Sreedharan Sajikumar
Ted Abel *Editors*

Synaptic Tagging and Capture

From Synapses to Behavior

Second Edition

Springer

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Editors
Sreedharan Sajikumar
Department of Physiology
and Healthy Longevity Translational
Research Programme
Yong Loo Lin School of Medicine
National University of Singapore
Singapore, Singapore

Ted Abel
Iowa Neuroscience Institute
University of Iowa
Iowa City, IA, USA

ISBN 978-3-031-54863-5 ISBN 978-3-031-54864-2 (eBook)
https://doi.org/10.1007/978-3-031-54864-2

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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Preface

In 2023, we celebrate the 25th anniversary of the discovery of Synaptic Tagging and Capture by Frey and Morris. Over this time, the field has broadened its scope, extending from cellular to behavioral levels. The initial release of the *Synaptic Tagging and Capture* book in 2015 achieved significant success, amassing over 12,000 downloads. The second edition features updated versions of previous chapters and introduces new ones, encompassing a wide range of research areas, including cellular, molecular, behavioral and system-level studies.

This book starts with the fundamentals in Chap. 1 by introducing the concept and basic principles of synaptic tagging and capture (STC) and then discusses functional and mechanistic aspects. The chapter by Prodan and Morris ends with a discussion of some outstanding questions which are taken up in greater detail in the other chapters of the book.

The next few chapters go in depth into the molecules and pathways involved in STC. In Chap. 2 by Koek et al., the role of NMDA and AMPA receptors in STC is described. This is followed in Chap. 3 by Hayashi et al., which presents data identifying the synaptic tag that in turn modifies the postsynaptic cytoskeletal structure to capture newly synthesized synaptic proteins, eventually resulting in memory persistence. The formation of long-lasting memories requires the de novo transcription of plasticity-related products (PRPs), and one of the PRPs is exemplified by Vesl-1S described in Chap. 5 by Okada and Inokuchi. They also put forth the hypothesis that the synaptic localization of PRPs is dependent on synaptic activity, suggesting that multiple cell biological activities underlie synaptic tagging, each of which is specific to a subset of PRPs and differently regulates synaptic localization and function of the PRPs at distinct times. Protein Kinase A (PKA), an important kinase for plasticity, is discussed in Chap. 6. This chapter describes how PKA modulates synapse-specific neuronal activity by coordinating signaling molecules and processes through PKA anchoring protein and the role of anchored PKA. Park also introduces new findings on PKA-dependent presynaptic mechanisms and in vivo signatures of STC. In Chap. 10, Korte introduces neurotrophins and their receptors as candidate molecules involved in the calibration and consolidation of memory, in particular BDNF and its TrkB receptor, as well as p75NTR with its various ligands.

With the former neurotrophin/receptor pair involved in mediating positive structural and functional plasticity and the latter involved in negative plasticity. These neurotrophins may also be of relevance to the age-related decline in memory function.

Chapters 4, 7, 8, 11 and 13 discuss the interactions between different neuronal inputs that enable memory formation and maintenance. Chapter 4 by Jones et al. links metaplasticity to STC. It reviews the evidence of the mechanisms of heterosynaptically and heterodendritically expressed metaplasticity and their potential roles in learning and memory. Chapter 7 by Alarcon is about the notion that STC processes can be compartment specific and that these compartments can work as the neuron's information integration units which determines whether incoming information will be associated or dismissed. This is a key strategy to organize multiple streams of neural activity and information to enhance the neuron's computing capability. Additionally, Fonseca discusses the evidence regarding the properties of synaptic cooperation and competition that contribute to the refinement of neural connections during development in Chap. 8. They also explain that similar rules apply during the induction and maintenance of synaptic plasticity which will allow us to further dissect the rules underlying associative learning. Chapter 11 by Maity and Connor reviews key intracellular signaling mechanisms that initiate lasting changes in the ability of synapses to undergo metaplasticity, priming future synaptic plasticity to enhance neuronal detection, encoding and association of salient future events which in turn facilitate the storage of detailed memories. The de novo transcription of PRPs involves the nucleus and its role in the synapse signaling pathway is presented in Chap. 13 by Farris and Dudek. They propose that the nucleus acts as a calculator of incoming signals from activated synapses. Additionally, 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.

Moving away from the basics, Ibrahim and Sreedharan explore emerging work studying STC beyond hippocampal area CA1, such as in the neighboring CA2 and lateral amygdala in Chap. 12. Exploring STC in different neuronal populations may reveal different plasticity rules which can in turn provide further insights into the STC model. This chapter also reviews behavioral tagging illuminating the relevance of STC in the reactivation and reconsolidation of memories. Most memories are only short-term and are forgotten with time. However, to be able to retain memories for extended periods, they have to be stabilized via cellular or initial memory consolidation. Takeuchi presents evidence in Chap. 14 that suggest the dopamine signaling via D1/D5 receptors in the hippocampus is crucial for the persistence of synaptic plasticity and memory, emphasizing the emerging role of the locus coeruleus (LC) in novelty-associated dopamine-dependent memory consolidation. Furthermore, this chapter discusses the ventral tegmental area (VTA)-hippocampal and LC-hipppocampal dopaminergic systems as well as their specialized mechanisms. With dopamine playing a crucial role in regulating functions ranging from motor control, mood, sleep, attention, rewards systems, reinforcing behavior to higher cognitive functions, Chap. 15 by Navakkode presents physiological and behavioral evidence that indicates dopamine-receptor signaling modulates

hippocampus-dependent synaptic plasticity and learning and memory. This chapter presents the dopaminergic neuromodulation required for the establishment of late-LTP (L-LTP) and STC in CA1 pyramidal neurons, as well as the mechanism by which dopamine neuromodulation induces the synthesis of PRPs. The discussions surrounding long-term synaptic plasticity and memory have been mainly about the roles played by neuronal elements. Chapter 16 by Li talks about the role of astrocytes, which are the most abundant glia cells in the brain, previously thought to only play a supportive role for neurons. However, evidence reveals that the coordinated action of neuron-glia networks results in synaptic plasticity and memory formation due to the ability of astrocytes to decode neural activity with elaborate Ca^{2+} dynamics. This in turn triggers the release of neuroactive molecules to drive synaptic plasticity and memory formation. Beyond the hippocampus, studies in the anterior cingulate cortex (ACC), a key cortical region for pain and emotion, have indicated that excitatory synapses are plastic, having the ability to undergo different forms of plasticity such as LTP, LTD and STC. In Chap. 17, Liu et al. review the current knowledge of ACC anatomy and the different forms of LTP and LTD as well as STC in this area, along with the role of ACC in pain and aging. Another region of the brain that is involved in modulating memory storage in other brain areas is the amygdala. Richter-Levin et al. in Chap. 18 introduce the concept of emotional tagging which states that the 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. Hence resulting in the formation of long-term memories. On the other hand, too intense of an emotional event can also result in the impairment of memory consolidation instead depending on a myriad of other factors. Elucidation of the mechanisms behind emotional tagging can provide insights into the neurobiology of affective disorders. With the synaptic plasticity changes that happen at the cellular level comes the behavioral changes that happen at the systems level. Extrapolating cellular theories such as LTP, LTD and STC to the in vivo model is achieved through the behavioral tagging model.

The next few chapters go into details about behavioral tagging. Chapter 19 by Moncada et al. details the postulates and predictions of the behavioral tagging hypothesis, deepens the mechanisms involved in the setting of the tag and the synthesis of proteins and revises the universe of experiments performed from rodents to humans to discuss its implications on learning and memory processing. Chapter 20 by Bae and Richardson examines whether behavioral tagging processes may also underlie the formation of long-term memories in infant animals, and whether these are the same processes that underlie behavioral tagging in the adult animal. As negative early life experiences have been suggested to form the foundation for later-life mental health function with many anxiety disorders having their onset during childhood or adolescence, insights gained can elucidate the mechanisms behind the disorders. Raghuraman and Hussaini discuss the implications of behavioral tagging on spatial memory in Chap. 21. Spatially tuned cells such as place and grid cells are involved in modulating spatial memory which is how the brain controls navigation. A spatial map is formed via the encoding of spatial memories by the

hippocampal-entorhinal system. It allows for representations of relations, in terms of the range and direction between locations and in discerning what exists where. This is made possible by PRPs orchestrated by spatially modulated cells. Jacob et al. in Chap. 22 provide a brief about cellular theories to understand the process of memory consolidation. This chapter also goes into detailed discussions on the studies on behavioral tagging and how they have established several molecular frameworks for a better understanding of fundamental brain functioning. Last but not least, Chap. 23 by Luboeinski and Tetzlaff reviews findings for the different computational studies of STC as well as presents their computational model of STC-based synaptic consolidation in recurrent networks of spiking neurons. Their previous studies suggest that STC can robustly implement cognitive memory functions such as memory improvement, selective consolidation, retroactive interference and priming of a particular memory. This hence provides a link between the physiological mechanisms of STC and the cognitive functions of long-term memory.

In this edition, we hope to provide readers with a comprehensive introduction to STC, and showcase how the field has grown and evolved over time. While preparing this second edition, it has been heartening to read from many leaders in this field about the latest research into the molecular details that underlie STC and synaptic mechanisms beyond the hippocampus, along with the employment of behavioral and computational tools for a more holistic understanding of STC. Many of these studies incorporated advanced modern techniques, theories and perspectives from adjacent disciplines, a trend that signifies the relevance and utility of STC research along the direction that modern neuroscience is taking. Nonetheless, there still exist many outstanding questions on STC that will continue to invigorate us. Firstly, the molecular identity of the synaptic tag has yet to be uncovered. Several candidates are identified, but the challenge will be to understand how they all work in concert to achieve the tag. Another challenge will be to delineate and integrate the role of other cell types beyond excitatory neurons into our current understanding of STC processes, which several teams have alluded to via their investigation into astrocytes and microglia. In particular, the question of whether inhibitory neurons exhibit or modulate STC mechanisms provides many exciting avenues of investigation. A third intriguing research direction lies in identifying and understanding the potential involvement of STC in memory processes post-consolidation. These questions, including the ramifications of STC on information storage and processing within the hippocampus and beyond at synaptic and behavioral levels, will continue to drive memory research as we uncover the inner workings of how the brain records, retains, recalls and modifies memory. As such, we invite readers to immerse themselves in the world of STC and join us in envisioning the road ahead.

Singapore, Singapore — Sreedharan Sajikumar
Iowa City, IA, USA — Ted Abel
23 January 2024

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About the Editors

Sreedharan Sajikumar (Saji) obtained his Ph.D. in 2005 from the Leibniz Institute for Neurobiology (LIN), Otto-von-Guericke University, Magdeburg, Germany. He conducted his Ph.D. research on the fundamental mechanisms of memory under the supervision of Professor Julietta U. Frey. During his postdoctoral training in Professor Martin Korte's laboratory at the Technical University, Braunschweig, Germany, he investigated the role of metaplasticity in 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 and memory. Since 2012, he has been a faculty member at the Department of Physiology and Research Director (since 2021) of the Healthy Longevity Translational Research Programme at the Yong Loo Lin School of Medicine, National University of Singapore. His research areas involve aging and neurodegeneration, synaptic tagging and capture (STC) as an elementary mechanism for storing long-term memory (LTM) in healthy and aging neural networks, and metaplasticity as a compensatory mechanism for improving memory in aging and neurodegenerative neural networks. He serves on the editorial boards of prestigious international journals such as *Neurobiology of Learning and Memory*, *Experimental Brain Research*, *Frontiers in Molecular Neuroscience*, and *Oxford Open Neuroscience*, among others. Since October 2017, he has been a faculty member in the Cognitive Neuroscience category on F1000. He is a recipient of the Singapore Neuroscience Association Young Investigator Award

(2017), Faculty Research Excellence Award (2017), and Graduate Mentor Award (2021) from the National University of Singapore. He was elected as a fellow of the Indian Academy of Science in 2023 and has been invited to deliver talks at prestigious organizations, including The Royal Society, London. His work has received consistent support from prestigious funding bodies, including the Alexander von Humboldt Foundation, Germany, Deutsche Forschungsgemeinschaft, Germany, the National Medical Research Council, Singapore, the Ministry of Education in Singapore, and the National Institute of Aging (NIA), NIH, USA.

Ted Abel, Ph.D., is the Director of the Iowa Neuroscience Institute, Roy J. Carver Chair in Neuroscience, and Chair of the Department of Neuroscience and Pharmacology in the University of Iowa Carver College of Medicine. A member of the National Academy of Medicine and a Fellow of the American Association for the Advancement of Science, his research focuses on the molecular mechanisms of memory storage and the molecular basis of neurodevelopmental and psychiatric disorders. He has been a pioneer in the use of molecular and genetic approaches to define how neural circuits mediate behavior. He co-directs the Hawkeye Intellectual and Developmental Disabilities Research Center, launched in 2021, which is focused on the diagnosis, prevention, treatment, and amelioration of intellectual and developmental disabilities.

Chapter 1
Synaptic Tagging and Capture: Functional Implications and Molecular Mechanisms

Alex Prodan and Richard G. M. Morris

Abstract This chapter introduces the concept of synaptic tagging and capture, which was first identified in electrophysiological studies in hippocampal brain slices. It outlines the basic principles of the concept and then moves on to discuss functional as well as mechanistic aspects. The former relates to behavioral and cognitive studies, showing that weakly encoded memories, which ordinarily decay rapidly and are, therefore, forgotten, may be retained for much longer if they occur around the time of strongly encoded memories associated with novelty or surprise. The mechanistic section outlines work seeking to identify the molecular basis of this apparently synergistic effect on memory, specifically the molecular mechanisms of synaptic tagging and the nature of plasticity-related proteins. It ends with a discussion of some outstanding questions, with many themes taken up in greater detail in other chapters of the book.

Keywords Synaptic Tagging · Synaptic Tagging and Capture (STC) · Memory · Hippocampus · LTP · Synaptic Plasticity

1.1 Introduction

The central principle of "synaptic tagging and capture" can be likened to two-factor authentication systems that we frequently use when shopping online. We place an order from our computer and provide information about how we wish to pay, likely

A. Prodan
Centre for Discovery Brain Sciences, Edinburgh Neuroscience, The University of Edinburgh, Edinburgh, UK

Wolfson Institute for Biomedical Research, University College London, London, UK

R. G. M. Morris (✉)
Centre for Discovery Brain Sciences, Edinburgh Neuroscience, The University of Edinburgh, Edinburgh, UK
e-mail: r.g.m.morris@ed.ac.uk

S. Sajikumar, T. Abel (eds.), *Synaptic Tagging and Capture*,
https://doi.org/10.1007/978-3-031-54864-2_1

using a credit card. Our bank then sends a message to a *different* device such as our mobile phone, providing a one-time password (OTP). Once we enter this six-digit number on our computer, our order is confirmed and successfully placed. The system of using two different devices provides greater security than the use of a single password. Think of this now from the perspective of the nervous system trying to organize the selective retention of some information but not others. The signal to "retain" may involve neuronal activation triggering RNA translation to provide the necessary plasticity-related proteins (PRPs) that mediate the stabilization of structural synaptic changes between neurons. However, the PRPs are likely distributed diffusely across even small segments of the dendritic compartments where they are synthesized. How is the selectivity of such stabilization to be secured? One mechanism that has evolved provides a second signal from a different source – akin to an OTP – which interacts with these diffusely distributed PRPs to anchor the exact location where the retention of information is to occur. The analogy is not exact, but we can think of the upregulation of available PRPs as "placing the order" and the existence of a synaptic tag as the "provision of an OTP." Synaptic tags are like OTPs only lasting a short time. More broadly, synaptic tagging and capture is a neuronal instantiation of a myriad of protein–protein and RNA–protein interactions that occur across different cell types, including numerous non-neuronal cells studied extensively across the life sciences (Alberts et al. 2017).

The phenomenon of synaptic tagging and capture (STC) was first proposed as an account of certain mammalian electrophysiological brain-slice experiments conducted in Magdeburg, Germany, and reported by Frey and Morris (1997). A protein synthesis inhibitor – anisomycin – was used to block PRP synthesis but, in the critical study of a series, was only applied when one pathway within the hippocampus was strongly activated to induce long-term potentiation (LTP) shortly after stimulation of another pathway; thus, the common pool of neurons had been activated beforehand in the absence of anisomycin. The key result was that *both* pathways showed long-lasting protein synthesis-dependent potentiation despite the second pathway being activated during the inhibition of protein synthesis. The suggestion was made that the diffuse availability of PRPs was triggered by the first activation but that both pathways created synaptic tags through a protein synthesis-independent process. These tags captured the diffusely traveling PRPs and thus both pathways showed synaptically localized long-lasting LTP. This study was followed by others, establishing the same result after strong and weak activation of independent pathways (Frey and Morris 1998a); the theoretical concept of STC was then developed (Frey and Morris 1998b). The hypothesis has developed over the years and now incorporates the notion that much RNA translation is local in dendritic domains rather than somatic (Holt and Schuman 2013).

There are intriguing *functional aspects* of STC – one theme of this chapter. As introduced here, one possibility is that it is a type of security system that ensures some pathways get to keep information but not others. While security might be beneficial, the analogy to two-factor authentication is potentially misleading as synaptic tagging differs in its underlying mechanism from a six-digit OTP; it is, of course, not a digital code. Nonetheless, the principle of two things coming together

within a temporal window for a specific outcome to happen is an economical concept that the nervous system appears to use to restrict action locally. Algorithmically, it is a logical AND gate with association across time in which two inputs are required to trigger an outcome. In this chapter, we consider other functional implications that are closer to the immediate needs of a memory system processing new information: what to keep and what to lose?

The *mechanistic aspects* of STC – the second theme – are diverse and include the synthesis of PRPs, the site(s) in an excitatory neuron where this may happen, the molecular nature of synaptic tags, and the types of interaction between PRPs and tags. STC is a multi-dimensional process whose study involves a variety of physiological, pharmacological, microscopy, and molecular biology methods – with all of these sometimes deployed in conjunction with behavioral work. As the study of LTP is central to STC, we make a note here on terminology. Post-translational (protein synthesis-independent) LTP is induced rapidly and typically short-lasting (3–6 h). It is often referred to as "early LTP" or LTP1. Protein synthesis-dependent LTP is induced more slowly and is longer-lasting, and often referred to as "late LTP," "L-LTP," or LTP2. We use here the latter terminology introduced by Graham Collingridge and now encapsulated in the second edition of "The Hippocampus Book" (Morris et al. 2024). This distinguishes post-translational, translational, and transcription-dependent components as LTP1, LTP2, and LTP3, respectively (Bliss et al. 2023). The scope of this book testifies to the diverse and fascinating avenues that have been explored to date by numerous laboratories, with our aim being to introduce only a subset of what has been explored, notably in several recent studies. For comprehensive reviews from the past few years, see Moncada et al. (2015), Nomoto and Inokuchi (2018), and Pinho et al. (2020).

1.2 Long-Term Potentiation and STC

Two cellular models of lasting synaptic plasticity have been identified, namely, LTP and long-term depression (LTD) (Hebb 1949; remastered in 2005). The structural and functional changes associated with synaptic plasticity may strengthen or weaken the connection between neurons in an activity-dependent manner (LTP and LTD, respectively). This has been predominantly studied in ex vivo hippocampal slices subjected to presynaptic electrical stimulation using both high- and low-frequency stimuli (Bliss and Lømo 1973; Bliss and Collingridge 1993; Andersen, 2008; Morris 2006); however, recent work has extended this to studies using patch recording and dendrite imaging (Scanziani and Häusser 2009; Häusser 2021). While LTP, as it is generally induced in physiological studies, may not occur in behaving animals (e.g., activation of a pathway at 100 Hz for 1 s), there are strong grounds for believing that the underlying plasticity mechanisms engaged by LTP play a role in memory (Martin et al. 2000). This link is supported by studies showing that properties such as associativity, cooperativity, and input specificity are implicated in both LTP and

memory formation (Sajikumar et al. 2008; Luscher and Malenka 2012; Hao et al. 2018).

The molecular cascade-mediating LTP can be divided into an early, transient, protein synthesis-independent phase (LTP1) and a late, more robust, protein synthesis-dependent phase (LTP2). While LTP1 can be artificially induced through a single train of high-frequency stimulation, one of the main synaptic changes that take place during LTP1 is the activation of N-Methyl-D-aspartate (NMDA) glutamate receptors, leading to an initially transient increase in the surface expression of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) glutamate receptors (Malenka and Bear 2004; Choquet and Hosy 2020). There are also concomitant presynaptic changes (Bliss et al. 2023). In contrast, LTP2 can involve neuromodulatory as well as glutaminergic inputs, and in addition to the functional changes associated with LTP1, structural changes such as the stabilization of synaptic strengthening is facilitated via upregulated de novo protein synthesis (Frey and Morris 1998b; Kandel 2012). LTP2 maintenance depends on processes which degrade and rebuild the actin cytoskeleton to allow for the structural expansion of dendritic spines as well as the stabilization of newly introduced AMPA receptors at the postsynaptic membrane (Korobova and Svitkina 2010; Bosch et al. 2014; Rudy 2015; Nakahata and Yasuda 2018). Previous studies investigating LTP-associated spine enlargement proposed the accumulation of F-actin and cofilin to be crucial for the maintenance of LTP2-induced structural changes (Bosch et al. 2014; Okamoto et al. 2009). The relevance of F-actin has also been highlighted through both electrophysiological and behavioral studies, which showed that the pharmacological disruption of F-actin hinders LTP maintenance and learning (Ramachandran and Frey 2009; Fonseca 2012). LTP3 induction, involving transcription, can be initiated by a CREB-regulated transcriptional activator (CRTC1), which translocates to the nucleus following calcium influx-induced neuronal depolarization (Bito et al. 2011; Ch'ng et al. 2015). Subsequently, CRTC1 binds to the cAMP response element-binding protein (CREB), which acts as a transcription factor and promotes the expression of a set of genes collectively referred to as immediately early genes (IEGs). IEG expression is essential for LTP3 induction (Kandel 2012).

Turning to data, when strong tetanic stimulation is used to induce synaptic potentiation, LTP2 occurs and can remain stable for many hours (Fig. 1.1a). This form of LTP is sensitive to the protein synthesis inhibitor anisomycin (Fig. 1.1b). When weak tetanic stimulation is used for LTP induction, only LTP1 occurs, which decays to baseline over a few hours (Fig. 1.1c). However, if strong tetanic induction precedes weak tetanization by, for example, 40 min, the weak pathway fails to decay to baseline and remains stable (individual experiment shown in Fig. 1.1d; group data in Fig. 1.1e). The consequence of this, 10 h after LTP induction, is that LTP may be stable and lasting or decay to baseline in the different conditions outlined in Fig. 1.1f. One technical point to note about these brain slice experiments is the care that needs to be taken to maintain the slices at a stable 32 °C, for the experiments to be of long duration (8 h or more), for there to be long incubation periods for the slices before the study starts, and for there to be two or three independent pathways. Shetty et al. (2015) outline the relevant issues in detail.

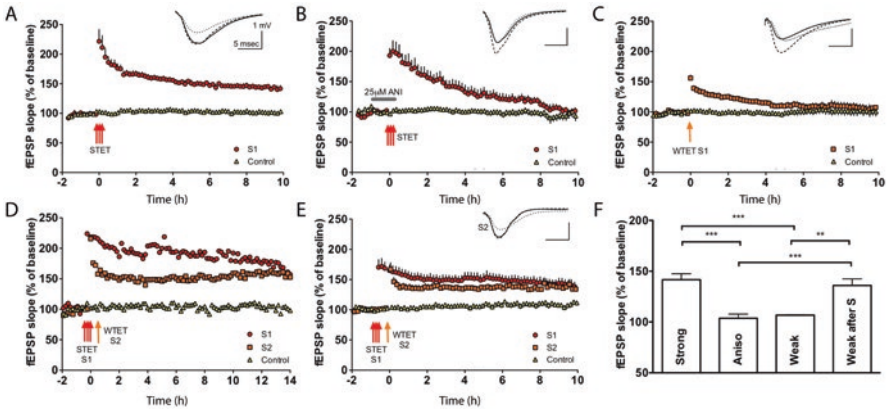

Fig. 1.1 Electrophysiological evidence of synaptic and tagging and capture. (**a**) Normalized data of 12-h study of long-term potentiation (LTP) in hippocampus brain slices maintained at 32 °C with a stable baseline for 2 h followed by tetanization with strong tetanus (100 Hz, 1 sec, repeated 3 times with 10 min intervals, red symbols). Approximately, 50% synaptic potentiation is observed, which is stable from approximately 3–4 h until the end of the experiment (10 h). (**b**) Equivalent strong tetanization but in the presence of the protein synthesis inhibitor anisomycin. The decay to baseline by 10 h establishes that the strong tetanization in panel A led to LTP2. (**c**) Weak tetanization (20 pulses in 4 theta bursts of 100 Hz stimulation for 200 ms) induces LTP at an initial level of 50% but this decays to baseline within 4–5 h. Weak tetanization only induces LTP1 in this case. (**d**) Strong tetanization in a single hippocampal brain slice of one pathway (multiple afferents) 40 min prior to weak tetanization of an independent pathway to a common pool of target neurons ("Strong-before-weak"). Note that the weak pathway is now stable for over 10-h post-tetanization. (**e**) Averaged data from multiple brain slices of the strong-before-weak protocol, plotted with standard errors of the mean. These data establish the conversion of LTP1 to LTP2 in the weakly tetanized pathway. **(f)** Averaged data at 10 h for panels **a**, **b**, **c**, and **e**, respectively (± SEM). (Data derived from Fig. 1.2 of Redondo et al. (2010) by permission)

Interestingly, synaptic plasticity is bidirectional, and LTD engages synaptic mechanisms that may be similar to those involved in forgetting-induced synaptic weakening (Tsumoto 1993; Citri and Malenka 2008). LTD is divided into two subtypes, one being dependent on NMDA receptors (Dudek and Bear 1995), while the other relying on the activation of metabotropic glutamate receptors (mGluR-LTD; Lüscher and Huber 2010). LTD induces the endocytosis of AMPA receptors from the postsynaptic terminal, alters the efficacy of the remaining receptors, and modulates presynaptic neurotransmitter release prior to protein synthesis-dependent structural changes. LTD can also be divided into an early and late phase, analogous to those observed during LTP (Sajikumar and Frey 2003; Sajikumar 2004).

While LTP and LTD involve structural alterations that take place within a homosynaptic environment, these physiological phenomena do not directly address memory associativity. Generally, different plasticity events are associated with different populations of synapses. However, under specific circumstances, different sets of synapses associated with distinct experiences can influence each other. The original STC hypothesis of Frey and Morris (1998b) offered a framework wherein memory

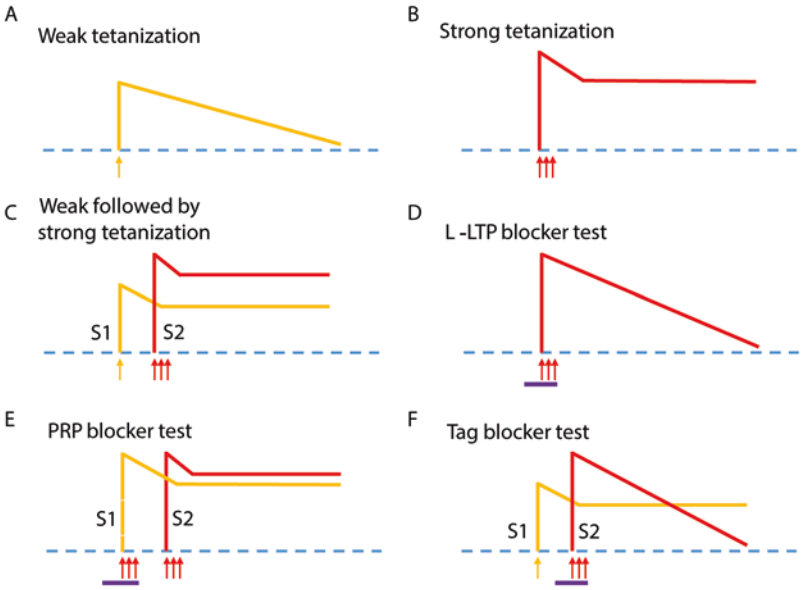

Fig. 1.2 Logic of searching for signal transduction pathways putatively involved in synaptic tagging or the synthesis of plasticity-related proteins. (**a**) Blue-dotted line represents the baseline EPSP. Weak tetanization (yellow) gives rise to a transient increase in EPSP. (**b**) Strong tetanization produces a persistent increase in EPSP. (**c**) Potentiation triggered by weak tetanization can be stabilized if followed by a strong tetanization. (**d**) If a drug blocks tag-setting, even strong tetanization results in LTP that decays to baseline. (**e**,**f**) Bottom row of panels shows two-pathway protocols that can be used to identify drugs that, given one tetanization but not the other, block PRP synthesis (**e**) or synaptic tag-blocking candidates (**f**). Number of arrows indicates the strength of the tetanization. The horizontal purple bars indicate the presence of an inhibitor

associativity over time could be addressed. Specifically, it is likely that STC is involved in a facet of memory consolidation called "cellular consolidation" (Dudai and Morris 2000; Dudai 2004). In vitro, ex vivo, and in vivo studies have suggested that the cellular consolidation process might act as a filter that separates daily, relevant, consolidation-worthy memories from irrelevant ones, the latter of which are forgotten (McNamara et al. 2014; Squire et al. 2015; Kempadoo et al. 2016; Takeuchi et al. 2016; Yamasaki and Takeuchi 2017; Duszkiewicz et al. 2019). Furthermore, the selection of relevant information is strongly dependent on the temporally proximal release of the neuromodulator dopamine. Such findings support a version of the STC hypothesis, suggesting that a potentiated synapse is considered tagged when it exhibits the molecular environment necessary to capture the available PRPs and utilizes them to stabilize structural synaptic changes in an input-dependent manner (Frey and Morris 1997). This idea was also discussed extensively in the first edition of this book.

The reliability and reversibility of manipulations intended to identify molecular candidates as synaptic tags or PRPs are essential. A common approach for assessing tag candidates is the experimental use of a weak followed by a strong tetanization

protocol to separate pathways. Critically, the putative plasticity protein of interest is inhibited during the strong tetanization but not during the weak. The conceptual importance of the "weak-before-strong" order is worth noting. Strong tetanization may alter parameters of cell activity, such as excitability, which could conceivably last long enough for the immediate effects of weak tetanization to have induction effects that would not be seen against a baseline background (Fig. 1.2a,b). The "strong-before-weak" ordering is still important of course, but "weak-before-strong" is arguably easier to interpret with respect to underlying mechanisms (Fig. 1.2c). A molecule's role in the different facets of STC can be inferred based on its ability to block LTP2 (Fig. 1.2d), but careful experimental design is required to distinguish a role in blocking the synthesis of PRPs or the setting of a synaptic tag. If only a transient change in synaptic efficacy on the strong pathway is observed, as in this case, the drug in question may be affecting either PRP synthesis or tagging. However, in a double tetanization study, if the drug is applied during weak tetanization and then washed out prior to strong tetanization of the other pathway, interest centers on whether LTP1 or LTP2 prevails. If only LTP2 is seen on both pathways, the drug likely affected PRP synthesis (Fig. 1.2e). However, if the drug is instead applied during the second strong tetanization, a different outcome may prevail if the drug is selectively affecting the synaptic tagging process (Fig. 1.2f). This example is particularly powerful because the strongly tetanized pathway paradoxically *fails* to show LTP2, whereas the weakly tetanized pathway *does*. In this and other protocols, careful experimental design helps to zero in on the putatively distinct molecular basis of synaptic tags and PRPs.

These observations further highlight the essential role of PRPs in synaptic plasticity and the capacity of potentiated synapses to capture the available resources necessary for plasticity stabilization. However, this study as well as most others were carried out using an ex vivo experimental framework, which is associated with reduced spontaneous activity, altered PRP baseline levels, and most often, severed dopaminergic afferents. Frey and colleagues initially demonstrated the modulatory effects of dopamine D_1/D_5 receptor activation for plasticity stabilization in pyramidal neurons from CA1 (Frey et al. 1991; Frey et al. 1993). Subsequent research showed that dopamine D_1/D_5 receptor agonists such as chloro-PB could promote the protein synthesis-dependent phase of synaptic plasticity in vivo, and weak stimulation of one pathway could be sufficient for achieving LTP2 when a neighboring synapse underwent D1/D5 receptor agonist-induced synaptic strengthening (Lemon and Manahan-Vaughan 2006; Navakkode et al. 2010). Furthermore, Wang and colleagues used the two-pathway experimental framework to show that STC-characteristic synaptic cooperation did not occur when weak tetanization was followed by strong tetanization in the presence of SCH23390 (a dopamine D_1/D_5 receptor antagonist) or anisomycin (a protein synthesis inhibitor), further highlighting the central roles of dopaminergic signaling and protein synthesis in synaptic plasticity and STC (Wang et al. 2010). This indicates that the activation of dopamine D1/D5 receptors is one step in triggering PRP synthesis rather than the tag generation process.

A distinct new development is underlined by the proposal that STC itself or a closely related phenomenon could arise through the insertion of calcium-permeable AMPA receptors (CP-AMPARs) (Park et al. 2016). The key finding is the observation that CP-AMPARs are required for the induction of LTP2 but not LTP1 (Park et al. 2018). It seems likely that CP-AMPARs can trigger de novo protein synthesis, via activation of PI3K and the MAPK cascade (Asrar et al. 2009). This creates the possibility of a strictly glutamatergic manner in which STC might happen. Specifically, tetanization of a strong pathway would both induce the insertion of CP-AMPARs and trigger PRP synthesis. If a separate but nearby pathway were then to be subject to LTP1 induction, the tags associated with that induction would capture the available PRPs and cause LTP2 to be observed. It is not yet clear if this mechanism would permit STC after both strong-before-weak (as described) and weak-before-strong protocols (Park et al. 2019). The duration of the intriguing CP-AMPAR experiments, to date, have only assessed the presented mechanism for 3 h post-LTP.

What about the situation in vivo*?* Although the controlled environment provided by ex vivo brain slice experiments allows for unraveling crucial molecular actors potentially involved in STC, the reductionist nature of this framework does not provide a complete picture of the neural mechanisms at play. Shires et al. (2012) tested the STC hypothesis in vivo by adapting the two-pathway protocol for highly collateralized axons of the ipsilateral and contralateral CA3 area in rats and examined two independent pathways that converge in the ipsilateral CA1 area. They confirmed that the transient LTP1 induced by weak stimulation could be stabilized when strong stimulation was applied to the neighboring pathway. These in vivo findings provide one bridge between the molecular/systemic aspect of the STC hypothesis and later behavioral studies.

At the opposite end of the spectrum, there are studies examining the phenomenon of STC at the level of single synapses. This was first achieved for synaptic plasticity observed in *Aplysia* neurons in culture (Martin et al. 1997) and in the hippocampus by Govindarajan et al. (2011). In this heroic study, two separate lasers were used to direct light to caged molecules of glutamate near dendritic spines in culture and to monitor changes in the size of dendritic spines (as a proxy for synaptic weight). As this work was performed in culture, neuromodulatory afferents were absent, and forskolin, an adenylyl cyclase activator, was used. Two main findings were observed. First, STC can be seen at individual synapses when "strong" activation was mimicked by the conjunction of caged release of glutamate and the presence of forskolin at one set of synapses but just glutamate at other nearby synapses. Second, the use of confocal microscopy to monitor dendrites and their associated spines enabled an estimate of the distance over which the local PRP synthesis could affect other activated synapses. This proved to be quite a short distance and largely within individual dendritic domains, leading to the concept of "clustered plasticity" (Govindarajan et al. 2006).

1.3 Functional Implications

LTP is a physiological phenomenon but widely thought to utilize the same or similar mechanisms to those used by the nervous system for memory formation and retention. From the perspective of STC, a weakly encoded memory would decay over time because there would have been no concomitant up-regulation of PRP synthesis (Fig. 1.3a). In contrast, a strongly encoded memory would not only be associated with synaptic potentiation but also RNA translation that may occur in the dendrites of cells following RNA translocation from the perikaryon permitting a temporal distribution of PRPs (Fig. 1.3a, b). When two memories are encoded within a short window of time, and in a common pool of neurons, the PRPs synthesized from dendritic RNA can be shared (Fig. 1.3c).

The first study to investigate whether STC occurs in vivo in behaving animals was a study of inhibitory avoidance learning by Moncada and Viola (Moncada and

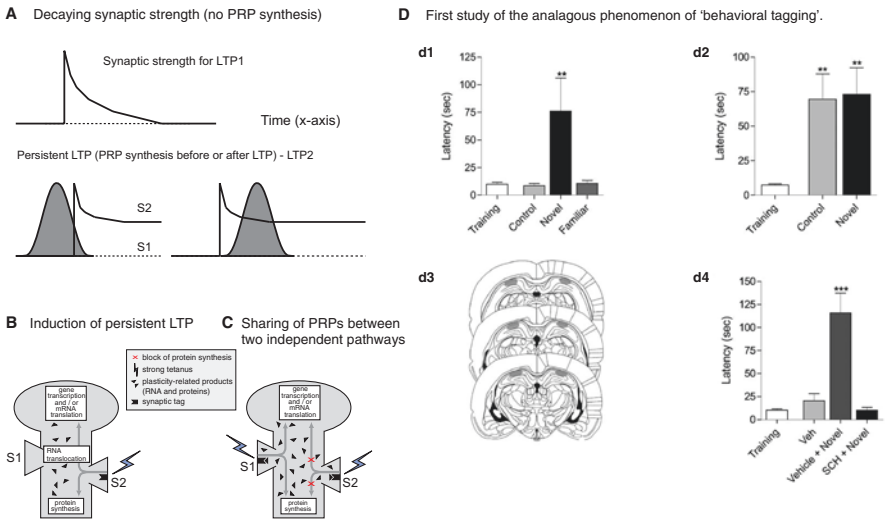

Fig. 1.3 Theoretical framework and discovery of behavioral tagging. (**a**) LTP1 induces a decaying form of LTP. If this overlaps in time with PRP synthesis and distribution (grey Gaussian) shortly before the induction of LTP1 (or shortly after), such that LTP1 is converted to LTP2. The x axis (time) illustrates a possible time scale of the neuronal distribution of PRPs. (**b**, **c**) Cartoon indicating a somatic site of gene transcription, mRNA translocation, and local dendritic mRNA translation to create plasticity-related proteins. (**d**) Application of these ideas to the behavioral domain. ***d1:*** Encoding of inhibitory avoidance behavior with a weak unconditional stimulus in association with task-unrelated environmental novelty causes greatly enhanced memory at 24 h (longer step-through latency); ***d2:*** encoding of inhibitory avoidance behavior with a strong unconditional stimulus causes greatly enhanced memory at 24 h irrespective of novelty; ***d3:*** coronal sections showing sites of infusion of drugs intended to block dopaminergic transmission; ***d4:*** when infused into the hippocampus shortly before novelty exposure, the D1/D5 antagonist SCH23390 blocks the long-term retention of inhibitory avoidance caused by novelty. (From Moncada and Viola (2007), by permission)

Viola 2007). They examined memory retention over time periods of 15 min to 24 h after training with a very weak shock, which was just sufficient to successfully trigger avoidance (Fig. 1.3d). Excellent memory was observed after 15 min, which decayed to a baseline level within 24 h. However, when 5-min exploration of a novel arena preceded such training, a good 24 h memory of the inhibitory avoidance task was seen when the novelty exploration was scheduled to occur 60 min earlier. The supposition was that this novelty exploration served as a "strong" event as it is known to upregulate IEGs, leading to RNA transcription and translation; it also seems likely that novelty will directly trigger the translation of RNAs that are already available near relevant synaptic sites through the influence of neuromodulatory activation of the relevant brain structure (such as hippocampus). Moncada and Viola (2007) showed that this enhancement of memory retention was blocked by the D1/D5 antagonist SCH23391 and the protein synthesis inhibitor anisomycin. A final study showed that offering novelty exploration 15 and 60 min after training also enhanced retention, pointing to symmetry with respect to the sequential activation of the synaptic tag and the upregulation of PRPs. A series of studies from the Viola lab built on these findings, establishing their generality to other learning tasks such as novel object-place tasks (hippocampal) and conditioned taste aversion (Neocortical) (e.g., Ballarini et al. 2009). A similar approach has been taken by others including a group in India, which has explored the impact of novelty on retention in a variety of spontaneous novelty tasks (Vishnoi et al. 2022).

The Viola group in Buenos Aries coined the term "behavioral tagging" to describe their findings (Ballarini et al. 2009; Viola et al. 2014), and this term is now widely used. However, we have slight reservations about the term as the physiological tag does not really tag a behavior. On the contrary, in a cognitive system such as the hippocampus, what is tagged for approximately an hour or so are the population of synapses across a set of neurons whose activation corresponds to activity in a Hebbian cell assembly or, in contemporary language, an engram circuit. The later reactivation of such neurons is a component of memory recall, which may then elicit specific patterns of behavior – such as long-term retention.

Wang et al. (2010) conducted a similar study that also used novelty exploration to enhance the availability of PRPs; however, they used a definitively hippocampal-dependent task in an event arena. The key feature was that animals had already long become familiar with the context through repeated daily experiments, but each day they were faced with creating a new memory of the most recent place where food reward is available. The task was, therefore, less of a test of long-term spatial memory than of "recent event memory" and how long such a memory might last. It is more episodic-like than earlier tasks used to investigate behavioral tagging. This is because the retrieval process cannot merely access long-term memory, it has to select the most recent memory in that context (see also Prodan et al. 2022). The results of Wang et al. (2010) were, nonetheless, much the same with enhanced retention at 24 h contingent on novelty exploration before or after memory encoding, and sensitivity to SCH23390 and anisomycin in association with the novelty component (Fig. 1.4a). Interestingly, if memory encoding occurred in the presence of SCH23390, forgetting was rapid, even with multiple encoding trials; however, if

this training/drug condition was preceded by novelty exploration, long-term retention for 24 h was rescued. This implies that the upregulation of PRPs by novelty exploration approximately 1 h before the single trial of spatial recency encoding was sufficient to compensate for the lack of PRPs when spatial encoding occurred in the presence of the D1/D5 antagonist.

A limitation of using behavioral studies, pharmacological antagonists, and antisense oligonucleotides is that they do not identify the specific cells whose activation by novelty mediates the upregulation of PRPs. Specifically, if dopamine release is involved, which "parent" neurons are responsible? A likely candidate was thought to be the ventral tegmental area (VTA), as suggested by Lisman and Grace (2005). Takeuchi et al. (2016) examined this possibility using an optogenetic approach in which the aim was to substitute novelty exploration by direct activation of the VTA and other candidate neurons. However, in designing their study, they took note of the suggestion of Robert Greene (Smith and Greene 2012) that other catecholaminergic neurons may also serve as sources of dopamine. Accordingly, they designed a study in which channelrhodopsin (ChR) was expressed selectively in VTA neurons and, additionally, in noradrenergic neurons of the locus coeruleus (LC) under the influence of the tyrosine-hydroxylase-1 (*thy-1*) promoter. The expectation was that VTA activation would be effective, while the LC activation would not be – even though both sets of cells expressed *thy-1*. In vivo electrophysiology was used to monitor the enhanced patterns of neuronal activity elicited by novelty exploration relative to that seen in a familiar environment (Fig. 1.4b, *b1*–*b3*). Thereafter, these patterns were mimicked by light pulses directed to thy-1-ChR expressing neurons in LC bilaterally. The surprising finding was that LC activation caused enhanced retention of spatial recency memory in the event arena, whereas VTA activation did not (Fig. 1.4b, *b4*). Interestingly also, this enhanced retention was, as in the Wang et al. (2010) pharmacological study, blocked by SCH23390. It follows that LC activation can substitute for novelty exploration to enhance the availability of PRPs and that it does so by an apparently D1/D5 sensitive mechanism. Duskiewicz et al. (2019) have taken this as evidence that LC presynaptic terminals can release DA as well as noradrenaline, a hypothesis that is consistent with high-performance liquid chromatography (HPLC) evidence (Kempadoo et al. 2016). However, this conclusion may be premature as the behavioral study performed by Takeuchi et al. (2016) relies on pharmacological evidence rather than direct observation of DA release from LC terminals over a very short-time scale, one much shorter than what can be analyzed using HPLC during a behavioral study. It is possible that there are interactions between rapid glutamate and noradrenergic release that, by some unknown mechanism, interact with postsynaptic DA receptors. The next step is, therefore, to use any of the rapid DA sensors in a manner that satisfactorily distinguishes between DA and NA release – but not all such sensors do so. The Takeuchi et al.'s (2016) study was conducted in mice, and a recent successful replication of the role of LC in memory enhancement has been conducted using *thy-1* expressing rats (Tse et al. 2023).

Nomoto et al. (2016) made use of two behavioral tasks with mice that either encountered novel objects (novel object recognition) or explored a new

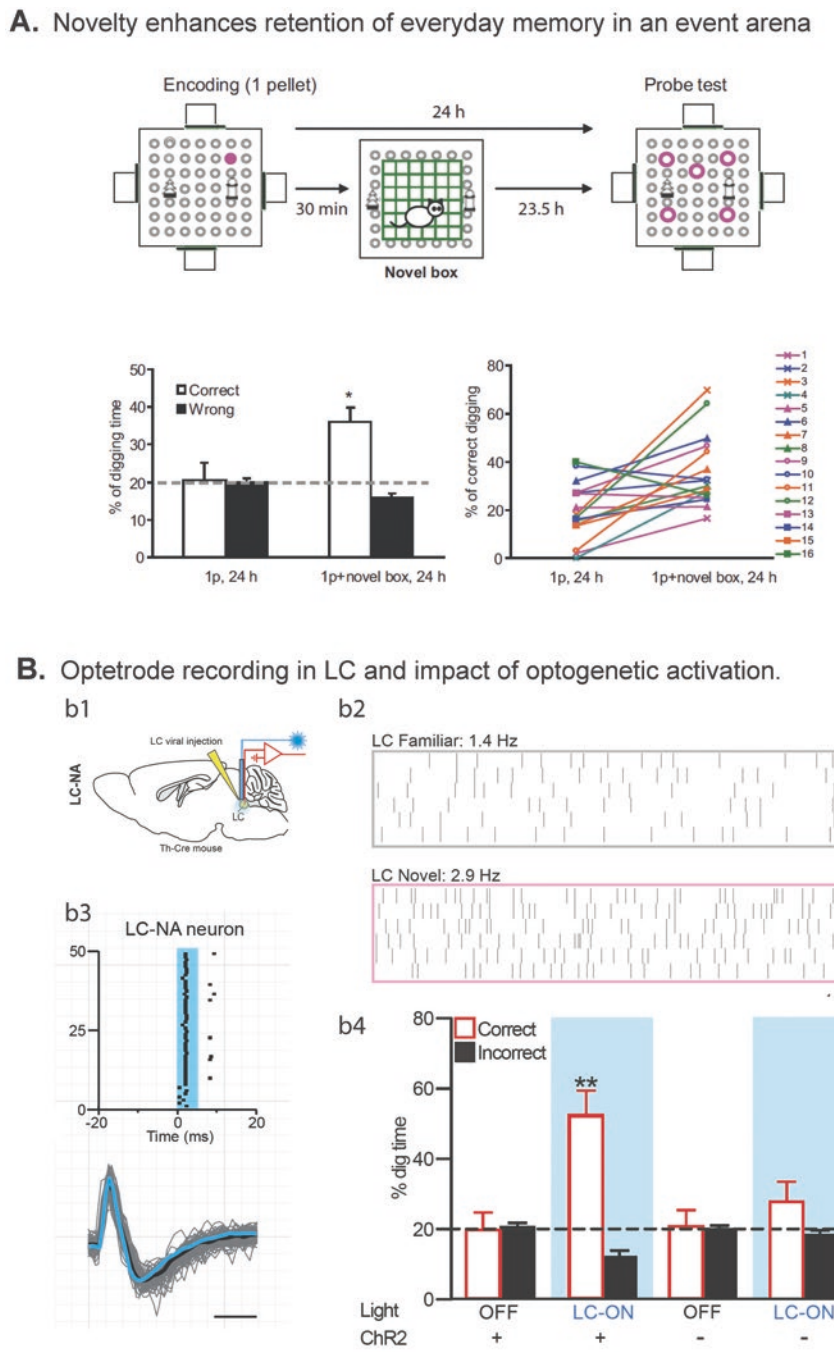

Fig. 1.4 Impact of novelty and optogenetic activation of the locus coeruleus on everyday memory. (**a**) Event arena experiment in which animals either experience post-encoding novelty (experimental condition) or not (control). Note enhanced memory retention at 24 h (digging in the correct location

environment (novel environment exploration). They showed that a 5-min exposure to novel objects led to the generation of a short-term memory (STM) that lasted 30 min and was independent of de novo protein synthesis, while the novel environment exploration task elicited LTM after 10-min sessions. The STM and LTM tasks were trained concurrently in other animals. A first finding was that a 10-min session of novel environment exploration 60 min before or after the exploration of novel objects allowed for LTM of novel object recognition (Nomoto et al. 2016). Subsequently, they performed a cell compartment analysis using fluorescent in situ hybridization targeted at nuclear and cytoplasmic Arc mRNA. Since Arc is present in the nucleus approximately 5 min after its translation and then is located in the cytoplasm 20–30 min later, the location of Arc within hippocampal neurons was used to distinguish the neurons involved in novel object recognition from those involved in novel environment exploration (Guzowski et al. 1999; Nomoto et al. 2016). Some CA1 neurons exhibited both cytoplasmic and nuclear Arc, indicating that a certain population of neurons was activated during both behavioral tasks. Furthermore, the enhancement of novelty-induced memory (i.e., the STM to LTM transition) was associated with an increase in the number of neurons displaying an active IEG signal during both tasks. It was further shown that anisomycin infusion impaired novelty-induced memory enhancement (Nomoto et al. 2016). These findings indicate that the effect of novelty on memory maintenance is dependent on de novo protein synthesis and further encourages the identification of the molecular mechanisms behind behavioral tagging.

There has been interested in seeking behavioral analogs of certain facets of STC established in vitro. One example concerns the phenomenon of "competitive maintenance" in which two or more independent pathways from early-LTP are induced under regimes of limited PRP availability, whereas the transition of one pathway from early- to late-LTP (LTP1 to LTP2) is at the expense of another (Fonseca et al. 2006; Sajikumar et al. 2014). One behavioral study successfully examined the interaction of weakly induced inhibitory avoidance learning with novelty exploration itself (Martínez et al. 2012). Using the novelty exploration time observed during a second session of exploration (i.e., a measure of how well the familiarity of the novel environment was retained), it was observed that enhanced retention of inhibitory avoidance could be at the expense of memory of the arena whose very novelty on first exposure would have triggered the PRPs that could have enhanced retention in both tasks. The use of antisense techniques also established a role for Arc as a possible molecule-mediating PRP. Other properties of STC shown in vitro include activity-dependent "resetting" of synaptic tags by depotentiating stimulation soon

Fig. 1.4 (continued) during a probe test). The effect is apparent across the majority of the individual animals in the study. (**b**) Cartoon showing optetrode targeting LC (b1). Raster plot of single neuron firing during exposure of the animals to a familiar or novel environment in which the rate of firing more than doubles during novelty (b2). Waveforms showing that the natural cell firing and optogenetic activation are equivalent (b3). Optogenetic activation of LC mimics the impact of novelty in enhancing memory retention (b4). (Based on Wang et al. (2010) and Takeuchi et al. (2016))

after LTP induction and "cross-capture" whereby strongly induced LTP enhances the retention of weakly induced LTD and vice versa (Sajikumar and Frey 2004).

Human studies have also been conducted, the pioneering work again being from the Buenos Aries group using the weak-before-strong protocol (Ballarini et al. 2013). Children were read a short story. A short time later, they either had an interactive music lesson, which was novel, or a familiar music lesson. Experiencing the novel music lesson enabled the children to better remember the short story 24 h later relative to those who heard the familiar music. The same result prevailed with visuospatial memory encoded 1 h before or after a novel science lesson in 12–15-year-old school children (Ramirez Butavand et al. 2020). A similar body of work has continued, and this reflects that a behavioral tagging/STC-like process akin to that seen in animals appears to work also in humans and may sometimes be relevant or even useful in school learning.

New research in humans is, however, taking such "cognitive tagging" a step further into the dimension of category-specificity. Does the capture process rely only on the neural consequences of novelty or can the consequences of context-specific or category-specific novelty be to enhance only relevant weak stimuli around the same time? Dunsmoor et al. (2015) used a hybrid Pavlovian fear conditioning and episodic memory design (Dunsmoor et al. 2022) in which the conditioned stimuli of the Pavlovian component consisted of different pictures from two different non-overlapping semantic categories (i.e., animals and tools). After an initial habituation phase involving similar pictures, new pictures from one category were paired with a shock to the wrist while new pictures from the other category were never shocked. This discriminative Pavlovian fear conditioning might have caused an upregulation of PRPs (difficult to measure in humans), but the more interesting possibility is that the use of a discriminative procedure could have allowed category-specific rescue. Later recognition memory results, conducted after a delay, indeed showed selectively enhanced performance for pictures from the shocked category that were encoded before, during, and after fear conditioning, relative to pictures from the other category. Dunsmoor et al. (2022) reviewed this and other studies raising the intriguing idea of STC in humans, which have an added categorical dimension that either is not seen in animals or has not yet been investigated.

1.4 Mechanistic/Molecular Aspects

1.4.1 Synaptic Tags

Based on previous studies investigating the molecular actors involved in the STC process, Redondo and Morris (2011) proposed several criteria that the molecular basis should fulfill:

1. To accommodate the compartmentalized nature of the STC, synaptic tags should be synapse-specific and exhibit a restricted spatial diffusion potential (~50 μm) (Govindarajan et al. 2011).

2. Since protein synthesis-independent processes such as LTP1 were shown to be sufficient for synaptic tagging, the molecules underlying the synaptic tag should not require de novo protein synthesis to be supplied.
3. The time interval within which a tagged synapse can capture PRPs and the capacity of certain drugs to reverse the tagged state of a synapse indicate that tags must be transient as well as reversible.
4. The tag must enable the interaction with PRPs in a synapse-specific manner with that interaction enabling synaptic stabilization.

Although numerous molecules have been examined as potential candidates underlying synaptic tags, none of them fulfill all these criteria. Multiple candidates may be necessary, suggesting a more complex mechanism than that of a single tag. That is, the idea of a "tagged state" generated by multiple factors may be preferred over the hypothesis that a single molecule represents the tag. Table 1.1 lists the most relevant molecular candidates involved in the synaptic tagging process.

1.4.2 CaMKII

Among the four isoforms of CaMKII (CaMKIIα, CaMKIIβ, CaMKIIγ, and CaMKIIδ), CaMKIIα and CaMKIIβ are the most abundant and have been shown to accumulate in active synapses (Bennett et al. 1983; Tobimatsu and Fujisawa 1989; Peng et al. 2004; Mikuni et al. 2016). Since actin represents the main cytoskeletal protein in dendritic spines, and CaMKII plays a crucial role in actin remodeling

Table 1.1 Synaptic tagging candidate molecules

Molecule	Function	Two-pathway experiments	Behavioral tagging experiments
CaMKII	Essential for actin remodeling, which in turn is indispensable for the synaptic tagging process	Sajikumar et al. (2007) and Redondo et al. (2010)	Moncada et al. (2011)
PKA	Phosphorylation of AMPA and NMDA receptors. Essential for setting the synaptic tag	Young et al. (2006) and Skeberdis et al. (2006); Sajikumar et al. (2007) and Park et al. (2014)	Moncada et al. (2011)
TrkB	A main BDNF receptor upstream of the MAPK pathway. Essential for the transition from S-LTP to L-LTP	Lu et al. (2011)	Lu et al. (2011)
AKAP	Important regulator of PKA and involved in protein scaffolding	Huang et al. (2006)	–
Neuropsin	Important functions in activity-induced cleavage of synaptic proteins	Ishikawa et al. (2008)	–

(Dillon and Goda 2005), an emerging question is whether CaMKII activation provides one facet of the environment associated with the tagged state. Once a synapse receives a supra-threshold stimulation, Ca^{2+} enters the synapse via NMDARs, which leads to the formation of Ca^{2+}/calmodulin (CaM) complex. CaM binds to the regulatory domain of CaMKIIα; thus, allowing the autophosphorylation of threonine 286 which activates the kinase (Thiel et al. 1988; Colbran et al. 1989; Ikeda et al. 1991). Although the CaMKIIα does not directly interact with actin filaments, CaMKIIβ harbors a domain that allows the binding of actin. Within synaptic terminals, actin exists in a dynamic equilibrium between its monomeric globular form (G-actin) and the filamentous form (F-actin) (Dillon and Goda 2005). Following activation of CaMKIIβ by CaMKIIα, the former dissociates from the F-actin and allows its interaction with proteins such as cofilin and Cdc42. Cofilin can cleave the actin filaments and remodel the cytoskeleton (Fig. 1.5). However, its activity is modulated by calcineurin as well as LIM kinase, and the different expression of these modulators can lead to opposite effects on cofilin (Racz and Weinberg 2006; Medvedeva et al. 2010). Nevertheless, the cleavage of F-actin by cofilin increases the availability of G-actin, which ultimately can promote spinogenesis and spine enlargement (Xie et al. 2007). Additionally, CaMKII triggers alternative pathways that converge and activate small GTPases, such as RhoA, Rac1, and Cdc42. In addition to the roles of these GTPases in synaptic crosstalk (see Outstanding Issues below), CaMKII activation of Rac1 via kalirin-7 ultimately leads to LIMK, p21-activated kinase (PAK), and cofilin activation in sequential order (Hall 1998; Symons and Settleman 2000). Alternatively, RhoA has been shown to be activated following CaMKII-induced phosphorylation of spinofilin (Ryan et al. 2005). RhoA exerts its influence on actin via cofilin and profilin, the latter of which acts as a G-actin-binding protein that facilitates polymerization by adding actin monomers to F-actin (Sarmiere and Bamburg 2004). Contrary to RhoA and Rac1, Cdc42 leads to the inactivation of cofilin through actin polymerization, which, in turn, inactivates CaMKIIβ, and F-actin is reestablished (Jędrzejewska-Szmek and Blackwell 2019). Lastly, CaM has been shown to promote the binding of T-lymphoma invasion and metastasis-inducing protein 1 to CaMKII and the formation of a Ca^{2+}-independent reciprocally activating kinase-effector complex (RAKEC), which provides a continuous activation of Rac1 and is hypothesized to aid LTP structural maintenance (Saneyoshi et al. 2019).

Due to the proclivity of CaMKII to accumulate in active spines as well as its location relative to the NMDARs and the actin filaments it influences (Mikuni et al. 2016), it can be concluded that this molecule satisfies the first important criterion of synaptic tags. Furthermore, Redondo and colleagues showed that the involvement of CaMKII in the tag generation process is independent of de novo protein synthesis, which provides sufficient grounds for satisfying the second criterion of synaptic tags (Redondo et al. 2010). The third criterion addresses the transient and reversible nature of the synaptic tag. Although Cdc42 can inactivate CaMKII, thus making its activation reversible, previous studies suggest that the CaMKII activity is important only for the first minute following the induction of functional and structural LTPs (Buard et al. 2010; Murakoshi et al. 2017; Chang et al. 2017). While CaMKII can

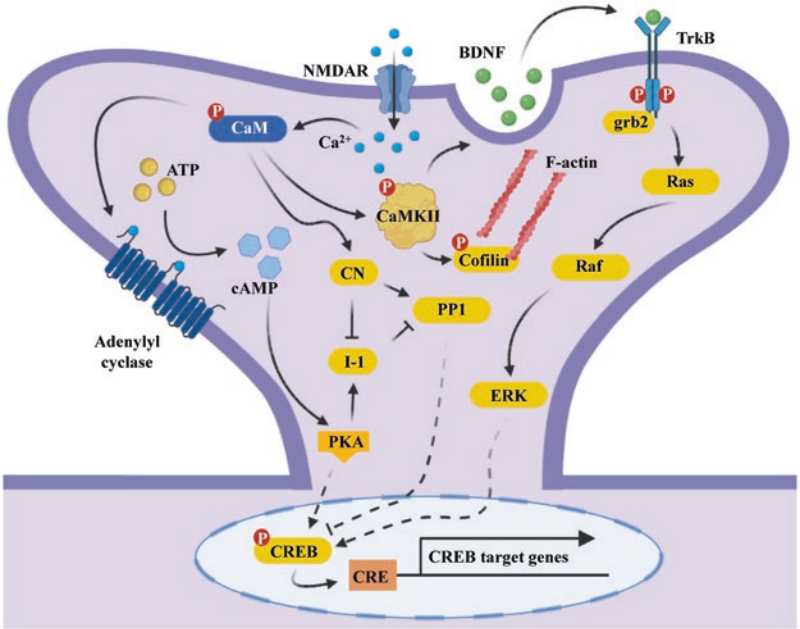

Fig. 1.5 NMDA- and TrkB-dependent signaling involved in functional and structural synaptic changes underlying learning and memory. Following activation of NMDA receptors, Ca^{2+} enters the postsynaptic terminal where it binds to CaM, which, in turn, gets phosphorylated and promotes the activation of adenylyl cyclase and the phosphorylation of CaMKII. CaM promotes the activation of CN, which indirectly inhibits CREB phosphorylation and the expression of immediately early genes. The effects of CN are counteracted by PKA and TrkB activation. The active form of CaMKII leads to actin remodeling as well as exocytosis of endogenous BDNF, the latter of which will bind the TrkB receptor. BDNF binding to TrkB leads to the activation of the Ras-Raf-ERK pathway, which culminates in due course with immediately early gene transcription. Similarly, adenylyl cyclase activation leads to increased production of cAMP, which, in turn, activates PKA and indirectly promotes the expression of immediately early genes. CN, calcineurin; I-1, inhibitor 1; PP1, protein phosphatase 1; PKA, protein kinase A; CaM, calmodulin; CaMKII, calcium/calmodulin-dependent kinase II; BDNF, brain-derived neurotrophic factor; and TrkB, tropomysin-related kinase B. (Figure created using Biorender)

exert some of its effect on actin filaments via RAKEC for up to 30 min, this is still incompatible with most two-pathway and in vivo studies that observed the synaptic capture after more than 60 min from the LTP induction (Saneyoshi et al. 2019). Thus, CaMKII only partially satisfies the third criterion. Lastly, there is no evidence that CaMKII can directly interact and capture PRPs; however, the increase in the number of binding sites associated with the newly formed F-actin following CaMKII activation might represent a method of capturing PRPs, as both CaMKII activation as well as new F-actin formation are essential for synaptic tagging (Sajikumar et al. 2007; Ramachandran and Frey 2009).