences compete for the common pool of PRPs.

Thus, if different learning are being consolidated into LTM, intracellular competition for PRPs will define which of the memory traces becomes stabilized in the neuronal network recruited by the learning tasks. This hypothesis has been put forward through different approaches such as computational models and electrophysiological experiments. Notably, sharing, crosstalk and capture are important mechanisms for the consolidation of plastic changes, at least at a cellular level. In consequence, a similar phenomenon could be taking place after the acquisition of new information.

Based in the protocols of the first BT experiments (Moncada and Viola 2007), this has been put to test by combining wIA and novel OF, two tasks that are dependent on hippocampus processing. The results show that under regimes of reduced protein resources, but not when resources are available in larger amounts, wIA learning task can impair the LTM formation of the OF because of their common requirement of PRPs. If rats are sequentially exposed to two different memory tasks under limited protein synthesis, LTM for one of them is formed in detriment of the formation of the other. In such scenario, the levels of PRPs may be insufficient to satisfy the LTM requirements of the two behavioral tasks because, for instance, the weak IA training that does not induce PRPs' synthesis uses the resources derived from the OF (Martinez et al. 2012).

Another question derived from these results is: which are the PRPs that are involved in this process? Activity-regulated cytoskeletal-associated protein (Arc) has been shown to be involved in the formation of several types of memories and has an important role in synaptic plasticity (Tzingounis and Nicoll 2006; Bramham et al. 2010; Wibrand et al. 2012). In particular, limiting the amount of available Arc (through the use of Arc mRNA antisense oligonucleotides delivered into the dorsal hippocampus) induced after a novel OF exploration session was shown to have deleterious effects in novelty promoted IA-LTM formation, suggesting that this is one of the PRPs necessary for the consolidation of both types of memory and for which learning tags competed for (Martinez et al. 2012). Latest research on Arc's role suggests that this protein is captured by CaMKIIβ, which induces an "inverse synaptic tagging process", recruiting Arc in the less active terminals. Arc, in turn, down-regulates the amount of GluA1 at individual synapses, therefore operates as a

specific sensor mediating the inactive synapse-specific control of AMPA-R clearance at weaker synapses in potentiated neurons, depending on the local history of both activity and inactivity (Okuno et al. 2012). Even though, Arc is an attractive candidate as a PRP that could be disputed between memory traces, other PRPs related to plastic changes in synaptic terminals should be considered as well (Barco et al. 2008).

Thus, competition between two memory traces for their consolidation can take place if protein resources are limited. This gives rise to an interesting observation: memory interference could be explained in terms of competition for protein resources between different learning tags, being Arc one of the PRPs required for the consolidation of both memory traces.

Centenary observations postulate that retrograde interference (RI), a phenomenon that alters LTM formation when an interpolated material (such as a different behavioral experience) is presented after learning, increases with the proximity of these events. Memory traces become less vulnerable to empirical forgetting, brain damage or retroactive interference as they consolidate with the passage of time (Ribot 1881; Jost 1897; Müller and Pilzecker 1900). Skaggs (1925) suggested that the interpolated task causing RI could be a similar material to be encoded, being the RI effect reduced when tasks are highly similar or, on the contrary, when they are markedly different. This could be reinterpreted considering the BT hypothesis involving capture of PRPs by different kinds of learning tags. If the interpolated material is identical to the original, it can represent a retraining and reinforce almost the same learning tags set for the original task. In such case, there would be no different kinds of learning tags capturing the PRPs. In contrast, a high dissimilarity of the material could imply its processing in different brain regions; thus, the respective learning tags would not interfere because they do not show spatial overlapping (Ballarini et al. 2009; Redondo and Morris 2011).

The BT model proposes a cellular mechanism to explain amnesia by retrograde and also anterograde interference, focusing on the competitive capture of proteins required for the consolidation of those memory traces. The work performed on this topic (Martinez et al. 2012) provided the first evidence of molecular events underlying memory competition that could explain how some information is stored while other is lost or impaired.

14.7 Concluding Remarks

In this chapter we have focused on the synaptic consolidation theory, commenting on factors that could affect this process blocking or improving the LTM formation. In particular, we discussed about the effect of a novel experience occurring close to a weak training session and how it could promote LTM formation for this late-associated learning. The effect was explained using the BT hypothesis, which postulates that PRPs are used to originate LTM when they are captured by specific learning tags. These PRPs could be provided by the same event that sets the tag (strong learning) or by an independent one, within a proper time-space association.

We have also enumerated the requirements to operate a BT mechanism in LTM formation: the setting of a transient tag, the utilization of PRPs at these sites, place and time convergence of tag and PRPs regardless if they are synthesized before or after the setting of the tag. In the past 5 years, several research groups have worked on the BT process demonstrating that operant and Pavlovian aversive paradigms, spatial object recognition, and other tasks based on spatial and appetitive learning, as well as memory extinction allow the consolidation of lasting mnemonic traces through BT mechanism (Moncada and Viola 2007; Ballarini et al. 2009; Wang et al. 2010; Lu et al. 2011; Moncada et al. 2011; Dong et al. 2012; Cassini et al. 2013; de Carvalho Myskiw et al. 2013). Moreover, a similar phenomenon was observed also in children who learnt about a tail or a drawing in school hours, suggesting the generality of the process in the formation of LTM (Ballarini et al. 2013). In addition, some aspects about the nature of the learning tag and the identification of PRP involved in the process are beginning to be unveiled (Lu et al. 2011; Moncada et al. 2011; Martinez et al. 2012; de Carvalho Myskiw et al. 2013). However, further studies are needed to fully understand the mechanism and the molecules involved, and ultimately to visualize the PRPs capture process at synaptic level.

In this chapter we summarized the experiments and results supporting the predictions related to generality of the BT phenomenon in diverse memory paradigms as well as temporal and region specificity of BT process. We have also described the requirements for the setting of tag and the PRPs synthesis. Finally we have demonstrated the existence of competition for PRPs leading to memory interference as well as LTM improvements triggered by providing more PRPs through multiple strong events associated to a weak training. However, in our opinion some hypothetical predictions of the BT hypothesis still remain to be addressed:

- Do different learning tasks set different learning tags? In other words, does each learning experience set a characteristic mark? Are there any differences in the quality and/or quantity of learning tags between different experiences?
- Does a metaplastic phenomenon affecting the duration of learning tags exist in a similar way to the observations made in synaptic plasticity experiments (Li et al. 2012)?
- Are learning tags being set and PRPs captured effectively at synaptic level?
- If a learning task is able to set a larger number of tags across a wider network, will this lead to the formation of a stronger LTM?
- Is there any other event capable of inducing protein synthesis without disrupting the tag able to promote the formation of LTM? Most BT experiments were performed using a novel exploration to an open field as an event of strong saliency. In that sense, it was recently demonstrated that PRPs could be provided by a CFC reconsolidation process (Cassini et al. 2013). However, are there other events such as cognitive reinforces or motivational situations capable to facilitate memory formation through a BT mechanism?
- Beyond the role played by learning tags in LTM formation, are there other kinds of tags mediating different aspects of memory, like for example the persistence of the mnemonic trace?

– Moreover, could memory reactivation or retrieval induce a re-tagging of the activated inputs? Is this mechanism involved in the reconsolidation of a memory trace?
– Given the remarkable degree of conservation of memory mechanisms observed in different brain regions and across species widely separated by evolution, does BT also operate in invertebrates as well?

These and many other questions will be probably answered in the near future. By now, BT hypothesis represents a wide framework to study and analyze memory processes, offering a consistent structure able to explain promotions, modulations, and interferences in the formation of lasting memories.

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