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SPECIAL ISSUE REVIEW

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Initial memory consolidation and the synaptic tagging and capture hypothesis

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Abstract

Everyday memories are retained automatically in the hippocampus and then decay very rapidly. Memory retention can be boosted when novel experiences occur shortly before or shortly after the time of memory encoding via a memory stabilization process called "initial memory consolidation." The dopamine release and new protein synthesis in the hippocampus during a novel experience are crucial for this novelty-induced memory boost. The mechanisms underlying initial memory consolidation are not well-understood, but the synaptic tagging and capture (STC) hypothesis provides a conceptual basis of synaptic plasticity events occurring during initial memory consolidation. In this review, we provide an overview of the STC hypothesis and its relevance to dopaminergic signalling, in order to explore the cellular and molecular mechanisms underlying initial memory consolidation in the hippocampus. We summarize electrophysiological STC processes based on the evidence from two-pathway experiments and a behavioural tagging hypothesis, which translates the STC hypothesis into a related behavioural hypothesis. We also discuss the function of two types of molecules, "synaptic tags" and "plasticity-related proteins," which have a crucial role in the STC process and initial memory consolidation. We describe candidate molecules for the roles of synaptic tag and plasticity-related proteins and interpret their candidacy based on evidence from two-pathway experiments *ex vivo*, behavioural tagging experiments *in vivo* and recent cutting-edge optical imaging experiments.

Abbreviations: 5-HT, Serotonin; ACVR1C, Activin A receptor type 1C; AKAP, A-Kinase anchoring protein; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; ARNT1, Aryl hydrocarbon receptor nuclear translocator 1; BDNF, Brain-derived neurotrophic factor; Ca^{2+}, Calcium; CaM, Calmodulin; CaMKII, Ca^{2+}/CaM-dependent protein kinase II; CaMKIV, Ca^{2+}/CaM-dependent protein kinase IV; CaMKK, Ca^{2+}/CaM-dependent protein kinase kinase; cAMP, Cyclic adenosine monophosphate; catFISH, Cellular compartment analysis of temporal activity using fluorescence *in situ* hybridization; CREB, cAMP response element binding protein; E-LTD, Early-LTD; E-LTP, Early-LTP; EPSP, Excitatory postsynaptic potential; FRET, Fluorescence resonance energy transfer; $GABA_A$, γ-aminobutyric acid type A; GluA1/2, AMPA receptor subunit 1 or 2; GluN2, NMDA receptor subunit 2B; LC, Locus coeruleus; LIM, LIM domain; L-LTD, Late-LTD; L-LTP, Late-LTP; LTD, Long-term depression; LTM, Long-term memory; LTP, Long-term potentiation; NMDA, *N*-methyl-D-aspartate; NPAS4, Neuronal PAS domain-containing protein 4; PKA, Protein kinase A; PKC, Protein kinase C; PKMζ, Atypical protein kinase C; PRP, Plasticity-related protein; PSD, Postsynaptic density; RacGEF, Rac-specific guanine exchange factor; RAKEC, Reciprocally activating kinase-effector complex; STC, Synaptic tagging and capture; STM, Short-term memory; TARP γ-2, Transmembrane AMPA receptor regulatory protein γ-2; TH, Tyrosine hydroxylase; Tiam1, T-lymphoma invasion and metastasis-inducing protein 1; TrkB, Tropomyosin receptor kinase B; VTA, Ventral tegmental area; ZIP, ζ inhibitory peptide.

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Lastly, we discuss the direction of future studies to advance our understanding of molecular mechanisms underlying the STC process, which are critical for initial memory consolidation in the hippocampus.

KEYWORDS

dopamine, hippocampus, memory consolidation, novelty, synaptic tagging and capture hypothesis

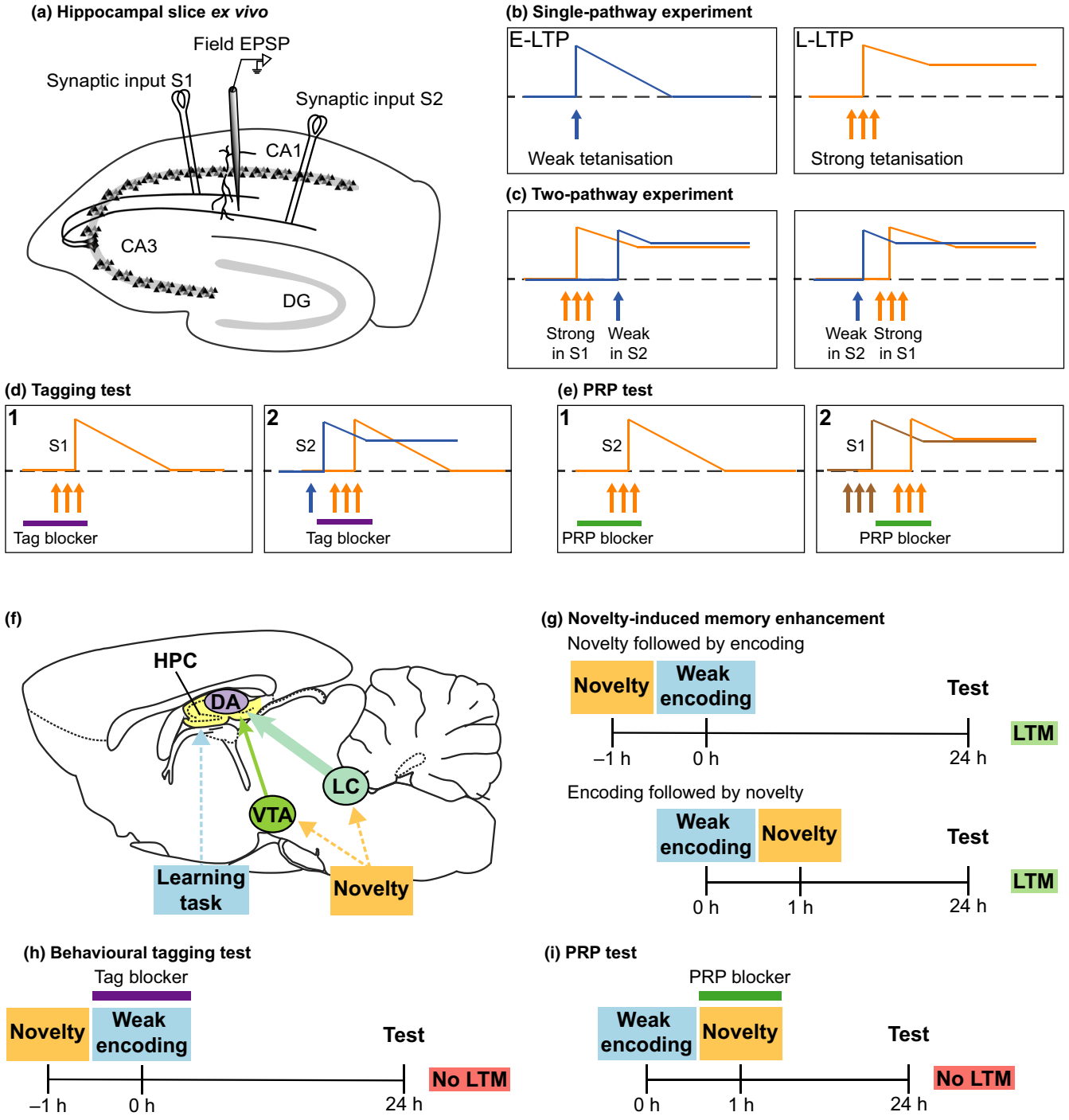
1 | INTRODUCTION

In our daily life, we are continuously bombarded with new experiences and facts. Trivial everyday memories are stored automatically in the hippocampus. However, they are all initially labile and only a selection of them becomes resistant and persist over time. The process by which this transition occurs is carried out in the hippocampus and is called "initial (or cellular) memory consolidation" (Takeuchi et al., 2013; Squire et al., 2015). In this way, our brains select which memories will be consolidated into long-lasting memories to be stored long term in the neocortical network. This happens through a process called "systems memory consolidation" (Takehara-Nishiuchi, 2020) which enables later recalled of the memory. However, the mechanisms underlying initial memory consolidation are not well-understood.

It is well known that the retention of trivial everyday memory can be boosted when novel or unexpected experiences happen shortly before or after the time of memory encoding (Brown & Kulik, 1977). This suggests that novelty triggers initial memory consolidation and converts trivial everyday memories into long-lasting, stable memories. Over the last several decades, studies using *in vitro* and *ex vivo* electrophysiological experiments, along with *in vivo* animal models, have widely contributed and supported the understanding of the mechanisms underpinning novelty-triggered initial memory consolidation, highlighting a crucial role for the concomitant release of dopamine into the hippocampus during novel experiences (Lisman & Grace, 2005; McNamara et al., 2014; Takeuchi et al., 2016; Kempadoo et al., 2016; Yamasaki and Takeuchi, 2017; Duszkiewicz et al., 2019). Importantly, this hippocampal dopamine-dependent novelty-induced memory boost fits well with the synaptic tagging and capture (STC) hypothesis postulated by Frey and Morris (1997) and provides a strong framework. The results obtained using the framework will allow us to explain how two independent events can be associated to enhance the retention of trivial memory.

In this review, we first give an overview of synaptic plasticity followed by the STC hypothesis, outlining important concepts such as heterosynaptic interaction, cooperation and competition, and compartmentalization. We then provide its relevance to dopamine signalling in order to explore the cellular and molecular mechanisms underlying initial memory consolidation in the hippocampus. We summarize electrophysiological STC processes based on the evidence from the two-pathway experiment *ex vivo* and *in vivo*, and a behavioural tagging hypothesis, which proposes a behavioural correlate for the STC hypothesis. We also discuss the function of two types of molecules that have a crucial role in the STC process and initial memory consolidation that are called "synaptic tags" and "plasticity-related proteins (PRPs)." We describe candidate molecules for the roles of synaptic tag and PRPs, and interpret their candidacy based on the evidence from two-pathway experiments *ex vivo*, behavioural tagging experiments *in vivo* and recent cutting-edge optical imaging experiments *in vitro* and *ex vivo*. Lastly, we discuss the future direction of studies that would advance our understanding of

FIGURE 1 Two-pathway experiment and behavioural tagging protocols. (a) Schematic representation of a rodent hippocampal slice *ex vivo* with electrode location to stimulate two separate synaptic inputs S1 and S2 in stratum radiatum of the CA1 region and the location of recording electrodes for the field EPSP from a single, stimulated neuronal population. (b) E-LTP and L-LTP induction by weak (blue arrow) and strong tetanizations (orange arrows), respectively, of a single pathway. (c) Two-pathway experiment. Strong tetanization of the S1 pathway before weak tetanization of the S2 pathway shifts E-LTP induced by weak tetanization in the S2 pathway to L-LTP (left). Similarly, strong tetanization of the S1 pathway after weak tetanization of the S2 pathway shifts E-LTP to L-LTP in the S2 pathway (right). (d) Tagging test. If the candidate blocker that inhibits L-LTP in the S1 pathway (panel 1) allows E-LTP in the S2 pathway to be converted to L-LTP (panel 2), this blocker would be a tag blocker and the tested molecule would be a candidate for synaptic tag. (e) PRP test. If the inhibition of L-LTP in the S2 pathway by the candidate blocker (panel 1) is reversed by a strong tetanization in the S1 pathway (panel 2), this blocker would be a PRP blocker and the tested molecule would be a candidate for PRP. (f) Schematic representation of novelty-induced activation of LC and VTA, resulting in dopamine (DA) release in the hippocampus (HPC). (g) Behavioural tagging protocol. Environmental novelty before (top) or after (bottom) weak encoding of a learning task that normally results in STM can be transformed into LTM. (h) A possible set-up for behavioural tagging test. If blocking of a candidate molecule during weak encoding inhibits novelty-induced enhancement of memory persistence, this molecule would be a candidate for behavioural tag. (i) A possible set-up for PRP test. If blocking of a candidate molecule during novelty inhibits novelty-induced enhancement of memory persistence, this molecule would be a candidate for PRP. (b-e) Adapted from Redondo and Morris (2011). (h and i) The set-ups are adapted from Moncada and Viola (2007) and Moncada et al. (2011)



the molecular mechanisms on STC process that are critical for initial memory consolidation in the hippocampus.

2 | SYNAPTIC PLASTICITY: CELLULAR MECHANISMS UNDERLYING LEARNING AND MEMORY

There is wide agreement in contemporary neuroscience that the processes of learning and memory are supported by changes in the strength of synapses and that synapses are the core location where information is stored in the brain. A mechanism by which changes in synaptic strength may occur was first proposed by Hebb (1949) and is known as "Hebb's rule." This states, "When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased." Or, put simply, "Neurons that fire together, wire together."

Synapses can change their structure and function, strengthening or weakening over time in an activity-dependent

manner. This ability, known as synaptic plasticity, is believed to be a cellular correlate of learning and memory processes and has been predominantly investigated experimentally in *ex vivo* hippocampal slices by stimulating populations of presynaptic fibres with short-duration trains of high/low-frequency stimulation. The basic cellular models of synaptic plasticity are two phenomena known as long-term potentiation (LTP) and long-term depression (LTD). Whilst LTD mechanisms correspond to a weakening of synaptic strength, LTP is a long-lasting increase in synaptic strength that occurs in response to brief high-frequency stimulation (Bliss & Lomo, 1973; Andersen, 1977; Bliss & Collingridge, 1993; Malenka & Bear, 2004; Morris, 2006).

The synaptic strengthening occurring during LTP has for a long time been inferred as a putative cellular mechanism, which may engage the same synaptic mechanisms as those involved in learning-related strengthening. Typically, LTP is studied by replacing a potential learning experience with high-frequency electrical stimulation of a neural pathway, or repeated pairings of presynaptic and postsynaptic cell firing.

LTP is traditionally distinguished as early-LTP (E-LTP) and late-LTP (L-LTP), where E-LTP refers to synaptic strengthening that usually decays in few hours, and which can be induced by either a single train of high-frequency stimulation or by a single theta-burst stimulation (weak tetanus) (Figure 1b). E-LTP is protein synthesis-independent and changes in synaptic strength occurring during this phenomenon involve transient increases in both the electrical current and the number of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-type glutamate receptors (AMPA receptors) at the postsynaptic site (Malenka & Bear, 2004; Choquet & Hosy, 2020). Conversely, L-LTP indicates a long-lasting enhancement of synaptic strength lasting for at least 6–10 hr. This form of LTP is usually induced by repeated trains of high-frequency stimulation (strong tetanus) (Figure 1b), which trigger gene transcription and protein synthesis within the hippocampal neurons that are responsible for the stabilization of synaptic strengthening (Frey & Morris, 1998; Kandel, 2012). Furthermore, L-LTP is caused by the activation of both glutamatergic and neuromodulatory inputs, which is not the case with E-LTP. For this reason, L-LTP is referred to as "heterosynaptic plasticity" or "modulatory input-dependent plasticity."

The functional changes that occur during L-LTP undergo different phases that are believed to be supported by changes in synapse architecture. The induction and maintenance of L-LTP depend on processes that degrade, rebuild, stabilize and consolidate an enlarged actin cytoskeleton (Bosch et al., 2014; Rudy, 2015; Nakahata & Yasuda, 2018). Recent advancements in the optical imaging of single spines, combined with glutamate uncaging, have allowed us to study the signalling mechanisms of L-LTP with single synapse resolution (Kruijssen & Wierenga, 2019). L-LTP is accompanied by two distinguishable processes: "functional plasticity," thought to increase the number of AMPA receptors at the postsynaptic site and "structural plasticity" by spine enlargement. Bosch et al. (2014) investigated spine structural expansion during LTP and proposed a model for spine reorganization that occurs after L-LTP induction, in which a state of binding capacity of the actin cytoskeleton increases due to a persistent increase in F-actin. This persistent increase in F-actin results not only in the number of F-actin docking sites, but also changes the biochemical composition of F-actin as the binding capabilities of cofilin complex increases, thereby allowing the capture of constituent proteins, which are indispensable for the maintenance of the potentiated state (Okamoto et al., 2009). This is consistent with the previous electrophysiological studies, suggesting that pharmacological disruption of F-actin prevents L-LTP (Ramachandran & Frey, 2009; Fonseca, 2012).

3 | SYNAPTIC TAGGING AND CAPTURE HYPOTHESIS

In 1997, Frey and Morris (1997) provided the first evidence for the STC hypothesis in rat hippocampal slices. Using a two-pathway framework, they stimulated two independent sets of synaptic inputs that presumably targeted the same population of neurons in the CA1 area of the hippocampus (Figure 1a). They observed that E-LTP resulting from weak stimulation of one pathway could lead to L-LTP if a strong stimulation was applied to the other pathway within a specific time window before or after the weak stimulation (Frey & Morris, 1997, 1998) (Figure 1c). Briefly, in the Frey and Morris framework, weak stimulation of one pathway activates glutamatergic inputs to create "synaptic tags," which modify only potentiated synapses. Strong stimulation of the other pathway is thought to activate both independent glutamatergic inputs and neuromodulatory inputs, such as dopaminergic inputs. Activation of dopaminergic inputs causes increased availability of PRPs, either in the soma or dendrites, which are then transported sparsely to the dendrites. Subsequently, tagged spines capture PRPs allowing conversion of the labile strengthening of these synapses (E-LTP) into a stable and long-lasting synaptic strengthening (L-LTP) (Figure 2) (Frey & Morris, 1997, 1998). Interestingly, Sajikumar and Frey (2004a) showed that the induction of protein synthesis-dependent L-LTD is also characterized by the STC process. In brief, they observed that early-LTD (E-LTD) in one pathway was transformed into L-LTD if L-LTP was induced in another pathway, in a process called "cross-tagging." The framework used in STC became a good model to study the input specificity and also a model to understand the heterosynaptic interaction between different groups of synaptic inputs. Martin et al. (1997) observed STC processes in *Aplysia* sensory neurons branching on two

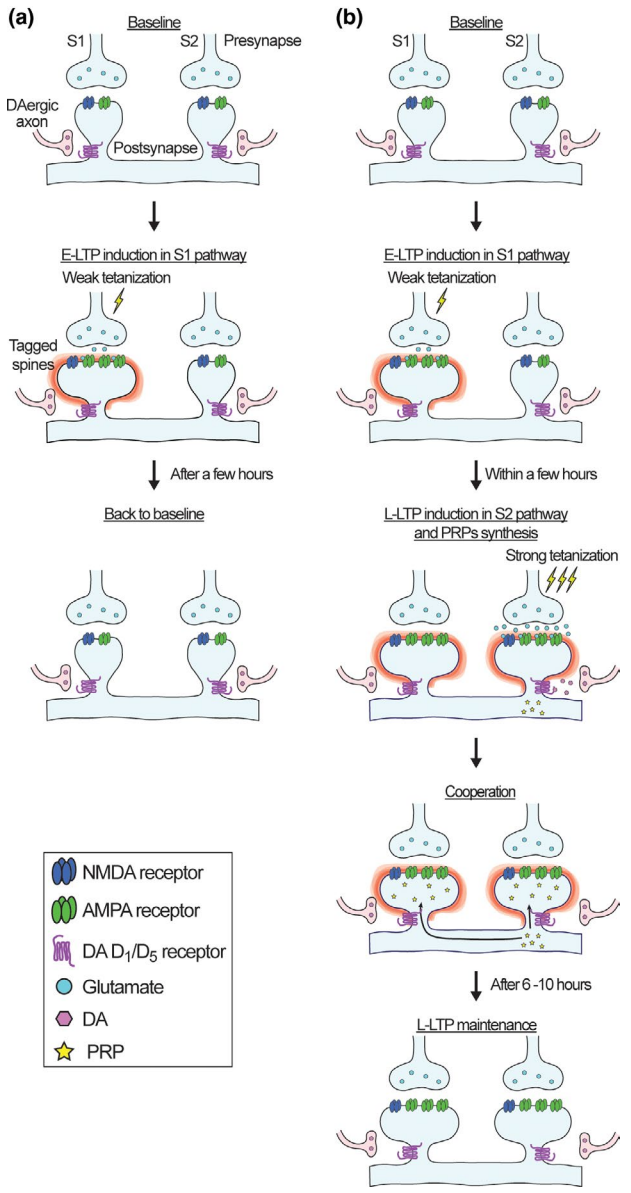


FIGURE 2 Molecular mechanisms of synaptic plasticity and dopamine signalling in the STC hypothesis. (a) Weak tetanization in the S1 pathway activates only glutamatergic input, which sets the synaptic tag (red line) in the potentiated spine and induces early-LTP (E-LTP) in the S1 pathway. Changes in the tagged spines during E-LTP involve both an increase in number of synaptic AMPA receptors and in the volume of spines. After a few hours, the tagged state and E-LTP in the S1 pathway return back to baseline. (b) In the STC processes, weak tetanization in the S1 pathway and strong tetanization in the S2 pathway, inducing late-LTP (L-LTP), are independently applied to the same neuron within a 1–2 hr timeframe. Strong tetanization in the S2 pathway activates both glutamatergic and dopaminergic inputs, which induces not only the setting of synaptic tag in potentiated spine, but also synthesis of plasticity-related proteins (PRPs). Newly synthesized PRPs can be captured by the tagged spines in the S2 pathway as well as in the S1 pathway, which is called "cooperation." The captured PRPs stabilize both increased number of synaptic AMPA receptors and the increased volume of spines in tagged spines, leading to conversion of E-LTP into L-LTP that lasts for at least 6–10 hr in the S1 pathway

independent motor neurons. They showed that the strong facilitation of one branch by five pulses of serotonin (5-HT) application resulting in long-term facilitation of the amplitude of excitatory postsynaptic potentials (EPSPs) promotes the transition of a short-term facilitation of EPSPs in the other branch, which received only weak facilitation by single-pulse 5-HT application, to long-term facilitation in a presynaptic protein synthesis-dependent manner. These results imply that the STC process is a general phenomenon that can be observed across species.

To summarize the Frey and Morris framework, two conditions are indispensable for the STC process: (a) local setting of synaptic tags generated by appropriate synaptic activity and (b) availability of PRPs that are synthesized as a result of the activation of dopamine D_1/D_5 receptors in a time-dependent manner (1–2 hr before or after the setting of synaptic tags). These PRPs can then be captured by potentiated spines that are in "a tagged state," leading to L-LTP (Frey & Morris, 1998; Redondo & Morris, 2011). Experimental evidence implies that the STC phenomenon is based on the sharing of intracellular PRPs among potentiated synapses. In fact, during the STC process, two distinct inputs, a weak and a strong stimulation, presumably converging on the same population of neurons, cooperate for the maintenance of the long-lasting synaptic strengthening of both synapses. This cooperative interaction between the two synapses, referred as "synaptic cooperation," mainly relies on the increased availability of PRPs occurring during the strong stimulation (Pinho et al., 2020). Conversely, when PRPs are limited, the potentiated synapses "fight for protein" and this situation is referred as "synaptic competition." Sajikumar et al. (2014) performed three-pathway experiments to increase synaptic competition among potentiated synapses. They first showed that, in the presence of a third pathway that did not receive any tetanization, a strong tetanization followed by a weak tetanization converted E-LTP to L-LTP in the second weakly tetanized pathway by virtue of the availability of PRPs from first strongly tetanized pathway. Surprisingly, strong tetanization followed by a second and third weak tetanizations may trigger sufficient competition to prevent L-LTP in all pathways. The increased need for PRPs within a specific time frame created competition among the three potentiated synapses because the amount of available PRPs were no longer enough to satisfy their requirement (Sajikumar et al., 2014). This evidence demonstrates that the availability of PRPs represents an essential component of the STC hypothesis.

Most studies investigating the STC hypothesis were predominantly carried out using *ex vivo* recordings of hippocampal synaptic plasticity. However, the necessarily artificial *ex vivo* recording conditions induce reduced spontaneous activity levels, severed dopaminergic afferents and altered PRP baseline levels. Results generated using these methods thus

require interpretational caution. Consequently, *in vivo* studies are generally accepted to be better suited for the study of translationally relevant neural mnemonic mechanisms. Using a novel two-pathway framework *in vivo*, Shires et al. (2012) showed that the STC hypothesis is a "living phenomenon." Briefly, they stimulated the highly collateralized axons of ipsilateral and contralateral CA3, and monitored two independently converging inputs to the ipsilateral CA1 using anaesthetized rats *in vivo*. Similarly to STC process *ex vivo*, they observed that E-LTP, induced by a weak stimulation, could be converted into L-LTP, if a strong stimulation was applied 30 min after. This approach provided a better link between *ex vivo* observations of protein synthesis-dependent synaptic strengthening at the cellular level and behavioural aspects of initial memory consolidation. Moreover, behavioural studies have also attempted to examine the STC hypothesis *in vivo*. This is referred to as "behavioural tagging" and is described below in Section 4.

3.1 | Compartmentalization

Initially, STC processes were studied at the apical dendrites in the CA1 (Frey & Morris, 1997; Barco et al., 2002). However, pyramidal neurons in the CA1 have both apical and basal dendrites (Amaral & Witter, 1989). Alarcon et al. (2006) performed two-pathway recordings both from apical and basal dendrites. Their findings showed that the STC process was similar in both dendritic compartments. Surprisingly, when they crossed the synaptic input between basal and apical dendrites, having one stimulation in the basal dendrites and another stimulation in the apical dendrites, this configuration failed to induce the STC process across dendritic compartments when they applied the same electrical stimulation to both compartments.

At the structural level, new dendritic spines preferentially grow in close proximity to activated synapses and become functional, suggesting that learning-related paradigms (e.g. LTP) play a major role in the reorganization of synaptic networks (Harvey & Svoboda, 2007; De Roo et al., 2008). This concept is known as "compartmentalized synaptic plasticity." In another study by Govindarajan et al. (2011) using two-photon glutamate uncaging combined with perforated patch-clamp electrophysiology, they investigated the relationship between spines that take part in the STC process. They showed that the STC process is temporally asymmetric, spatially localized and preferentially occurs between stimulated spines that reside in the same dendritic branch. Moreover, they also demonstrated that the STC process is induced following bath application of dopamine D_1/D_5 receptor agonist along with pseudo-synchronous glutamate uncaging of multiple spines on the same branches, supporting a "clustered plasticity model," reviewed by Govindarajan et al. (2006). The clustered plasticity model addresses an issue concerning the transport rates of proteins, which are not sufficient to reach multiple dendritic branches within the 1-hour time window of the synaptic tag (Washbourne et al., 2002). The proposed answer for this conundrum lies with the discovery of dendritic ribosomes (Steward & Levy, 1982). These ribosomes are capable of dendritic protein synthesis, which appears to be activity-dependent (Smith et al., 2005). Of note, the synthesis of the PRP candidate, Homer1a, in the soma, and subsequent transportation to tagged spines, has been observed (Okada et al., 2009). This discrepancy between dendritic and somatic protein syntheses is still up for debate, and more experiments are required before these processes are fully understood.

3.2 | The role of dopamine in the STC hypothesis

Converging evidence has indicated that dopamine signalling in the hippocampus is required for the persistence of synaptic plasticity and memory. Several *ex vivo* studies revealed an important modulatory effect of dopamine D_1/D_5 receptors on the persistence of glutamatergic synaptic plasticity in the CA1 (Frey et al., 1991; Frey et al., 1993). The activation of dopamine D_1/D_5 receptors also causes an LTP-like enhancement of glutamatergic synaptic transmission that is dependent on cyclic adenosine monophosphate (cAMP) signalling (Frey et al., 1990; Monsma et al., 1990; Frey et al., 1991; Angenstein et al., 1992; Frey et al., 1993; Huang et al., 1994). More evidence from electrophysiological recordings *in vivo* supports the role of dopamine in persistence of synaptic plasticity (Swanson-Park et al., 1999; Lemon & Manahan-Vaughan, 2006). The activation of dopamine D_1/D_5 receptors *in vivo* is crucial for L-LTP in the CA1, confirming *ex vivo* results (O'Carroll & Morris, 2004; Navakkode et al., 2007). Conversely, blockade of dopamine D_1/D_5 receptors prevents the maintenance of L-LTP (Frey et al., 1990; Huang et al., 1994).

Beneficial effects of dopamine on synaptic strength are mainly heterosynaptic rather than homosynaptic (Sajikumar et al., 2007). It has also been shown that dopaminergic modulation takes part in STC processes (Navakkode et al., 2010). Navakkode et al (2010) showed that, whilst a dopamine D_1/D_5 receptor agonist induced enhancement of synaptic strength in one pathway, the E-LTP induced by weak tetanization in a second pathway was transformed into L-LTP, showing an STC phenomenon in which the dopamine signalling facilitated the transformation of E-LTP to L-LTP. Interestingly, studies from the 1990s indicate that activation of dopamine D_1/D_5 receptors is critical for the PRP synthesis required for L-LTP (Frey et al., 1991). More recently, Wang et al. (2010) brought forward this idea performing the two-pathway

experiments in rat hippocampal slices *ex vivo*. They showed that the STC phenomenon no longer occurred in first pathway with weak tetanization if subsequent strong tetanization was applied to the second pathway in the presence of either a dopamine D_1/D_5 receptor inhibitor or a protein synthesis inhibitor, showing that synthesis of *de novo* PRPs mediated by the activation of dopamine D_1/D_5 receptors in the second pathway is critical for conversion of E-LTP into L-LTP in the first pathway. Together, these results suggest that inhibition of dopamine D_1/D_5 receptors prevents STC process by blocking the synthesis of PRPs rather than affecting the setting of synaptic tags.

3.3 | Two-pathway experimental set-up for identifying candidates for the synaptic tag and PRP

The two-pathway set-up has been the predominant experimental method used to study the STC process (Frey & Morris, 1997). With this method, the individual input can be recorded specifically and manipulated independently, thus providing the opportunity to study specific factors and timings involved in the process. Before doing a two-pathway experiment to evaluate potential candidates for the synaptic tag or PRP, it is important to set up a highly responsive and specific system to reliably manipulate the candidate molecule, such that it can be "turned on/off." Traditionally, this has been done using reversible pharmacological inhibitors (Sajikumar et al., 2007; Redondo et al., 2010). In addition, genetically modified animal models, such as the tropomyosin receptor kinase B $(TrkB)^{F616A}$ knock-in mouse can be used. (Chen et al., 2005; Lu et al., 2011). This $TrkB^{F616A}$ knock-in mouse has a modified ATP-binding site and this allows highly specific inhibition of the tyrosine kinase activity of $TrkB^{F616A}$ using 1NMPP1. Recently, additional alternatives have been developed, such as optogenetic control of activity for candidate molecules, either with light-inducible inhibition of calcium/calmodulin (Ca^{2+}/CaM)-dependent protein kinase II (CaMKII) or light-inducible-activation of TrkB (Chang et al., 2014; Murakoshi et al., 2017). The reversibility of manipulation is important, as reliable manipulation at specific timings is crucial to target a single pathway.

To assess synaptic tag candidates, a weak followed by a strong tetanization protocol is used with manipulation (inhibition) of the candidate during the second, strong tetanization (Figure 1d). Theoretically, if the candidate is responsible for setting the synaptic tag, the first weak tetanization should be able to capture (or hijack) PRPs produced from the second strong tetanization and successfully convert E-LTP to L-LTP. In contrast, the second strong tetanization should fail to express L-LTP.

To assess PRP candidates, a strong followed by strong tetanization protocol is used together with the manipulation (inhibition) of the candidate (Figure 1e). Manipulation should be applied during the tetanization of the second pathway. If the manipulation occurs during the initial tetanization of the first pathway, this should inhibit PRP production not only in the first pathway but also in the second pathway. If the second tetanization with manipulation is able to induce L-LTP in the second pathway, then the candidate is considered to be involved in synthesis of PRPs, rather than setting of synaptic tags.

4 | BEHAVIOURAL TAGGING

The behavioural tagging hypothesis, proposed by Moncada and Viola (2007), translates the STC hypothesis into a behavioural paradigm. The hypothesis postulates that a weak encoding of a learning task that normally results in short-term memory (STM) can be transformed into long-term memory (LTM) when an unrelated behavioural event that induces protein synthesis occurs shortly before or after the time of the weak encoding. The mechanisms underlying this phenomenon are thought to be based on the same mechanisms as the STC hypothesis, such that, whilst the weak encoding sets the "behavioural tag," the concurrent behavioural event provides the *de novo* PRPs to be captured by the behavioural tag (Moncada et al., 2011). In accordance, the synaptic tag is believed to underlie the "behavioural tag" on the cellular level. However, as the precise mechanisms are not yet identified, the behavioural tag replaces the synaptic tag in the context of behavioural tagging hypothesis. The concurrent behavioural event is generally introduced in terms of spatial, environmental or object novelty in behavioural tagging experiments.

In Moncada and Viola's study (2007), the STM of a weak encoding during a hippocampal-dependent inhibitory avoidance task in rodents (Whitlock et al., 2006) was consolidated to LTM (tested 24 hr after the weak encoding) following 5 min of novel open-field exploration before or after the weak encoding (Figure 1g). In the years following this study, the novelty-induced enhancement of retention of memory has been demonstrated in other hippocampus-dependent tasks such as spatial object recognition, contextual fear conditioning, novel object recognition, the everyday memory task in the event arena (Ballarini et al., 2009; Wang et al., 2010; Nomoto et al., 2016; Takeuchi et al., 2016) and in the hippocampus-independent conditioned taste-aversion task (Ballarini et al., 2009). Memory enhancement effects were not observed when animals were familiarized to the open field (or to any other form of novelty) or when the novelty was presented too far, temporally, from the weak training, pointing out the novel characteristics of the concurrent event and the existence of a critical time window, respectively

(Moncada & Viola, 2007; Ballarini et al., 2009; Moncada et al., 2011). Interestingly, a study conducted on elementary school children showed that novel science, music or visual lessons given within a critical time window (1 hr before or after the actual lesson) enhanced subsequent LTM of the actual lesson material obtained by reading a story (Ballarini et al., 2013). A similar effect was observed in another study (Ramirez Butavand et al., 2020), in which high school students who experienced a novel science or sex education lesson one hour before or after initial exposure to a geometric figure presented during a regular class were significantly better at replicating the geometrical figure, tested at 2 and 45 days, compared with students not exposed to the novel lesson. In summary, behavioural tagging stands strong as a general mechanism for initial memory consolidation in the brains of rodents and humans.

As the associativity of LTP in the STC phenomenon occurs between the two independent pathways converging on the same population of neurons, the associativity of LTM in behavioural tagging experiments requires activation of overlapping neuronal populations by weak encoding and novel experience for memory enhancement. Accordingly, Ballarini et al (2009) showed that STM of a hippocampus-*dependent* task could not be transformed into LTM using novelty by a hippocampus-*independent* task and vice versa. Here, the novelty introduced was a novel taste and did not convert STM to LTM in spatial object recognition. These results indicate that neuronal populations in different brain regions activated by weak encoding and novelty cannot benefit from associative properties of LTM formation as the PRPs induced by novelty in one set of neurons cannot be captured by the behavioural tag in the other set of neurons. Indeed, a recent study supports this idea (Nomoto et al., 2016). Using "cellular compartment analysis of temporal activity using fluorescence *in situ* hybridization" (catFISH), the neuronal populations activated by weak encoding in a novel object recognition task and novel context exploration were identified separately by the detection of cytoplasmic and nuclear *Arc* RNAs, respectively. The coexistence of both cytoplasmic and nuclear *Arc* RNAs in a single neuron indicated common activation of this neuron during both novel object recognition task and novelty exploration. The number of overlapping neurons in the CA1 area of the hippocampus increased significantly following a novelty-induced memory boost in a novel object recognition task. Furthermore, optogenetic inhibition of the neuronal population engaged by novelty exploration impaired the retrieval of LTM in a novel object recognition task.

Several methods are currently being used to selectively interfere with candidate molecules or the specific circuit responsible for either of two important processes involved in behavioural tagging experiments, namely encoding and initial memory consolidation (Figure 1h, i). This enables the separate investigation of the two important processes of LTM formation and identification of underlying circuits and molecular mechanisms. It was shown that behavioural tag setting is a process independent of protein synthesis (Ballarini et al., 2009). Novelty-induced enhancement of the transient memory of weak training was not impaired, even though the protein synthesis inhibitor anisomycin was infused into the CA1 area of the hippocampus 15 min before the weak training. Instead, various candidate molecules such as CaMKII, protein kinase A (PKA) (Moncada et al., 2011) and TrkB (Lu et al., 2011) were suggested to contribute to behavioural tag setting in accordance with the candidates from the STC phenomenon (Table 1) as discussed in Section 5.

The process through which novelty promotes LTM formation is typically investigated in a similar way as in electrophysiological two-pathway experiments. Infusion of the protein synthesis inhibitor anisomycin before or immediately after novelty has been shown to impair consolidation of a transient weak memory into LTM (Moncada & Viola, 2007; Ballarini et al., 2009; Wang et al., 2010). This indicates that the novelty-induced memory enhancement is dependent on *de novo* protein synthesis in contrast to the setting of a behavioural tag. Recent evidence has highlighted possible side effects of the commonly used anisomycin, which may be responsible for its amnestic effects rather than protein synthesis inhibition. Neuronal silencing (suppressed local field potentials) (Sharma et al., 2012) and altered monoaminergic transmitter release at anisomycin injection sites (Qi & Gold, 2009) are among the speculated side effects. However, it is also suggested that the amnestic effect of anisomycin can be attributed solely to protein synthesis inhibition in a dose-dependent manner. Shires et al. (2012) reported no significant changes in the baseline field EPSPs at doses of anisomycin used in their experiments, ruling out the undesired effect of neuronal silencing.

Existing evidence shows that novelty acts through dopaminergic neuromodulation and particularly through hippocampal dopamine D_1/D_5 receptor activation. When animals were injected with dopamine D_1/D_5 receptor antagonist or protein synthesis inhibitor in the dorsal hippocampus before novelty, transition from STM to LTM mediated by novelty was prevented (Moncada & Viola, 2007; Wang et al., 2010; Moncada et al., 2011; Takeuchi et al., 2016). These results suggest that novelty-induced memory boosts are mediated by dopamine-dependent protein synthesis. In addition to dopaminergic regulation, the activity of β-adrenoceptors has been shown to be indispensable for novelty-induced memory enhancement of inhibitory avoidance in rats, as the infusion of an antagonist of these receptors (propranolol) into the dentate gyrus of the rats 10 min before novelty inhibited a novelty-induced memory boost (Moncada et al., 2011). However, contradictory results were presented by Takeuchi et al. (2016) in which propranolol injections

TABLE 1 Synaptic tag contributors from electrophysiological and behavioural studies

Candidates	Function/ relevance	Limitations	Two-pathway experiments	Electrophysiology article	Behavioural tagging experiments	Behavioural tagging article
Actin remodelling	Indispensable for LTP	Structural protein	Yes	Ramachandran & Frey (2009), Fonseca (2012)	No	–
AKAP	Scaffolding protein Important for regulating PKA	Only regulates to set the tag	Yes	Huang et al. (2006)	No	–
AMPA receptor	Stimulate PRP synthesis Ca^{2+}-permeable AMPA receptors would be synaptic tags	No clear pathway	Yes	Park et al. (2019)	No	–
CaMKII	Indispensable for synaptic tagging process Kinase activity might "set the tag" Stimulates actin remodelling	Short-lived, no known interactions with PRPs	Yes	Sajikumar et al. (2007,), Redondo et al. (2010)	Yes	Moncada et al. (2011)
Neuropsin	Provides evidence about different synaptic tagging mechanisms	Not required for all "types" of tagging	Yes	Ishikawa et al. (2008)	No	–
NCAM	Required for the induction of LTP	Not validated using the two-pathway experimental set-up	No	Muller et al. (2000)	No	–
PKA	Important for tagging Kinase activity might "set the tag" Phosphorylation of NMDA and AMPA receptors Presynaptic PKA is necessary for STC, presumably via regulation of synaptic vesicle protein 2	No known PRP capture mechanism	Yes	Young et al., (2006), Sajikumar et al. (2007), Skeberdis et al. (2006), Park et al. (2014)	Yes	Moncada et al. (2011)

(Continues)

TABLE 1 (Continued)

Candidates	Function/ relevance	Limitations	Two-pathway experiments	Electrophysiology article	Behavioural tagging experiments	Behavioural tagging article
Protein degradation	Important for protein synthesis/ degradation equilibrium	Not the tag, but regulates the process	Yes	Cai, Frey, Sanna, and Behnisch (2010)	No	–
TrkB	BDNF receptor, activates MAPK pathway	Transmembrane receptor, no PRP interaction is known	Yes	Lu et al. (2011)	Yes	Lu et al. (2011)

into the dorsal hippocampus did not impair novelty-induced memory enhancement in the everyday memory task in mice. The contrasting effects of β-adrenoceptor blockage on novelty-induced memory boost may be due to variations in the timing of drug injections with respect to the novelty and the behavioural task, which were presented in different orders and intervals in both studies. Another factor that may have influenced results is the different target regions within the hippocampus that were used. Whilst drug injections were aimed at the dentate gyrus in the Moncada et al. (2011) study, the injections were aimed at the CA1 area in the study by Takeuchi et al. (2016). The target locations are innervated in different densities by noradrenergic axons in different species used in both studies. The highest noradrenergic innervation density is reported in the hilus of the dentate gyrus of rats (Foote et al., 1983), whilst noradrenergic axons are more evenly innervated throughout the dorsal hippocampus in mice (Takeuchi et al., 2016; Kempadoo et al., 2016). Finally, although the learning tasks used in these studies, which were inhibitory avoidance in Moncada et al. (2011) and the everyday memory task in Takeuchi et al. (2016), are both hippocampus-dependent, they may each depend more strongly on different hippocampal areas. Hence, further studies are required to provide insight on the regulation of memory enhancement by β-adrenoceptors.

Another discrepancy in literature involves the brain regions responsible for the dopaminergic innervation of the hippocampus. The ventral tegmental area (VTA) had long been the brain region largely held responsible for novelty-induced dopaminergic transmission in the hippocampus (Lisman & Grace, 2005; McNamara et al., 2014; Moncada, 2017). However, evidence from recent studies has instead emphasized the influence of the locus coeruleus (LC), which has been shown to have dense tyrosine hydroxylase-positive (TH^+) axons projecting to the dorsal hippocampus, which corelease dopamine as well as noradrenaline (Figure 1f) (Takeuchi et al., 2016; Kempadoo et al., 2016; Moncada, 2017). A combined view on the differential roles of VTA and LC in the dopaminergic regulation of novelty-mediated memory consolidation was recently proposed (Duszkiewicz et al., 2019). Here, it was suggested that the VTA-hippocampus system regulates initial memory consolidation as well as system memory consolidation through hippocampal reactivation (McNamara et al., 2014) accompanied by a "common novelty," which is related to prior knowledge and hence can be integrated into existing memory traces. In addition, Duszkiewicz et al. (2019) suggested that the LC-hippocampus system regulates initial memory consolidation in the case of "distinct novelty," which is not related to prior knowledge and hence cannot be integrated into existing memory traces. As demonstrated by Takeuchi et al. (2016), optetrode recordings from VTA-TH^+ and LC-TH^+ neurons presented a response to novelty, introduced by five minutes of open-field exploration with novel floor substrates, in terms of increased firing rates. However, the change in firing rates of LC-TH^+ neurons was much more pronounced than that of VTA-TH^+ neurons. The responses of LC-TH^+ neurons eventually habituated to the novelty in contrast to VTA-TH^+ neurons, which is an effect in support of the behavioural tagging experiments showing the indispensability of novel characteristics of the open field in order to have the memory enhancement effect (Moncada & Viola, 2007). In addition, the time window for novelty-induced enhancement of memory persistence might be different between the VTA-hippocampus and LC-hippocampus systems. The LC-hippocampus system produces a more widespread memory enhancement within a few hours' time window, in line with the STC hypothesis. In contrast, the VTA hippocampus might produce a narrower time window for memory enhancement (McNamara et al., 2014; Moncada, 2017). Recently, Moncada (2017) showed that electrical stimulation of VTA and/or LC before or after a weak memory encoding had impact on enhancement of memory persistence in an inhibitory avoidance and a spatial object recognition task in rats. In contrast, Takeuchi et al. (2016) showed that optogenetic activation of VTA was not able to mimic the beneficial effect of novelty, and pharmacological blockade of VTA during novelty exploration did

not have any effect on novelty-induced memory boosts in the everyday memory task in mice (Takeuchi et al., 2016). One possible explanation of this discrepancy is based on the interesting results of a recent study (Weidner et al., 2019), which showed that electrical stimulation of VTA induces much higher self-stimulation pressing rates in mice compared with optogenetic stimulation at same intensities. However, they reported that under effectively matched (iso-behavioural) stimulation strengths, the activity pattern of both stimulation methods yields very similar results. Hence, the usage of different stimulation methods with unmatched intensities and hence perhaps resulting in under- or over-stimulation of VTA in either one of the studies could be potentially responsible for this discrepancy. Finally, it is worth mentioning that both methods carry the risk of causing unspecific neuronal activation. Electrical stimulation at high intensities is likely to result in widespread stimulation without cell-type specificity in the brain, whilst optogenetic stimulation, which is believed to be restricted to the specific neuron types, may cause unexpected neuronal activation in the case of Th-Cre mice, as Cre-mediated recombinations have also been detected in the inhibitory γ-aminobutyric acid-containing (GABAergic) neurons of the VTA in addition to dopaminergic neurons (Lammel et al., 2015). Thus, for both methods, the results should be interpreted carefully. Keeping these facts in mind, further studies with more precise approaches will be helpful for identifying the different roles of VTA and LC.

Although behavioural tagging continues to be a solid explanation for novelty-induced LTM formation, there have been arguments against the hypothesis. An alternative view on the observations regarding behavioural tagging is discussed in the paper by Korz (2018), who argues that this phenomenon can be explained instead by changes in neuronal intrinsic excitability, unless the behavioural observations are not supported by electrophysiological evidence of synaptic changes. It is argued that increased intrinsic excitability of a group of neurons due to learning and memory consolidation of the first behavioural event (novelty in case of behavioural tagging) could make these neurons more prone to be chosen for memory allocation for the second learning task. However, a study by Rossato et al. (2018) effectively disproves the intrinsic excitability explanation for LTM formation with interesting electrophysiological and behavioural results, showing that LTP and encoding of spatial memory in the delayed-matching-to-place task in the water maze are not impaired in the presence of low doses of the GABA type A ($GABA_A$) receptor agonist muscimol, which in fact blocks cell firing (blocks neuronal excitability) and retrieval of spatial memory. Hence, observations from behavioural tagging experiments cannot be simply attributed to changes in neuronal intrinsic excitability.

5 | SYNAPTIC TAG

According to the STC hypothesis, the synaptic tag must fulfil several criteria (Redondo & Morris, 2011). As previously described, the "synapse is tagged" during E-LTP. As such, some defining aspects must be shared between the induction of E-LTP and the tagging of the synapse. (a) Both LTP and STC processes are highly compartmentalized and thus the synaptic tag must be synapse-specific/locally restricted. (b) A synapse can become tagged during E-LTP; thus, the process must be protein synthesis-independent. (c) A time window has been observed during which tetanization of the secondary independent pathway results in the conversion of E-LTP into L-LTP, and thus, the tag must be transient and reversible. In addition, certain cellular conditions can "reverse the tag" (Sajikumar & Frey, 2004b). (d) Lastly, and perhaps most importantly, the tag must be able to interact with or capture PRPs. However, this criterion is not based on empirical data. Rather, it is a logical conclusion when defining a factor capable of converting E-LTP into L-LTP and the indispensable function of a candidate for such a process.

Over the last 20 years, *ex vivo* experiments using the two-pathway set-up in hippocampal slices, combined with behavioural tagging experiments *in vivo,* have revealed many factors involved in the setting of a synaptic tag (Table 1). However, many of these do not satisfy all criteria proposed above. Indeed, without a single candidate that fulfils all necessary criteria, the initial STC hypothesis prediction of a single-synaptic tag molecule has been challenged. This challenge is further supported by an increasing number of factors that are indispensable for STC process (Martin & Kosik, 2002). It is becoming increasingly evident that a multitude of factors form the synaptic tag. Thus, a potentiated synapse might be considered as being in a "tagged state" rather than being "tagged." Among the candidates most central to the synaptic tags are CaMKII, TrkB and PKA.

5.1 | CaMKIIα/β

The Ca^{2+}/CaM-dependent protein kinase family consists of four types (I, II, IV and K). The catalytic domain is highly conserved between these, making distinction between them using a pharmacological inhibitor difficult. Furthermore, CaMKII exists in four different isoforms (α, β, γ and δ) (Swulius & Waxham, 2008). Redondo et al. (2010) solved the issue of specific inhibition of CaMKII using KN-93 at an optimized concentration. Therefore, they were able to shed light on the distinct roles of CaMKII and Ca^{2+}/CaM-dependent protein kinase kinase (CaMKK) and it became clear that CaMKII was a candidate for a specific synaptic tag . This is further supported by behavioural tagging studies (Moncada et al., 2015).

Several criteria for being the synaptic tag are met by CaMKII. [1] Firstly, CaMKIIα and β have been shown to accumulate in activated spines. This effect is highly specific to the activated spine and lasts for more than 30 min (Mikuni et al., 2016). This is further supported by the highly localized gradient of Ca^{2+} near an activated synapse (Rios & Stern, 1997; Zaccolo et al., 2002) and the close proximity of CaMKII to both local actin filaments and NMDA receptors (Shen & Meyer, 1999; Otmakhov et al., 2004). These two factors make CaMKII location and activation specific to the PSD. [2] The involvement of CaMKII in the synaptic tagging process has been shown to be protein synthesis-independent (Redondo et al., 2010). [3] The activation of CaMKII is involved in the induction of both functional LTP and structural LTP (Harward et al., 2016; Saneyoshi et al., 2019). It was initially believed that CaMKII, through autophosphorylation, could remain activated, independent of Ca^{2+}/CaM, for up to one hour (Barria et al., 1997; Lisman et al., 2002). Further support for the importance of CaMKII autophosphorylation has been shown; for example, when removing the autophosphorylation site, functional LTP is inhibited. Further, the phosphorylation site is indispensable in behavioural experiments as without it, memory is impaired (Giese et al., 1998). However, later studies have shown that autophosphorylation is not important for maintaining either functional LTP or structural LTP. Rather, CaMKII activation is essential only during the induction of both functional LTP and structural LTP, and inhibition after induction has no effect (Buard et al., 2010; Chang et al., 2017). In fact, CaMKII activation was shown by Chang et al. (2017) to only be active for less than one minute. This was further refined by applying optogenetic inhibition of CaMKII using the light-inducible CaMKII inhibitor "PaAIP2," which binds to the kinase domain of CaMKII and inhibits kinase activity. Murakoshi et al. (2017) show that CaMKII activation is only important for functional LTP and structural LTP during the first minute after LTP induction. There is a clear discrepancy between earlier pharmacological studies and later studies, but on the whole, later findings suggest that the activation of CaMKII is important for induction but not for the maintenance of functional LTP and structural LTP.

A recent study suggested that Ca^{2+}/CaM-activated CaMKII can form a reciprocally activating kinase-effector complex (RAKEC) with T-lymphoma invasion and metastasis-inducing protein 1 (Tiam1), which stably activates Rac1, a factor required for maintaining structural LTP (Saneyoshi et al., 2019). This complex is formed when Ca^{2+}/CaM activation of CaMKII relieves the binding of the autoinhibitory domain, thus allowing the binding of a pseudo-autoinhibitory domain of Tiam1. This results in a constitutively active form of CaMKII. Whilst the activation of CaMKII was traditionally thought to be dependent on autophosphorylation (Lisman et al., 2002), the RAKEC activation of CaMKII was observed to be independent. The RAKEC has been shown to increase the duration of CaMKII activity to last for 10 min, but with a fraction remaining active after 30 min. Activated CaMKII phosphorylates and activates Tiam1, which is the actin regulating Rac-specific guanine exchange factor (RacGEF), resulting in its persistent activity, which, in turn, causes activation of Rac1 subsequently resulting in actin remodelling to maintain structural LTP.

Interestingly, it has been shown that, whilst CaMKII and Tiam1 form RAKEC, CaMKII becomes less sensitive to the light-inducible CaMKII inhibitor PaAIP2 and this is caused by Tiam1 blocking the binding site. This effect was not observed using a fluorescence resonance energy transfer (FRET) probe for CaMKII (Takao et al., 2005; Lee et al., 2009), and this is likely due to the large quantity of CaMKII in PSD and the relatively small amount of Tiam1. Due to the large difference in CaMKII and Tiam1 concentrations and a one-to-one interaction between them, only a fraction of CaMKII will form RAKEC. As new studies that utilize novel methods reveal more information about the distinct temporal roles of CaMKII activation, we learn more of the complexity of the process. It is difficult to conclude with certainty due to this complexity. However, based on the latest research, two independent signalling pathways for CaMKII are involved in functional LTP and structural LTP. One is essential for LTP induction, is short-lived and dependent on autophosphorylation. Another is autophosphorylation-independent and is facilitated through the RAKEC formation, which stays active for at least 10 min after LTP induction. Based on this, the activity of CaMKII/Tiam1 appears to fit well with the time window for the STC hypothesis.

CaMKII does not fit the final criterion [4] as there is no evidence that the active form of CaMKII captures any plasticity-related proteins. Concluding on CaMKII's role as a synaptic tag becomes more interesting, and difficult, as more information reveals independent pathways with different activation time windows. Taken together, these findings suggest that CaMKII is involved in setting the synaptic tag, during a short burst in CaMKII activation, which mediates the signal and activates downstream targets, whilst a fraction of CaMKII forms a RAKEC, lasting for a longer activation period of time, and facilitates the reorganization of the actin cytoskeleton. This indicates that CaMKII activity is at the centre of both the initial changes occurring during LTP induction, whilst also remaining active after induction and facilitates more changes that are indispensable for persistence of structural LTP.

As KN-93 is believed to bind to the catalytic domain specifically (Sumi et al., 1991), two-pathway experiments and behavioural studies do not give any information about the involvement of specific CaMKII isoforms as it is preserved between isoforms. Whereas CaMKIIγ and δ-isoforms have been shown to be associated with the nucleus (Zalcman et al.,

2018), the α- and β-isoforms are mostly compartmentalized to PSD (Liu & Murray, 2012) and have been shown to co-localize (Ochiishi et al., 1994) and form heteromeric holoenzymes in excitatory synapses (Shen et al., 1998; Brocke et al., 1999). There is one major aspect by which CaMKIIα and β differ: CaMKIIβ contains a protein scaffolding domain, capable of binding other proteins, including actin filaments (Sanabria et al., 2009). The binding of CaMKIIα/β complex to actin filaments has been shown to greatly increase filament stability (Okamoto et al., 2007; Lin & Redmond, 2008). Upon tetanization, the heteromeric CaMKIIα/β complex releases from the actin filaments. The CaMKIIα/β complex then translocates to the postsynaptic membrane and forms the RAKEC with Tiam1 (Strack et al., 1997; Otmakhov et al., 2004; Hudmon et al., 2005; Saneyoshi et al., 2019). This frees up the tightly bound actin filaments, which allow interactions with Rho-GTPases, such as Rac1, leading to remodelling of the cytoskeleton (Okamoto et al., 2007; Okamoto et al., 2009; Zalcman et al., 2018).

Although these functional properties of the CaMKIIα and β-isoforms have not been confirmed using two-pathway experiments, the findings suggest a possible mechanism in which the cooperation of CaMKIIα and β-isoform is implicated in the STC process. Interestingly, an inactive form of CaMKIIβ was shown to interact with Arc, which is involved with weakening synapses, by the removal of AMPA receptors from the postsynaptic site (Okuno et al., 2012). This implies that the active form of CaMKIIα and β is involved in strengthening the synapse upon LTP induction and the inactive form of CaMKIIβ is involved in weakening the synapse if it remains inactive, suggesting that CaMKII is right at the centre of synaptic tag regulation.

5.2 | TrkB

TrkB, the receptor for brain-derived neurotrophic factor (BDNF), was proposed by Lu et al. (2011) as a potential candidate for the synaptic tag following experiments using the $TrkB^{F616A}$ knock-in mouse line. Here, the $TrkB^{F616A}$ protein was specifically and reversibly inhibited by the compound 1NMPP1 (Chen et al., 2005) showing that TrkB is required for converting E-LTP and STM into L-LTP and LTM, respectively (Lu et al., 2011). TrkB fulfils several criteria for being a candidate for the synaptic tag. [1] TrkB activation is spatially limited in two ways: BDNF signalling is local with limited diffusion (Nagappan et al., 2009), and TrkB phosphorylation is restricted to BDNF release sites (Lu et al., 2011). [2] Inhibiting TrkB with anisomycin showed that TrkB can be activated in a protein synthesis-independent manner (Lu et al., 2011). [3] The activation of TrkB, through transient phosphorylation, was shown to last for at least 60 min, matching the temporal window of the synaptic tag (Lu et al., 2011; Harward et al., 2016). [4] The known PRP candidate BDNF is a biological ligand for the TrkB receptor (Barco et al., 2005; Lu et al., 2008). During induction of structural LTP by glutamate uncaging, BDNF released from postsynaptic spines and postsynaptic TrkB became activated within minutes (Harward et al., 2016). This suggests that the existing pool of available BDNF in postsynaptic spines is involved in the synaptic tagging process, rather than newly synthesized BDNF. The dual function of pre-existing BDNF and newly synthesized BDNF as a PRP has been described by Barco et al. (2005). Here, they characterize an important role for BDNF in both induction and maintenance of functional LTP at CA3-CA1 synapses. These results suggest that an early initial release of BDNF could be involved with setting the tag, whilst the later effect might require *de novo* BDNF synthesis and be involved in converting E-LTP into L-LTP. However, known TrkB and BDNF interactions are extracellular receptor-ligand binding and do not provide an explanation of synapse-specific transport and capture of BDNF in the potentiated spines. Thus, this should not be thought of as evidence that TrkB captures newly synthesized PRPs. Therefore, further studies are required to provide evidence of the synapse-specific transport and capture of newly synthesized BDNF by TrkB in tagged spines.

The activation of TrkB in a single dendritic spine via glutamate uncaging is dependent on activation of NMDA receptors and CaMKII, suggesting that TrkB activation occurs downstream of CaMKII (Harward et al., 2016). Although these findings provide much evidence for interactions between candidates for the synaptic tag during structural LTP, they do not demonstrate heterosynaptic LTP similar to that found in the two-pathway experiments. A further two-pathway experiment would be necessary for determining whether interaction between TrkB and CaMKII is indispensable for the synaptic tagging process to occur.

5.3 | Protein kinase A

Two-pathway experiments that use the adenylate cyclase inhibitor KT 5720 to indirectly inhibit PKA activity results in impaired conversion of E-LTP to L-LTP (Young et al., 2006). Several criteria for the synaptic tag are filled by PKA. [1] The activity of PKA is compartmentalized by two factors. Firstly, the local production of cAMP limits the activation to a specific area (Tasken & Aandahl, 2004), and secondly, A-kinase-anchoring proteins (AKAPs) play a major role in regulating PKA activity. In fact, two-pathway experiments using AKAP-specific inhibitors revealed that microdomains of PKA signalling are indispensable for the synaptic tagging process (Huang et al., 2006). Recently, Tang and Yasuda (2017) reported that glutamate uncaging in single dendritic spines of a CA1 pyramidal neuron induced transient PKA

activation (~5 min) during induction of structural LTP. They also observed that PKA activation spread more than 10 μm. [2] The activation of PKA appears to be protein synthesis-independent, but PKA is required for L-LTP presumable via regulation of protein synthesis (Kandel, 2001; Abel & Nguyen, 2008). [3] By binding of cAMP, PKA is reversibly activated, thereby providing a restriction of PKA activation to cAMP availability. [4] There is no evidence that PKA captures PRPs at the potentiated spines.

The roles of PKA in the synaptic tagging process are interesting as PKA interacts with several other candidates. PKA indirectly enhances the kinase activity of CaMKII by phosphorylating inhibitor-1, which is a potent inhibitor of protein phosphatase-1 (PP1), thus prolonging the effects (Blitzer et al., 1998). PKA also regulates synaptic strength by phosphorylation of AMPA and NMDA receptors (Raman et al., 1996; Lee et al., 2000; Esteban et al., 2003; Skeberdis et al., 2006). Phosphorylation of AMPA receptors also regulates receptor sorting, which determines whether the receptor will be reinserted or degraded (Ehlers, 2000). Phosphorylation of stargazin [transmembrane AMPA receptor regulatory protein γ-2 (TARP γ-2)] affects both the trafficking and channel activity of AMPA receptors (Chetkovich et al., 2002; Choi et al., 2002; Shaikh et al., 2016). In addition, PKA regulates the LIM domain kinase (LIMK) pathway, which is involved in actin remodelling (Nadella et al., 2009). Furthermore, TrkB phosphorylation is gated by cAMP, implicating a role for PKA (Ji et al., 2005) and activation of PKA increases TrkB phosphorylation (Patterson et al., 2001). These findings suggest that PKA is involved in the functional and structures changes that occur during E-LTP. Park et al. (2014) discovered another critical function of the compartmentalization of PKA, and by extension of AKAPs. In their study, it was observed that both presynaptic PKA and postsynaptic PKA are indispensable for the STC process. This is presumably mediated by postsynaptic release of pre-existing BDNF, which activates presynaptic PKA via presynaptic TrkB. Presynaptic PKA activity is important for maintaining the presynaptic pool of readily releasable synaptic vesicles.

6 | PLASTICITY-RELATED PROTEINS

According to the STC hypothesis (Redondo & Morris, 2011), PRPs must also fulfil several criteria to be considered as candidates. Candidate PRPs should [1] be synthesized and distributed by strong tetanization, especially in a manner dependent on activation of dopamine D_1/D_5 receptors, [2] be captured by tagged synapses and [3] contribute to the ultimate stabilization of functional and structural synaptic changes.

6.1 | The synthesis of PRPs

PRPs are translated from mRNA by tetanization or activation of dopamine D_1/D_5 receptors. It is still unclear whether this mRNA is newly synthesized by transcription and/or is pre-existing. The transcription inhibitors actinomycin D and 5,6-dichloro-1-β-D-ribofuranosyl-1H-benzimidazole both inhibit homosynaptic L-LTP in electrophysiological experiments (Nguyen et al., 1994; Frey et al., 1996). Likewise, actinomycin D inhibits heterosynaptic L-LTP in two-pathway experiments. The application of actinomycin D during strong tetanization prevented L-LTP expression, but not during weak tetanization (Young & Nguyen, 2005). In addition, the CaMKK inhibitor, STO-609, can inhibit L-LTP in two-pathway experiments (Redondo et al., 2010). The CaMKK-"Ca^{2+}/CaM-dependent protein kinase IV" (CaMKIV) pathway mediates a cAMP response element binding protein (CREB) phosphorylation followed by the initiation of gene transcriptions. Therefore, it is predicted that gene transcription via the CaMKK-CaMKIV pathway might be required for the synthesis of PRPs. However, there is no evidence as to whether PRP synthesis requires only new gene transcription. Future studies should elucidate whether newly synthesized and/or pre-existing mRNA is required for PRP synthesis.

It is also still unclear where within the neuron PRPs are translated by tetanization or activation of dopamine D_1/D_5 receptors. There are two possibilities: translation in the soma or local translation in the dendrite close to tagged synapses. Local translation close to tagged synapses is predicted to have an important role in PRP synthesis (Govindarajan et al., 2011). In fact, Govindarajan et al. (2011) reported that the efficiency of the STC phenomenon was negatively correlated with the distance between two synapses stimulated with the combination of caged glutamate and forskolin on the same dendritic branch. In addition, the efficiency of STC phenomenon between two synapses stimulated with caged glutamate and forskolin independently on different dendritic branches was lower, compared with the same dendritic branch. On the other hand, there is a report that a PRP candidate, Homer1a, is synthesized in the soma and is subsequently transported to a tagged spine stimulated by microperfusion of glutamate (Okada et al., 2009). The methods for stimulation of spines and the protocol for preparing cultures were not unified in these experiments. Although it may be necessary to consider its effect, both local translation in close proximity of tagged synapses and translation in the soma might be important for *de novo* PRP synthesis.

Recently, Brigidi et al. (2019) reported that EPSPs in CA1 pyramidal neurons in mice *ex vivo* or novel experiences *in vivo* led to dendritic translation of neuronal PAS domain-containing protein 4 (NPAS4) and Aryl hydrocarbon receptor nuclear translocator 1 (ARNT1), which is of the Arnt subfamily of transcription factors, within five minutes and steadily

TABLE 2 PRP candidates from electrophysiological and behavioural studies

Candidates	Molecular function relevant to functional or structural plasticity	Two-pathway experiments	Electrophysiology article	Behavioural tagging experiment	Behavioural tagging article	Optical imaging experiments	Optical imaging article
BDNF	Modulate NMDA receptor, AMPA receptor and PSD-95 Spine enlargement	Yes	Barco et al. (2005)	No	–	No	–
ACVR1C	–	Yes	Park et al. (2017)	No	–	No	–
PKMζ	Regulation of GluA2-containing AMPA receptor trafficking and degradation	Yes	Sajikumar & Korte (2011)	No	–	No	–
Homer1a	Interactions with PSD scaffolding proteins Interferes with spine enlargement	No	–	No	–	Yes	Okada et al. (2009)
Arc/Arg3.1	F-actin stabilization Induction of AMPA receptor endocytosis at inactive synapses via interaction with inactive CaMKIIβ (Inverse tagging)	No	–	Yes	Tomaiuolo et al. (2015)	Yes	Okuno et al. (2012)

increased to 90 min. Dendritically synthesized NPAS4 dimerized with ARNT1 was transported to the nucleus and engaged the enhancers at the upstream side of genes relevant to synaptic plasticity. In stark contrast, action-potential-induced NPAS4 required *de novo* transcription in the nucleus and association with ARNT2 to regulate the promoters of housekeeping genes. This study warns us that artificial manipulation of the membrane potential can undoubtedly skew the ratio of NPAS4/ARNT1 and NPAS4/ARNT2, obscuring molecular mechanisms of the STC process, especially the identification of PRPs.

Several molecules that have been suggested as candidate PRPs by various studies are listed in Table 2. However, not all of them fulfil the PRP criteria and some lack evidence from two-pathway and/or behavioural tagging experiments. In the following section, we briefly discuss the most likely candidates.

6.2 | BDNF

One of the most likely PRPs is BDNF as it has been implicated in synaptic plasticity and learning and memory (Lu et al., 2008). Furthermore, its receptor, TrkB, is assumed to be one of the contributors of synaptic tagging processes, hence making BDNF a target for PRP studies. However, the interaction between BDNF and its receptor TrkB should be interpreted with caution as discussed in Section 5.2.

In the two-pathway experiments by Barco et al. (2005), strong tetanization of the first pathway followed by weak tetanization of the second pathway was performed on hippocampal slices *ex vivo* using two different lines of genetically modified mice. One BDNF knockout mouse line in which the forebrain, including the CA3 and CA1 areas, lacked BDNF resulted in a significant decrease in the induction of E-LTP and STC process-mediated maintenance of L-LTP in the second pathway. In contrast, the induction of E-LTP was not affected using postsynaptic CA1 area-specific BDNF knockout mice, yet STC process-mediated maintenance of L-LTP was reduced in the second pathway. These results suggest that BDNF may play a dual role in the STC process. First, newly synthesized postsynaptic BDNF might be responsible for STC process-mediated maintenance of L-LTP. Second, the presynaptic release of BDNF into the synaptic cleft after tetanic stimulation may participate in the synaptic tagging in postsynaptic potentiated spines in addition to postsynaptic autocrine BDNF (Harward et al., 2016). Interestingly, strong tetanization in the first pathway produced normal L-LTP in both BDNF knockout mice lines reported by Barco et al. (2005), raising the possibility that there can be different tagged states and/or different kind of PRPs that are captured by tagged spines depending on the tetanization pattern. Evidence from behavioural studies points to the role of BDNF

in expression and persistence of LTM, as demonstrated in inhibitory avoidance (Alonso et al., 2002) and contextual fear conditioning (Liu et al., 2004). Further evidence is required to confirm BDNF as a PRP regardless of its influence as a ligand of TrkB.

It has been suggested that BDNF modulates NMDA receptors (Alonso et al., 2002) and PSD-95 (Yoshii & Constantine-Paton, 2007) as well as influences the expression and phosphorylation of AMPA receptors (Carvalho et al., 2008) in the postsynapse through TrkB activation. As shown by Tanaka et al. (2008), using two-photon glutamate uncaging with the application of postsynaptic spikes for single identified spines of CA1 pyramidal neurons in hippocampal slice culture, BDNF induces spine enlargement in manner dependent on both postsynaptic activity and protein synthesis, accompanied by an increase in glutamate-induced currents.

6.3 | ACVR1C

The activin A receptor type 1C (ACVR1C), a component of the transforming growth factor-β receptor superfamily, is a candidate that fulfils PRP criteria [1] and [3]. It is a receptor complex that phosphorylates the transcription factor Smad upon activation (Bondestam et al., 2001). Park et al. (2017) nicely demonstrated the role of ACVR1C by using two-pathway experiments combined with pharmacological interventions and translin knockout mice. Translin/trax is an RNA-binding complex, which is stable under the presence of both proteins. It has been implicated in dendritic trafficking and translation of RNAs (Swulius & Waxham, 2008), but recent studies have revealed its role in suppressing microRNA-mediated translational silencing (e.g. Asada et al. (2014)). Translin KO mice were not able to maintain either heterosynaptic L-LTP in two-pathway experiment or LTM in an object-location memory task. In addition, translin KO mice did not exhibit synaptic upregulation of ACVR1C protein levels following training in an object-location memory task compared with wild-type littermates, suggesting that translin/trax protein complex mediates the suppression of the microRNA silencing of ACVR1C expression. Furthermore, pharmacological inhibition of ACVR1C was shown to mimic phenotypes of translin KO mice: deficits in both maintenance of heterosynaptic L-LTP of a weekly tetanized pathway in the two-pathway experiment and LTM in the object-location memory task. Interestingly, the inhibition of ACVR1C did not affect L-LTP of strongly tetanized pathway, similar to two-pathway experiments using BDNF knockout mice in which the forebrain, including the hippocampal CA3 and CA1 areas, lacked BDNF (Barco et al., 2005). Further studies of ACVR1C and its potential role as a PRP are required, given that there are no behavioural tagging experiments currently reported.

6.4 | PKMζ

Another candidate PRP that has been addressed by many studies is the atypical protein kinase C (PKC) isoform, PKMζ. Studies show that PKMζ is indispensable for the persistence of L-LTP (Sajikumar et al., 2005) and LTM in a place-avoidance task (Pastalkova et al., 2006) and an object-location task (Migues et al., 2010) as inhibition of PKMζ by "ZIP," which is a synthetic cell-permeable peptide designed based on the endogenous PKCζ pseudosubstrate sequence (Sadeh et al., 2015), impairs these processes. It has been suggested that PKMζ exerts its LTP- and LTM-persistence effects through the regulation of trafficking of AMPA receptors containing the glutamate receptor subunit 2 (GluA2) in activated synapses (Ling et al., 2006; Migues et al., 2010). Studies that have assessed PKMζ functions have all used ZIP. However, there is no good evidence of ZIP selectivity and several studies show nonselective effects of ZIP, such as neuronal silencing, (LeBlancq et al., 2016). Furthermore, results from those studies that used ZIP have been contradicted by studies using PKMζ knockout mice (Lee et al., 2013; Volk et al., 2013). Volk et al. (2013) reported that PKMζ was not indispensable for either induction or maintenance of LTP in the CA1 area of the hippocampus and that application of ZIP reduced baseline transmission in PKMζ knockout mice. In addition, the knockout mice showed normal memory in hippocampus-dependent contextual and trace fear conditioning tests (Lee et al., 2013; Volk et al., 2013). Similarly, Lee et al. (2013) reported that knockout of PKMζ had no impact on learning and memory including cued fear conditioning, object-location recognition, novel object recognition and motor learning. Although a possible explanation of the results of knockout and acute knockdown by PKMζ antisense studies came from Tsokas et al. (2016), who suggested that PKMζ function may be compensated by a related protein PKC ι/λ in PKMζ knockout mice, further studies using various methods are required to validate PKMζ as a PRP. Hence, it is important not to rely on one single method to assess functions of candidate PRPs.

6.5 | Homer1a

Homer1a (Vesl-1S), a short isoform of Homer1 that is known to be upregulated following LTP (Kato et al., 1997), is a candidate PRP. A study by Okada et al. (2009) used optical imaging of rat hippocampal primary cultures to demonstrate that soma-derived Homer1a was captured by activated spines in an input-specific manner, fitting the STC hypothesis. However, the evidence from two-pathway experiments and behavioural tagging studies is still lacking, making it difficult to address Homer1a as a PRP. Although behavioural tagging studies on Homer1a are missing, there is evidence showing

that its expression is upregulated upon contextual fear learning and novel context exploration (Clifton et al., 2017), and that it is required for successful LTM formation but not for STM in Home1a-specific knockout mice (Inoue et al., 2009).

Homer1a is implicated in the regulation of synaptic plasticity, acting as a dominant-negative regulator, where it disrupts interactions between long, constitutively expressed Homer scaffolding proteins and their effector proteins and affects Ca^{2+} gating (Clifton et al., 2019). Further evidence suggests that Homer1a reduces the density and size of dendritic spines in cultured hippocampal neurons and diminishes AMPA- and NMDA-receptor-mediated postsynaptic currents (Sala et al., 2003). As Homer1a was chronically overexpressed in this study, it is difficult to attribute this downscaling effect of Homer1a as the function of PRPs that are acutely synthesized after a strong tetanization. Further studies using methods to control the temporal expression of Homer1a are required to confirm Homer1a as a PRP.

6.6 | Arc

There is evidence that Arc/Arg3.1 is essential for LTP maintenance and memory consolidation (Guzowski et al., 2000; Plath et al., 2006). In Plath et al. (2006), Arc knockout mice had impaired maintenance of L-LTP and L-LTD and LTM formation in an object recognition task, whilst the expression of E-LTP, E-LTD and STM was intact. Another study (Messaoudi et al., 2007) showed that Arc inhibition *in vivo* by Arc antisense oligodeoxynucleotides infusions into the dentate gyrus 2 hr, but not 4 hr, after high-frequency stimulation reversed E-LTP. The significance of the time point of antisense oligodeoxynucleotides application, with relevance to the critical time window described in the STC hypothesis, might address the involvement of Arc in L-LTP expression as a PRP even though the results were not demonstrated in two-pathway experiments. This same study further reported that Arc inhibition dephosphorylated cofilin, the major actin regulator protein, and impaired F-actin stabilization in postsynaptic sites. Actin dynamics are known to be crucial for maintaining structural plasticity changes in the synapse, and this is listed as one of the major synaptic tagging components in Table 1. Evidence for Arc as a potential PRP has also been demonstrated in a behavioural tagging study (Tomaiuolo et al., 2015). Here, Arc expression in the dorsal hippocampus was shown to be indispensable for novelty-induced memory enhancement of weak inhibitory avoidance encoding as the memory-boosting effect of novelty was prevented by intrahippocampal CA1 infusions of Arc antisense oligonucleotides.

In contrast to studies suggesting a promoting role for Arc in synaptic plasticity, and learning and memory, there are also studies that attribute a demoting role. Several studies (Chowdhury et al., 2006; Shepherd et al., 2006) speculate that Arc may instead be involved in homeostatic downregulation by inducing glutamate-receptor-subunit-1-(GluA1)-containing AMPA receptor endocytosis. In fact, optical imaging studies by Okuno et al. (2012) report that Arc proteins, which were newly synthesized upon BDNF stimulation in cultured hippocampal CA1/CA3 neurons *in vitro*, accumulated in dendritic shafts, whilst inhibition of synaptic activity in these previously active spines resulted in Arc accumulation in these inactive synapses via the preferential binding of the inactivated form of CaMKIIβ. In a complex with CaMKIIβ, it has been suggested that Arc induces AMPA receptor endocytosis and synapse weakening in a process called "inverse tagging."

Finally, a very recent study (Kyrke-Smith et al., 2020) investigating the role of Arc in L-LTP *ex vivo* and *in vivo* by using two different Arc knockout mice lines ($Arc^{GFP/GFP}$ and $Arc^{-/-}$) and a conditional Arc knockout mouse line ($Arc^{fl/fl}$) reported that Arc is dispensable for the maintenance of L-LTP in the CA1 and the dentate gyrus. Theta-burst stimulation at CA3-CA1 synapses *ex vivo* in both $Arc^{GFP/GFP}$ and $Arc^{-/-}$ mice, which induced L-LTP, resulted in significantly higher L-LTP magnitude compared with wild-type control. In contrast, the conditional Arc knockout in CA1 excitatory neurons did not show any significant differences in L-LTP magnitude compared with wild types. This result possibly indicates compensatory mechanisms in conventional Arc knockout mice. They also investigated the effect of Arc knockout ($Arc^{GFP/GFP}$) on L-LTP induced by theta-burst stimulation in medial perforant path-granule cell synapses in the dentate gyrus *in vivo*. The results were in accordance with the previous ones, such that Arc knockout mice did not show impairment in the maintenance of L-LTP. It is hard to conclude on the exact role of Arc based on the current results that used different approaches. The most likely case is the involvement of Arc in the reduction in synaptic strength, perhaps through "inverse tagging," thus likely contributing to synaptic plasticity and learning.

7 | CONCLUSION AND FUTURE PERSPECTIVES

We have reviewed a large number of studies on the STC hypothesis that used electrophysiological two-pathway and optical imaging of single spine experiments *in vitro* and *ex vivo* and behavioural tagging experiments *in vivo*. We then followed this by evaluating current candidates for both synaptic tags and PRPs.

Although two-pathway and behavioural tagging experiments have been the predominant methods used to determine the particular factors involved in STC process, there are some limitations to them. Using a simple system where

a reversible pharmacological inhibitor is applied during the tetanization has proven to be a comprehensive task. Additional creativity is required to specifically manipulate candidates, like CaMKII, who share large similarities in catalytic domain with other kinases. Utilizing different inhibitor affinities at various concentrations, Redondo et al. (2010) overcame this issue. However, with recent research revealing insights regarding the specific function of an inhibitor, questions have arisen. For example, KN-93, which is thought to bind the CaMKII's catalytic domain, seems to bind CaM (Wong et al., 2019). This changes the ability of CaM to bind to downstream factors, which might be associated with unforeseen biological effects of the drug. However, there is currently no empirical evidence that this is the case. This is not the case for the PKMζ and the inhibitor ZIP, traditionally used to interfere with its function. As discussed earlier, the lack of specificity of ZIP-mediated inhibition has been addressed with substantial discrepancies between ZIP-mediated inhibition and knockout studies (Lee et al., 2013; Volk et al., 2013; LeBlancq et al., 2016; Tsokas et al., 2016). To avoid similar issues in the future, methods used should be given good consideration and single-method approaches should be avoided. Integrating other methods for manipulation of candidates, both synaptic tags and PRPs, should be considered too. The utilization of the $TrkB^{F616A}$ mouse line is a good example of a system with which the activity of the candidate can be controlled with a high specificity. Future studies may utilize other cutting-edge experimental tools, such as optical methods for rapidly controlling the induction or degradation of specific proteins (Zhou et al., 2015).

Furthermore, two-pathway experiments have been vital for investigating the heterosynaptic LTP associated with the STC process. However, information obtained has been mostly limited to identification of candidates, compartmentalization and timing, but not molecular mechanisms. Among the factors that have been identified as indispensable for the STC process, most of them are considered upstream or downstream of setting the synaptic tag. In fact, most well-studied candidates, such as the protein kinase CaMKII and PKA, are believed to be involved in setting the synaptic tag rather than *being* the actual tag. Despite the many interesting findings and candidates that fulfil several of the original synaptic tag criteria, many candidates only fulfil criteria related to temporal and spatial regulation. Whilst these capacities are important for a functional tag, candidates fulfilling the criteria of the synaptic tag, which interact with and capture PRPs in potentiated spines, remain elusive.

One possible direction to elucidate molecular mechanisms of the STC process is through studies designed to identify PRPs that have increased availability due to dopaminergic signalling. In these studies, PRP candidates should be screened by cell-type-specific isolation of ribosome-associated mRNA (Sanz et al., 2009) with RNA sequencing to identify CA1 pyramidal neuron-specific *de novo* translation of mRNAs induced by novelty exploration or optogenetic activation of LC that have already established the enhancement of memory persistence. The molecular functions of PRP candidates should then be assessed by the instantaneous loss-of-function tools using optical methods for rapidly controlling the induction or degradation of specific proteins (Zhou et al., 2015) that could allow the functional assay with high spatial and temporal resolution. In the functional assay, there is an increasing trend for inducing single-synaptic LTP with glutamate uncaging, and measuring AMPA receptor trafficking and spine volume with optical imaging techniques in rat slice cultures *ex vivo* (Bosch et al., 2014), as it is difficult to measure EPSCs in the single neuron stimulated by glutamate uncaging. However, most of these studies did not use two-pathway or behavioural tagging experiments, thereby leaving a gap in knowledge between these results and the STC hypothesis. Although Govindarajan et al. (2011) reported heterosynaptic LTP by combining the glutamate uncaging with the two-pathway set-up, they did not assess the molecular function of candidates for PRP using their combined two-pathway set-up yet. In the future, functional assays should follow an integrative approach that combines the two-pathway experiment using electrophysiological and optical imaging techniques *ex vivo*, and behavioural tagging experiments *in vivo* with the instantaneous loss-of-function tools using optical methods to manipulate PRP candidates. These experiments could provide crucial information about molecular mechanisms underlying the STC process that are critical processes for initial memory consolidation in the hippocampus.

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CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

TT conceptualized the idea for the manuscript. KO, KH, LP and GB collaborated in writing the first draft. TT made comments and modifications/amendments. All authors edited, read and then approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and that further information with respect to the search process, study identification and reporting will be made available upon request.

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