COMPUTATIONAL EXPLORATIONS OF MEMORY CONSOLIDATION, MEMORY RECONSOLIDATION, AND RELATED PHENOMENA

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Contents

ABSTRACT

The term *memory consolidation* is used to describe two different groups of phenomena, on the one hand a family of fast intra-cellular processes believed to stabilize new memory traces, and on the other hand larger-scale and slower processes whereby new memory traces, initially hippocampus-dependent, are reorganized and gradually become independent of the hippocampus. To avoid confusion, the former type is referred to as *synaptic* consolidation and the latter as *systems* consolidation. A related term, *memory reconsolidation*, refers to a temporary instability that memories undergo after retrieval. Like consolidation, reconsolidation has also been observed at both the synaptic and the systems level. An enormous effort has been channeled into understanding these phenomena, and a large volume of data has been collected. Nevertheless, the underlying mechanisms are only partially understood and different explanations have been suggested for many findings. In this dissertation I present two computational models designed to investigate proposed mechanisms of memory consolidation and reconsolidation. The first model concerns mechanisms at the synaptic level and the second addresses systems consolidation and reconsolidation. Both models incorporate mechanisms inspired by recent neuroscience discoveries, allowing them to capture findings not covered by previously published works. Predictions are derived from the models, suggesting experiments that may test their correctness.

RÉSUMÉ

Le terme *consolidation de la mémoire* est utilisé pour décrire deux groupes de phénomènes différents: d'une part une famille de processus intracellulaires rapides censés stabiliser de nouvelles traces de mémoire, et d'autre part des processus à plus grande échelle et plus lents par lesquels de nouvelles traces de mémoire, dépendantes initialement de l'hippocampe, sont réorganisées et deviennent progressivement indépendantes de l'hippocampe. Pour éviter toute confusion, le premier type est appelé consolidation *synaptique* et le second, consolidation *des systèmes*. Un terme apparenté, *reconsolidation de mémoire*, fait référence à une instabilité temporaire que subissent les souvenirs après leur récupération. Une reconsolidation a également été observée au niveau synaptique et au niveau des systèmes. Des efforts considérables ont été consacrés à la compréhension de ces phénomènes, et un grand volume de données a été recueilli. Néanmoins, les mécanismes sous-jacents ne sont que partiellement compris et différentes explications ont été suggérées. Dans cette thèse, je présente deux modèles informatiques conçus pour étudier les mécanismes proposés de consolidation et de reconsolidation de la mémoire. Le premier modèle concerne les mécanismes au niveau synaptique et le second concerne la consolidation et la reconsolidation des systèmes. Les deux modèles incorporent des mécanismes inspirés de découvertes récentes en neuroscience, permettant de reproduire des résultats ne faisant pas partie de travaux publiés antérieurement. Des prévisions sont dérivées de ces modèles, suggérant des expériences susceptibles de vérifier leur exactitude.

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CHAPTER ONE: INTRODUCTION

Memory consolidation is the name given to neural processes that stabilize new memories after acquisition, making them persist for long periods of time. *Memory reconsolidation* refers to processes that restabilize consolidated memories when retrieval has made them temporarily unstable. The subject of this dissertation is the investigation of possible neural mechanisms underlying these phenomena, using methods of computational modeling.

OVERVIEW

The dissertation is organized as follows: The remainder of this chapter introduces the concepts of memory consolidation and reconsolidation at the synaptic and systems levels. This is followed by two chapters each presenting a computational model, the first of synaptic-level phenomena and the second at the systems level. Each of these chapters is introduced by a review of the major scientific discoveries that established our current understanding of synaptic and systems consolidation and reconsolidation, respectively; the presentations of the models themselves are in the form of journal articles, the first published in PLOS Computational Biology (Helfer & Shultz, 2018), the second submitted for publication (Helfer & Shultz, submitted for publication).

MEMORY CONSOLIDATION

Newly acquired memories can be disrupted by a number of different kinds of manipulations including interference training, certain pharmaceuticals, electroconvulsive shock, and surgical procedures, but as time passes memories gradually become resistant to such interventions (Dudai, 2004). The neural processes responsible for stabilizing new memories are collectively known as *memory consolidation*.

Neuroscientists distinguish between two different, though related, types of memory consolidation. The first type concerns subcellular biochemical processes that stabilize enhancements of synaptic strength that come about due to recent neural activity, modifications that are believed to be important mechanisms for memory storage (Cajal, 1894; Kandel, Dudai, & Mayford, 2014; Sossin, 2008). Initially, such activity-induced modifications are labile and susceptible to disruption, but under the right circumstances they may stabilize and persist for very long periods of time (Abraham, 2003; Davis & Squire, 1984; Duncan, 1949). The stabilization of synaptic modifications is called *synaptic* (or *cellular*) consolidation (Dudai, 2004).

The second sense of the term memory consolidation relates to the time-limited involvement of the hippocampus in the life of a memory. Bilateral damage to the hippocampi, areas in the medial temporal lobes of the mammalian brain, has been shown to cause profound loss of recent memories while leaving older memories largely unaffected (Kim & Fanselow, 1992; Scoville & Milner, 1957; Squire & Zola-Morgan, 1991; Winocur, 1990). The observation that memories thus seem to depend on the hippocampus for a limited time after acquisition has inspired the idea that they are initially captured by the hippocampus and then gradually established in other brain areas (McClelland, McNaughton, & O'Reilly, 1995; Milner, 1989; Nadel & Hardt, 2010; Squire & Zola-Morgan, 1991). The putative process responsible for this reorganization is called *systems* memory consolidation, the "systems" qualifier reflecting the notion that this process involves interaction between different brain systems. (An alternative view of systems consolidation, the "multiple trace theory", is discussed in Chapter Three.) In addition to the findings from work with human patients, a time-limited dependence on the

hippocampus for memory recall has been documented in monkeys, rabbits, rats, and mice (Kim, Clark, & Thompson, 1995; Raybuck & Lattal, 2014; Squire, 1992).

The time scales of the two types of consolidation are very different: systems consolidation may take weeks or months to complete (even longer in humans), whereas the process of synaptic consolidation is usually understood to complete within hours (Dudai, 2004; Nader & Einarsson, 2010) – although some cellular changes may continue for days (Dudai, 2012).

MEMORY RECONSOLIDATION

Although the two terms systems and synaptic memory consolidation describe quite different phenomena, they both denote processes that create long-lasting memory traces, and interventions that interfere with either of these processes can cause memory impairments. Once consolidation of a memory trace has completed, however, it is generally no longer vulnerable to such interventions. Consolidation was once thought to be permanent, but interestingly, it has been shown that reactivation (retrieval) of a memory can trigger a temporary return to a labile state in which it is again susceptible to disruption. This state is normally followed by spontaneous restabilization, but interference during the period of instability can produce lasting memory impairments. Forms of such post-reactivation instability have been demonstrated at both the synaptic and the systems levels; the neural processes that destabilize memories after retrieval and then restabilize them are called *synaptic* and *systems memory reconsolidation*, respectively. Synaptic reconsolidation thus denotes a retrieval-induced transient sensitivity to disruptive treatments, and systems reconsolidation refers to reactivation temporarily placing a memory in a state of vulnerability to hippocampal damage.

Although both synaptic and systems memory consolidation and reconsolidation have been the subject of intense investigation for decades, the underlying mechanisms, being unavailable for direct observation, remain to a large extent unknown. As in most of science, researchers investigate these phenomena by formulating hypotheses about the mechanisms at work, deriving predictions from the hypotheses, and designing experiments that can confirm or refute the predictions. This process can be aided by computational modeling. Specifically, computational models can be used to test the feasibility of proposed mechanisms, and to investigate how well they can account for empirical results. A computational model may also uncover properties or behaviors that have not previously been observed in the target system, which may suggest new hypotheses and ideas for future experiments.

In this dissertation I will describe two computational models of memory consolidation and reconsolidation, and discuss the results obtained from running simulations in these models. The first model addresses phenomena at the synaptic level and the second at the systems level.

CHAPTER TWO: A MODEL OF SYNAPTIC CONSOLIDATION AND RECONSOLIDATION

BACKGROUND

As background for the first paper, this section describes the fundamentals of synaptic memory consolidation and reconsolidation, including the underlying neural phenomenon, long-term potentiation of glutamatergic synapses.

MEMORY CONSOLIDATION

It has long been known that recently acquired memories are more susceptible to disruption than remote ones. Ribot (1882) observed that trauma-induced retrograde amnesia affects new memories more than older ones, and Müller and Pilzecker (1900) found that new learning is more likely to interfere with recent memories than with older ones. They coined the

term *consolidation* (Consolidirung, modern Ger. Konsolidierung) to refer to a putative process that gradually stabilizes new memories. Since then, a large body of evidence has accumulated in support of the existence of neural processes that stabilize memory traces over time (see Dudai, 2004, for a review).

Several studies from the 1940s and 1950s demonstrated that electroconvulsive shock (ECS) could interfere with the establishment of long-term memory in rodents (Duncan, 1949; Gerard, 1955; Thompson & Dean, 1955) and humans (Cronholm & Lagergren, 1959; Kehlet & Lunn, 1951), but only when applied shortly after acquisition. Memory loss in humans after head injury was similarly known to affect recent memories more than remote ones (Russell & Nathan, 1946). These results gave further support to Müller and Pilzecker's idea that memories undergo a transition from an initially vulnerable and labile state to a long-lasting consolidated state.

THE DISCOVERY OF RECONSOLIDATION

In 1968, Misanin et al. reported that ECS could impair not only newly acquired memories, but also 24-hours-old, i.e. consolidated, memories – but only when the convulsive treatment was administered immediately after a "memory reactivation" (Misanin, Miller, & Lewis, 1968). In the experiment, rats were presented with a white noise signal (conditioned stimulus, CS) followed by an electric foot shock (unconditioned stimulus, US). After training, the CS alone would elicit a fear response (conditioned response, CR) from the rats. The following day, one group of rats were given a CS presentation followed by ECS treatment; control groups received only CS, only ECS, or neither. On the third day, the rats were tested by presenting the CS and measuring their fear response. The results showed that ECS, when preceded by the CS presentation, produces a reduction of fear response comparable to that of ECS given immediately after training. Neither ECS alone nor CS alone produces this effect.

Post-reactivation susceptibility to ECS in rats was also demonstrated by Schneider and Sherman (1968) and Lewis, Mahan and Bregman (1972).

Similarly, Judge and Quartermain (1982) reported that the protein synthesis inhibitor anisomycin, which was known to produce memory deficits in mice when injected systemically immediately after training (for a review, see Davis & Squire, 1984), could also impair expression of older memories if given 30 minutes or less after reactivation.

Przybyslawski and Sara (1997) achieved similar results using the drug MK-801, an antagonist of the N-Methyl-D-aspartate (NMDA) synaptic receptor. MK-801 was known to prevent rats from learning a maze task if injected systemically pre-training. Przybyslawski and Sara's study showed that MK-801 could also produce significant amnesia for the task if administered 24 hours after training, but only if the injection was given 90 minutes or less after a maze run. They proposed that memory reactivation (in this case triggered by returning the rat to the maze in which it had previously been trained) temporarily returns a memory to a labile state, and that it subsequently spontaneously restabilizes. Further, this restabilization involves some or all of the same processes that are needed for consolidation when a memory is first acquired, specifically processes involving the NMDA receptor, as this is the target of MK-801. They introduced the term *memory reconsolidation* to describe this process (Przybyslawski & Sara, 1997).

In 2000, Karim Nader and coworkers (Nader, Schafe, & Le Doux, 2000) demonstrated that anisomycin infusion into the amygdala could also disrupt an established fear conditioning memory, but – again – only if performed shortly after reactivation. Consistent with Judge and Quartermain (1982), this suggested that both the consolidation and reconsolidation processes depend on synthesis of new proteins.

Taken together, these studies supported the notions that (a) newly acquired memories undergo a protein-synthesis-dependent consolidation process that takes an hour or less to complete, (b) that reactivation can return a memory that has undergone consolidation to a labile state, and (c) that an NMDA-receptor-dependent process involving protein synthesis is required to subsequently restabilize it. The phenomenon, now known as *synaptic memory reconsolidation,* has attracted much interest in the wake of the Przybyslawski and Sara (1997) and Nader et al. (2000) papers, and a large literature now exists (for reviews, see Baldi & Bucherelli, 2015; Besnard, Caboche, & Laroche, 2012; Nader & Einarsson, 2010).

In parallel with these advances in behavioral neuroscience, insights about the underlying neural and biochemical mechanisms were gained through a series of discoveries in the fields of neuroanatomy and molecular neuroscience.

THE NEURON DOCTRINE AND THE HEBBIAN SYNAPSE

The 1906 Nobel Prize in Physiology or Medicine was shared by two pioneers of neuroscience, Santiago Ramón y Cajal and Camillo Golgi, "in recognition of their work on the structure of the nervous system" (Grant, 2007). This was a controversial decision as the two recipients found themselves on opposing sides of a fundamental disagreement about the nature of the central nervous system. Golgi favored the hypothesis that the brain consists of a single continuous network, the reticulum, whereas Cajal championed the neuron doctrine, which maintained that the brain is made up of individual nerve cells, or neurons, and that the networks of nerve fibers that can be observed under the microscope consists of protrusions from such neurons. Based on observations made using a staining technique developed by Golgi, Cajal concluded that nerve fibers terminated “in contiguity but not in continuity” with the “nerve arborizations” (dendrites) and cell bodies of other neurons (Cajal, 1894). Although the neuron

doctrine soon gained the upper hand, the issue was not finally settled until the 1950s, when the electron microscope made possible direct observation of the synaptic cleft (Guillery, 2005). Cajal further introduced the idea that learning new skills involves strengthening of existing connections between neurons as well as the creation of new connections (Cajal, 1894, pp. 466–467), thereby laying the foundation for the now almost universally accepted synaptic theory of memory (Langille & Brown, 2018) and formulating the idea of activity-driven synaptic plasticity.

Half a century later, Donald Hebb (1949) refined this idea by articulating what we now call Hebb's Rule, that when one neuron repeatedly participates in exciting a second neuron so that it fires, this will cause a strengthening of the connection from the first neuron to the second, such that the ability of the first neuron to contribute to the firing of the second is increased.

LONG-TERM POTENTIATION

Another 24 years would pass before the first evidence of Hebbian synaptic plasticity was published in a landmark paper by Timothy Bliss and Terje Lømo (Bliss & Lømo, 1973). They demonstrated that repeated high-frequency stimulation of neural pathways in the rabbit hippocampus would cause them to subsequently exhibit an elevated response to stimulation. The effect was observed to persist for at least 10 hours, in contrast to previously known forms of synaptic enhancement or facilitation, which only lasted a few minutes at most (Andersen, 2003). The authors alternately called the effect *long-lasting potentiation* or *long-term potentiation*; with time, the latter designation took hold, perhaps due to its easy-to-pronounce acronym, LTP (Andersen, 2003).

The Bliss and Lømo paper triggered an explosion of LTP research with thousands of papers published to date (Nicoll, 2017). LTP has now been observed in a number of brain areas

in addition to the hippocampus (Citri & Malenka, 2008) and it has been suggested that it is present at all excitatory synapses in the brain (Malenka & Bear, 2004). LTP is widely considered to be a cellular model of memory, in the sense that the cellular changes observed in LTP are believed to mirror those that occur in neurons when memories are formed. (Frankland & Bontempi, 2005; Kandel et al., 2014; Lisman, Cooper, Sehgal, & Silva, 2018; Lynch, 2004; Sossin, 2008).

PHASES OF LTP

There are several phases of LTP. Early-phase LTP (E-LTP) results from moderately strong stimulation and lasts from minutes to hours (Abraham, 2003, p. 203; Malenka & Bear, 2004). Late-phase LTP (L-LTP) requires more intense stimulation and can persist for weeks, months or even years (Abraham, Logan, Greenwood, & Dragunow, 2002). Sometimes L-LTP is further divided into two separate phases, LTP-2 and LTP-3 (E-LTP being referred to as LTP-1 in this scheme), which differ in that LTP-3 requires gene transcription, in addition to the RNA translation required for LTP-2 (Abraham, 2003).

THE GLUTAMATERGIC SYNAPSE AND GLUTAMATE RECEPTORS

LTP is observed in glutamatergic synapses, by a wide margin the most abundant type of excitatory synapse in the brain, named after its neurotransmitter, glutamate (Meldrum, 2000). In a glutamatergic synapse, when the presynaptic neuron fires, it releases glutamate molecules into the narrow synaptic cleft. The glutamate molecules diffuse across the cleft and attach to specific binding sites on receptors in the membrane of the postsynaptic neuron, thereby activating the receptors. Of the several kinds of glutamate receptors, AMPA[1] receptors (AMPARs) are

[1] α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

primarily responsible for synaptic transmission and NMDA[2] receptors (NMDARs) are involved with regulatory functions, including the regulation of synaptic strength. These are both ionotropic receptor types, meaning that they are gated ion channels which, when activated, permit specific types of ions to pass through the cell membrane. To understand the mechanisms of LTP, it is necessary to go into some detail about these two types of glutamate receptors.

NMDARs are permeable to positive ions such as sodium (Na^{2+}), potassium (K^{+}) and calcium (Ca^{2+}). Ca^{2+} influx through NMDARs is of particular interest, as it is the trigger for a number of important signaling pathways (chains of biochemical reactions) in the neuron. Although these pathways are only partially known, it has been shown that some of them are critical for LTP induction, because infusing neural tissue with drugs that inhibit NMDAR activity can block induction of LTP (Collingridge, Kehl, & Mclennan, 1983). NMDAR activity is also crucial for reconsolidation, and different subtypes of NMDARs are known to be involved with the destabilization and restabilization of L-LTP that follows memory reactivation (Milton et al., 2013).

In the years following Bliss and Lømo's discovery of LTP, other researchers investigated its properties and demonstrated that it indeed has the characteristics postulated by Hebb (1949) for a memory mechanism (Nicoll, 2017): the (combined) stimulation of the postsynaptic neurons had to be strong ("cooperativity"), and only participating neural pathways were potentiated ("input specificity") (Levy & Steward, 1979; McNaughton, Douglas, & Goddard, 1978). Weak stimulation of synapses would not by itself cause potentiation, but when several synapses were simultaneously stimulated such that the combined stimulation was above a certain threshold, the weakly stimulated synapses were also potentiated ("associativity") (McNaughton et al., 1978).

[2] N-methyl-D-aspartate

The mechanism behind the Hebbian characteristic of LTP remained a mystery until 1984, when a remarkable property of the NMDA receptor was simultaneously discovered by two teams, Mayer et al. (1984) and Nowak et al. (1984): ion conductance through the NMDA receptor is strongly voltage-dependent. When the receptor is activated by glutamate, it does not become permeable to ions unless the membrane in which it is inserted is depolarized. The reason for this is that its channel has a binding site for a magnesium ion, Mg^{2+}. When the membrane is at its resting potential, its interior is at a negative potential with respect to the exterior; the resulting electrostatic field attracts an Mg^{2+} ion and holds it in place, blocking the channel. But if the cell membrane is depolarized, which happens when the neuron fires, then the Mg^{2+} ion is ejected, allowing the influx of Ca^{2+} (Mayer et al., 1984; Nowak et al., 1984). As a result, the NMDAR functions as a coincidence detector: it conducts ion current only upon near simultaneous firing of the presynaptic neuron (required for glutamate release) and the postsynaptic neuron (required for membrane depolarization). This property of the NMDAR provides an explanation for the Hebbian nature of LTP induction: when the presynaptic and postsynaptic neurons both fire, the ion channels of NMDA receptors on the postsynaptic neuron become permeable, calcium ions flow into the synaptic compartment and activate signaling pathways that promote insertion of AMPA receptors.

AMPA receptors are composed of four subunits, each of which may belong to any of four different types, designated GluA1, GluA2, GluA3 and GluA4 (Isaac, Ashby, & McBain, 2007; Kessels & Malinow, 2009). AMPARs that contain the GluA2 subunit are impermeable to calcium ions, whereas those lacking GluA2 are calcium-permeable (Isaac et al., 2007). In the following, the two types will be referred to as CI-AMPARs and CP-AMPARs, respectively. Both kinds are permeable to sodium and potassium ions, the main carriers of synaptic current, and are

therefore able to contribute to synaptic strength when inserted in the post-synaptic density (PSD), the specialized area of cell membrane that makes up the post-synaptic component of a synapse.

Experiments have indicated that E-LTP and L-LTP are mediated by different AMPAR types. E-LTP induction is associated with a transient increase in the number of CP-AMPARs in the synapse, whereas L-LTP is characterized by a persistently elevated number of CI-AMPARs (Clem & Huganir, 2010; Kessels & Malinow, 2009; Plant et al., 2006). In addition, memory reactivation has been shown to trigger a temporary return to an E-LTP-like state with high CP-AMPAR and low CI-AMPAR counts (Clem & Huganir, 2010; Hong et al., 2013).

The overall picture, then, is that moderate stimulation of a glutamatergic synapse triggers NMDAR-dependent signaling that induces E-LTP, characterized by an increased level of CP-AMPARs that persists for about an hour at most. Stronger stimulation can activate signaling pathways that induce long-lasting L-LTP, characterized by an elevated CI-AMPAR count. Memory retrieval triggers processes, also NMDAR-dependent, that first destabilize, then restabilize participating synapses. The period of temporary instability, known as the reconsolidation window, is characterized by high CP-AMPAR and low CI-AMPAR counts.

A number of different sub-cellular biochemical processes have been suggested to explain these events (Malenka & Bear, 2004). In the following paper I present a computational model of a set of molecular mechanisms proposed to explain the trafficking and maintenance of the GluA2-containing AMPARs (CI-AMPARs) in the context of late-phase LTP. Specifically, the model centers around the putative role of PKMζ (protein kinase M zeta) in L-LTP in these processes. PKMζ is an atypical isoform of protein kinase C with the unusual property that it is constitutively active (Sacktor et al., 1993), which makes it an interesting candidate for a role in the maintenance of long-term memory traces (Sacktor, 2011). As described in the paper, a

growing body of evidence supports the hypothesis that PKMζ does play a role in the trafficking of CP-AMPARs into potentiated synapses. The model was developed to investigate the computational feasibility of this hypothesis.

CONTRIBUTION TO ORIGINAL KNOWLEDGE

The present model of late long-term potentiation (L-LTP) is built around two coupled positive feedback loops of molecular reactions. The first feedback loop arises from PKMζ catalyzing translation of its own RNA, and has figured in previously published computational models. In addition, our model incorporates a second feedback relationship involving interaction between PKMζ and GluA2-containing AMPA receptors. This allows the model to reproduce a number of empirical findings related to inhibition of PKMζ activity and blocking of AMPAR endocytosis by means of the synthetic peptides ZIP and $GluA2_{3Y}$, respectively. We also demonstrate that the system of coupled feedback loops can produce the robust bistability observed in L-LTP, without the need for postulating an ultrasensitive relationship between PKMζ concentration and RNA translation rate, an advance over previous models.

CONTRIBUTION OF AUTHORS

Conceptualization: Peter Helfer (PH), Thomas R. Shultz (TRS).
Formal analysis: PH.
Funding acquisition: TRS.
Investigation: PH.
Methodology: PH.
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Supervision: TRS.
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Visualization: PH.
Writing – original draft: PH.
Writing – review & editing: TRS & PH.

PAPER #1

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Data Availability Statement: All computer program files are available from the ModelDB database, accession number 239541 (http://modeldb.yale.edu/239541).

Coupled feedback loops maintain synaptic long-term potentiation: A computational model of PKMzeta synthesis and AMPA receptor trafficking

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Abstract

In long-term potentiation (LTP), one of the most studied types of neural plasticity, synaptic strength is persistently increased in response to stimulation. Although a number of different proteins have been implicated in the sub-cellular molecular processes underlying induction and maintenance of LTP, the precise mechanisms remain unknown. A particular challenge is to demonstrate that a proposed molecular mechanism can provide the level of stability needed to maintain memories for months or longer, in spite of the fact that many of the participating molecules have much shorter life spans. Here we present a computational model that combines simulations of several biochemical reactions that have been suggested in the LTP literature and show that the resulting system does exhibit the required stability. At the core of the model are two interlinked feedback loops of molecular reactions, one involving the atypical protein kinase PKMζ and its messenger RNA, the other involving PKMζ and GluA2-containing AMPA receptors. We demonstrate that robust bistability – stable equilibria both in the synapse's potentiated and unpotentiated states – can arise from a set of simple molecular reactions. The model is able to account for a wide range of empirical results, including induction and maintenance of late-phase LTP, cellular memory reconsolidation and the effects of different pharmaceutical interventions.

Keywords: LTP, PKMζ, PKMzeta, AMPAR, synaptic stability, reconsolidation, computational model

Author Summary

The brain stores memories by adjusting the strengths of connections between neurons, a phenomenon known as synaptic plasticity. Different types of plasticity mechanisms have either a strengthening or a weakening effect and produce synaptic modifications that last from

milliseconds to months or more. One of the most studied forms of plasticity, long-term potentiation, is a persistent increase of synaptic strength that results from stimulation and is believed to play an important role in both short-term and long-term memory. Researchers have identified many proteins and other molecules involved in long-term potentiation and formulated different hypotheses about the biochemical processes underlying its induction and maintenance. A growing number of studies support an important role for the protein PKMζ (protein kinase M Zeta) in long-term potentiation. To investigate the explanatory power of this hypothesis, we built a computational model of the proposed biochemical reactions that involve this protein and ran simulations of a number of experiments that have been reported in the literature. We find that our model is able to explain a wide range of empirical results and thus provide insights into the molecular mechanisms of memory.

Introduction

The brain stores memories by adjusting the strengths of connections between neurons. Such synaptic plasticity comes in different forms that strengthen or weaken synapses and range from very short-lived to long-lasting. One of the most well-studied forms of plasticity is long-term potentiation, LTP, a phenomenon whereby synaptic strength is persistently increased in response to stimulation. Different forms of LTP are known to play important roles in both short-term and long-term memory.

Many different proteins have been identified in the sub-cellular molecular processes that are involved in LTP. An important question is how these proteins, with lifetimes measured in hours or days, can maintain memories for months or years. We present a computational model that demonstrates how this problem can be solved by two interconnected feedback loops of molecular reactions.

We begin with an overview of LTP with emphasis on the empirical findings that our model aims to explain. This is followed by a description of the model, an account of our results, and discussion of their implications.

Glossary

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, an artificial glutamate analog
AMPAR	AMPA receptor, a glutamatergic receptor that is activated by AMPA, in addition to glutamate.
anisomycin	an antibiotic that inhibits protein synthesis by blocking the formation of peptide bonds
BRAG2	a protein that plays a key role in AMPAR endocytosis
CA1, CA3	cornu ammonis area 1 and 3, regions of the hippocampus
consolidation	conversion of synaptic potentiation from a short-lived to a lasting form; formation of L-LTP
dendrite	protrusion on a neuron where most of the incoming synapses are located
E-LTP	early-phase LTP
endocytosis	of receptors: removal from the cell membrane by internalization
EPSP	excitatory postsynaptic potential
GluA2	one of four AMPA receptor subunit types
$GluA2_{3Y}$	(also known as $GluR2_{3Y}$) a synthetic peptide that blocks endocytosis of GluA2-containing AMPARs
glutamate	the most common excitatory neurotransmitter in the central nervous system
hippocampus	a structure in the mammalian brain
kinase	an enzyme that catalyzes phosphorylation
L-LTP	late-phase LTP
LTM	long-term memory
LTP	long-term potentiation
mRNA	messenger RNA, a molecule that specifies the sequence of amino acids in a protein
NMDA	N-Methyl-D-aspartic acid, a synthetic glutamate analog
NMDAR	NMDA receptor, a glutamatergic receptor that is activated by NMDA, in addition to glutamate
peptide	short chain of amino acids. Chains longer than about 50 amino acids are called proteins.
phosphorylation	addition of a phosphate group to a molecule, e.g. a protein, which may thereby be activated or deactivated
PKC	a family of protein kinases
PKMζ	"PKM zeta", a constitutively active PKC isoform found in brain tissue
protein kinase	a kinase that phosphorylates proteins
PSD	postsynaptic density, a region of cell membrane that forms the receiving side of a synapse
PSI	protein synthesis inhibition (or inhibitor)
reconsolidation	restabilization of LTP after reactivation-induced destabilization
RNA	ribonucleic acid, a molecule produced by transcription of DNA
ZIP	zeta-inhibitory peptide, a molecule that inhibits PKMζ activity

Background

In his address to the Royal Society in 1894, Santiago Ramon y Cajal hypothesized that the brain stores information by adjusting the strengths of associations between neurons, as well as by growing new connections [1]. In the years since, the existence of both of these mechanisms, now known as synaptic plasticity and synaptogenesis, respectively, has been well established, and there is ample evidence that synaptic plasticity plays an important role in learning and memory [2–4].

Neurons communicate by transmitting signals across chemical synapses, where presynaptic axon terminals connect to postsynaptic neurons, most often on their dendrites. When a nerve impulse (action potential) arrives at the axon terminal, neurotransmitter molecules are released into the synaptic cleft, a narrow gap between the two neurons, where they activate receptors in the membrane of the postsynaptic neuron. This sets in motion a series of biochemical events in the postsynaptic neuron, the details of which depend on the type of receptor, among other factors. Synaptic strength depends both on the amount of transmitter that is released by the arrival of a nerve impulse at the axon terminal and on the number and sensitivity of the receptors. It may thus be regulated on either the pre- or postsynaptic side, and mechanisms of synaptic plasticity have been shown to operate in both compartments [3]. Plasticity may either strengthen or weaken a synapse, and the effect may be short-lived or long-lasting. Short-term synaptic plasticity, lasting from milliseconds to minutes, is primarily due to presynaptic mechanisms that adjust the amount of transmitter release, whereas postsynaptic modifications that adjust the number and sensitivity of receptors are important for long-term plasticity [4]. In particular, this is true of long-term potentiation (LTP), a type of persistent strengthening of synapses in response to stimulation [5,6], which has been studied extensively in the CA3-CA1 synapses of the rodent

hippocampus [4] and is known to depend on an increase in the number of receptors inserted in the postsynaptic membrane [7].

There are at least two forms of LTP: Moderately strong stimulation induces early-phase LTP (E-LTP), which persists for at most a few hours. When the stimulation is stronger, E-LTP may be followed by late-phase LTP (L-LTP), which can last for days, months or longer [7,8] and is believed to be an important mechanism for the storage of long-term memories [9,10]. The establishment of L-LTP, known as synaptic or cellular memory consolidation, is a process that takes less than an hour [11,12] and requires synthesis of new protein. This has been demonstrated by showing that infusion of protein-synthesis-inhibiting drugs such as anisomycin can prevent establishment of L-LTP [12–15]. On the behavioral level, protein synthesis inhibition (PSI) has been shown to impair the formation of long-term memory, consistent with the notion of L-LTP as a memory mechanism [16].

Once long-term memory is established, it is in general no longer vulnerable to infusion of a protein synthesis inhibitor [16]. However, memory retrieval can induce a state of transient instability, during which the memory is again susceptible to protein synthesis inhibition [17–19]. This susceptibility of memory to post-retrieval PSI infusion has been shown to correlate with instability of L-LTP at the neural level [20,21], providing further evidence of the importance of LTP as a mechanism of long-term memory. The synaptic destabilization that is triggered by memory retrieval is followed by a period of restabilization which has similarities with the initial synaptic consolidation that follows memory acquisition. It has therefore become known as *memory reconsolidation* [19], more specifically *synaptic* (or *cellular*) *reconsolidation*, to avoid confusion with the related but distinct phenomenon *systems reconsolidation*, a temporary dependence on the hippocampus for restabilization of a memory after reactivation (retrieval). For

reviews of reconsolidation research, see [22–24]. For a computational model of systems consolidation and reconsolidation, see [25].

Glutamatergic Synapses

In this report, we focus on L-LTP induction and maintenance at glutamatergic synapses, the most abundant type of synapse in the vertebrate nervous system [26,27]. Glutamatergic synapses contain several kinds of receptors that are activated by the neurotransmitter glutamate. Of particular interest for LTP are the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA receptor or AMPAR), which mediates synaptic transmission [28], and the N-methyl-D-aspartate receptor (NMDA receptor or NMDAR), which is involved with regulatory functions including the regulation of synaptic strength [29,30].

AMPARs are ion channels that open when activated by the neurotransmitter glutamate. The opening of the channel allows positively charged ions, mainly sodium and potassium, to flow through the cell membrane [31]. This causes a partial depolarization of the membrane, which at rest is polarized by a net negative charge inside the cell. The partial depolarization is known as an excitatory postsynaptic potential, or EPSP, and the amplitude of the EPSP produced by a single action potential arriving at a synapse is a measure of synaptic strength. Among other factors, the EPSP amplitude depends on the number of AMPARs inserted in the postsynaptic density (PSD), the area of cell membrane that constitutes the receiving side of the synapse [31]. Thus mechanisms that control the trafficking of AMPARs into and out of the PSD play an important part in the regulation of synaptic strength.

AMPARs are heterotetramers, i.e. they consist of four non-identical subunits. The subunits are of four different kinds, named GluA1, GluA2, GluA3 and GluA4, and AMPARs can be made up of different combinations of these [32]. GluA2 is of particular interest here, because L-LTP is

associated with an increase in the number of GluA2-containing AMPARs inserted in the PSD [20,33,34].

AMPA receptors are not permanently inserted in the PSD, but are constantly being recycled. Certain proteins transport AMPARs into the PSD from pools maintained in adjacent areas, while others remove them (a process known as internalization or endocytosis) and either recycle them to stand-by pools or mark them for degradation [35,36].

Protein Kinase M zeta (PKMζ)

Many proteins have been implicated in the induction and maintenance of LTP, including CaMKII, PKA, MAPK and several isoforms of PKC (for a review, see [7]). An atypical isoform of PKC, protein kinase Mζ (PKMζ), is believed to play an important role for L-LTP. The level of PKMζ has been shown to increase as the result of NMDA receptor stimulation [37,38], consistent with its proposed role in L-LTP induction. Inhibition of PKMζ activity results in disruption of established L-LTP [39–41], and perfusion of PKMζ into a neuron can induce L-LTP [39] . PKMζ activity is believed to increase the number of inserted GluA2-containing AMPARs at the synapse both by facilitating the trafficking of these receptors into the PSD and by inhibiting their removal [42]. GluA2-containing AMPARs are held at extrasynaptic pools by the protein PICK1 which binds to the GluA2 subunit [34]. PKMζ facilitates interaction between the trafficking protein NSF and the GluA2 subunit, which results in its release from PICK1, freeing the AMPARs to diffuse laterally into the PSD [34]. Furthermore, once GluA2-containing AMPARs are inserted in the PSD membrane, PKMζ prevents their removal by inhibiting the interaction between the protein BRAG2 and the GluA2 subunit [43], an interaction that plays a key part in endocytosis of GluA2-containing AMPARs [42,44].

While GluA2-containing AMPARs are important for the stabilization of L-LTP, there is evidence that GluA2-lacking AMPARs play an important role in the induction of early-phase LTP (E-LTP), and also in reconsolidation. Several studies have shown that GluA2-lacking AMPARs are initially inserted at the time of memory acquisition or LTP induction, and then gradually replaced by GluA2-containing AMPARs during consolidation [45–47]. Hong et al. [20] showed that memory reactivation triggers an abrupt replacement of GluA2-containing AMPARs by GluA2-lacking AMPARs. This is followed by a gradual reversal, i.e. the GluA2-containing AMPARs are restored and the number of GluA2-lacking AMPARs declines, as the potentiated state of the synapse is restabilized [20]. Because the temporary removal of GluA2-containing AMPARs is compensated for by an increase in GluA2-lacking AMPARs, the synaptic strength remains more or less constant during the period of instability [20]. Rao-Ruiz et al. [21] reported similar results, although they observed a brief period of reduced synaptic strength between the GluA2-containing AMPAR removal and GluA2-lacking AMPAR insertion. Taken together, these results suggest that the stabilization of LTP, both initially during consolidation, and after reactivation-induced destabilization, requires insertion of GluA2-containing AMPARs, and that PKMζ plays an important role in maintaining the GluA2-containing AMPARs at the synapse.

An important question is how L-LTP, which can last for months or longer [8], can be maintained by a protein like PKMζ, with a half-life that probably does not exceed several hours or at most a few days [48–51]. A proposed answer to this question involves local translation of messenger RNA (mRNA) in or near dendritic spines. Most synapses are formed at dendritic spine heads, with one synapse per spine [52]. It has been shown that PKMζ mRNA is transported from the cell body to dendrites [53,54], but the mRNA in its basal state is translationally repressed by

molecules that bind to it, or to the complex of proteins required to initiate translation [50,53,55]. There is evidence that PKMζ catalyzes reactions that lift this translational block [49,56], possibly through inhibition of the PIN1 protein [42], resulting in a positive feedback loop [49]. By promoting its own synthesis in this manner, PKMζ may be able to remain at an increased level, and thus maintain L-LTP, for a long time, perhaps indefinitely.

It has also been suggested that the increased amount of inserted GluA2-containing AMPARs at a potentiated synapse captures the PKMζ molecules and keeps them from dissipating away from the synaptic compartment [42]. This hypothesis is supported by several studies that show that blocking endocytosis of GluA2-containing AMPARs can prevent depotentiation under protocols that otherwise cause disruption of L-LTP [21,33,57]. Together with PKMζ's inhibiting effect on AMPAR endocytosis this constitutes a second feedback loop, a reciprocal relationship in which PKMζ and GluA2-containing AMPARs prevent each other's removal from the synapse. As we shall see, the interaction between these two feedback loops plays a central role in our explanation of synaptic bistability, that is that synapses have two stable equilibrium states, unpotentiated and potentiated. Transient stimuli can cause a synapse to transition between these two states, but in the absence of such signals it tends to remain in one state or the other.

L-LTP, LTM and Pharmacological Interventions

The notion that L-LTP is an important neural correlate of long-term memory (LTM) has been supported experimentally by demonstrating that pharmacological interventions that block L-LTP induction also interfere with the establishment of LTM [58], and that interventions that disrupt established L-LTP also impair consolidated memories [59]. Here we consider three types of pharmaceuticals that have been shown to produce significant results with respect to both L-LTP induction and maintenance, and to related behavior-level memory phenomena.

Protein synthesis inhibitors. Infusion of protein synthesis inhibitors (PSIs) such as anisomycin into brain tissue can prevent the induction of L-LTP [58], and also interferes with memory consolidation, the establishment of LTM [60,61]. Once L-LTP is established, it becomes resistant to infusion of anisomycin [11,12]. This does not mean that L-LTP can be maintained indefinitely without ongoing protein synthesis, but rather that it can tolerate an interruption of protein synthesis for the amount of time that anisomycin remains active after infusion.

Reactivation of a consolidated memory, e.g. by a reminder, can temporarily return it to a labile state in which it is again vulnerable to PSI infusion [18,60]. The putative molecular process underlying this phenomenon has been termed *cellular* or *synaptic memory reconsolidation* [18,62]. Concordant with the hypothesis that L-LTP is the neural correlate of LTM, the temporary post-reactivation vulnerability of LTM to PSI infusion can be explained as destabilization of L-LTP, followed by a restabilization phase that requires protein synthesis, hence the susceptibility to PSI. The destabilization has been shown to require the activity of NMDA receptors [29], and to depend critically on endocytosis of GluA2-containing AMPARs [57,63].

Thus protein synthesis inhibition is known to both prevent establishment of L-LTP and to block reconsolidation, i.e. block restabilization of L-LTP after retrieval-induced destabilization.

ZIP. Much of the work demonstrating the role of PKMζ in L-LTP is based on administration of the synthetic peptide ZIP (zeta-inhibitory peptide), which binds to the catalytic region of the PKMζ molecule, thus blocking its enzymatic activity [41]. On the behavioral level, infusion of ZIP into brain tissue has been shown to impair consolidated LTM [59]. On the neural level, ZIP is known to disrupt established L-LTP when applied during the maintenance phase [39–41,64]. These results are consistent with the notion of a positive feedback loop: Inhibiting PKMζ's

enzymatic activity prevents it from catalyzing its own synthesis; the PKMζ concentration then drops, the AMPAR endocytosis rate increases, and the synapse returns to its basal state. On the other hand, ZIP does not prevent L-LTP induction when applied only during or immediately after stimulation. This was demonstrated by Ren et al. [65] in an in-vitro experiment where onset and duration of ZIP application were precisely controlled.

GluA2$_{3Y}$. GluA2$_{3Y}$ is a synthetic peptide that blocks regulated endocytosis of GluA2-containing AMPARs [66,67]. Infusion of GluA2$_{3Y}$ has been shown to block both the destabilizing effect of PSI infusion after memory reactivation [20,57] and the depotentiating effect of ZIP during L-LTP maintenance [33]. The GluA2$_{3Y}$ peptide is modeled on a sequence of the GluA2 subunit's carboxyl tail and its endocytosis-inhibiting effect is believed to be due to competitive disruption of the binding of endocytosis-related proteins to this sequence on GluA2 subunits [68].

Computational Model

The findings described above suggest a model of L-LTP maintenance with two connected feedback loops: (1) PKMζ maintains its own mRNA in a translatable state and translation of the mRNA in turn replenishes PKMζ. (2) PKMζ maintains GluA2-containing AMPARs at the synapse, and these in turn keep PKMζ molecules from dissipating away from the synaptic compartment. Below we describe a computational model that incorporates these relationships and investigate its ability to account for results reported in the empirical literature.

Methods

Deterministic vs. Stochastic Simulation

Systems of chemical reactions can be modeled either by deterministic methods based on ordinary differential equations (ODEs) or by stochastic simulation. When the numbers of molecules are

small, stochastic simulation is the better choice, because random fluctuations then have significant effects that are not captured by deterministic methods [69]. In particular, random fluctuations can cause a small system to spontaneously transition from one steady state to another; the resulting impact on system stability can be studied in a stochastic simulation, but not in a deterministic model [70], because the latter only accounts for average reaction rates over a large number of molecules.

The molecules of interest for our simulation are present in small numbers in a dendritic spine head, e.g. fewer than a hundred PKMζ molecules (see S1 Text) and at most ca 150 AMPARs [71,72]. This is well below the size of system that can be realistically simulated by deterministic methods [70,73]. We therefore base our simulation on the Gillespie algorithm [74], a well-established and widely used approach to discrete and stochastic simulation of reaction systems [69,70,73].

Model Description

The model consists of four inter-dependent pairs of processes (see Fig 1):

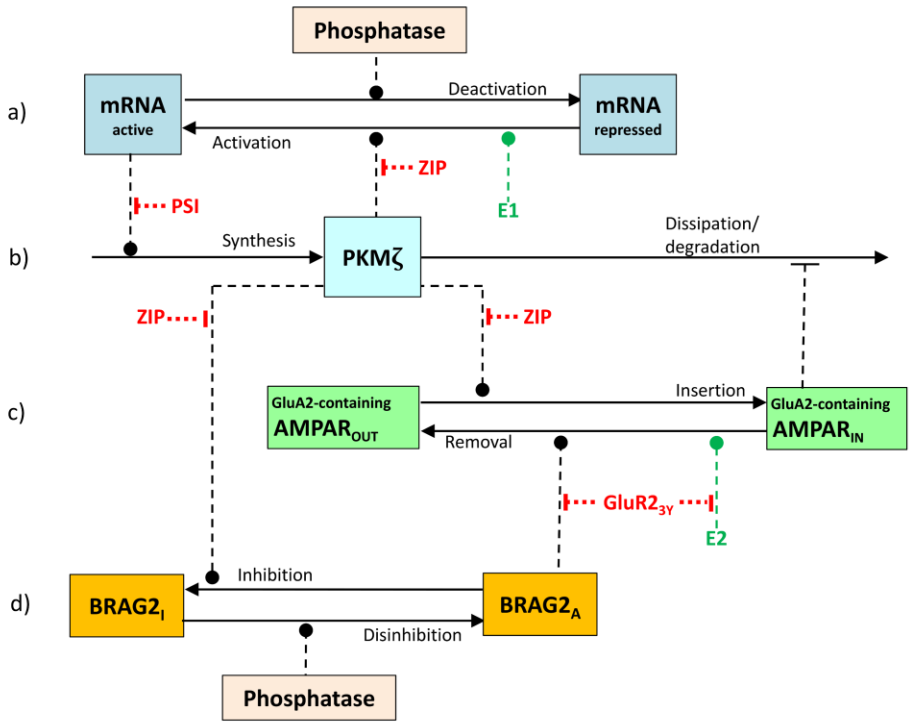

Fig 1: Process diagram. a) Activation/deactivation of PKMζ mRNA (blue). Translational repression of mRNA is lifted by catalytic activity of PKMζ, possibly by phosphorylation of mRNA-binding proteins. A phosphatase (pink) dephosphorylates the same proteins, returning mRNA to its repressed state. b) Synthesis and degradation/dissipation of PKMζ (cyan). Synthesis consists in local translation of PKMζ mRNA. Degradation and/or dissipation away from the synaptic compartment is inhibited by inserted GluA2-containing AMPARs. c) Trafficking of GluA2-containing AMPARs (green) into and out of the PSD. Insertion is facilitated by PKMζ, and removal (endocytosis) by the BRAG2 protein. d) Inhibition/disinhibition of BRAG2-GluA2 interaction. Inhibition is modeled as phosphorylation of BRAG2 (orange) catalyzed by PKMζ, and disinhibition as dephosphorylation catalyzed by a phosphatase. E1 and E2

(dark green) are enzymes activated by NMDAR stimulation at L-LTP induction and memory reactivation, respectively. The effects of PSI, ZIP and GluA23Y (red) are modeled by disabling the indicated catalytic reactions. Solid arrows represent chemical reactions and receptor trafficking. Dashed lines with filled circles represent catalytic activity. Dashed lines with crossbars represent inhibition.

Activation/deactivation of PKMζ mRNA. PKMζ lifts the constitutive translational repression of PKMζ mRNA by phosphorylating some substrate, possibly mRNA-binding proteins attached to the mRNA. The mRNA molecule with attached proteins and ribosomes (polysome) is represented as a single molecule in the model, and de-repression is modeled as phosphorylation of (some component of) this molecule by PKMζ. The opposite reaction, dephosphorylation by a phosphatase assumed to be present at fixed concentration, returns the mRNA to its repressed state.

Synthesis and degradation/dissipation of PKMζ. Synthesis consists in local translation of PKMζ mRNA. (This is somewhat speculative: PKMζ mRNA has been shown to be present in dendrites [53,54], but not specifically in dendritic spines.) Inserted GluA2-containing AMPARs inhibit degradation and/or dissipation of PKMζ away from the synaptic compartment by binding PKMζ molecules [42], probably via a scaffold protein such as PICK1 or KIBRA [75]. This is modeled as an affinity of PKMζ for inserted GluA2-containing AMPARs, with a reduced dissipation/degradation rate while so attached.

GluA2-containing AMPAR trafficking into and out of the PSD. The model includes a fixed-size population of GluA2-containing AMPARs. At any time, a subset of the AMPARs are inserted in the PSD while the remainder are maintained in extrasynaptic pools. Transport of AMPARs into the PSD is facilitated by PKMζ and removal (endocytosis) is enabled by the

protein BRAG2. In addition to these two regulated processes, constitutive processes traffic AMPARs into and out of the synapse at lower rates.

Inhibition and disinhibition of BRAG2-GluA2 interaction. The mechanism by which PKMζ inhibits the interaction between BRAG2 and the GluA2 subunit to block AMPAR removal from the PSD is not known, but presumably involves phosphorylation of some substrate. We model the inhibition as phosphorylation of the BRAG2 molecule itself; other possibilities include phosphorylation of a site on the GluA2 subunit or of another participating protein. The BRAG2-GluA2 interaction is restored through dephosphorylation of the same substrate by a phosphatase, which is assumed to be present in fixed concentration.

Although the increase in PKMζ level that is associated with L-LTP induction is known to depend on NMDAR activation [38], the underlying biochemical pathways are unknown. In the model this mechanism is represented by an unspecified enzyme that we call E1 which, when activated by a reaction cascade triggered by NMDAR activation, has the ability to lift the translational block on PKMζ mRNA, thereby enabling PKMζ synthesis.

Similarly, the destabilizing effect of memory reactivation has been shown to depend on NMDAR activity and on endocytosis of GluA2-containing AMPARs [20,57,76], but the biochemical cascades that connect these event have not yet been identified. In our model, reactivation is simulated as an increase in the level of a second unspecified enzyme E2 with the ability to catalyze endocytosis of GluA2-containing AMPAR.

In addition to these processes, the model includes simulation of the effects of the three pharmaceuticals described in the introduction. The time intervals that these drugs remain at a high enough concentration to inhibit their targets depend on the doses infused and also on their

specific rates of decay or metabolism. The intervals used here are based on activity periods reported in the cited references:

PSI: Infusion of a protein synthesis inhibitor is simulated by disabling PKMζ synthesis for nine hours, the amount of time that the protein synthesis inhibitor anisomycin remains active after infusion into brain tissue [77].

ZIP: Administration of the ZIP peptide is simulated by disabling PKMζ's enzymatic activity – catalysis of mRNA activation, facilitation of GluA2-containing AMPAR trafficking into the PSD and inhibition of BRAG2-GluA2 interaction – for twelve hours [78].

GluA2$_{3Y}$: Perfusion of GluA2$_{3Y}$ is simulated by disabling regulated endocytosis of GluA2-containing AMPAR for twelve hours [76]. (GluA2$_{3Y}$ does not affect constitutive endocytosis of GluA2-containing AMPAR [67].)

Table 1 lists the molecule species included in the model, including complexes formed during enzymatic reactions. All simulations begin in the lower (unpotentiated) steady state with the indicated initial molecule counts.

Table 1: Molecule species

Symbol	Description	Initial count
P	Unbound PKMζ	0
R_I	unphosphorylated PKMζ mRNA (inactive)	100
R_A	phosphorylated PKMζ mRNA (active)	0
PP	phosphatase	100
PP•R_A	PP + R_A complex	0
$E1_A$	E1 enzyme, active	0
$E1_I$	E1 enzyme, inactive	100
$E1_A$•R_I	$E1_A$ + R_I complex	0

A_U	Uninserted GluA2-containing AMPAR	100
A_I	Inserted GluA2-containing AMPAR	0
$A_I \bullet P$	PKMζ bound to inserted AMPAR	0
$P \bullet R_I$	P + R_I complex	0
$A_I \bullet P \bullet R_I$	A_I + P + R_I complex	0
B_A	Active BRAG2	100
B_I	Inactive BRAG2	0
$PP \bullet B_I$	PP + B_I complex	0
$P \bullet B_A$	P + B_A complex	0
$A_I \bullet P \bullet B_A$	A_I + P + B_A complex	0
$B_A \bullet A_I$	B_A + A_I complex	0
$B_A \bullet A_I \bullet P$	B_A + A_I + P complex	0
$E2_A$	E2 enzyme, active	0
$E2_I$	E2 enzyme, inactive	100

Simulated Reactions

Activation of PKMζ mRNA. PKMζ mRNA is present in dendritic spines, but is translationally repressed in its basal state [42,53] due to mRNA-binding proteins that prevent translation from being initiated [55]. PKMζ is able to lift the repression, possibly by phosphorylating these proteins, thus catalyzing its own synthesis in a positive feedback loop. We model mRNA with its associated proteins as a single molecule, represented by R_I in its inactive repressed state, and by R_A when activated. Activation is modeled using Michaelis-Menten kinetics [73], i.e. a PKMζ molecule (P) and an inactive mRNA molecule (R_I) form a complex $P \bullet R_I$. The complex may then either dissociate (reaction 2) or the catalytic reaction (3) may take place, producing active mRNA (R_A):

$$P + R_I \xrightarrow{c_1} P \bullet R_I \quad (1)$$

$$P \bullet R_I \xrightarrow{c_2} P + R_I \quad (2)$$

$$P \bullet R_I \xrightarrow{c_3} P + R_A \quad (3)$$

Deactivation of PKMζ mRNA. The PKMζ mRNA returns to its repressed state when the mRNA-binding proteins are dephosphorylated by a phosphatase which we denote by PP. This is also modeled with Michaelis-Menten kinetics (as are all enzymatic reactions in the model):

$$PP + R_A \xrightarrow{c_4} PP \bullet R_A \quad (4)$$

$$PP \bullet R_A \xrightarrow{c_5} PP + R_A \quad (5)$$

$$PP \bullet R_A \xrightarrow{c_6} PP + R_I \quad (6)$$

PKMζ synthesis and degradation/dissipation. PKMζ is synthesized by local translation of active mRNA (reaction 7). Over time PKMζ degrades or diffuses away from the synaptic compartment. Reaction 8 represents the combined effect of these two processes. The model is unspecific with respect to their relative importance for PKMζ turnover.

$$R_A \xrightarrow{c_7} R_A + P \quad (7)$$

$$P \xrightarrow{c_8} 0 \quad (8)$$

Inhibition/disinhibition of BRAG2. BRAG2 is inhibited by PKMζ and reactivated by phosphatase. Both processes are described by Michaelis-Menten kinetics. B_A and B_I denote active and inhibited BRAG2, respectively:

$$P + B_A \xrightarrow{c_9} P \bullet B_A \quad (9)$$

$$P \bullet B_A \xrightarrow{c_{10}} P + B_A \quad (10)$$

$$P \bullet B_A \xrightarrow{c_{11}} P + B_I \quad (11)$$

$$PP + B_I \xrightarrow{c_{12}} PP \bullet B_I \quad (12)$$

$$PP \bullet B_I \xrightarrow{c_{13}} PP + B_I \quad (13)$$

$$PP \bullet B_I \xrightarrow{c_{14}} PP + B_A \quad (14)$$

AMPA receptor trafficking. Transport of GluA2-containing AMPARs into the PSD has been shown to involve a trafficking process that is facilitated by PKMζ [34]. Because the details of this process are unknown, including which substrate of PKMζ mediates it, we model it as a

simple enzymatic reaction wherein PKMζ catalyzes the conversion of an uninserted GluA2-containing AMPAR, $A_{U,}$ to an inserted one, A_I.

$$P + A_U \xrightarrow{c_{15}} A_U \bullet P \quad (15)$$

$$A_U.P \xrightarrow{c_{16}} P + A_U \quad (16)$$

$$A_U.P \xrightarrow{c_{17}} P + A_I \quad (17)$$

The protein BRAG2 catalyzes endocytosis of GluA2-containing AMPARs, removal from the PSD.

$$B_A + A_I \xrightarrow{c_{18}} B_A \bullet A_I \quad (18)$$

$$B_A \bullet A_I \xrightarrow{c_{19}} B_A + A_I \quad (19)$$

$$B_A \bullet A_I \xrightarrow{c_{20}} B_A + A_U \quad (20)$$

A pair of unregulated processes maintain background cycling of GluA2-containing AMPARs into and out of the PSD:

$$A_U \xrightarrow{c_{21}} A_I \quad (21)$$

$$A_I \xrightarrow{c_{22}} A_U \quad (22)$$

Sequestering of PKMζ in the synaptic compartment. Our model implements the notion suggested by Sacktor [42] and supported by empirical results [20,33,57], that GluA2-containing AMPARs, when inserted in the PSD, prevent diffusion of PKMζ molecules away from the synapse and/or slows down degradation of PKMζ. We model this as a PKMζ molecule binding to an inserted GluA2-containing AMPAR to form a complex $A_I \bullet P$ (reaction 23) and by bound PKMζ having a much lower rate of dissipation/degradation than free PKMζ ($c_{24} << c_8$):

$$P + A_I \xrightarrow{c_{23}} A_I \bullet P \quad (23)$$

$$A_I \bullet P \xrightarrow{c_{24}} A_I \quad (24)$$

The $A_I \bullet P$ complex is dissolved if the GluA2-containing AMPAR is removed from the membrane by BRAG2 or constitutively:

$$B_A + A_I \bullet P \xrightarrow{c_{25}} B_A \bullet A_I \bullet P \quad (25)$$

$$B_A \bullet A_I \bullet P \xrightarrow{c_{26}} B_A + A_I \bullet P \quad (26)$$

$$B_A \bullet A_I \bullet P \xrightarrow{c_{27}} B_A + A_U + P \quad (27)$$

$$A_I \bullet P \xrightarrow{c_{28}} A_u + P \quad (28)$$

PKMζ remains catalytically active while sequestered by GluA2-containing AMPARs, thus the reactions catalyzed by free PKMζ (reactions 1-3 and 9-11) are also catalyzed by PKMζ when it is bound to A_I:

$$A_I \bullet P + R_I \xrightarrow{c_{29}} A_I \bullet P \bullet R_I \quad (29)$$

$$A_I \bullet P \bullet R_I \xrightarrow{c_{30}} A_I \bullet P + R_I \quad (30)$$

$$A_I \bullet P \bullet R_I \xrightarrow{c_{31}} A_I \bullet P + R_A \quad (31)$$

$$A_I \bullet P + B_A \xrightarrow{c_{32}} A_I \bullet P \bullet B_A \quad (32)$$

$$A_I \bullet P \bullet B_A \xrightarrow{c_{33}} A_I \bullet P + B_A \quad (33)$$

$$A_I \bullet P \bullet B_A \xrightarrow{c_{34}} A_I \bullet P + B_I \quad (34)$$

NMDAR stimulation. The mechanism by which NMDAR activation causes an increase in PKMζ is unknown. We model the effect of strong NMDAR stimulation as a rapid increase in the number of active molecules of an unspecified enzyme E1 which, like PKMζ, activates PKMζ mRNA. $E1_I$ and $E1_A$ represent the E1 enzyme in its active and inactive states, respectively:

$$E1_A + R_I \xrightarrow{c_{35}} E1_A \bullet R_I \quad (35)$$

$$E1_A \bullet R_I \xrightarrow{c_{36}} E1_A + R_I \quad (36)$$

$$E1_A \bullet R_I \xrightarrow{c_{37}} E1_A + R_A \quad (37)$$

The E1 enzyme spontaneously deactivates at a rate that is specified by the reaction constant c_{38}:

$$E1_A \xrightarrow{c_{38}} E1_I \quad (38)$$

Reactivation. Reactivation of a consolidated memory causes it to become destabilized [18,79,80]. The molecular mechanism underlying this destabilization is not well understood, but has been showed to depend critically on endocytosis of GluA2-containing AMPAR [20,57,63].

We model the destabilizing effect of reactivation as an increase in the number of molecules of a second unspecified enzyme, E2, which catalyzes AMPAR endocytosis:

$$E2_A + A_I \xrightarrow{c_{39}} E2_A + A_U \quad (39)$$

$$E2_A + A_I \bullet P \xrightarrow{c_{40}} E2_A + A_U + P \quad (40)$$

As in the case of BRAG2-catalyzed endocytosis (reaction 27), the AMPAR/PKMζ complex dissolves when the AMPAR is endocytosed (reaction 40).

The E_2 enzyme spontaneously deactivates at a rate that is specified by the reaction constant c_{41}:

$$E2_A \xrightarrow{c_{41}} E2_I \quad (41)$$

Protein synthesis inhibition. The effect of PSI infusion is simulated by disabling synthesis of PKMζ (reaction 7).

Inhibition of PKMζ by ZIP. The effect of ZIP infusion is simulated by disabling all PKMζ enzymatic activity (reactions 1, 9, 15, 29 and 32).

Inhibition of AMPAR endocytosis by GluA2$_{3Y}$. The effect of GluA2$_{3Y}$ infusion is simulated by disabling regulated AMPAR endocytosis, whether catalyzed by BRAG2 (reactions 18 and 25) or by the E2 enzyme (reactions 39 and 40).

The simulated reactions are summarized in Table 2. Reaction rates are controlled by Gillespie reaction constant, c_1, c_2, etc., such that $c_i\,dt$ is the average probability that a particular combination of the reactant molecules of reaction i will react during the next infinitesimal time interval dt [74]. The values for the reaction constants have been selected so that the model's behavior approximates the observed time courses of the simulated experiments; see cited references in the description of each simulation.

Table 2: Simulated reactions

	Reaction	Description	c_i (s^{-1})
1	$P + R_I \xrightarrow{c_1} P \bullet R_I$	Formation of P•R_I complex	10.0
2	$P \bullet R_I \xrightarrow{c_2} P + R_I$	Dissolution of P•R_I complex	400.0
3	$P \bullet R_I \xrightarrow{c_3} P + R_A$	Activation of PKMζ mRNA, catalyzed by PKMζ	100.0
4	$PP + R_A \xrightarrow{c_4} PP \bullet R_A$	Formation of PP•R_A complex	4.0
5	$PP \bullet R_A \xrightarrow{c_5} PP + R_A$	Dissolution of PP•R_A complex	400.0
6	$PP \bullet R_A \xrightarrow{c_6} PP + R_I$	Deactivation of PKMζ mRNA, catalyzed by phosphatase	100.0
7	$R_A \xrightarrow{c_7} R_A + P$	Translation of PKMζ mRNA	0.2
8	$P \xrightarrow{c_8} 0$	PKMζ degradation or dissipation	0.65
9	$P + B_A \xrightarrow{c_9} P \bullet B_A$	Formation of P•B_A complex	1.0
10	$P \bullet B_A \xrightarrow{c_{10}} P + B_A$	Dissolution of P•B_A complex	400.0
11	$P \bullet B_A \xrightarrow{c_{11}} P + B_I$	Inhibition of BRAG2, catalyzed by PKMζ	20.0
12	$PP + B_I \xrightarrow{c_{12}} PP \bullet B_I$	Formation of PP•B_I complex	1.0
13	$PP \bullet B_I \xrightarrow{c_{13}} PP + B_I$	Dissolution of PP•B_I complex	400.0
14	$PP \bullet B_I \xrightarrow{c_{14}} PP + B_A$	Disinhibition of BRAG2, catalyzed by phosphatase	0.06
15	$P + A_U \xrightarrow{c_{15}} A_U \bullet P$	Formation of P•A_U complex	0.4
16	$A_U.P \xrightarrow{c_{16}} P + A_U$	Dissolution of P•A_U complex	400.0
17	$A_U.P \xrightarrow{c_{17}} P + A_I$	PKMZ-catalyzed trafficking of GluA2-containing AMPAR into the PSD	20.0
18	$B_A + A_I \xrightarrow{c_{18}} B_A \bullet A_I$	Formation of B_A•A_I complex	10.0
19	$B_A \bullet A_I \xrightarrow{c_{19}} B_A + A_I$	Dissolution of B_A•A_I complex	400.0
20	$B_A \bullet A_I \xrightarrow{c_{20}} B_A + A_U$	BRAG2-catalyzed endocytosis of GluA2-containing AMPAR	4.0
21	$A_U \xrightarrow{c_{21}} A_I$	Unregulated trafficking of GluA2-containing AMPAR into the PSD	0.05
22	$A_I \xrightarrow{c_{22}} A_U$	Unregulated removal GluA2-containing AMPAR from the PSD	0.005
23	$P + A_I \xrightarrow{c_{23}} A_I \bullet P$	Inserted GluA2-containing AMPAR binds PKMζ	1.0
24	$A_I \bullet P \xrightarrow{c_{24}} A_I$	Degradation of PKMζ bound to inserted AMPAR	0.0001
25	$B_A + A_I \bullet P \xrightarrow{c_{25}} B_A \bullet A_I \bullet P$	Formation of B_A•A_I•P complex	10.0

26	$B_A \bullet A_I \bullet P \xrightarrow{c_{26}} B_A + A_I \bullet P$	Dissolution of BA•AI•P complex	400.0
27	$B_A \bullet A_I \bullet P \xrightarrow{c_{27}} B_A + A_U + P$	BRAG2-catalyzed endocytosis of GluA2-containing AMPAR with bound PKMζ.	4.0
28	$A_I \bullet P \xrightarrow{c_{28}} A_u + P$	Unregulated endocytosis of GluA2-containing AMPAR with bound PKMζ.	0.005
29	$A_I \bullet P + R_I \xrightarrow{c_{29}} A_I \bullet P \bullet R_I$	Formation of A_I•P•R_I complex	10.0
30	$A_I \bullet P \bullet R_I \xrightarrow{c_{30}} A_I \bullet P + R_I$	Dissolution of A_I•P•R_I complex	400.0
31	$A_I \bullet P \bullet R_I \xrightarrow{c_{31}} A_I \bullet P + R_A$	Activation of PKMζ mRNA, catalyzed by AMPAR-bound PKMζ	100.0
32	$A_I \bullet P + B_A \xrightarrow{c_{32}} A_I \bullet P \bullet B_A$	Formation of A_I•P•B_A complex	1.0
33	$A_I \bullet P \bullet B_A \xrightarrow{c_{33}} A_I \bullet P + B_A$	Dissolution of A_I•P•B_A complex	400.0
34	$A_I \bullet P \bullet B_A \xrightarrow{c_{34}} A_I \bullet P + B_I$	Inhibition of BRAG2, catalyzed by AMPAR-bound PKMζ	20.0
35	$E1_A + R_I \xrightarrow{c_{35}} E1_A \bullet R_I$	Formation of $E1_A$•R_I complex	10.0
36	$E1_A \bullet R_I \xrightarrow{c_{36}} E1_A + R_I$	Dissolution of $E1_A$•R_I complex	400.0
37	$E1_A \bullet R_I \xrightarrow{c_{37}} E1_A + R_A$	Activation of PKMζ mRNA, catalyzed by E1 enzyme	100.0
38	$E1_A \xrightarrow{c_{38}} E1_I$	Spontaneous deactivation of E1 enzyme	0.3
39	$E2_A + A_I \xrightarrow{c_{39}} E2_A + A_U$	Endocytosis of GluA2-containing AMPAR, catalyzed by E2 enzyme	0.1
40	$E2_A + A_I \bullet P \xrightarrow{c_{40}} E2_A + A_U + P$	E2-catalyzed endocytosis of GluA2-containing AMPAR with bound PKMζ	0.1
41	$E2_A \xrightarrow{c_{41}} E2_I$	Spontaneous deactivation of E2 enzyme	0.5

Simulation environment

The model is implemented as a C++ program and all simulations were executed on an Intel i5-2400 computer running the Debian Linux 8.4 operating system.

Objectives

Our computational model simulates the regulation of PKMζ concentration at the postsynaptic density and its role in the induction and maintenance of L-LTP. The goal for the model is to simulate the empirical results described in the introduction and summarized in Table 3 below. Most of the cited results are from studies of Schaffer collateral synapses on CA1 pyramidal

neurons in the rat or mouse hippocampus, a few refer to unspecified hippocampal regions or amygdala of rat or mouse.

Table 3: Simulation objectives

	Result	Description	Citations
1	**Induction by NMDAR stimulation**	Strong NMDAR stimulation induces L-LTP	[40,81]
2	**PSI blocks NMDAR-triggered L-LTP induction**	Infusion of protein synthesis inhibitors prevents L-LTP induction by NMDAR stimulation	[9,82]
3	**ZIP during stimulation does not prevent L-LTP induction**	ZIP treatment during and immediately after stimulation does not prevent establishment of L-LTP	[65]
4	**Induction by PKMζ perfusion**	Perfusion of PKMζ into a neuron induces L-LTP	[39,83]
5	**PSI does not disrupt established L-LTP/LTM**	Application of a protein synthesis inhibitor during L-LTP maintenance (without preceding reactivation) does not cause disruption of L-LTP	[12,18,79]
6	**Reactivation does not disrupt LTM**	Memory reactivation does not by itself disrupt LTM	[18,79]
7	**Reactivation followed by PSI infusion does disrupt LTM**	PSI administered within a time window after reactivation disrupts LTM	[18,79]
8	**GluA2$_{3Y}$ blocks the LTM-disrupting effect of PSI**	GluA2$_{3Y}$ administered together with PSI after reactivation blocks the LTM-disrupting effect of PSI	[20,57,63]
9	**ZIP disrupts established L-LTP**	Infusion of ZIP during the maintenance phase disrupts L-LTP	[39–41]
10	**GluA2$_{3Y}$ blocks the depotentiating effect of ZIP**	GluA2$_{3Y}$ infused together with ZIP prevents depotentiation of established L-LTP	[33]

Results

In the following plots of simulation results, P denotes the total number of PKMζ molecules in the synaptic compartment, whether free or bound to a substrate or to an AMPAR (see Table 1), and A_I denotes the number of AMPARs inserted in the PSD, with and without bound PKMζ molecules. Reaction numbers refer to the reactions described in Table 2.

NMDAR Stimulation Induces L-LTP

We model the result of strong NMDAR stimulation as a rapid increase of the population of active E1 enzyme molecules. This causes the translational repression of PKMζ mRNA to be lifted (reactions 35-37) and synthesis of PKMζ to start (reaction 7). Fig 2 shows a trace of the number of PKMζ molecules, active PKMζ mRNA molecules and GluA2-containing AMPARs inserted in the PSD during a single simulation run.

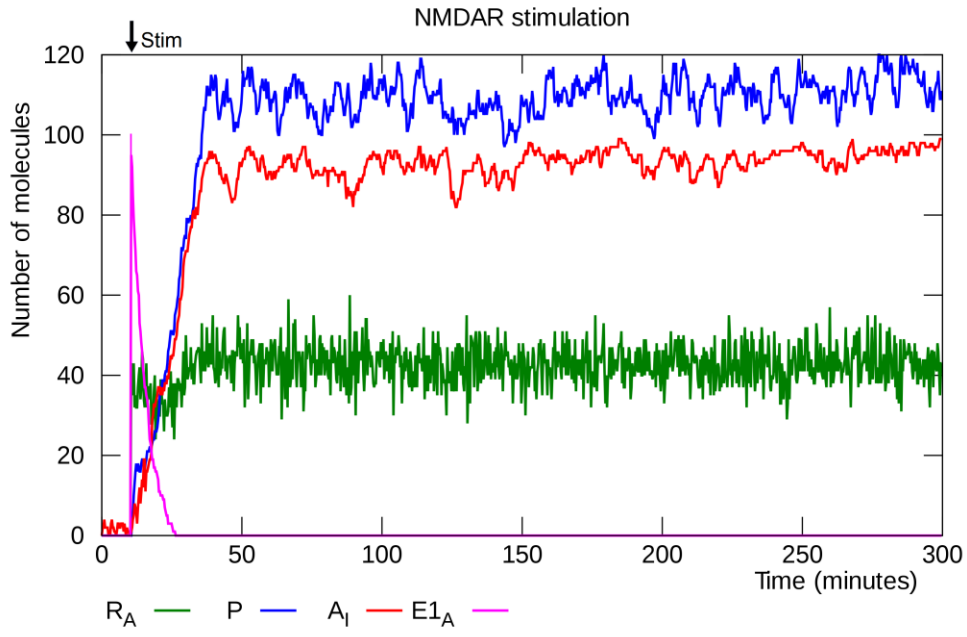

Fig 2: L-LTP induction, single simulation trace. NMDAR stimulation is simulated by instantaneous activation of 100 E1 molecules at "Stim". E1 lifts the translational

inhibition of PKMζ mRNA, synthesis of PKMζ starts, PKMζ drives up the number of inserted GluA2-containing AMPARs, and the synapse switches to its potentiated steady state. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme.

The model has two stable states: an unpotentiated state in which there are very few active mRNA molecules, PKMζ molecules and inserted GluA2-containing AMPARs, and a potentiated state with significantly higher levels of each of these molecules. The brief spike of E1 enzyme lifts the translational repression of enough PKMζ mRNA molecules to trigger a transition to the potentiated state. Although the molecule numbers fluctuate in the potentiated state, it is in fact very stable: No spontaneous depotentiation events are observed even when the model is allowed to run for a full year of simulated time. Fig 3 shows mean molecule counts for 100 simulations of L-LTP induction. It takes the model between 30 and 60 minutes of simulated time to complete the switch to its upper (potentiated) steady state in which there is a high number of inserted GluA2-containing AMPARs. This is consistent with the observed duration of the cellular consolidation window [16,58].

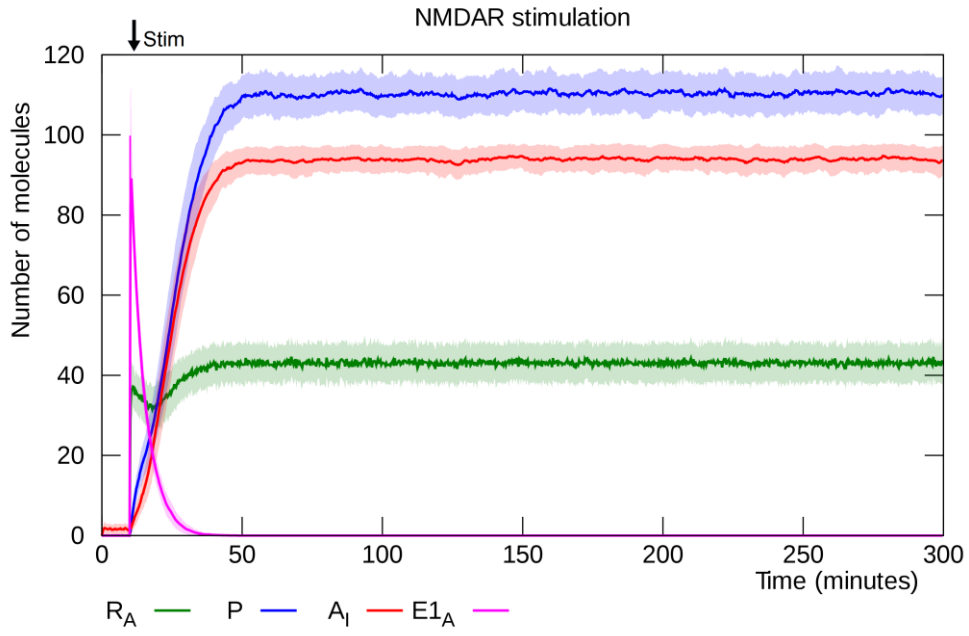

Fig 3: L-LTP induction. The same simulation as in Fig 2, but here solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. NMDA stimulation triggers a brief spike of E1 activity that activates PKMζ mRNA. This is followed by a slight decline in the number of active mRNA molecules, until the growing amount of PKMζ drives it back up and an equilibrium is reached. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme.

PSI Prevents L-LTP Induction

Simulated PSI infusion prevents NMDAR stimulation from inducing L-LTP, (Fig 4).

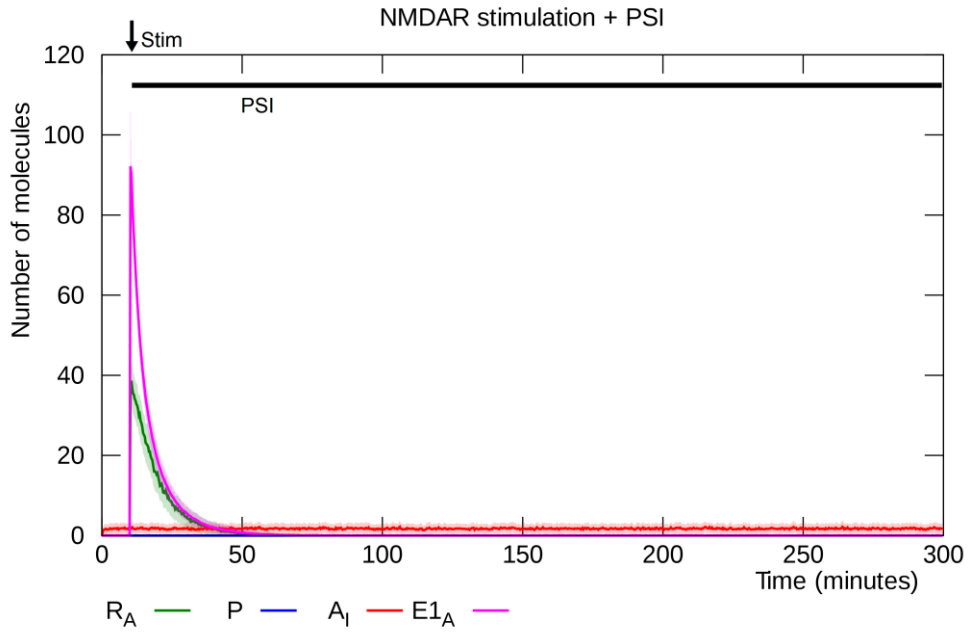

Fig 4: PSI prevents NMDAR stimulation from inducing L-LTP. $E1_A$ enzyme activates PKMζ mRNA, but PSI prevents PKMζ synthesis and when the E1 enzyme becomes inactivate, phosphatase returns the mRNA to its inhibited state. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme.

Although the spike of activated E1 enzyme releases the translational block of mRNA, resulting in a high level of activated PKMζ mRNA (R_A in the model), translation is prevented by the protein synthesis inhibitor, and PKMζ synthesis is not initiated [9,37]. When the E1 enzyme returns to its inactive form the mRNA becomes repressed again, and the model remains in its unpotentiated state. Like the potentiated state, the unpotentiated state is very stable: No spontaneous potentiation events are observed even when running the model for a year of simulated time.

By introducing a variable delay between stimulation and PSI infusion, we can study the model's consolidation window, the time interval after induction during which PSI prevents establishment of L-LTP. As shown in Fig 5, when the delay before PSI infusion is 20 minutes or less, the model consistently settles in the lower (unpotentiated) steady state with zero or very few inserted GluA2-containing AMPARs. When the delay is 50 minutes or more, the model settles in the upper (potentiated) state where the number of inserted GluA2-containing AMPARs fluctuates between ca 60 and 100 (cf. Fig 2). With intermediate delays, the probability of settling in the upper state gradually increases with increasing delay. The model's consolidation window is thus in the range 30 to 45 minutes, consistent with empirical results [11,12]. Fig 5 illustrates the model's bistable character: It settles either in the unpotentiated or potentiated state, never in the region with intermediate numbers of inserted GluA2-containing AMPARs. See also Fig 3 and Fig 4.

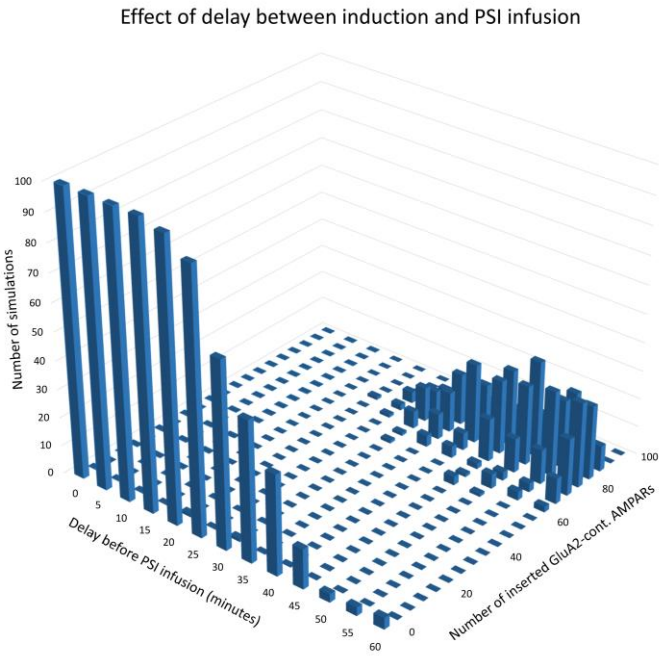

Fig 5: Consolidation window. Results of simulated NMDAR stimulation followed by PSI infusion after a delay varying from 0 to 60 minutes in 5-minute steps. One hundred simulations were run with each value for the delay. The number of inserted GluA2-containing AMPARs was recorded twenty hours after stimulation. For each value of the delay, the heights of the columns indicate the number of simulations that terminated with the corresponding numbers of inserted GluA2-containing AMPARs.

ZIP During and Immediately After Stimulation does not Prevent L-LTP induction

ZIP application during stimulation and the first 10 minutes thereafter after does not prevent L-LTP induction, (Fig 6).

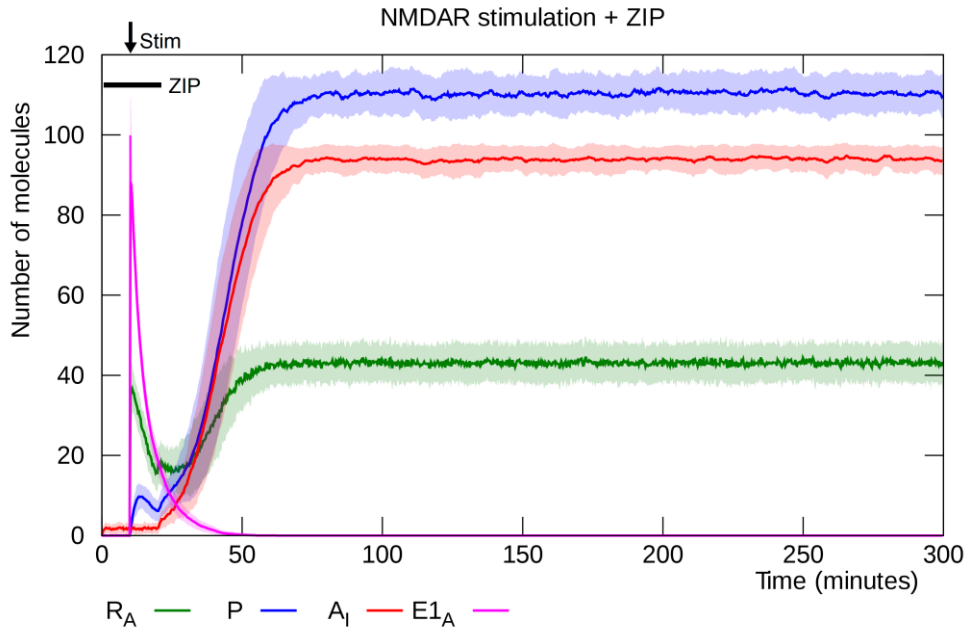

Fig 6: ZIP immediately after stimulation does not prevent L-LTP induction. In this simulation, ZIP inhibits PKMζ activity during the first 10 minutes after stimulation. L-LTP induction is delayed somewhat compared to Fig 3, but enough active PKMζ mRNA remains when the ZIP is removed to trigger a transition to the potentiation state. Solid

lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme.

Presence of ZIP during the first ten minutes after stimulation does not prevent L-LTP induction [65]. The stimulation lifts the translational block and PKMζ production gets started. Even though PKMζ's enzymatic activity is inhibited, the mRNA stays activated long enough to ride out the ZIP activity. When the ZIP is washed out, PKMζ becomes active and drives the synapse into its potentiated state.

PKMζ Infusion Induces L-LTP

L-LTP can be induced by diffusion of PKMζ into a neuron [39,41]. We simulate infusion by rapidly increasing the number of PKMζ molecules in the synaptic compartment to 100. This causes the model to settle into its potentiated state, (Fig 7).

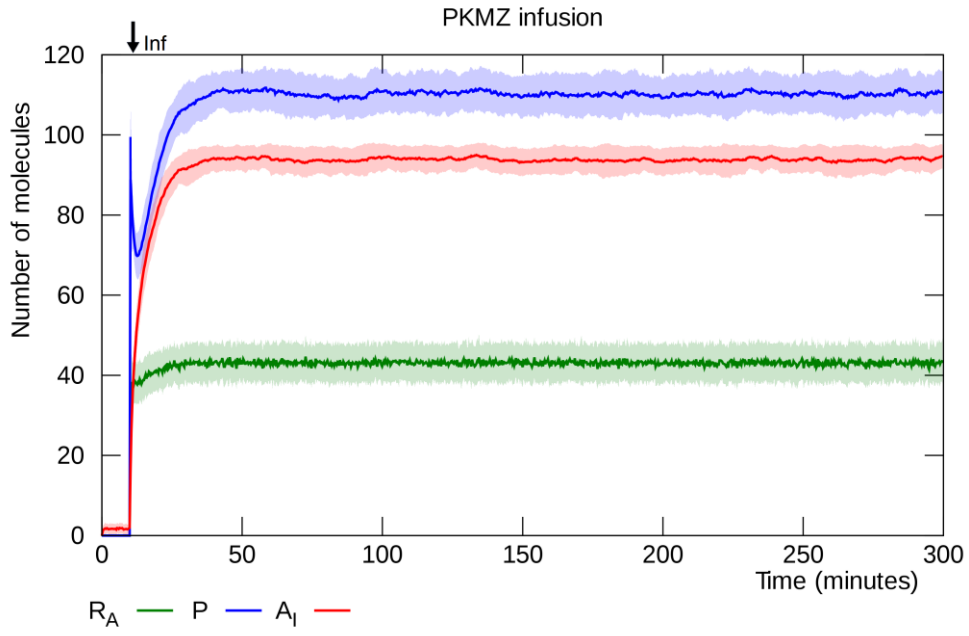

Fig 7: PKMζ infusion induces L-LTP. Infusion is simulated by stepping the PKMζ molecule count to 100 at "Inf". The PKMζ lifts the translational inhibition of PKMζ mRNA, synthesis starts and the synapse switches to its potentiated state. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs.

PSI Blocks PKMζ-Infusion-Induced Potentiation

The same level of PKMζ infusion that induces L-LTP in the previous experiment (100 molecules) fails to do so in the presence of PSI (Fig 8). Although the PKMζ infusion initially causes a temporary increase in the number of inserted GluA2-containing AMPARs, the PSI prevents replenishment to compensate for PKMζ degradation and dissipation and the model returns to its unpotentiated state. This result, though plausible, has not been demonstrated in a published experiment. It thus constitutes a prediction of the model.

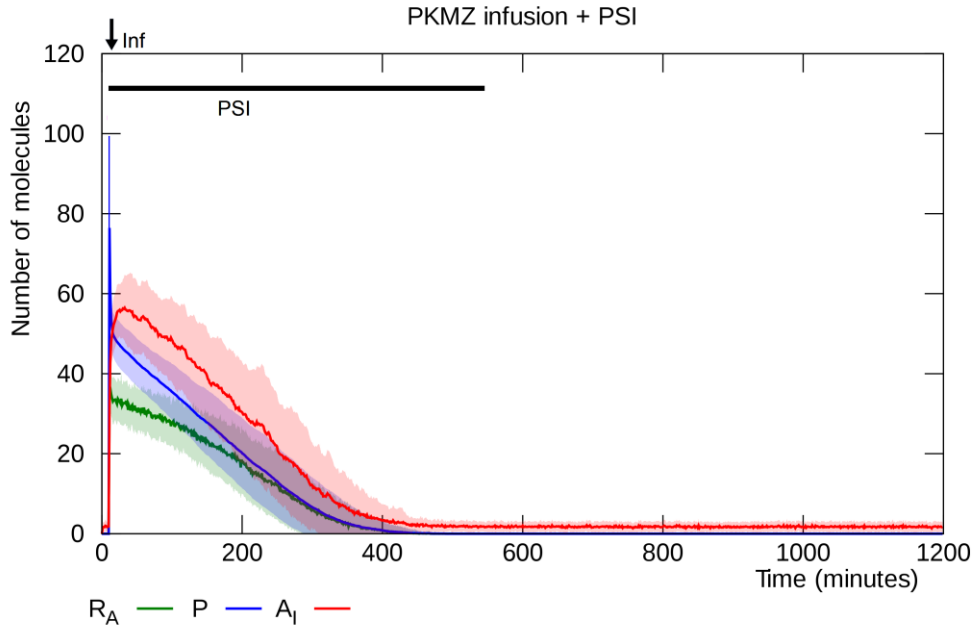

Fig 8: PSI blocks L-LTP induction by PKMζ infusion. As in Fig 7, infusion of PKMζ is simulated at "Inf". PKMζ triggers activation of PKMζ mRNA as well as an increase of inserted GluA2-containing AMPARs, but in the absence of PKMζ synthesis (blocked by PSI), the PKMζ level declines and the synapse settles back into its unpotentiated state. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs.

PSI does not Disrupt Established L-LTP

Fonseca et al. [12] demonstrated that suppressing protein synthesis for 100 minutes by bath application of anisomycin did not disrupt established L-LTP. Fig 9 shows the results of simulating this experiment in our model. The interruption of protein synthesis causes the number of PKMζ molecules to drop, which in turn leads to a transient decline in the number of inserted GluA2-containing AMPARs, but the system recovers when the PSI is removed.

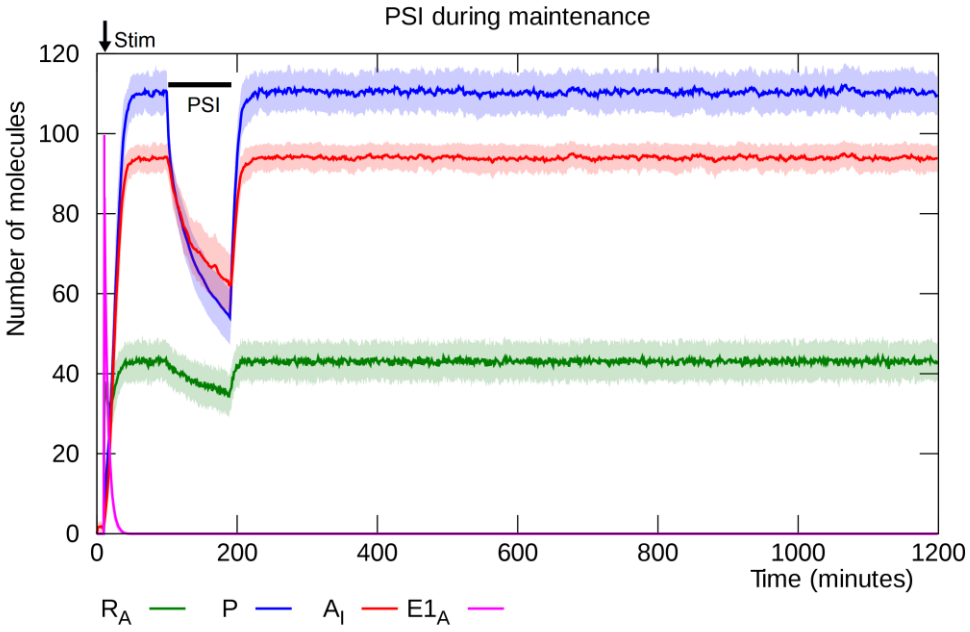

Fig 9: PSI infusion during the maintenance phase does not disrupt established L-LTP. L-LTP is induced by NMDAR stimulation at "Stim". Once L-LTP is established (100 minutes after induction), protein synthesis inhibition is applied for 100 minutes. The interruption of kinase synthesis causes a decline in the levels of PKMζ and inserted GluA2-containing AMPARs, but the synapse recovers when the PSI is removed. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. RA: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme.

If the model is correct, then the transient decrease in the number of GluA2-containing AMPARs may be detectable as a reduced EPSP current after PSI application. However, it is possible that the temporary removal of GluA2-containing AMPARs is compensated for by insertion of GluA2-lacking AMPARs, similarly to what has been shown to happen during retrieval-induced destabilization [45], in which case the synaptic strength would be maintained. If this is the case, then it may instead be possible to detect a transient increase in rectification index, because GluA2-lacking AMPARs, but not GluA2-contaning ones, are characterized by a slight inward rectification [20,45]. Our model thus predicts that one or the other of these two effects (EPSP reduction or rectification) should be detectable after PSI application during L-LTP maintenance.

Reactivation Destabilizes, but does not Disrupt, L-LTP

The effect of memory reactivation is simulated as a brief spike in the amount of active E2 enzyme (Fig 10). This results in rapid endocytosis of the inserted GluA2-containing AMPARs [20,21] and release of the bound PKMζ molecules which then start to dissipate. However, due to continued synthesis, the PKMζ level is kept from dropping below threshold and the model settles back into the potentiated steady state [18,79].

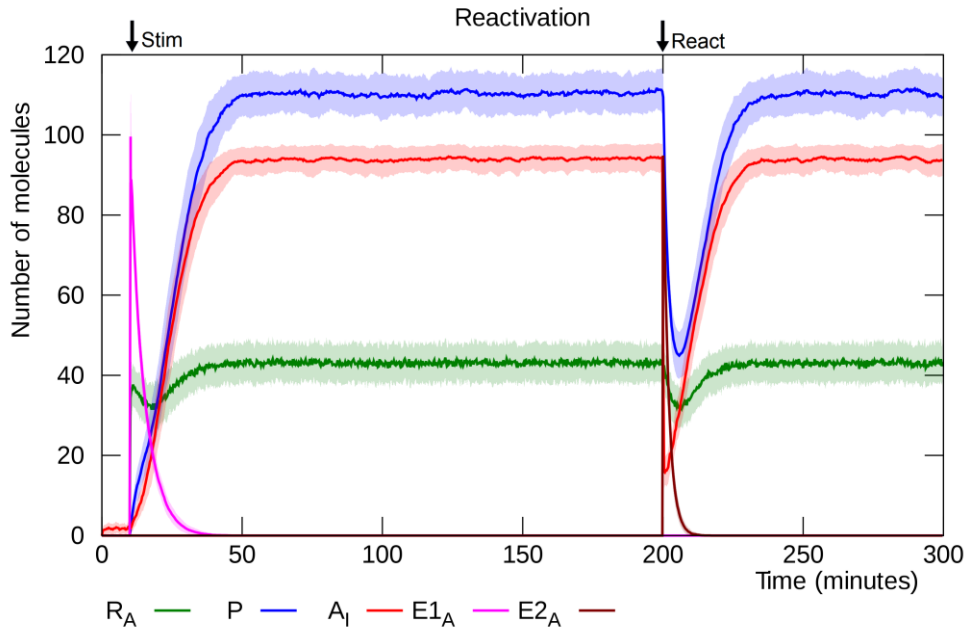

Fig 10: Reactivation. NMDAR stimulation is simulated by a pulse of active E1 enzyme at "Stim", and reactivation by a pulse of active E2 enzyme at "React". $E2_A$ causes rapid endocytosis of GluA2-containing AMPARs, which in turn leads to PKMζ depletion. PKMζ mRNA only declines slowly, however, and the synapse returns to its potentiated state when the E2 enzyme deactivates. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme, $E2_A$: activated E2 enzyme.

Although the population of inserted GluA2-containing AMPARs is almost completely depleted after reactivation, the levels of PKMζ and active PKMζ mRNA stay well above their depotentiation thresholds and the model reliably recovers from post-reactivation instability (reconsolidation), unless challenged by simulated pharmacological interventions (see below). As mentioned earlier, Hong et al. demonstrated this abrupt decrease of inserted GluA2-containing

AMPARs after memory retrieval, as well as a corresponding transient increase of GluA2-lacking AMPARs, which maintained the synaptic strength during the labile period [20].

Reactivation Followed by PSI Disrupts L-LTP

Simulation of PSI infusion simultaneously with reactivation, or shortly thereafter, causes disruption of L-LTP (Fig 11).

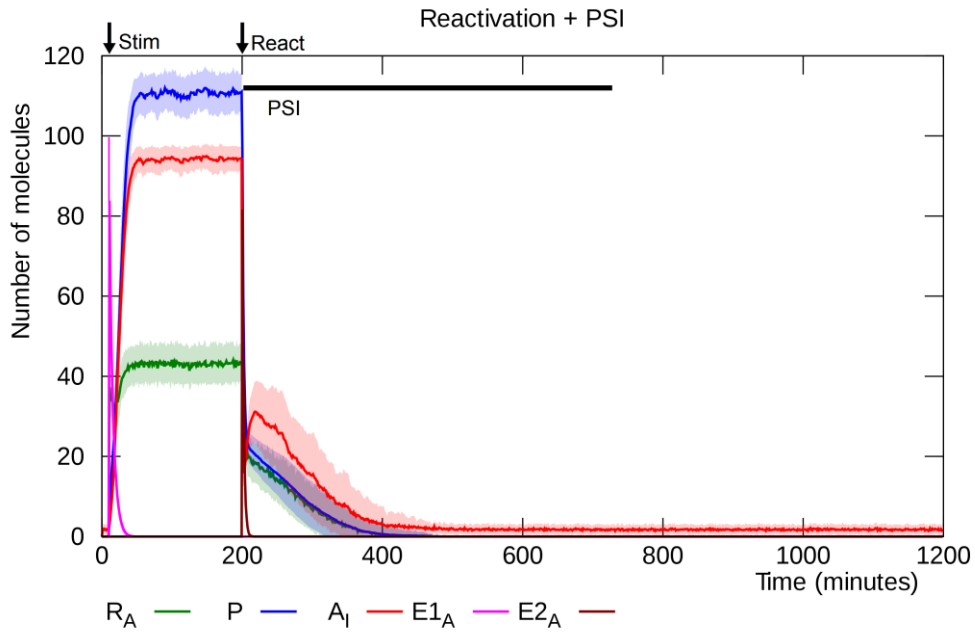

Fig 11: Reactivation with simultaneous PSI infusion. As in Fig 10, reactivation is simulated as a pulse of active E2 enzyme at "React", but here the presence of PSI prevents recovery and L-LTP is disrupted. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme, $E2_A$: activated E2 enzyme.

In the absence of new protein synthesis, the PKMζ level drops below threshold and the model settles into its unpotentiated state [18,79]. By varying the delay between reactivation and PSI infusion, we can establish the model's reconsolidation window, the time interval after reactivation during which L-LTP is vulnerable to PSI. As shown in Fig 12, if PSI infusion is applied 15 minutes or less after reactivation, then the model reliably switches to its lower (unpotentiated) steady state with few inserted GluA2-containing AMPARs, but with a delay of 30 minutes or more, L-LTP disruption does not result: the model remains in its potentiated state where the number of inserted GluA2-containing AMPARs fluctuates in the 60-100 range. The model's reconsolidation window is thus in the range 20 to 30 minutes, consistent with empirical results [18,84].

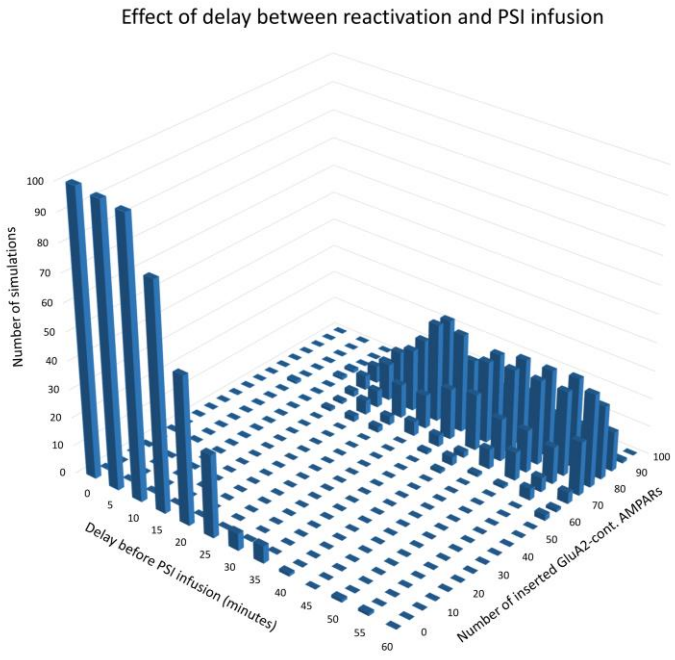

Fig 12: Reconsolidation window. Results of simulated reactivation followed by PSI infusion. The delay between reactivation and PSI infusion is varied from 0 to 60 minutes

in 5-minute steps. One hundred simulations were run with each value for the delay. The number of inserted GluA2-containing AMPARs was recorded twenty hours after stimulation. For each value of the delay, the heights of the columns indicate the number of simulations that terminated with the corresponding numbers of inserted AMPARs.

GluA2$_{3Y}$ Blocks Post-Reactivation PSI-Infusion From Causing Depotentiation

When the GluA2$_{3Y}$ peptide is infused together with PSI after reactivation, it prevents the disruption of L-LTP that PSI otherwise causes [57,63].

As before, reactivation triggers activation of the E2 enzyme, but here the GluA2$_{3Y}$ peptide blocks its endocytotic effect. As a result, the GluA2-containing AMPARs remain inserted and although the PSI stops synthesis of new PKMζ, the existing population of PKMζ molecules, bound to the inserted GluA2-containing AMPARs, declines at a slow enough rate to maintain the synapse in its potentiated state while the PSI wears off (Fig 13).

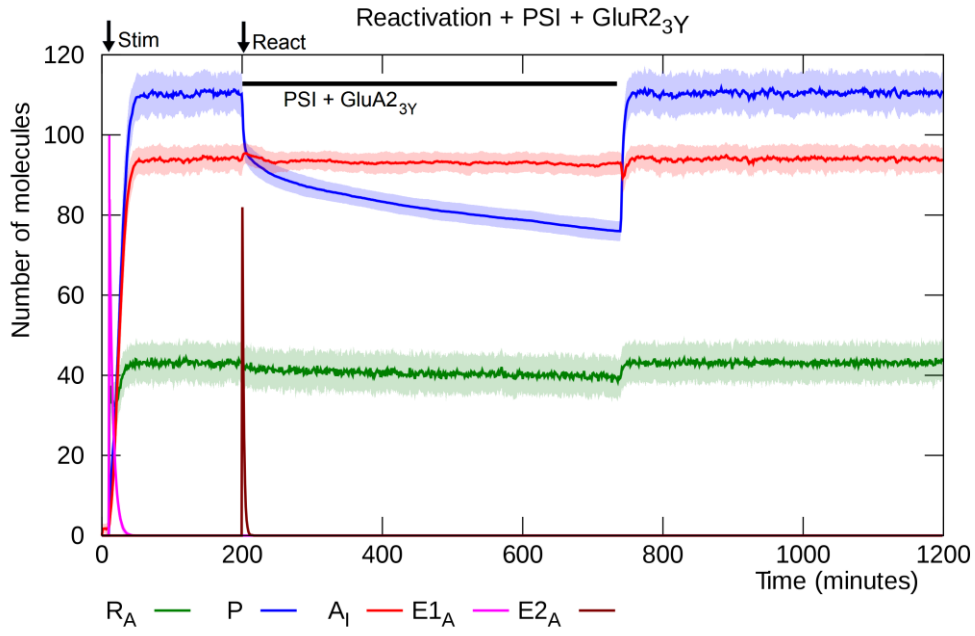

Fig 13: Infusion of PSI and GluA2$_{3Y}$ immediately after reactivation. In this simulation the endocytotic effect of the reactivation-triggered pulse of active E2 enzyme is blocked by GLUA2$_{3Y}$. As a result, the GluA2-containing AMPARs remain inserted and continue to sequester PKMζ molecules. The post-reactivation application of PSI still causes a decline in the level of PKMζ, but because of the low dissipation/degradation rate, the PKMζ level remains high enough that the L-LTP survives until the PSI wears off. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme, $E2_A$: activated E2 enzyme.

ZIP Infusion Disrupts Established L-LTP

Infusion of ZIP during L-LTP maintenance causes rapid depotentiation [39–41]. ZIP inhibits PKMζ enzymatic activity, including both the catalysis of its own synthesis and the maintenance of an increased level of inserted GluA2-containing AMPARs in the PSD. The result is rapid removal of GluA2-containing AMPARs and depletion of PKMζ, and the synapse quickly settles into its unpotentiated state (Fig 14). The minimum duration of ZIP application needed to reliably disrupt L-LTP in the model is around 30 minutes.

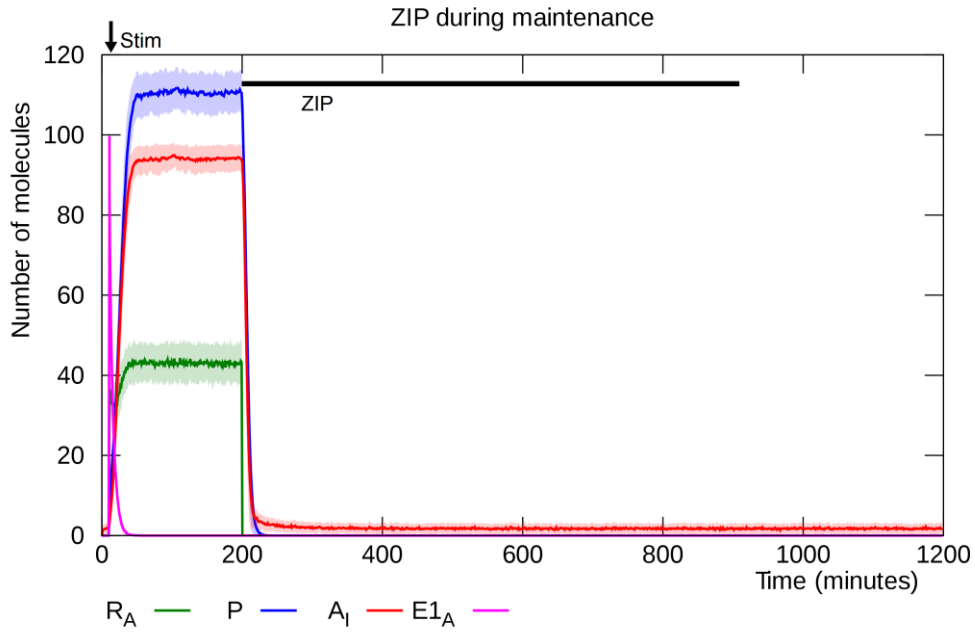

Fig 14: ZIP infusion during L-LTP maintenance. Application of ZIP inhibits PKMζ's enzymatic activity, leading to rapid depotentiation. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme.

GluA2$_{3Y}$ Blocks Depotentiation by ZIP Infusion

When the GluA2$_{3Y}$ peptide is infused together with ZIP during L-LTP maintenance, the disruptive effect of ZIP is blocked [33].

As before, ZIP inhibits PKMζ's catalysis of its own synthesis as well as its facilitation of AMPAR trafficking into the PSD and its blocking effect on BRAG2-induced endocytosis of GluA2-containing AMPAR. But in this case, even though BRAG2 remains active, the presence of GluA2$_{3Y}$ prevents it from inducing endocytosis of the inserted GluA2-containing AMPARs. As a result, the GluA2-containing AMPARs remain in the PSD and continue to maintain the

PKMζ molecules at the synapse. The number of PKMζ molecules declines only slowly and the potentiation is able to survive through the 12-hour period of ZIP activity (Fig 15).

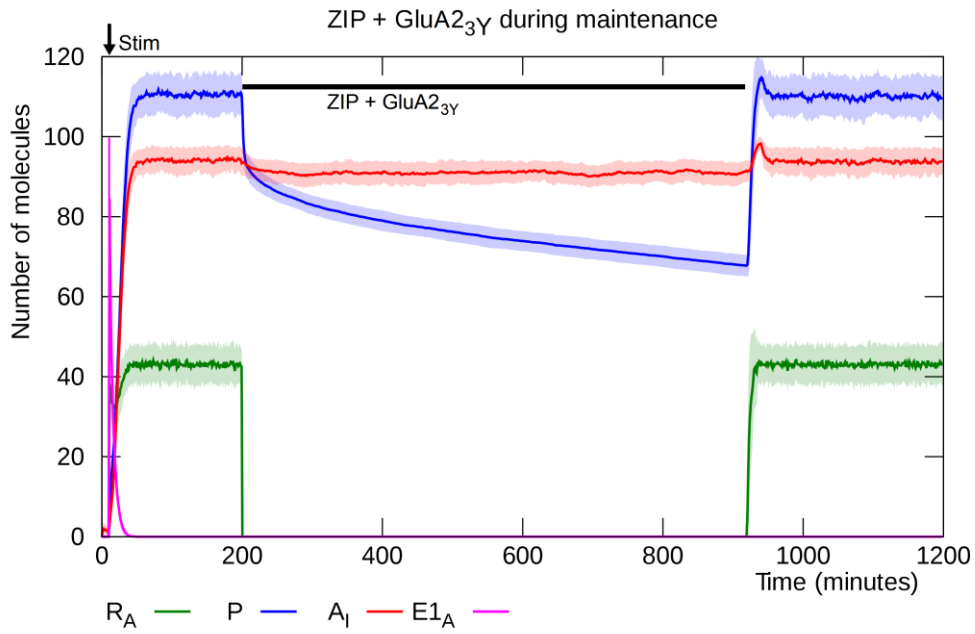

Fig 15: Infusion of ZIP and GluA2$_{3Y}$ during L-LTP maintenance. ZIP blocks PKMζ's enzymatic activity: PKMζ mRNA returns to its untranslatable state, and BRAG2 becomes active. However, GluA2$_{3Y}$ prevents BRAG2 from inducing GluA2-containing AMPAR endocytosis, the PKMζ molecules remain attached to the inserted AMPARs, and the catastrophic disruption of L-LTP seen in Fig 14 is averted. Solid lines represent mean molecule counts for 100 simulations. Lightly colored bands indicate standard deviation. R_A: active PKMζ mRNA, P: PKMζ, A_I: inserted GluA2-containing AMPARs, $E1_A$: activated E1 enzyme.

Discussion

The model presented here is able to explain a range of results relating to the role of PKMζ in late-phase long-term synaptic potentiation, including L-LTP induction by NMDAR stimulation

or by PKMζ infusion and the findings that whereas PSI, but not ZIP, can block induction of L-LTP, the reverse is true for disruption of established L-LTP. In addition, it accounts for cellular reconsolidation, reconsolidation blockade by PSI infusion and prevention of ZIP- or PSI-induced depotentiation by infusion of the $GluA2_{3Y}$ peptide. While subsets of these results have been covered by earlier models [85–89], ours is the first to account for all of them. A further distinguishing feature of our model is that it demonstrates that a wide range of empirical findings described in the LTP literature can be accounted for by simple molecular reactions whose rates are governed only by the law of mass action, i.e. without postulating cooperative binding or other non-linear dependencies on reactant concentrations.

Our model demonstrates that a bistable mechanism for synaptic potentiation can arise from the interaction of two coupled feedback loops, neither of which needs itself be bistable. One of these, the mutual reinforcement between PKMζ and PKMζ mRNA, has been featured in previously published models of L-LTP maintenance [85–88]. The second positive feedback relationship in our model is between PKMζ and inserted GluA2-containing AMPARs, which mutually maintain each other by inhibiting each other's removal from the synapse [42]. The ability of inserted AMPARs to sequester PKMζ molecules at the synapse allows the model to account for findings involving the inhibition of regulated endocytosis of GluA2-containing AMPAR [33,57].

Our model exhibits robust bistability; when left to run for a full year of simulated time in either the potentiated or depotentiated state, no spontaneous transitions between the steady-states were observed. The source of this bistability can be understood by considering the interaction between the two feedback loops. The PKMζ-mRNA interaction is a positive feedback loop: A greater number of PKMζ molecules will keep more mRNA molecules in an unrepressed state and more unrepressed mRNA results in a higher rate of PKMζ synthesis. This subsystem has two steady

states: a lower steady state with zero PKMζ molecules and zero unrepressed mRNA molecules, and a higher state at a level that depends on the reaction rates, in particular PKMζ's dissipation rate, because at equilibrium the synthesis and dissipation rates are equal. The lower steady state is unstable; the introduction of just a few PKMζ molecules can cause a switch to the upper state. The PKMζ – mRNA feedback loop thus has only a single stable steady state which depends on the PKMζ dissipation rate, as illustrated in Fig 16.

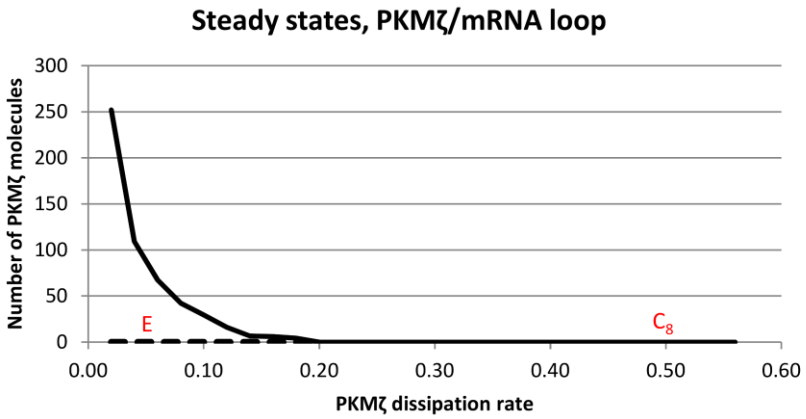

Fig 16: PKMζ level at steady state as a function of dissipation rate. The solid line represents a stable steady state, and the dashed line an unstable steady state. The x-axis represents the reaction constant for PKMζ dissipation/degradation. "C_8" indicates the value used for the reaction constant of reaction 8, dissipation/degradation of unbound PKMζ. "E" indicates the effective dissipation rate in the potentiated state, when a large proportion of the PKMζ molecules are bound to inserted GluA2-containing AMPARs.

Bistability arises because of the influence of the second feedback loop, the interaction between PKMζ and GluA2-containing AMPARs. In the unpotentiated state, the PKMζ dissipation/degradation rate is controlled by the reaction constant c_8, which has a value of 0.5. As

seen in Fig 16, the steady state at this rate has zero PKMζ molecules. In the potentiated state, an increased number of GluA2-containing AMPARs in the PSD bind PKMζ molecules; this results in a reduction of the effective PKMζ dissipation/degradation rate to a value where the steady state has ca 100 PKMζ molecules (indicated by 'E' in Fig 16).

Comparison With Previous Computational Models of PKMζ Regulation

Clopath et al. [89] describe a mathematical model of synaptic tagging and capture (STC) [90], wherein mechanisms of tag-setting and triggering of protein synthesis interact with a bistable process that maintains potentiation. Although the authors suggest that one of the model's parameters may represent the level of PKMζ activity, the mechanisms of the process are unspecified, and the model therefore cannot account for the results targeted by our model: the effects of PSI, ZIP and $GluA2_{3Y}$ in the contexts of L-LTP induction and maintenance, or of memory reactivation.

A simple model by Ogasawara and Kawato [86] simulates L-LTP induction and maintenance as well as reconsolidation based on the interactions of only three molecules: PKMζ, PKMζ mRNA and F-Actin. It is, however, not able to account for most of the results addressed in this paper.

A paper by Zhang et al. [88] features a dual-loop model of LTP that exhibits windows of susceptibility to PSI after induction and reactivation as well as vulnerability to a kinase inhibitor in the maintenance phase. The relationship between the kinase and AMPA receptors is not modeled, and thus the ability of an endocytosis blocker like $GluA2_{3Y}$ to rescue L-LTP is not accounted for. Also, the kinase modeled in [88] is unnamed but characterized by auto-activation rather than persistent activity, and should therefore probably not be interpreted as PKMζ.

Smolen et al. [87] model synaptic tagging and capture, including "cross-tagging" between LTP and LTD. As in our model, synaptic stability is based on PKMζ's ability to catalyze its own

synthesis. Unlike our model, [87] does not account for the effects of protein synthesis inhibition, kinase inhibition, reactivation or the ability of endocytosis blocking to rescue L-LTP.

A paper by Jalil et al. [85] models PKMζ regulation at the synapse, with a focus on compensatory interactions between PKMζ and a second atypical PKC isoform, $PKC_{\iota/\lambda}$. Bistability is achieved by combining the PKMζ auto-catalytic synthesis feedback loop with auto-phosphorylation. The model predicts the differential effects of ZIP and PSI at L-LTP induction and maintenance, but does not account for L-LTP rescue by AMPAR endocytosis blocking, nor for reconsolidation.

Limitations

Our model represents a subset of the mechanisms believed to be involved in LTP induction and maintenance [3,91]. Some processes not included in our model are:

- the induction and stabilization of early LTP, which likely involves GluA2-lacking AMPARs [45], the MAPK/ERK signaling pathway and the proteins PKA, CaMKII [91] and PKCλ [65,92]
- a later phase of L-LTP, sometimes called LTP3, which requires gene transcription as well as mRNA translation [93] and may involve a "tagging and capture" mechanism for selectively targeting gene products to potentiated synapses [40,90].
- polymerization/depolymerization of actin and restructuring of the cytoskeleton [94,95]

The processes that we have modeled thus form a subset of a more complex machinery. Nevertheless, it is interesting to note that this relatively simple model is able to account for many of the empirical findings regarding the role of PKMζ in L-LTP induction and maintenance, and

to exhibit the degree of stability required for a neural mechanism to support long-lasting memories.

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Supporting Information

S1 Text. Estimating the number of PKMζ molecules in a spine head.

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S1 Text. Estimating the number of PKMζ molecules in a spine head

Zeta-inhibitory peptide (ZIP) disrupts the ability of PKMζ to potentiate synaptic transmission when applied extracellularly in concentrations around 1 μM [1]. According to Serrano et al. [2], 5 μM completely blocks potentiation and 1 μM reduces the increase in AMPAR response by 50%.

Assuming that ZIP molecules enter neurons by diffusion only (i.e. they are not actively transported into cells), 1 μM would be an upper limit on the intracellular ZIP concentration.

1 μM = 10^{-6} * 6.022 * 10^{23} molecules/liter = 10^{-6} * 6.022 * 10^{23} * 10^{3} ≈ 6 *10^{20} molecules/m^3.

The volume of a dendritic spine head is between 0.01 and 0.1 μm^3 [3,4]. If we conservatively use the upper limit of this range, 10^{-1} μm^3 = 10^{-19} m^3, then the maximum number of ZIP molecules that would be present in a spine head due to a 1 μM bath concentration would be ≈ 6 *10^{20} * 10^{-19} = 60.

Because ZIP's inhibitory action results from ZIP molecules binding to PKMζ molecules in a one-to-one ratio, we may assume that for ZIP to significantly disrupt PKMζ activity, the number of ZIP molecules must be at least of the same order of magnitude as the number of PKMζ molecules.

Consequently, the number of PKMζ molecules at a potentiated synapse may be estimated to be fewer than about one hundred.

Note: this estimate does not take into account that ZIP is unevenly distributed in the spine head due to myristoylation, which gives it an affinity for the cell membrane. The same applies to other molecules in the simulation: the Gillespie algorithm assumes even distribution in the reaction vessel.

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CHAPTER THREE: A MODEL OF SYSTEMS CONSOLIDATION AND RECONSOLIDATION

The second paper addresses memory consolidation and reconsolidation on the systems level, with an emphasis on their relationships to the synaptic phenomena investigated in the first paper. In particular, it explores the question of whether LTP-like mechanisms at the synaptic level can explain findings from memory research at the systems level.

BACKGROUND

In this section I describe the series of discoveries that led to our current understanding of systems consolidation and reconsolidation.

SYSTEMS CONSOLIDATION

Scoville and Milner (1957) discovered in their work with the late patient Henry Molaison (known widely as H. M.) that bilateral damage to the hippocampus caused dense anterograde amnesia, the inability to acquire new memories, as well as profound retrograde amnesia, i.e. impaired recall of previously acquired memories. The retrograde amnesia was temporally graded: recently learned memories were more severely impaired than older ones. Such a temporal gradient has typically (though not always) been observed in subsequent studies of bilateral hippocampal damage in humans (Dudai, 2004; Squire & Alvarez, 1995), and has also been reported in behavioral experiments with animals, including mice (Kitamura et al., 2009), rats (Kim & Fanselow, 1992; Winocur, 1990), rabbits (Kim et al., 1995) and monkeys (Zola-Morgan & Squire, 1990). The observation that recent memories are more vulnerable than older ones to hippocampal damage gave rise to the notion that the hippocampus plays a crucial role not only in acquisition, but also in the maintenance and recall of new memories, but over time these functions are taken over by the neocortex (Marr, 1970, 1971; McClelland et al., 1995; Milner, 1989; Nadel & Hardt, 2010; Squire & Zola-Morgan, 1991).

THE STANDARD THEORY OF SYSTEMS CONSOLIDATION

What could the benefit be of first encoding memories in the hippocampus and then gradually establishing them in the neocortex? According to what has become known as the standard theory of systems consolidation (Frankland & Bontempi, 2005; McClelland et al., 1995; Squire & Alvarez, 1995), the neocortex is a "slow learner", unable to record memories in real time, whereas the hippocampus, in contrast, is able to quickly create memory traces of events as they happen. Subsequently, hippocampal memory traces are spontaneously and repeatedly reactivated over an extended period of time, perhaps primarily during slow-wave sleep (Langille, 2019; Leonard, McNaughton, & Barnes, 1987; Squire & Alvarez, 1995). Such replay events, so the theory goes, causes the original activation patterns to be reinstated in the neocortex, allowing the more time-consuming establishment of intra-neocortical connections.

Several explanations have been proposed for why the creation of memory traces takes longer in the neocortex than in the hippocampus. McClelland, McNaughton and O'Reilly (1995) suggested that the neocortex has a lower "learning rate" than the hippocampus, and that this serves to avoid "catastrophic interference", a phenomenon observed in some artificial neural networks, whereby new learning tends to destroy older memories. In McClelland et al.'s model, the hippocampus learns quickly, then repeatedly replays both new and – for some time – older memories to the neocortex. Because of the low learning rate in neocortical circuits, this procedure allows new patterns to be gently integrated instead of clobbering previously learned material. Another explanation is based on the observation that neurons in the hippocampus are much more densely interconnected than those in the neocortex (Chklovskii, Mel, & Svoboda, 2004; Frankland & Bontempi, 2005; Lisman & Morris, 2001). Creation of a new hippocampal memory trace is therefore thought to consist mostly in adjusting the strengths of existing

synapses, whereas laying down a trace in the neocortex requires the more time-consuming processes of axonal growth and synaptogenesis to create new connections between neurons (Chklovskii et al., 2004; Lisman & Morris, 2001; Maviel, Durkin, Menzaghi, & Bontempi, 2004). Moreover, creation of a neocortical memory is a complex process of integration with pre-existing memories that involves reorganization and reclassification of information (Frankland & Bontempi, 2005; Marr, 1970).

The idea that hippocampal "replay" can trigger reinstatement of activation patterns in neocortex raises the question of what kind of information is stored in the hippocampus. McClelland et al., in their "two memory systems" proposal (1995), suggested that the hippocampus stores "compressed representations" of cortical patterns, that could be decoded to reconstruct activation patterns in neocortex, but most authors favor a view where the hippocampus does not store memory content, but rather "indices" (Teyler & Discenna, 1986) or "links" to loci in the neocortex where components of memory traces are recorded (Alvarez & Squire, 1994; Frankland & Bontempi, 2005). In these accounts, the hippocampus plays a dual role: it supports, through replay, the establishment and/or strengthening of intra-neocortical connection, and it also assist in memory retrieval until those connections become strong enough to function independently.

In summary, the standard theory of systems consolidation maintains that memory traces in the hippocampus provide linkage between activation patterns in the neocortex, which together form a complete memory (Squire & Zola-Morgan, 1991; Teyler & Discenna, 1986). But this role is temporary; over time the establishment of intra-neocortical connections makes memories independent of the hippocampus (Squire & Zola-Morgan, 1991).

THE MULTIPLE-TRACE THEORY

Nadel and Moscovitch (1997) proposed an alternative explanation for the temporally graded retrograde amnesia produced by hippocampal damage. According to their Multiple Trace Theory (MTT), hippocampal traces are created not only when an episode is experienced, but also on each occasion when it is recalled. Older memories thus come to be represented by a greater number of traces distributed throughout the hippocampal complex, and because of this redundancy will be less affected than new memories by any partial hippocampal damage. MTT also posits that episodic (temporal and spatial) aspects of a memory remain hippocampus-dependent indefinitely, whereas semantic information (knowledge about the world) is stored in the neocortex and other extra-hippocampal structures.

In more recent statements of this theory (Moscovitch, Cabeza, Winocur, & Nadel, 2016; Nadel, Winocur, Ryan, & Moscovitch, 2007; Winocur, Sekeres, Binns, & Moscovitch, 2013), the multiple-trace aspect has been de-emphasized, while the idea of differential treatments for semantic and episodic memories has been retained: semantic memories become hippocampus-independent through a process of systems consolidation in the way the standard theory suggests (Nadel et al., 2007), whereas episodic information remains dependent on the hippocampus. The quality of autobiographical memories is therefore transformed over time as detailed spatiotemporal information is gradually lost or degraded in the hippocampus while "gist-like" or semantic representations persist in neocortex (Moscovitch et al., 2016; Winocur, Moscovitch, & Bontempi, 2010). Thus the theory in its current form, now known as the Transformation Hypothesis (Winocur, Frankland, Sekeres, Fogel, & Moscovitch, 2009; Winocur et al., 2010), agrees with the standard theory about the temporary role of the hippocampus in the acquisition and consolidation of semantic memory, but differs from it in positing that episodic-

type (spatial and temporal) information remains hippocampus-dependent for as long as it is retained (Winocur & Moscovitch, 2011). Empirical support for the notion that spatial specificity is lost as memories age includes rodent studies by Wiltgen et al. (2010), Gafford, Parsons, & Helmstetter (2013) and Einarsson et al. (2015). On the other hand, Dede and Smith, in a comprehensive review of human cases of temporal lobe damage (Dede & Smith, 2016), conclude that there is no evidence for loss of remote episodic information after hippocampal damage, contradicting the transformation hypothesis and supporting the standard theory.

While the role of the hippocampus in episodic memory thus remains controversial, there is good evidence that semantic information does undergo systems consolidation. In addition to the lesion studies that demonstrated the requirement for hippocampal involvement in the establishment of independent neocortical memory traces, experiments using inactivation of selected brain areas have shown that memories over time become less dependent on the hippocampus for recall and more dependent on neocortical structures, in particular the anterior cingulate cortex (Einarsson et al., 2015; Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004; Sierra et al., 2017; Wiltgen et al., 2010).

SYSTEMS RECONSOLIDATION

Systems reconsolidation was first reported by Land et al. (2000), and subsequently by Debiec et al. (2002) and Winocur et al. (2009; 2013). These studies showed that hippocampal lesions produced significant impairment of consolidated fear memories if performed immediately after memory reactivation, but not later, and not without reactivation.

An inactivation study by Einarsson et al. (2015) has also demonstrated that reactivation triggers a temporary reengagement of hippocampus. A fear memory that could be suppressed by pharmaceutical inactivation of the anterior cingulate cortex (ACC) before reactivation, became

insensitive to the same treatment for a limited time after reactivation. At six hours after reactivation, neither inactivation of the ACC or the hippocampus could suppress the memory, but simultaneous inactivation of both did suppress it. At 24 hours, ACC-dependence had returned. The interpretation is that while reactivation leaves the strength of the ACC trace intact, it results in the creation of a short-lived hippocampal trace, such that either trace alone is capable of supporting recall. However, as the lesion studies had shown, while the ACC trace remains viable during the "reconsolidation window", it is temporarily in a vulnerable state, subject to impairment if deprived of hippocampal support.

To summarize, new memories depend on the hippocampus for recall, possibly because it provides linkage between neocortical patterns whose simultaneous activation is required for recall of the memory. Subsequently, memories are made hippocampus-independent by a consolidation process that itself requires hippocampal participation. Reactivation can trigger a temporary reengagement of the hippocampus and destabilization of the cortical trace; restabilization requires hippocampal involvement.

THE MODEL

The computational model presented in the following paper demonstrates that systems-level consolidation and reconsolidation phenomena can be explained in terms of the lower-level mechanisms of synaptic consolidation and reconsolidation discussed in the first section of this dissertation. It is inspired by previous models of systems consolidation, in particular Alvarez and Squire's model from their seminal 1994 paper (Alvarez & Squire, 1994), but adds to it a more neurally plausible connection model based on potentiation and AMPA receptor exchange. This allows the model to reproduce a wider range of phenomena, including systems reconsolidation as well as a number of findings reported in lesion and inactivation studies.

CONTRIBUTION TO ORIGINAL KNOWLEDGE

This is the first computational model of systems reconsolidation to be published. It demonstrates that neurally plausible mechanisms of synaptic potentiation are computationally sufficient to explain a wide range of findings from the systems consolidation and reconsolidation literature.

The paper clearly distinguishes between three different aspects of hippocampus-dependence that are often conflated in the literature – dependence on the hippocampus for memory retrieval, for establishing a neocortical memory trace, and for recovery from retrieval-induced instability – and shows how each of them can be explained by the proposed underlying mechanisms.

A number of predictions are derived from the model, suggesting experiments that may test its correctness.

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Formal analysis: PH.
Funding acquisition: TRS.
Investigation: PH.
Methodology: PH.
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Supervision: TRS.
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Visualization: PH.
Writing – original draft: PH.
Writing – review & editing: TRS.

PAPER #2

Helfer, P., & Shultz, T. R. (submitted for publication). *A Computational Model of Systems Memory Consolidation and Reconsolidation*.

A Computational Model of Systems Memory Consolidation and Reconsolidation

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Abstract

In the mammalian brain, newly acquired memories depend on the hippocampus for maintenance and recall, but over time the neocortex takes over these functions, rendering memories hippocampus-independent. The process responsible for this transformation is called *systems memory consolidation*. However, reactivation of a well-consolidated memory can trigger a temporary return to a hippocampus-dependent state, a phenomenon known as *systems memory reconsolidation*. The neural mechanisms underlying systems memory consolidation and reconsolidation are not well understood. Here, we propose a neural model based on well-documented mechanisms of synaptic plasticity and stability and describe a computational implementation that demonstrates the model's ability to account for a range of findings from the systems consolidation and reconsolidation literature. We derive several predictions from the computational model, and suggest experiments that may put them to the test.

Keywords: memory reconsolidation; artificial neural network; AMPA receptor exchange; neural plasticity; computational model

A Computational Model of Systems Memory Consolidation and Reconsolidation

The neural processes that transform memories from short-term to long-term storage are collectively known as *memory consolidation*. They include *synaptic consolidation*, relatively rapid intra-cellular changes that stabilize synaptic potentiation, and *systems consolidation*, slower and larger-scale processes that reorganize and restructure memory traces across brain systems. Specifically, systems consolidation refers to mechanisms that gradually make memories independent of the hippocampus, a structure in the medial temporal lobe of the mammalian brain.

Whereas new memories are susceptible to disruption by a number of different types of interventions (e.g. electroconvulsive shock, certain pharmaceuticals, surgical procedures, and interference from new learning), consolidated memories are resistant to these treatments. However, retrieval of a consolidated memory can trigger a process in which it transiently becomes vulnerable to such interventions again, but subsequently restabilizes into a consolidated state. This is known as *reconsolidation*, and like consolidation it can be observed both at the synaptic and systems level. While much has been learned about the molecular underpinnings of synaptic consolidation and reconsolidation, the mechanisms responsible for the systems-level phenomena remain largely unknown [1,2].

Here, we present an artificial neural network model that includes connection dynamics based on mechanisms of synaptic plasticity and demonstrate how these low-level processes can account for systems consolidation and reconsolidation.

We begin with overviews of synaptic and systems memory consolidation and reconsolidation, and of previously published computer simulations. Next, we describe our model, report on simulation results and discuss their implications.

Synaptic Consolidation and Reconsolidation

Synaptic transmission. Neurons generate electrical signals called action potentials (APs) that travel along nerve fibers (axons) toward synapses where connections are made with other neurons. When an action potential reaches a synapse, neurotransmitter is released into the *synaptic cleft*, a narrow gap between the presynaptic *active zone* and the *post-synaptic density* (*PSD),* specialized areas of neuronal cell membrane that together make up the synapse. The neurotransmitter molecules bind to receptor proteins in the PSD, thereby triggering a response in the postsynaptic neuron. Depending on the type of neurotransmitter and the type of receptor, the response may be excitatory or inhibitory (making the postsynaptic neuron more or less likely to generate an AP), or have some other, e.g. regulatory, function. The average size of the excitatory or inhibitory response that is generated by the arrival of an action potential at a particular synapse is a measure of synaptic strength, and it depends both on the amount of transmitter released and on the numbers and types of receptors in the PSD [3,4]. Most neuroscientists believe that memories are stored in the strengths of synapses [4,5], an idea first articulated by Santiago Ramón y Cajal in the late 19th century [6] and known as *the synaptic theory of memory* [7].

Glutamate receptors. The amino acid glutamate is the most abundant neurotransmitter in the vertebrate nervous system [8]. There are several types of glutamate receptors, among which the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA receptor or AMPAR) is chiefly responsible for mediating excitatory synaptic transmission [3,9], and the N-methyl-D-aspartate receptor (NMDA receptor or NMDAR) is involved with regulatory functions including the regulation of synaptic strength [3,10,11].

Long-term potentiation. When a neuron is stimulated strongly enough to make it fire (generate an AP), participating glutamatergic synapses are strengthened by a process called long-term potentiation (LTP) [12], which is associated with an increase in the number of AMPARs inserted in the PSD [13]. There are different stages of LTP. Moderately strong stimulation gives rise to early-phase LTP (E-LTP), which lasts for at most a few hours. More intense stimulation can trigger the induction of late-phase LTP (L-LTP), which can persist for months or longer [14].

Many researchers believe that LTP as studied in the laboratory is a cellular model for learning and memory, i.e. that it replicates the synaptic changes that occur during memory formation [14–18], although conclusive proof for this has proved difficult to obtain [19,20].

Consolidation. Induction of L-LTP – but not E-LTP – is believed to require RNA translation (synthesis of new proteins), based on experiments with protein synthesis inhibiting drugs (PSIs) such as anisomycin or cycloheximide. Infusion of such drugs before or immediately after stimulation can prevent establishment of L-LTP [21–24]. However, once L-LTP has been established, a process that takes on the order of one hour, it is no longer vulnerable to PSI infusion [18,21,25].

At the behavioral level, PSI injection within the first hour after training, but not later, has been shown to cause memory impairments [26–29], suggesting that the formation of long-term memory similarly requires protein synthesis (but see Canal and Gold [30], who argue that, at least in the case of anisomycin, the memory impairments may be caused by other effects of the drug, not by protein synthesis inhibition).

Reconsolidation. Several studies from the 1940s and 1950s demonstrated that electroconvulsive shock (ECS) could interfere with the establishment of long-term memory in rodents [31–33] and in humans [34,35], but only when applied within an hour or two after

acquisition. However, in 1968, Misanin et al. reported that ECS could impair 24-hour-old, i.e. consolidated, memories of fear conditioning in rats - but only if the convulsive treatment was immediately preceded by memory "reactivation", i.e. retrieval cued by presentation of the conditioned stimulus [36]. Post-reactivation susceptibility to ECS was also demonstrated by Schneider and Sherman [37] and Lewis, Mahan and Bregman [38]. Judge and Quartermain [28] reported that injection of the protein synthesis inhibitor anisomycin, which was known to produce memory deficits when administered to mice immediately after training, could also impair older memories if given 30 minutes or less after reactivation. Przybyslawski and Sara [39] showed that the NMDA receptor antagonist MK-801 could induce memory deficits in rats that had been trained on a maze-running task, if injected up to 90 minutes after a maze run, but not after 120 minutes. The authors proposed that reactivation returns a well-established memory to a labile state from which it normally restabilizes spontaneously, and that this restabilization requires some or all of the same NMDA receptor-dependent events that are needed for consolidation of new memories. They therefore referred to the process as *memory reconsolidation* [39], a term first introduced by Spear [40]. Nader et al. [41] demonstrated that anisomycin infusion into the amygdala of rats could disrupt an established fear-conditioning memory if performed immediately after reactivation, but not six hours later. Taken together, these studies support the notion that reactivation can render a memory trace that has undergone synaptic consolidation labile, and that an NMDA-dependent process, likely involving protein synthesis, is required to subsequently restabilize it. The phenomenon, known as *synaptic memory reconsolidation*, has attracted much interest in the wake of the Przybyslawski and Sara [39] and Nader et al. [41] papers, and a large reconsolidation literature now exists [for reviews, see 42–45].

A reconsolidation-like phenomenon has also been observed at the synaptic level: protein synthesis inhibition normally does not disrupt established (i.e. several hours old) L-LTP, but if administered together with low-frequency electric stimulation, it can cause depotentiation [21,46].

The function of reconsolidation. It is generally believed that the function of post-retrieval plasticity is to permit memory modification or updating when new information is encountered [44,47,48]. Several studies with human subjects have shown that new training material is more likely to interfere with an established memory if presented after reactivation of the original memory [49–51], although some authors have questioned whether such results really are evidence of a reconsolidation process [52,53]. Post-reactivation memory updating has also been demonstrated in rodents [54,55].

The molecular underpinnings of LTP. The biochemical basis of LTP is not completely understood, but intense research efforts during the last several decades have begun to throw light on some of the underlying molecular mechanisms [4,56]. One significant discovery is that different types of AMPA receptors are of importance for the induction and maintenance of the early and late phases of LTP [57–59]. An AMPA receptor is made up of four subunits, each of which can be of several different kinds. Depending on its subunit composition, an AMPA receptor may or may not permit calcium ions to pass through the cell membrane, and is accordingly designated as calcium-permeable (CP) or calcium-impermeable (CI) [60]. E-LTP induction is characterized by a rapid increase in the number of CP-AMPARs, while the establishment of L-LTP requires insertion of CI-AMPARs [57,61–63]. This discovery is significant because the increased CP-AMPAR count is relatively short-lived, whereas an elevated level of CI-AMPARs can be sustained for a long time [57,61,63]. The ability of CI-

AMPARs to remain at the PSD for a long time has been hypothesized to be due to molecular mechanisms that traffic CI-AMPARs to the potentiated synapse and protect them against removal [64–69]. Interestingly, memory retrieval has been shown to trigger a transient reversal to a state with high CP-AMPAR and low CI-AMPAR counts [57,59], providing a potential explanation for post-retrieval synaptic instability. As previously mentioned, infusion of a protein synthesis inhibitor can interfere both with the induction of L-LTP and with restabilization of L-LTP after reactivation. A proposed explanation for this is that proteins required to transport CI-AMPARs into the synapse, and to maintain them there, need to be synthesized in order for either of these processes to occur [67].

Additional evidence supporting the notion of LTP as a memory model includes other pharmacological interventions that affect both LTP and memory. For example, inhibition of NMDA receptors blocks both LTP and fear memory acquisition [70,71], inhibition of the protein kinase PKMζ with ZIP (zeta-inhibitory peptide) disrupts both L-LTP [72,73] and long-term memory (LTM) [16,74,75], and blocking endocytosis (removal from the cell membrane) of CI-AMPARs rescues both L-LTP and LTM from these effects of ZIP [76,77]. Also, optogenetic stimulation protocols that induce long-term depression (LTD) and LTP have been shown to inactivate and reactivate, respectively, a conditioned fear response [78].

To summarize, LTP is considered a cellular model of memory, because it is compatible with the synaptic theory of memory, and although a causal relationship has proved difficult to establish, many results support the notion that the synaptic changes that characterize LTP also play an important role in the cellular mechanisms of memory.

Systems Consolidation and Reconsolidation

Consolidation. Scoville and Milner [79] famously documented that bilateral hippocampal lesions in human patients resulted in profound memory loss for past events (retrograde amnesia), as well as a near complete inability to form new long-term memories (dense anterograde amnesia). The retrograde amnesia appeared to be graded: it was most severe for the period shortly before surgery, while older memories were relatively spared. These findings have been confirmed with other human patients [80–82], and reproduced in animal studies with primates [83,84] and rodents [85,86] (although a temporal gradient is not always observed [87,88]). The observation that recent memories are more vulnerable to hippocampal damage than older ones has given rise to the notion that the hippocampus plays a crucial role in the maintenance and recall of new memories, but over time memories become hippocampus-independent [83,89–92]. The process responsible for this putative reorganization is called *systems memory consolidation* [93]. According to what is called *the standard theory of systems consolidation* [94], the hippocampus quickly records information about events in real time, then repeatedly replays them, perhaps primarily during sleep [95], thereby driving a more time-consuming process of memory trace creation in the neocortex [89,90]. A possible explanation for why it takes longer to lay down a memory trace in neocortex is that it is more sparsely inter-connected than the hippocampus, and that creation of a memory trace therefore requires axonal growth and synaptogenesis [89,96].

While the standard theory is not universally accepted [97–99], it enjoys widespread support and is compatible with a large body of empirical evidence [80,100–103].

Several neocortical regions in the frontal and temporal lobes have been identified as locations where memories consolidate [89,104–106]; among these the anterior cingulate cortex

(ACC), part of the prefrontal cortex, has received particular attention because it has been shown to play an important role for recall of remote memories in several experimental paradigms including fear conditioning, conditioned taste aversion, trace eyeblink conditioning and spatial discrimination [89,104,107–109].

Experimental methods. Much information regarding the role of the hippocampus in systems consolidation comes from lesion experiments with rats or mice, where bilateral hippocampal lesions are performed at different intervals following training. In a common type of fear conditioning, a sound or a specific spatial context (conditioned stimulus) is paired with an electric foot shock or other aversive unconditioned stimulus. Recall is subsequently tested by presenting the conditioned stimulus alone and measuring the degree of fear response. Findings from this type of study indicate that hippocampal lesions immediately after training result in severely impaired recall, but longer delays between conditioning and lesions produce gradually less severe impairments. The time interval after conditioning during which hippocampal lesions result in significant memory impairment is called the *systems consolidation window*. For fear conditioning in rodents, most studies report a consolidation window of between three and four weeks [110–114]. Longer windows have been reported for primates: months in monkeys [84], and years in humans [79,115].

Systems consolidation has also been investigated by studying the effect of reversible inactivation of specific brain areas. Studies using pharmaceutical inactivation of the rodent hippocampus and/or ACC have shown that retrieval of a fear memory is hippocampus-dependent one or three days after acquisition, but not after 28 or 30 days. At this point it has instead become dependent on the ACC for retrieval [107,116–118].

Thus results from both lesion and inactivation studies are compatible with the standard theory, and similar results have also been obtained in studies measuring brain activity during retrieval of recent and remote memories [92,104,119].

Reconsolidation. Whereas synaptic reconsolidation – the transient post-reactivation susceptibility of memories to amnestic interventions like electro-convulsive shock or administration of protein synthesis inhibitors – has been studied since the 1960s [28,36,38], systems reconsolidation – the return of hippocampus-dependence after reactivation – was first described by Land et al. in 2000 [113], and subsequently by Debiec et al. [120] and Winocur et al. [121,122]. These researchers found that hippocampal lesions produced amnesia for 30- or 45-day old fear memories if performed immediately after reactivating the memories by presenting the conditioned stimulus. Hippocampal lesions did not produce memory impairments without preceding reactivation, nor if administered after the reactivated memory was allowed 48 hours to restabilize after reactivation [120]. It thus appears that reactivation renders a neocortical memory trace unstable and that a functioning hippocampus is needed for its restabilization.

Inactivation studies have provided additional information about the effect that reactivation has on remote memories. As noted above, 30-day old fear memories are strongly dependent on ACC for retrieval. In an elegant study, Einarsson et al. [116] showed that six hours after memory reactivation, ACC inactivation no longer impaired retrieval, but after 24 hours ACC-dependence had returned. At the six-hour time point, inactivation of the hippocampus also did not affect retrieval, but simultaneous activation of both hippocampus and ACC did block recall.

Together, the findings from post-reactivation lesion and inactivation studies suggest that reactivation triggers the creation of a short-lived hippocampal memory trace that is able to support recall and is also required for restabilization of the ACC trace.

An additional interesting finding is that PSI infusion into hippocampus immediately after reactivation blocks reconsolidation when performed three [120,123], five [124], or seven [125,126] days after training. These results suggest that recovery from the destabilization caused by reactivation depends on protein synthesis in the hippocampus.

Researchers differ regarding the maximum memory age at which reactivation can trigger systems reconsolidation. Whereas the lesion and inactivation studies indicate that memories are susceptible for at least a month after training [113,116,120–122], the results from post-reactivation PSI infusion into HPC are less clear. Debiec et al. [120] were able to demonstrate reconsolidation blockade with anisomycin as late as 45 days after training, whereas Frankland et al. [123] were unable to show the effect at 36 days. The reason for this discrepancy is not clear.

An interesting clue to a connection between synaptic and systems reconsolidation is provided by Ghazal [127]: reactivation of a 1-day-old fear memory triggered a strong reduction of CI-AMPARs in hippocampus but not in ACC, whereas at 30 days the opposite was true. This result is consistent with a transition from hippocampus to ACC engagement over a systems-consolidation timeframe and with synaptic reconsolidation taking place in whichever of the two systems is engaged in the retrieval.

In summary, retrieval of a new (e.g. 3-day-old) fear memory requires the hippocampus but not the ACC. Over time, a reversal takes place so that retrieval of a 30-day-old memory requires the ACC but not the hippocampus. Reactivation of a consolidated memory temporarily returns it to ACC-independence for retrieval. Systems consolidation (establishment of a

neocortical trace) and systems reconsolidation (restabilization after reactivation-induced destabilization of the neocortical trace) both require hippocampal involvement.

Model

Based on the findings described in the foregoing, we here present a model of systems memory consolidation and reconsolidation. Although the role of LTP in learning and memory remains conjectural, for the purpose of modeling we assume that LTP – or an LTP-like mechanism – is the cellular substrate of memory and investigate to what extent this type of synaptic mechanism can explain the systems-level phenomena.

Below we provide a conceptual description of the model; the computational implementation is described in the Methods section.

Synaptic level

- Moderately intense stimulation induces E-LTP, which involves the rapid insertion of CP-AMPARs. Constitutive processes subsequently remove these within hours.
- More intense stimulation sets in motion L-LTP induction (synaptic consolidation) which involves a state change in a bistable mechanism (molecular switch). When in the ON state, this mechanism maintains a high CI-AMPAR count in the synapse; when the switch is OFF, the CI-AMPAR count drifts towards the basal level characteristic of the unpotentiated synapse.
- Memory retrieval abruptly removes CI-AMPARs from the synapse and replaces them with CP-AMPARs, thus returning the synapse to an E-LTP-like state. The subsequent restoration of L-LTP is protein-synthesis-dependent and requires NMDAR activity, i.e. neural stimulation.

Systems level

- Stimulus presentations trigger patterns of activation in multiple ensembles of neurons in the neocortex (NC). These active neurons in turn project onto and activate neurons in the hippocampus (HPC), where a memory trace is quickly created, providing linkages between the activated NC ensembles. Linkages are also created through prefrontal cortex, in particular the ACC, but these connections are initially too weak to support memory retrieval without HPC support.
- Subsequently, the HPC memory trace is spontaneously and repeatedly reactivated which causes stimulation of these same NC neural ensembles through nerve fibers projecting back from the HPC to the NC. Over time, the repeated reactivation of the NC neural ensembles strengthens the ACC linkages, eventually to a point where they can support retrieval of the memory without assistance from the hippocampus.
- Meanwhile, the HPC trace is gradually weakened by constitutive decay processes [128,129].
- If a consolidated memory is reactivated by a reminder, then activity in the neocortical neural ensembles triggers re-establishment of the HPC linkage. Simultaneously, the retrieval causes transient destabilization of the synapses in the ACC linkage (synaptic reconsolidation).
- Following reactivation, hippocampal replay stimulates the now destabilized synapses of the ACC linkage. This activity drives restabilization of these synapses. Meanwhile, the reactivated HPC trace rapidly decays, leading to a return to ACC-dependence in 24 hours or less.

Temporal characteristics

The temporal characteristics of systems consolidation and reconsolidation provide some clues to the underlying mechanisms:

The reconsolidation window is shorter than the consolidation window.

Hippocampal lesion after training produces a memory impairment, more severe the shorter the delay between conditioning and lesion. Similarly, hippocampal lesion after reactivation also causes more severe impairment if performed early. However, the time scales are very different. New memories are vulnerable for at least several weeks after training [84,86,111,115], whereas the post-reactivation window of vulnerability only lasts for a few days [120]. Our model attributes this difference to the different natures of the processes being interrupted by HPC lesioning in the two scenarios. In the case of consolidation, what is being interrupted is the gradual and relatively slow establishment and strengthening of intra-neocortical connections; thus the earlier the intervention is performed, the weaker the partially consolidated memory trace will be. Reconsolidation blockade, on the other hand, interrupts the much faster process of re-stabilization of destabilized neocortical synapses. The earlier in the reconsolidation window hippocampal lesion is performed, the fewer synapses will have had time to restabilize, leading to more severe memory loss.

Memories become transiently ACC-independent after reactivation.

Whereas a consolidated fear memory depends strongly on the ACC for recall, reactivation triggers a brief period of ACC-independence, such that six hours after reactivation ACC inactivation has little or no effect on retrieval, but 18 hours later full ACC dependence has returned [116]. This finding suggests that reactivation

> triggers the creation of a short-lived hippocampal memory trace that is able to support recall six hours later, but not 24 hours later. For a memory trace to last for six hours or more, it must have undergone synaptic consolidation, yet 24 hours is a short lifetime for a consolidated trace – compare the situation after initial acquisition, where the hippocampal trace is able to support recall for at least several days. The reason why a post-reactivation hippocampal trace is so short-lived is not known. A possible explanation may be that memory retrieval activates an unidentified signaling pathway that accelerates depotentiation in the affected HPC synapses.

Computational Modeling

Several artificial neural network (ANN) models have simulated hippocampal-neocortical interaction [90,96,130]. These models all demonstrate how spontaneous reactivation of hippocampal traces can strengthen neocortical connections and are thus able to capture aspects of systems consolidation. However, a computational model of systems reconsolidation has not yet been published. Below, we present a computational implementation of the previously described model, and show that it is capable of reproducing both systems consolidation and reconsolidation, including effects of pharmaceutical and surgical interventions. The key to this capability is a more detailed connection design than is traditionally used in neural networks, Specifically, our connections simulate the AMPA receptor exchanges underlying synaptic consolidation and reconsolidation.

Methods

Simulation Targets

Table 1 summarizes the empirical findings described in the introduction, which our model aims to reproduce in simulations.

Table 1: Simulation targets

	Result	Description
1	Retrieval is HPC-dependent and ACC-independent at 3d [116,119,127,131,132].	Retrieval of a three-day old memory is impaired by HPC inactivation but unaffected by ACC inactivation.
2	Retrieval is ACC-dependent and HPC-independent at 30d [107,116,119,131,127].	Retrieval of a 30-day old memory is impaired by ACC inactivation but unaffected by HPC inactivation.
3	PSI during conditioning impairs LTM but not STM [29,132,124].	Systemic injection of PSI during training prevents LTM induction – but does not impair STM .
4	PSI infusion during maintenance does not cause memory impairment [29,132].	Systemic PSI injection does not impair a consolidated memory.
5	HPC lesion at 3d causes memory impairment [111,113].	HPC lesion at 3 days or less after training causes later recall to be severely impaired compared to non-lesioned animals.
6	HPC lesion at 30d does not cause memory impairment [120,111,113].	HPC lesion, when not preceded by memory reactivation, does not result in subsequently impaired recall.
7	Reactivation alone does not impair a consolidated memory [113,120].	Reactivation without subsequent HPC lesion or PSI infusion does not by itself impair a consolidated memory.
8	Reactivation + PSI causes memory impairment [120,123,124,125,126].	Reactivation immediately followed by PSI infusion into HPC causes impairment of a consolidated memory.
9	Delayed impairment effect of post-reactivation PSI infusion in HPC [120,124].	The impairment caused by post-reactivation PSI infusion in HPC does not manifest in retrieval test soon after lesion (4h) but only later (24h).
10	Reactivation + HPC lesion causes memory impairment [113,120,121].	Reactivation immediately followed by HPC lesion causes impairment of a consolidated memory.
11	Graded effect of post-reactivation HPC lesion [120].	The severity of memory impairment caused by post-reactivation HPC lesion diminishes with increasing reactivation-lesion delay.
12	Retrieval can be supported by either ACC or HPC 6h after reactivation [116].	Six hours after reactivation, neither ACC inactivation nor HPC inactivation alone impairs retrieval, but simultaneous inactivation of both does block retrieval.

Network Architecture

Like other artificial neural networks, ours consists of units and connections, where units are analogs of biological neurons (or ensembles of neurons) and connections model synapses.

Topology. We use a recurrent artificial neural network with four regions representing hippocampus (HPC), anterior cingulate cortex (ACC) and two sensory cortex areas, SC0 and SC1, to which stimuli are presented. Each region consists of 25 units. Each HPC and ACC unit is bidirectionally connected to all units in the other three regions, see Figure 1.

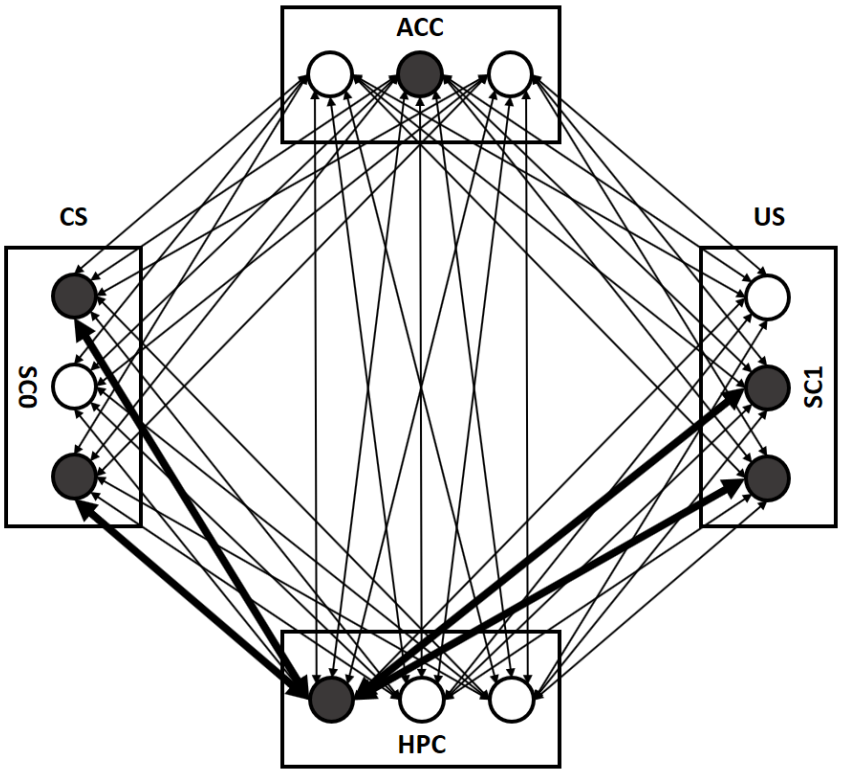

Figure 1: Network architecture. To reduce clutter only three of the twenty-five units in each region are shown. Each double-headed arrow represents two independent connections, one in each direction, between a pair of units. The diagram illustrates the state after initial acquisition: presentation of the unconditioned stimulus (US) and

conditioned stimulus (CS) has activated some units in SC0 and SC1 (filled circles) and fast learning has created strong linkages (bold lines) through HPC. Linkages through ACC are still weak.

Units. The units are bistable and stochastic; the probability that a unit will be active at any time *t* in the simulation is an asymmetric sigmoid function of net input,

$$P_j(t) = \frac{1}{1 + e^{-\frac{net_j(t)}{T}}} \quad (1)$$

where $net_j(t)$, the net input to unit *j* at time *t*, is the sum of the activity levels of units connected to unit *j*, weighted by inbound connection strengths.

Connections. The connections are abstract models of glutamatergic synapses, characterized by four variables:

- *capacity*: the maximum number of AMPARs that can be inserted, roughly equivalent to PSD size or number of receptor "slots" in the PSD.
- *numCpAmpars*: the number of currently inserted CP-AMPARs
- *numCiAmpars*: the number of currently inserted CI-AMPARs
- *isPotentiated*: a Boolean attribute that models the bistable nature of L-LTP

This combination of binary and continuously variable attributes makes it possible to model synapses where potentiation behaves like a bistable switch [66,134,135], yet synaptic strength is variable with many distinct levels [136].

Stimulation causes an increase of the *capacity* attribute, allowing more AMPARs to be inserted. A connection's weight at any moment is proportional to its total number of inserted AMPARs (*numCpAmpars* + *numCiAmpars*). The set of connections between any two regions, e.g. from HPC to SC1, is referred to as a *tract*.

Simulation

A simulation consists of a sequence of time steps. Various interventions may be scheduled for any time point during the simulation, and in addition several background processes execute at each time step. The scheduled event types are training, reactivation, HPC lesion, HPC inactivation and ACC inactivation. The background processes are consolidation, AMPAR trafficking and random depotentiation. In addition, a retrieval test can be executed at any time. The different interventions and background processes are described in the following.

Learning rule. The network learns activation patterns by a Hebbian learning rule [137] that increases the capacity of connections between simultaneously activated units, asymptotically towards a maximum value:

$$c_{ij}(t+1) = c_{ij}(t) + \mu(C_{\max} - c_{ij}(\mathrm{t})) \qquad (2)$$

where $c_{ij}(t)$ is the capacity of the connection between units i and j at time t, C_{max} is the maximum connection capacity (a global constant) and μ is a learning rate specific to the tract that connection ij belongs to.

Capacity growth is followed by an increase in the number of CP-AMPARs such that the total AMPAR count becomes equal to the connection capacity. This models the rapid CP-AMPAR influx during E-LTP induction. In addition, probabilistic induction of L-LTP in a connection is simulated by turning on its *isPotentiated* attribute with a probability that depends on the strength of the stimulation.

Learning takes place (a) when stimuli are presented for training, (b) at memory retrieval (reactivation), and (c) when patterns are spontaneously activated by the memory consolidation process. Descriptions of these mechanisms follow:

Training. To train an association, subsets of units in SC0 and SC1 representing an unconditioned stimulus, US, and a conditioned stimulus, CS, respectively, are activated. The network randomly selects and activates linkage units in HPC and ACC and then applies the learning rule to all connections that connect two simultaneously active units, i.e. connections that are in the Hebbian condition. The learning rate is defined to be relatively high in HPC, allowing rapid creation of linkages strong enough to support recall. The ACC learning rate is lower, hence linkages through the ACC are not strong enough to independently support recall immediately after training.

Retrieval. To test recall of a trained pattern, the CS units are activated in SC0, and the network is cycled by repeated application of the activation function in all units. The activity pattern that the SC1 region then settles on may be compared to the associated US pattern to calculate a recall test score.

Systems consolidation. At every simulation time step a randomly selected trained pattern is activated in HPC, after which the entire network is cycled in the same manner as for recall test (but without stimulus presentation). Whatever pattern the network settles into is then reinforced by application of the learning rule. Because the network is more likely to settle into trained patterns than other random states, this will tend to strengthen CS-US linkages through the ACC, eventually making recall of trained patterns HPC-independent.

AMPAR trafficking. At each time step, AMPAR trafficking is simulated by adjusting the numbers of AMPARs in all connections according to the following rules: (1) *numCpAmpars* declines exponentially towards zero, simulating CP-AMPARs' limited dwell time at the synapse. (2) If a connection's source and destination units are both active (Hebbian condition) and its *isPotentiated* attribute is true, then *numCiAmpars* grows asymptotically towards the number of

available slots in the connection (*capacity*), simulating activity-dependent trafficking of CI-AMPARs into a potentiated synapse, otherwise *numCiAmpars* also declines exponentially, simulating a depotentiated synapse's gradual return to baseline.

Depotentiation. Potentiated connections are subject to random depotentiation. This happens with higher probability in HPC than in the neocortical regions, modeling the observed faster decline of hippocampal traces over time.

Reactivation. Reactivation is modeled as an unreinforced CS presentation, i.e. a cued retrieval. The CS pattern is activated in the SC0 region, the network is cycled, and when it settles, AMPAR exchange is simulated in all connections between simultaneously active units (i.e. connections in the Hebbian condition): *numCiAmpars* is reduced to a configured minimum, and *numCpAmpars* is set to its maximum allowed value *(capacity – numCiAmpars).* This puts the ACC linkage connections in an unstable E-LTP-like state, modeling the post-reactivation instability documented in empirical studies. A randomly selected set of HPC linkage units is then activated, in the same manner as during initial acquisition, and a round of Hebbian learning takes place.

As noted in the introduction, the hippocampal engagement is much briefer after reactivation (less than 24h) than after initial training, when HPC can support recall for at least three days. The mechanism underlying this faster disengagement is not known. One possibility is that memory retrieval activates a signaling pathway that accelerates random depotentiation in the HPC links. To simulate such a mechanism, the probability of depotentiation is transiently increased in HPC connections that are potentiated at reactivation, and then decays exponentially back to its base value.

Hippocampal lesion. Hippocampal lesion is simulated by disconnecting the HPC region from the simulation.

PSI infusion. Infusion of PSI into a region – HPC or ACC – is simulated by disabling potentiation and CI-AMPAR insertion for nine hours of simulation time, corresponding to the amount of time that the protein synthesis inhibitor anisomycin remains active in brain tissue [138].

Inactivation. Reversible inactivation of HPC or ACC is modeled by transiently disabling activation of all units in the HPC or ACC region, respectively.

Simulation environment

The model is implemented as a C++ program and all simulations were executed on an Intel i5-2400 computer running the Debian Linux 8.4 operating system.

Model fitting

Simulations are controlled by the parameters indicated in the following table. Parameter values were manually chosen to make simulation time courses approximate those reported in the referenced literature. The "HPC" and "ACC" columns contains values for connections in the corresponding sub-networks.

Name	HPC	ACC	Description
acqLearnRate	0.2	0.01	Learning rate per training cycle for acquisition
consLearnRate24h	0.0	0.01	Learning rate per 24h of consolidation
psdDecayRate24h	0.2	0.2	PSD shrink rate when unpopulated
cpAmparRemovalRate01h	0.1	0.1	Constitutive removal rate for CP-AMPARs
ciAmparInsertionRate1h	2.0	2.0	CI-AMPAR insertion rate in potentiated synapse
ciAmparRemovalRate24h	0.3	0.3	CI-AMPAR removal rate in unpotentiated synapse
baseDepotProb01h	0.002	0.0	Probability of spontaneous depotentiation
maxE3DepotProb01h	0.05	0.05	Prob. of depotentiation induced when E3 is at max level

Results

After training the network with a CS-US association, recall is tested by presenting the CS in the SC0 region and comparing the resulting activation pattern in the SC1 region with the trained US (a score of 1.0 indicates a perfect match). The descriptions below compare the recall scores in simulation runs where an intervention (lesion or inactivation) is performed with simulation runs without the intervention (labeled "baseline" in the diagrams). The score values in all diagrams are means of 100 simulation runs. Error bars indicate standard deviation.

Consolidation and reconsolidation windows. HPC lesions produce memory deficits when performed in the consolidation or reconsolidation windows, but not otherwise. See Figure 2 and Figure 3. These simulations reproduce findings 5-7 and 10-11 of Table 1.

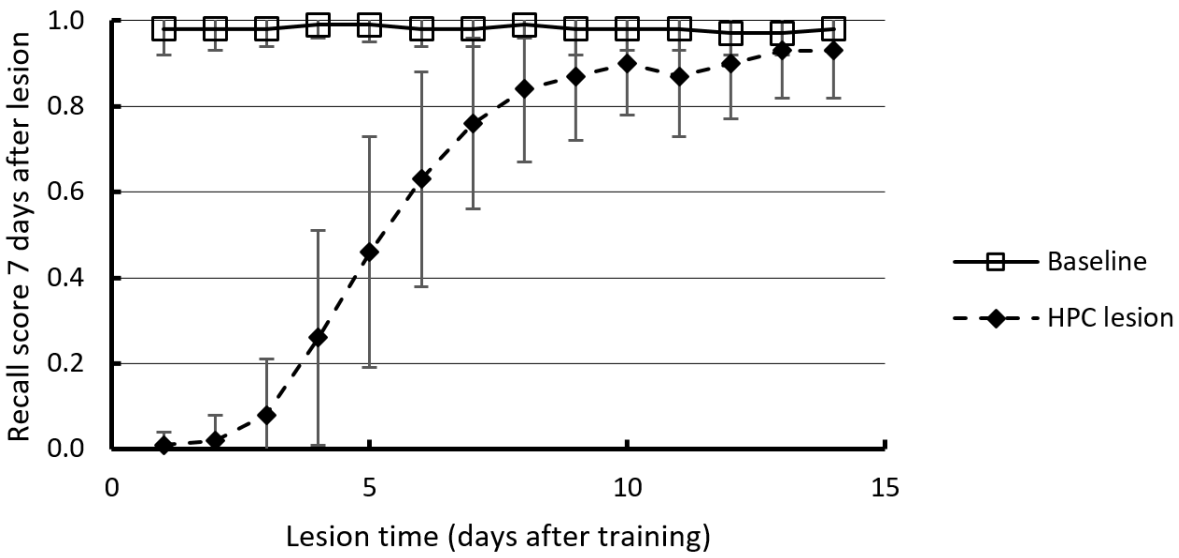

Figure 2: Consolidation window. Simulated HPC lesions produce severe impairment when performed shortly after training, but not later. Recall tests are performed 7 days after lesioning, e.g. the data point corresponding to lesions performed 5 days after conditioning reflects recall tests executed on day 12. This simulates delays used in behavioral experiments to allow the animals to recover from surgery before testing. The

baseline data points reflect recall tests at the corresponding time points, but without preceding lesions.

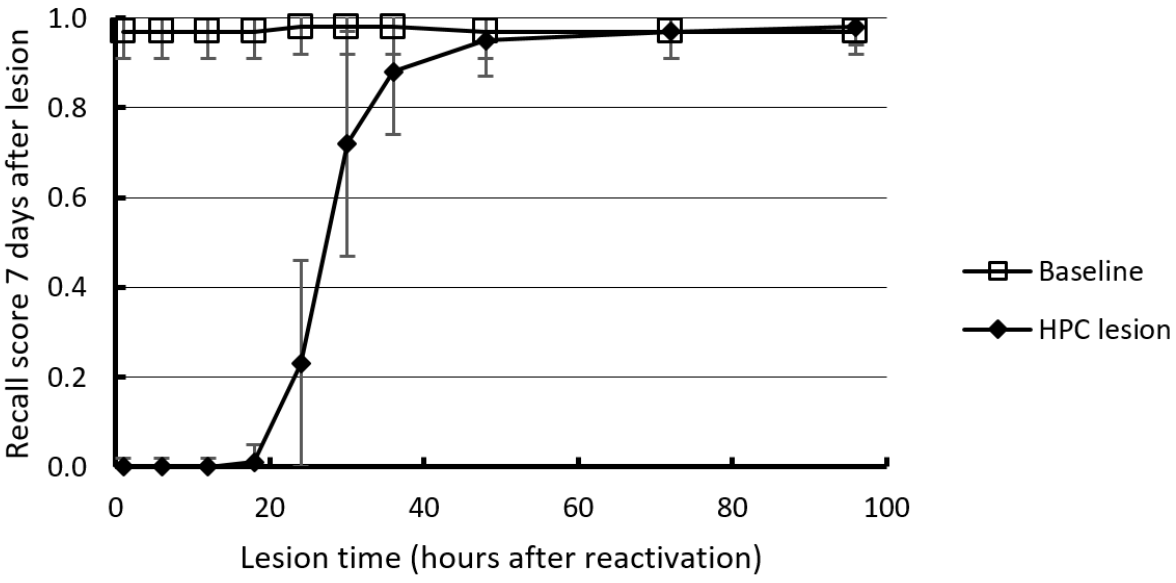

Figure 3: Reconsolidation window: Simulated HPC lesions produce severe impairment when performed shortly after reactivation, but not later. Recall tests are performed 7 days after lesioning, i.e. the data points corresponding to lesions performed 0-23 hours after reactivation reflect recall tests executed on day 7 after reactivation, etc. The baseline data points correspond to recall tests at the corresponding time points, but without preceding lesions.

Effect of systemic PSI infusion before training. Systemic PSI infusion before conditioning impairs formation of long-term memory but does not impair short-term memory, see Figure 4. This simulation reproduces finding 3 of Table 1.

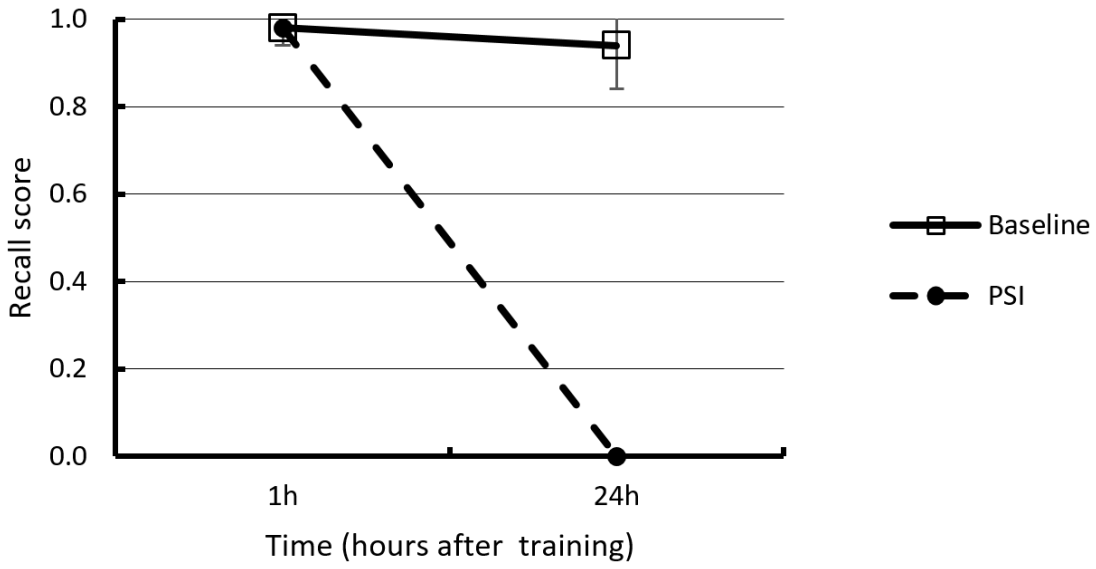

Figure 4: The effect of systemic PSI infusion before training. Short-term memory (1h) is unaffected, but long-term memory (24h) is severely impaired.

Effect of PSI on consolidated memory. Systemic PSI infusion during maintenance, i.e. after completed systems consolidation, does not impair subsequent memory retrieval, see Figure 5. This simulation reproduces finding 4 of Table 1.

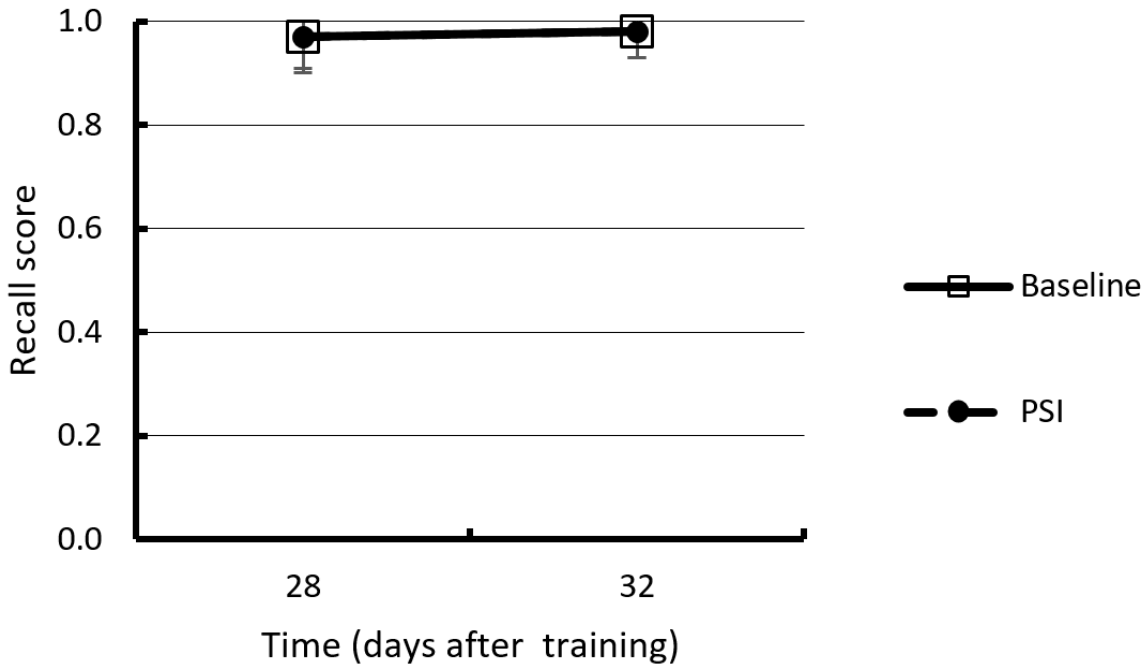

Figure 5: Effect of PSI on consolidated memory. Systemic PSI infusion is simulated on day 30 after conditioning. The diagram shows recall performance before and after the intervention. PSI infusion in the maintenance phase does not affect recall performance.

PSI infusion in HPC after reactivation. Post-reactivation PSI infusion in HPC does not cause immediate memory loss. Rather, the recall impairment develops over a period of more

than 4 but less than 48 hours [120], see Figure 6. This simulation reproduces findings 8 and 9 of Table 1.

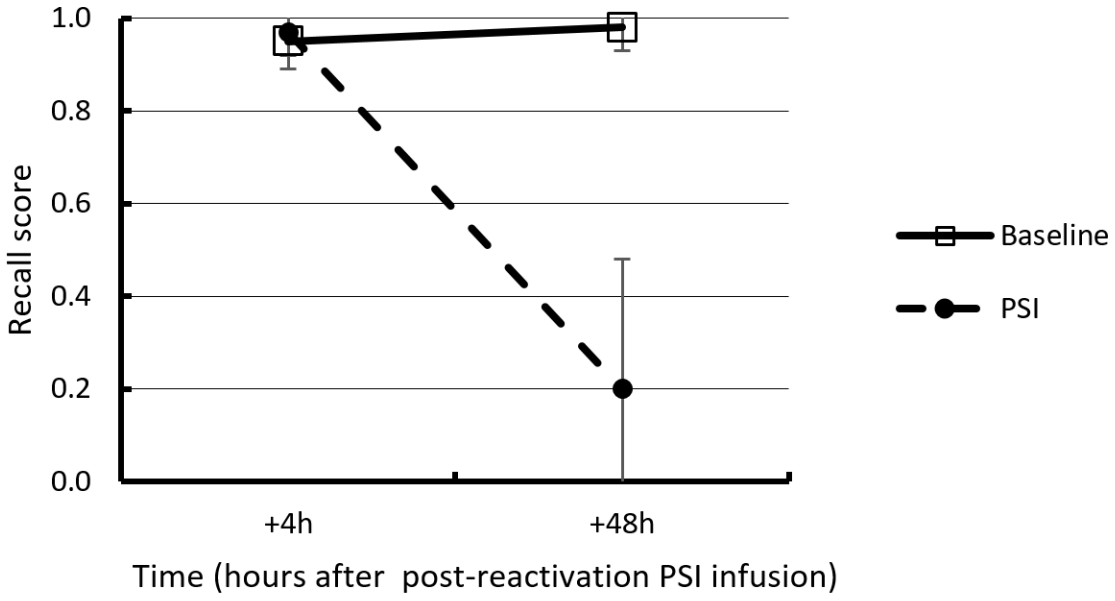

Figure 6: PSI infusion in HPC after reactivation. PSI is infused immediately after reactivation; recall tests are performed four hours later or 48 hours later. The impairment only manifests at the later time point.

HPC/ACC-dependence for recall. Consolidation transforms memories from being HPC-dependent to being ACC-dependent for recall, see Figure 7. These simulations reproduce findings 1 and 2 of Table 1.

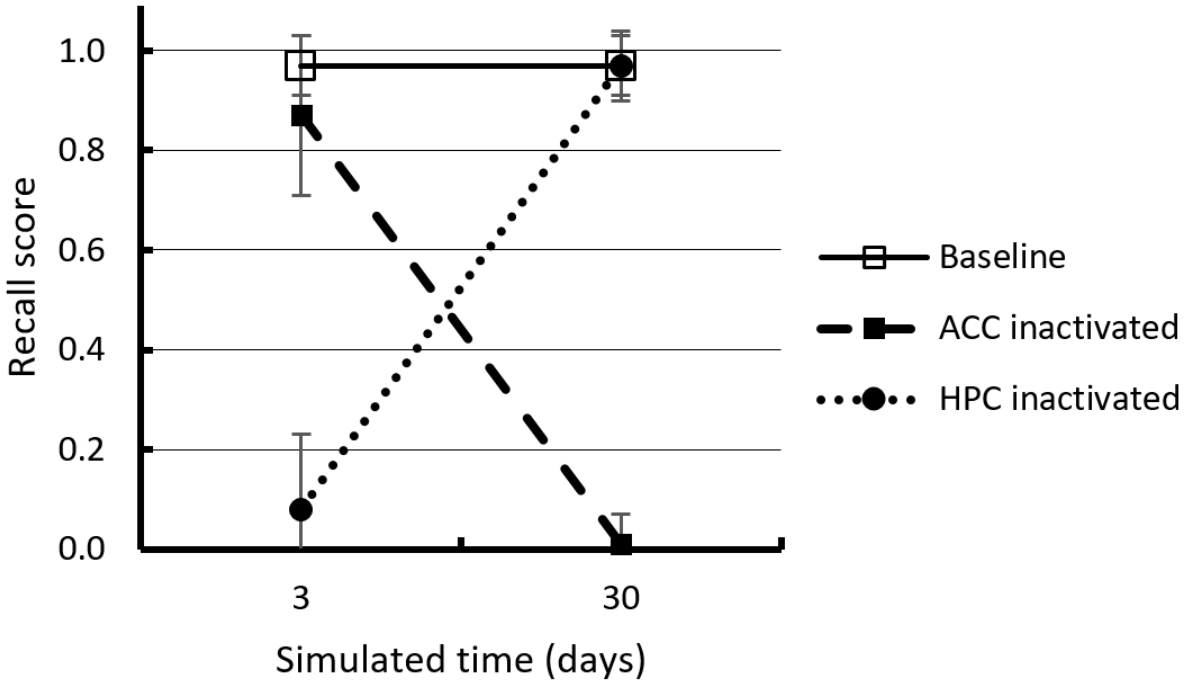

Figure 7: HPC/ACC-dependence for recall. HPC inactivation impairs recall 3 days after training, but not at 30 days. ACC inactivation does not affect recall 3 days after training, but causes severe impairment at 30 days.

Temporary ACC-independence after reactivation. Reactivation creates a transient HPC linkage which temporarily returns the memory to ACC-independence, see Figure 8. These simulations reproduce findings 12 and 13 of Table 1.

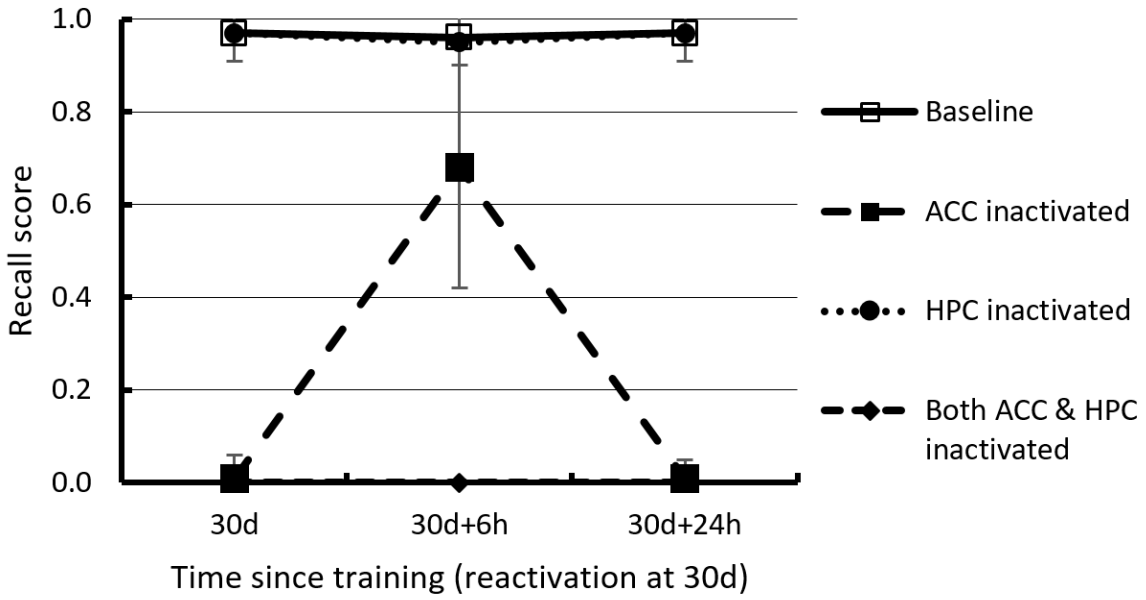

Figure 8: Temporary ACC-independence after reactivation. Before reactivation ACC inactivation severely impairs recall of a consolidated memory. Six hours after reactivation neither ACC inactivation nor HPC activation produces significant impairment. At 24h after reactivation, ACC dependence has returned.

Discussion

We have presented an artificial neural network model of systems memory consolidation and reconsolidation that accounts for a broad range of findings from the literature, including those from studies employing hippocampal lesions as well as ones using reversible inactivation of the hippocampus or anterior cingulate cortex. At the core of the model is a new connection design in which variable stability arises from simulation of receptor exchanges that have been observed in glutamatergic synapses.

It is worth noting that although the term “reconsolidation” suggests a recapitulation of consolidation, the model reflects our view that the two processes are quite different. Whereas systems consolidation is a gradual strengthening of the intra-neocortical connections that link

together the components of a memory, systems reconsolidation consists in the restoration of stability in such synapses following reactivation-induced destabilization. HPC lesions in the "consolidation window" (the first couple of weeks after training) and in the "reconsolidation window" (the first day or two after reactivation) both result in memory deficits – but for different reasons: HPC lesions in the consolidation window prematurely interrupts the hippocampal replay that drives establishment and strengthening of neocortical linkages, leaving a weak memory trace there. Loss of HPC in the reconsolidation window, in contrast, deprives neocortical neurons of the HPC stimulation that is needed to restabilize synapses that have been destabilized by reactivation-induced AMPAR exchange. Without such stimulation, the destabilized neocortical trace decays.

Our model is the first to demonstrate results analogous to both systems consolidation and systems reconsolidation in an artificial neural network, and also to reproduce the effects of a number of experimental interventions described in the empirical literature. This was made possible by the use of a connection model with a plasticity mechanism inspired by biological synapses.

In closing, we list a number of predictions that have been derived from the model and may be used to test its validity in future experiments.

Predictions

1. The model predicts that if reactivation is prevented from triggering AMPA receptor exchange in the ACC, then HPC lesion in the reconsolidation window will not impair recall. This could be tested by infusing a drug like $GluA2_{3Y}$ into the ACC before reactivation. $GluA2_{3Y}$ is a synthetic peptide that prevents endocytosis (removal) of CI-AMPARs from the synapse. In contrast, $GluA2_{3Y}$ should not be able to prevent the amnestic effect of hippocampal lesion

during the consolidation window, because in this case the impairment is not due to depotentiation but to interrupted consolidation.

2. For the same reason (destabilization depends on CI-AMPAR endocytosis), the model predicts that $GluA2_{3Y}$ infusion in ACC before reactivation would abolish the amnestic effect of post-reactivation PSI infusion in hippocampus.
3. Destabilization can also be prevented by selectively inhibiting GluN2B-containing NMDA receptors [10,139], cannabinoid receptor 1, or L-type voltage gated calcium channels [27]. The model predicts that inhibiting any of these receptors or channels in ACC during reactivation would reduce or eliminate the amnestic effect of post-reactivation hippocampal lesion or PSI injection.
4. The model attributes the short duration of post-reactivation ACC-independence to accelerated depotentiation of linkage synapses in hippocampus. Blocking AMPAR endocytosis in hippocampus, e.g. by local $GluA2_{3Y}$ infusion, should therefore lengthen the duration of post-reactivation ACC-independence.
5. If, as in our model, post-reactivation hippocampal lesion or PSI infusion causes memory impairments by depriving neocortical linkage synapses of the stimulation required to restabilize, then prolonged (several days) reversible inactivation of hippocampus should have the same effect.
6. In the model, all that is needed for restabilization of a reactivated memory trace is repeated activation of its ACC linkage. This suggests that it may be possible to compensate for an inactivated hippocampus (as in prediction 5) by triggering reactivations externally, i.e. by reminders. This idea is related to a result reported by Lehmann et al. [140], where repeated

conditioning sessions were shown to significantly speed up the development of hippocampus-independence.

Data availability statement

All computer program files are available from the ModelDB database, accession number TBD (http://modeldb.yale.edu/TBD).

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DISCUSSION

A scientific model aims to make some aspect of reality easier to understand by explaining empirical findings in terms of hypothetical underlying relationships which, while not empirically demonstrated, adhere to commonly accepted knowledge.

A model may be conceptual, consisting of verbal descriptions, perhaps augmented by graphical representations. While such models are useful for reasoning about explanations for observed phenomena, a mathematical model is a more powerful tool in that it enforces explicit expression of relationships and specification of parameter values, and can be tested to determine to what extent it is able to account for empirical results. A computational model is an implementation of a mathematical model that facilitates experimentation, exploration of parameter spaces, etc. This allows the modeler to evaluate how accurately the model reproduces empirical findings. In addition, computer simulations may exhibit behaviors not previously observed in the real-world system, which can be used to generate predictions for future experiments and to inspire new hypotheses. It is interesting to reflect on how these qualities of computational modeling are exemplified by the two models presented here.

The synaptic model simulates molecular reactions in a dendritic spine head, using the Gillespie algorithm for stochastic simulation of chemical reactions (Gillespie, 1977). It incorporates a set of molecular reactions proposed to explain the induction and maintenance of late long-term potentiation (L-LTP). L-LTP has a switch-like behavior: a brief stimulus can trigger a synapse to switch from the unpotentiated to the potentiated state and remain there for long periods of time, perhaps indefinitely. Empirical studies have indicated that the protein kinase PKMζ plays an important role in L-LTP, and there is evidence of a signaling pathway

through which PKMζ can lift the repression of mRNA translation (Westmark et al., 2010). This has suggested a conceptual model in which the switch-like behavior of L-LTP is due to a positive feedback loop in which PKMζ facilitates its own synthesis (Sacktor, 2011).

Any attempt to develop this idea into a quantitative model of PKMζ maintenance in L-LTP soon runs into the issue that a simple positive feedback loop does not in general produce a bistable system. (L-LTP is bistable: a synapse is stable both in the potentiated and unpotentiated states.) The reason why a simple molecular feedback loop cannot produce a bistable system is that chemical reaction rates are governed by the law of mass action, i.e. they are proportional to the products of concentrations of reactants, and a linear positive-feedback loop can only produce a monostable system (Tyson, Chen, & Novak, 2003). In a monostable synapse, either only the unpotentiated state would be stable, so that the synapse would depotentiate as soon as the stimulation ceases, or only the potentiated state would be stable, so that the slightest perturbation would irreversibly potentiate the synapse. Such synapses would not be able to store information. We thus have a situation where the conceptual model at first glance appears to explain the observed phenomenon (positive feedback enables long-lasting potentiation), but mathematical modeling reveals that a more elaborate explanation is required.

Previous computational models of PKMζ maintenance in L-LTP (Jalil, Sacktor, & Shouval, 2015; Smolen, Baxter, & Byrne, 2012; Zhang, Smolen, Baxter, & Byrne, 2010) have addressed this issue by postulating a non-linear ("ultrasensitive") relationship between mRNA translation rate and PKMζ concentration. While such nonlinearity can explain bistability, there is no evidence that the PKMζ – mRNA signaling pathway involves any ultrasensitive dependencies.

In our model of L-LTP, bistability is achieved without invoking ultrasensitivity, thanks to a second positive feedback loop that incorporates the attachment of PKMζ to GluA2-containing AMPA receptors. The motivation for modeling this relationship was to be able to reproduce findings involving PKMζ inhibition with ZIP and endocytosis blockade with $GluA2_{3Y}$, which had not been covered by previous models. The realization that it could also explain bistability without the need for ultrasensitivity provides an interesting example of how modeling can aid the discovery process.

The objective of the second model was to investigate whether systems reconsolidation can be explained in terms of LTP-like synapse-level processes. Previous models had reproduced systems consolidation, but not reconsolidation, using traditional neural networks where connections are described by a single attribute, strength. To model reconsolidation, a connection model was needed that could additionally represent synaptic stability. This was accomplished by modeling the AMPA receptor exchanges that have been observed in LTP. The resulting system demonstrates that both systems consolidation and reconsolidation can be explained in terms of low-level synaptic processes. In addition, a significant number of findings from lesion and inactivation studies are reproduced. The paper includes several predictions that may be used to test the model's correctness.

The two models may be said to form part of an explanatory hierarchy in that the first investigates the mechanisms underlying LTP and the second explores the potential role for LTP-like processes in explaining higher-level memory phenomena.

CONCLUDING REMARKS

The objective of the modeling work presented here was to investigate the plausibility of mechanisms that have been proposed to explain memory phenomena. At the synaptic level, I

asked whether a mathematical formulation of the PKMζ hypothesis would produce a computationally feasible explanation for the induction and maintenance of late-phase LTP, and at the systems level, whether an LTP-like model of synaptic plasticity could be shown capable of explaining systems consolidation and reconsolidation. In each case the model provided an affirmative answer to the question posed, and was able to reproduce, and thus explain, more empirical findings than previously published models have done. Furthermore, a number of testable predictions have been generated. The confirmation or refutation of these predictions will be valuable in guiding future modeling work.

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